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Combining natural enemies and selective pesticides in IPM programmes of exotic pests: the *Tuta absoluta* (Lepidoptera: Gelechiidae) case

Final dissertation

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"Human beings are not in control of nature,
but simply one of its parts.
The survival of one part depends
on the health of all in the web of life"

Rachel Carson

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Chapter I

1. Introduction

1. Introduction

1.1. Invasive species

Alien species are defined as entities whose presence in a region is attributable to human actions that enabled them to overcome fundamental geographical barriers (synonyms: exotic and nonnative species) (Pyšek and Richardson 2010). In the past, the geographical barriers had limited the entrance of exotic species, but they have been made mostly ineffective by commercial traffic, making climate mismatches the only natural barrier against the global spread of invasive species (Tatem and Hay 2007). Exotic organisms can be plants, animals, as well as other organisms, and they are generally unintentionally introduced *via* plant material or other goods imported from other geographic areas where the species is either native or a previous invader (Hanafi 2005). However, not all non-indigenous species are harmful. In fact the majority of species exploited in agriculture, forestry and fisheries are alien species (Wittenberg and Cock 2001).

A modest amount of alien species form self perpetuating populations in the new region; among these, a subset spreads, or has the capacity to spread, over substantial distances from the introduction sites. Depending on their status within the naturalization-invasion continuum, alien species may be termed casual, naturalized, or invasive (McNeely et al. 2001). Introductions of individuals in remote geographical areas can, in certain circumstances, lead to invasions, i.e., to their establishment, increase in number and geographical spread. Reasons for success or failure of such introductions remain unclear and constitute a significant research area in invasion biology (Kolar and Lodge 2001). The impacts of invasive species are sometimes rapid and dramatic, especially where they result in the transformation of ecosystems (Pimentel 2000).

The problem of alien species has intensified in the last few years, making it a serious challenge to tourism and to globalized trade transport, especially in the cases of trading of live animals, horticultural and raw animal products. These have dramatically enhanced the spread of invasive species over the past century, allowing them to surmount natural geographic barriers, even at long distances (Wittenberg and Cock 2001). Indeed, the increased spread of invasive species reflects the rapid globalization and trade liberalization (Evans 2003).

These organisms, non native to a given ecosystem, cause, or are likely to cause, economic or environmental injury or harm to human health (National Invasive Species Council of USA 1999). Indeed, they represent a serious global threat to both natural (Samways 2007) and agronomic (Haack et al. 2010) ecosystems. Invading species demand both (i) an ecological cost, by the reduction in local biodiversity particularly endemic species, owing to the absence of their coevolved natural enemies in the new environment and to their superior competitive ability (Clavero and Garcìa-Berthou 2005, Paini et al. 2008); and (ii) an economic cost mainly by damage to cropping systems (Vitousek et al. 1996).

The economic and ecological effects of invasive species, notably pests, are now widely recognized and the full range of economic costs of biological invasions goes beyond the immediate impacts on the affected agricultural producers (Mack et al. 2000, Olson 2006, Desneux et al. 2011). Invasive pests often include secondary and tertiary economic effects, such as shifts in consumer demands, changes in the relative prices of inputs, loss of important local biodiversity, and other natural resource and environmental amenities (Evans et al. 2002). The range of economic impacts can be broadly classified into two categories: direct and indirect impacts. The direct impacts reflect the effects of the particular pest or disease on the host, whereas the indirect impacts are non host-specific. The latter would be the general effects that are generated by the presence of a pest but not specific to the pest-host dynamics that could affect public health issues (such as compromising key ecosystem functions); general market effects (including possible changes in consumers' attitudes toward a given product); research requirements; market access problem and impacts on tourism and other sectors of an economy (Bigsby and Whyte 2001).

In agriculture, invasive species can notably reduce yields and increase the costs related to their management, generally resulting in increased reliance on pesticides (Desneux et al. 2011, Haack et al. 2010, Suckling and Brockerhoff 2010), which can disrupt previously developed Integrated Pest Management (IPM) systems (Thomas 1999, Pimentel 2000, Ragsdale et al. 2011). About one-third of insect pests in worldwide cropping systems are alien species (Ward and Masters 2007), and their economic impact has been considerable (Pyšek et al. 2008). Moreover, the establishment of an invasive exotic species has the potential to cause cascading ecological impacts that may extend into natural systems as well (Williamson 1996).

In addition to negative effects on human well-being (notably agricultural practices and food safety), which generally represent the main concern associated with biological invasions, they also have serious implications for biodiversity. In fact, along with other drivers of ecosystem degradation, such as habitat change and exploitation, environmental pollution, climate change, and associated effects (including the loss of keystone species, loss of pollinators and altered ecosystem functioning), biological invasions contribute to the decline of biodiversity worldwide, mainly by displacing native species occupying the same ecological niche (Paini and Roberts 2005, Pyšek and Richardson 2010).

The acreage of protected crops, grown in glasshouses, tunnels and screen houses, has also developed rapidly in many European and Mediterranean countries over the last four decades. These environments have proven ideal for the proliferation of exotic pests, many of which have established, either temporarily or permanently (Hanafi 2005, Ehlers 2011). Almost all European countries are signatories of the Convention on Biological Diversity (CBD) and are therefore obliged to "*prevent the introduction of alien species and, when prevention fails, to control as far as possible those exotic species that threaten indigenous ecosystems, habitats or species*" (CBD 1992). Management of the exotic species problem is neither easy nor cheap and requires international cooperation (Hanafi 2005). These actions should be aimed at collating available information at the global scale, analyzing the role of biological invasions as a driver threatening biodiversity and improving risk-assessment schemes (Baker et al. 2009). Thus, the initial step in a risk-assessment programme must be to distinguish the harmful from the harmless alien species and identify the impacts of the former on native biodiversity. Risk assessment is the first step in the risk management process to prevent or mitigate the spread of invasive species. Preventing the introduction of species with a high possibility of becoming invasive is, in theory, the most cost effective management strategy (Pyšek and Richardson 2010), therefore the pre-border risk assessment has the potential to intercept alien insects with potentially high economic impact. In many instances, the best or only way of reducing introductions is to manage vectors and pathways. By contrast, the multiple pathways of introduction and the huge volume of traded commodities make the interception of all potentially invasive alien species unrealistic.

Once an exotic species established in a new environment, early detection and rapid response strategies are crucial elements of integrated programmes for dealing with invasive species (Hulme et al. 2009). Early detection of a potential invasive species is often crucial in determining whether eradication of the species is feasible. The possibility of early eradication or at least of effectively containing a new colonizer makes investment in early detection worthwhile; and, in this contest, understanding the factors that influence a species' ability to invade is important for the prediction of future invasions and the management of present ones (Paini et al. 2008, Pyšek et al. 2008). When prevention has failed, eradication is the preferred course of action. Eradication can be a successful and cost-effective solution in response to an early detection of a non-indigenous species (Wittenberg and Cock 2001). Eradication provides a means to alleviate and/or remove the detrimental effects that exotic species typically have on their host ecosystems (Myers et al 2000). However, such programmes are not always successful and, when eradication is not feasible (i.e. negative results of the analysis of the costs and likelihood of success), the last step in the sequence of management options is the control of an invasive species. The aim of control is to reduce the density and abundance of an invasive organism in order to keep it below an acceptable economic threshold. There are numerous specific methods for controlling invasive species and many of them can be used in eradication programmes too. Physical control is highly specific to the target, but always very labour-intensive, therefore it is not economically feasible in countries where human labour is costly. Chemical control is often very effective as a short-term solution; by contrast, its major drawbacks are the high costs, the non-target effects, and the possibility of the pest species developing resistance (Weisenburger 1993, Feyereisen 1995, Desneux et al. 2007, Lu et al. 2012). In comparison with other methods, classical biological control, through the introduction of selected natural enemies from the native areas of the invasive pest, when successful and feasible is highly cost-effective, permanent, self-sustaining and ecologically safe because of the high specificity of the agents used (Mills 2005, Messing and Wright 2006, Cock et al. 2010). Biological control is particularly appropriate for use in nature reserves and other conservation areas because of its environmentally friendly nature and the increasing instances of prohibition of pesticide use in these areas (van den Bosch 1971, Symondson et al. 2002, Desneux et al. 2010, Pyšek and Richardson 2010). Whereas, IPM combining several methods often provides the most effective and acceptable control (van Lenteren and Woets 1988, Castle and Bentley 2009, Ragsdale et al 2011).

1.1.1. *Tuta absoluta* arrival and spread in the Mediterranean basin

The South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an invasive pest native to South America where it is considered one of the most devastating pests of tomato (Fernandez and Montagne 1990, Guedes and Picanço, 2012). The species has a particular preference for tomato (*Solanum lycopersicum* L.) although it is also able to attack and cause damage on different genera and species of the Solanaceae family. The larvae of the moth feed and develop inside tomato leaves, stems and fruits and commonly cause complete yield loss in greenhouse and open-field tomatoes in infested areas. The pest occurs throughout the entire growing cycle of tomatoes both for fresh market and processing, and larval feeding can cause losses of up to 100% (Desneux et al 2010) (see Chapter II for more details on its biology and damage).

This pest was reported for the first time outside its native continent in eastern Spain in late 2006 (Urbaneja et al. 2007) and has subsequently spread throughout the Mediterranean basin and Europe (37 counties). The exceptional speed, about 4,000 km in 5 years, of *T. absoluta* spreading in the Western Palaearctic region may be explained by intra-continental dispersal facilitation due to human transportation (as attributed in South America by Caceres 1992), but, as hypothesized by Desneux et al. (2011), it can also be caused by ecological or evolutionary phenomena. The

Mediterranean environment may be particularly suitable for the pest because of various factors including climatic conditions or tomato varieties (Desneux et al 2010).

The absence of co-evolved natural enemies may also explain why the dynamics in the newly invaded area looks so explosive in comparison to the native area where natural enemies are frequent (Luna et al. 2012). The tomato production system is more intensive and concentrated in Europe and North Africa and thus constitutes a richer and more homogeneous environment than in South America which may have a positive effect on growth and dispersion of *T. absoluta*. Another hypothesis is a rapid post-invasion evolution of the newly introduced pest population. Indeed, the literature on biological invasions shows that dispersal capabilities can increase during colonization (Phillips et al. 2006) because of a simple evolutionary process, namely best dispersers mate on the colonization front (Shine et al. 2011). A similar process may explain the increase in growth ability (evolution toward "r" strategy) of the invader during colonization (Burton et al. 2010). Besides, the artificial selection of insecticide resistant population may have enhanced *T. absoluta* rapid distribution over wide geographic areas (Siqueira et al. 2000, Gontijo et al. 2012, Haddi et al. 2012).

The impact of *T. absoluta* on tomato crops, during the first years after its detection, has led European and North African growers to use insecticides extensively (Sannino and Espinosa 2010, Speranza and Sannino 2012, Urbaneja et al. 2012). For example, at that time, up to 15 insecticide applications specifically targeting *T. absoluta* were added to Integrated Pest Management (IPM) schemes in Spain (Desneux et al. 2011). This was also the case in Brazil where the immediate consequence of *T. absoluta* introduction was the sudden increase in insecticide use in tomato fields from 10-12 applications per cultivation cycle to more than 30 applications, which required 4-6 weekly sprays (Guedes and Picanco 2012). Nevertheless, chemical approach may disrupt previous successful IPM procedures adopted during the last decades all over European countries for other pests in tomato cultivations; indeed it may disturb natural enemies because of multiple side effects and may lead to resistance, as occurred in the area of origin of this pest, causing multiple problems from economical, ecological, and environmental view points.

1.2. Integrated Pest Management

1.2.1. History and definition

Throughout the late nineteenth and early twentieth centuries, in the absence of powerful pesticides, crop protection specialists relied on knowledge of pest biology and cultural practices to produce multi-tactical control strategies that, in some instances, were precursors of modern IPM systems (Kogan 1998). The seed of the idea of integrated control appears in a paper by Hoskins et al. (1939):

"*...biological and chemical control are considered as supplementary to one another or as the two edges of the same sword (...) nature's own balance provides the major part of the protection that is required for the successful pursuit of agriculture (...) insecticides should be used so as to interfere with natural control of pests as little as possible...* "

Conceivably, "integrated control" was uttered by entomologists long before formally appearing in a publication, as it often happens with the nature of an expression before it is committed to paper by a then-recognized originator.

The *Integrated Control Concept* appears for the first time in 1959 in a publication by four authors from Riverside and Berkeley campuses of the University of California (Stern et al. 1959). They elaborated a new paradigm of pest control that serves as the foundation of arthropod IPM today. Their remarkable insight into the fundamental importance of 'environmental resistance' in regulating pest populations around a general equilibrium position was at the heart of the integrated control concept. They provided examples of arthropods, such as the Colorado potato beetle, for which the changed environments alter pest status, but particularly focused on management practices, such as chemical treatments, that potentially alter population equilibrium by impairing biological control, a key component of environmental resistance. Although there was already awareness in the 1950s of emerging problems associated with increasing reliance on pesticides, these authors developed the theoretical basis and put forth the necessary approaches in the field to enable the integration of biological and chemical control. Therefore, they defined Integrated Control as:

"*Applied pest control which combines and integrates biological and chemical control. Chemical control is used as necessary and in a manner which is least disruptive to biological control. Integrated control may make use of naturally occurring biological control as well as biological control effect by manipulated or induced biotic agents*".

The scientific basis of "Integrated Pest Control" evolved over a period of about 10 years, mainly among researchers at the University of California who established integrated control as a new trend in economic entomology. But, the desire to reconcile the use of insecticides with biological control transcended the US scientific community. For example, "complementary", "coordinated" and "harmonious" were used by Canadian and European entomologists to qualify "control" aimed at maximizing the impact of the combined methods (Kogan 1998). Unquestionably, the impression caused by publication of *Silent Spring* (Carson 1962) accelerated acceptance of the integrated control concept. Indeed, this book added an element of drama essential for the movement that was characterized by spokespersons of agrochemical industries as just another bandwagon (Jones 1970).The concept was explicitly defined in 1965 at a symposium organized by the Food and Agriculture Organization (FAO) (FAO 1967). It was attended by leading plant protection specialists from 36 countries and became a landmark in the advancement of the integrated control concept in its broadest sense. Indeed, the concept of "Integrated Control", originally limited to the combination of chemical and biological control methods, was greatly expanded in that symposium, and redefined to become synonymous with what we presently consider IPM:

"*Integrated control is a pest management system that in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains the pest populations at levels below those causing economic injury*".

Thus, the concept of *integration* stemmed from foundations established in the USA. Concurrently, however, the concept of "Pest Management" that had been proposed by Australian ecologists in 1961 (Geier and Clark 1961), started receiving greater recognition in the USA. Additionally, the Annual Review of Entomology publication of Geier in 1966 (Geier 1966) provided the impetus for that recognition. The convergence of the concepts of integrated control and pest management, and the ultimate synthesis into integrated pest management, opened a new era in the protection of agricultural crops, domestic animals, stored products, public health, and the structure of human dwellings against the attack of arthropod pests, plant and animal diseases, and weeds (Kogan 1998, Bajwa and Kogan 2002). IPM is the part of Integrated Production (IP) focusing on pest, disease and weed management (Boller et al. 2004), it is a concept of sustainable agriculture based on the use of natural resources and regulating mechanisms to replace potentially polluting inputs (Wijnands et al. 2012). The agronomic preventive measures and biological/ physical/chemical methods are carefully selected and balanced, taking into account the protection of health of both farmers, consumers and of the environment.

1.2.2. The principles of IPM strategies

The objective of IPM as a strategic, effective and feasible approach towards crop protection is to safeguard the quality and quantity of the production whilst minimizing the impact of pesticide use on human health and the environment. IPM applies to noxious species of phytophagous animals, plant pathogens and weeds. Noxious species are defined as those causing economic losses higher than their control costs (Ehler 2006, Wijnands et al. 2012).

Since almost all aspects of the management of a crop, or even a farm have a potential impact on the occurrence and development of pests, diseases and weeds, a fundamental approach towards crop protection starts with taking these interactions into account. Consequently, agroecosystems are the basis for planning the approach, i.e. working with natural processes and regulatory mechanisms rather than relying on interventions alone (Boller et al. 2004).

The basic IPM strategy focuses on minimizing the use and impact of pesticides. Therefore emphasis is given to preventive and/or indirect measures which must be utilized to the fullest extent before direct control measures are applied (Wearing 1988). Direct measures may only be taken if economically justified. All elements of the strategy should be carefully integrated in a coherent strategy, to be fully effective. For the IPM practitioner, this implies: (i) simultaneous management of multiple pests; (ii) regular monitoring of pests, and their natural enemies and antagonists as well; (iii) use of economic or treatment thresholds when applying pesticides; (iv) integrated use of multiple, suppressive tactics (Ehler 2006). In the long run, this holistic approach in dealing with pests should reduce pesticide use, provide economic savings for the farmer and protect both the environment and human health. Therefore, the term ‘integrated’ implies incorporation of natural enemy/antagonist levels into decision-making, and use of compatible, non-disruptive tactics that preserve these agents. Integration can be viewed as either vertical (i.e., within a class of pests) or horizontal (i.e., among all classes of pests).

The principles of IPM, its strategic objectives and management tactics are reported below (modified from Wijnands et al. 2012):

1. *Prevention and/or suppression of pests, diseases and weeds*:

The incidence and severity of most pest, disease and weed problems can be greatly lowered by applying agronomic practices that favour the competitive advantage of the crops against their harmful organisms, i.e. Good Agronomics Practices (GAP).

- Prevent build-up of pest, disease and weed populations by crop rotation.
- Escape periods of high pest, disease and weed pressure by timing of sensitive crop stages.

- Optimize crop fitness against attacks and make use of resistance, tolerance and competitive ability by fertilizing strategies, crop management and cultivar choice.
- Prevent spreading pest and diseases and weeds by field hygiene and adapted agricultural practice.
- Keep agro-ecosystem fit by supporting functional biodiversity enhancing and protecting beneficial organisms.
- Design of the complete agro-ecosystem by ecological infrastructures.

2. *Monitoring of pest organisms and application of economic damage thresholds*:

Assessing the necessity of intervention (control) based on knowledge about the real situation and the potential of losses leads to more targeted interventions. This step implies:

- Know the pests, diseases and weeds.
- Know the beneficial organisms by identifying the *site-specific* key pests, diseases and weeds that require regular interventions.
- Monitor pest, disease and weed incidence using monitoring traps and crop inspection;
- Define action threshold levels using intervention thresholds.
- Define early warning systems by forecasting models for pest and disease incidence.

3. *Non-chemical control methods*:

Many interventions with pesticides can be replaced or supported by non-chemical alternatives.

- Optimize interference with pest, disease and weed biology by mating disruption, sterile insect technique, use of bio-pesticides.
- Use physical interference: crop covers such as nets, exclusion fences, mechanical weed control.

4. *Chemical control methods*:

Pesticides chosen in function of their minimum side effects and minimal interference with preventive and non-chemical control methods.

- Select pesticides specifically targeted to harmful organisms and with minimal side effects, protect beneficial organisms: classify pesticides according to toxicity, ecotoxicology, persistence, etc. with special emphasis on protection of key beneficial organisms. Establish transparent criteria of preferred and less preferred pesticides.
- Optimize application technique and timing using well maintained and calibrated spraying equipment operated by trained persons.
- Optimize the dosage of pesticide using weather and efficacy forecasts when available to optimize timing and dosage. Consider row or spot applications.

- Prevent development of resistance: anti-resistance strategies based on sequence or combinations of active ingredients and alternation with other IPM methods. Adapt application rates and frequencies.
- Check efficacy comparing treated areas with small untreated ones.

5. *Forbidden methods*:

Some interventions (mostly chemical) are prohibited because they interfere with the agro-ecosystem in a way that prevents sustainability, such as chemical soil disinfection or broad spectrum pesticide applications.

1.3. Biological control of arthropods pests

Biological control can be defined as the use of an organism to reduce the population density of another organism and thus includes the control of animals, weeds and diseases (Bellows Fisher 1999). This section will be focused only on the biological control of arthropods, which DeBach (1964) defined as '*the study and uses of parasites, predators and pathogens for the regulation of host (pest) densities*'. This definition establishes two of the main principles of biological control. Firstly, in nature, most organisms are consumed by other organisms, which in many cases leads to drastic reductions in the population of the prey/host species; in biological control, man exploits this 'natural control' to reduce the pest population densities (Hodek 1967, Hawkins and Lawton 1987, Landis et al. 2000, Pennacchio and Strand 2006, Gardiner et al. 2009). Secondly, biological control reduces rather than eradicating pests, so that the pest and the natural enemy remain in the agro-ecosystem at low densities (Bellows Fisher 1999, Askew and Shaw 1986). A number of important pests can be kept at a low population density by biological control over long periods of time. In other cases, populations of pests are significantly reduced by natural enemies, but repeated releases or additional methods are needed to achieve an adequate level of control (van Lenteren and Woets 1988).

Many biological control strategies use predatory insects and mites, insects that attack other insects (parasitoids) or nematodes, targeted against insect and mite pests; these are the so-called "macrobial" agents. Biological control operates throughout the world as part of the management of pests in agriculture, forestry and greenhouse horticulture. Although biological control has sometimes been introduced to combat arthropod pests that have developed resistance to insecticides and acaricides, the first biological control programmes precede the modern pesticide era (Warton 1993, Viggiani 1984, van Lenteren and Godfray 2005, Bale et al. 2008).

Several approaches can be implemented to enhance the role of natural enemies in regulating population levels of insect species and preventing them from causing economic damage. Indeed, there are three main techniques of biological control: *classical*, *augmentative* and *conservation control* (van Lenteren 1993).

The first major successes in biological control occurred with exotic pests controlled by natural enemy species collected from the country or area of origin of the pest (named as classical control). Over the past 120 years, more than 5000 introductions of approximately 2000 non-native control agents have been made against arthropod pests in 196 countries or islands with remarkably few environmental problems (Bale et al. 2008, van Lenteren 2012). The cost benefit ratio for classical biological control is highly favourable (1:250) and for augmentative control higher to that of insecticides, but with much lower development costs (Collier and van Steenwyk 2004, Bale et al. 2008).

Classical biocontrol is used mainly against ‘exotic’ pests that have become established in new countries or regions of the world. Relatively, small numbers (usually less than 1000) of a certain natural enemy species are collected from the country or region of origin of the pest, ‘inoculated’ into the new environment, and allowed to build up the level of control, which can be maintained over very long periods of time (Ehler 2011). This type of biological control has been most successful with perennial crops, where the long-term nature of the ecosystem enables the interactions between pest and natural enemy to become fully established over a period of time (Viggiani 2000, Bale et al. 2008). By contrast, although biological control of exotic species using co-evolved natural enemies has long been considered a safe, cost effective, and environmentally benign tool, since Howarth’s (1991) publication, attention has been drawn to the risks involved in the import and introduction of exotic biological control agents into new natural environments, for having unintended side effects on non-target organisms (Simberloff and Stiling 1996, Messing and Wright 2006, Simberloff 2012).

Augmentative biocontrol refers to all forms of biological control in which natural enemies are periodically introduced, and usually requires the commercial mass production of the large numbers of the control agent to release. This strategy has been successfully applied against a range of open-field and greenhouse pests, whilst conservation biological control schemes have been developed with indigenous predators and parasitoids. The cultivation systems used for short-term annual crops destabilize the pest-natural enemy relationship, and thus often prevent the establishment of viable breeding populations of natural enemy species over several crop production cycles. The aim of inundative releases is to create an immediate massive ratio in favour of the natural enemy, analogous to the use of a pesticide, producing a rapid reduction or local extinction of the pest. Control is achieved mainly by the individuals that have been released rather than their offspring (Smith 1996, van Lenteren 2012).

Conservative biocontrol refers to the use of indigenous predators and parasitoids, against native or exotic pests. Various ecological measures are implemented to enhance the abundance or activity of the natural enemies, including manipulation of the crop microclimate, creation of overwintering refuges, increasing the availability of alternative hosts and prey, providing essential food resources such as flowers, applying only pesticides that are selective towards biocontrol agents (Croft and Brown 1975, van Lenteren and Woets 1988, Gurr et al. 2000; Landis et al. 2000, Harwood et al. 2007, Gardiner et al. 2009, Desneux et al. 2007, Lu et al. 2012).

1.3.1. Arthropod natural enemies

Insects and other organisms that use arthropods as a food resource resulting in their death, weakening, or reduced reproductive potential are called natural enemies, *biological control agents*, or *beneficials*. In agricultural systems, the most common natural enemies are insects and mites. There are two general types of functional groups of arthropods natural enemies: predators and parasitoids.

Predatory behaviour is widespread among insects (mainly belonging to the orders Odonata, Mantodea, Hemiptera, Neuroptera, Coleptera, Diptera and Hymenoptera), spiders and mites; they consume one or more living prey. While, parasitoids live in or on the body of their host insect during at least part of their life cycle, during the parasitisation. Parasitoids that insert their eggs into a host's body are called endoparasitoids; while those that lay their eggs outside the body the host body, and whose larvae develop on the outside of the host's body, are called ectoparasitoids. Usually the larval stage of the parasitoid is the parasitic life stage. The adult stage is typically free-living (not parasitic), and needs resources such as water, nectar, or pollen for survival. Most of the female parasitoid searches for a host and, depending on the species, deposits one or more eggs in or on the host. The eggs hatch and develop feeding on the host. When parasitoid development is complete, the parasitic larvae emerge and pupate outside the host, or, in the case of many parasitoids, pupate inside the host and emerge as adults. Parasitoids may be divided into *koinobionts* and *idiobionts* on the basis of whether or not they allow their host to feed and develop beyond the stage attacked (Askew and Shaw 1986; Pennacchio and Strand 2006). Most parasitoids belong to the orders Hymenoptera and Diptera. There are a few species of beetles (Coleoptera) that parasitize other insects. The small group of twisted-wing parasites (order Strepsiptera), consists entirely of parasitoids. Parasitic Hymenoptera are some of the most abundant natural enemies of all fruit pests. Most species belong to the Ichenumonoidea, Chalcidoidea and Proctotrupoidea. Of the Ichneumonoidea, the Ichneumonidae develop on largest pests (mainly larvae of moths and beetles). A very large group of Chalcidoidea is represented in many agroecosystems biocoenosis. The Aphelinidae, Encyrtidae, Eulophidae, Mymaridae and Trichogrammatidae are among the most active parasitoids of several pests. The majority of parasitoids in the agroecosystems, with some exceptions, are native species. Relatively, few of the accidentally or purposely introduced exotic species contribute to the natural pest control. Their different habits allow them to attack the hosts in almost all stages. The parasitization rate varies in relation to specific situations, but, in general, it is high for aphids, leafminers, leafhoppers, and scale insects. The role of the parasitoids is rather variable in relation to the type of agroecosystem. It appears particularly important in regulating the populations of some pests.

The entomophagous arthropods that attack insect herbivores can be divided broadly into specialist and generalist. *Specialist* species feed on one or a few prey species, and their dynamics is thus tightly linked to that of their prey. This close relationship with a prey species may allow specialists to mount a strong numerical response and thereby control the prey population (Hassell 1980, Turchin et al. 1999). The tight dynamical link between specialist natural enemies and prey populations has focused attention on specialists as potential biological control agents (DeBach 1964). In contrast, *generalist* species feed on many species and thus respond less strongly to density fluctuations of any single prey species. The lack of a close relationship with any single prey species may limit the ability of generalists to respond to and control herbivore population growth. On the other hand, generalist predator communities are widely acknowledged as providing valuable levels of regulation of several pests, such as for example aphids, in agroecosystems throughout the world (Symondson et al. 2002). Indeed, they are able to establish populations in highly disturbed ecosystems, such as annual cropping systems, by using alternative resources, *viz* plant material (Lundgren et al., 2009) or alternative prey (Harwood et al. 2007; Desneux and O'Neil 2008).

1.3.2. Selecting efficient arthropod biological control agents

The theoretical sequence of procedures in any biological control project is: (i) the search for and evaluation, selection, and collection of natural enemies; (ii) screening, and sometimes mass propagation; and (iii) release, colonization, and evaluation of consequences in the target crop (van Lenteren 1986). However, many biological control programmes are strongly influenced by pragmatic events. Therefore, the importance of the criteria when choosing natural enemies for artificial release programmes is essential for the full success of the programme itself. Some crucial features of an efficient natural enemy are listed below:

- The ability of the natural enemy to develop to the adult stage in or on the host is essential for ongoing control (Pennacchio and Strand 2006). If the enemy killed the host but did not develop, natural enemies would have to be reintroduced in each subsequent pest generation; therefore this would necessitate an inundative programme, usually unattractive because of excessive costs (Smith 1996). Furthermore, natural enemy population development should be synchronous with that of the pest organism to prevent cyclical pest outbreaks (Girardoz et al. 2006).

- At an early stage of pre introductory research, tests should be performed to determine whether the natural enemies are able to develop, reproduce, and disperse in the climatic conditions under which they are to be used (Jervis et al 2008).
- The natural enemy should not attack other beneficial organisms in the same environment or non pest organisms of importance or interest in the area where it is to be used (Harvey 2012).
- Good mass rearing methods for natural enemies are the basis for a successful biological control programme. Cultural methods largely determine the costs of the natural enemy and therefore the probability of application (van Lenteren 1986).
- Efficient natural enemies should have a pest kill rate equal to or greater than the potential maximum rate of population increase of the pest species. However, this criterion is not by itself sufficient for effectiveness, because at low pest densities the full reproductive and predatory potential of the natural enemy may not be achieved (van Lenteren and Woets 1988).
- Good searching efficiency is thought to be a necessity; natural enemies should be able to locate and reduce pest populations before they have crossed economic threshold densities (Vet and Dicke 1992).

1.4. Pesticide side effects on natural enemies

The conservation and enhancement of natural enemies in agriculture are increasingly important both economically and environmentally, thus making *pesticide risk assessments* on non-target arthropods crucial when these pesticides are included in IPM programmes (Desneux et al. 2007, Stark et al. 2007). Indeed, the importance of testing side effects on beneficials is also highlighted by the regulatory issues on the tests that are needed to authorize the use of a new pesticide. In Europe this subject is under a new regulation (EEC/CEE, 2009a) which comprises the decision making criteria for the authorization of plant protection products at member state level, including tests on natural enemies. These assays are based on standards, which settle the data requirements giving reliable decision criteria to assess the risk of pesticides, jointly defined by EPPO (European Plant Protection Organization), BART (Beneficial Arthropod Regulatory Testing) and IOBC/WPRS (International Organization for Biological and Integrated Control of Noxious Animals and Plants/West Palaearctic Regional Section) (Hassan 1992, Candolfi et al. 2000). The testing method comprises several trials organized in a tiered approach through laboratory, semi-field and field tests based on mortality rates and sub-lethal effects (Sterk et al. 1999).

Various methodologies have been developed for risk assessment of agrochemical on non-target arthropods according to the biological and/or behavioural traits of the organisms studied. Most of the studies tested field rates for one or several specific crops and for various targeted pests. In addition, a smaller number of studies also determined the median lethal dose, rate or concentration (LD/LR/LC_{50}) for various natural enemies and then assessed mortality induced by reduced concentrations (e.g.$<LC_{50}$) (Hassan 1992, Wennergren and Stark 2000).

Although most pesticide risk assessment experiments were usually based on acute toxicity tests, in the last two decades an increasing number of studies focused on the sublethal effect assessment. Evaluating of the sublethal effects of pesticides on natural enemies often aim to assess the suitability of pesticides for IPM. However, sublethal effects on natural enemies are rarely taken in account when IPM programmes are established (Stark et al. 2003, Desneux et al 2007, Urbaneja et al. 2008). To reduce non target effects of pesticides on natural enemies, selectivity tests are performed with the aim of choosing pesticides with a high degree of lethal toxicity against the target pests and minimal non target lethal toxicity (Croft 1990, Walthall and Stark 1997). However, given the potential importance of sublethal effects on natural enemies, pesticide choice should also consider those with minimal sublethal effects on key components of beneficial efficiency (Desneux et al. 2006a, 2006b, 2007, Stark et al. 2007). Indeed, some key physiological and behavioural traits could be impaired on those individuals that survive an exposure to a pesticide (the pesticide dose/concentration can be sublethal or lethal) and the consequent biological control services that natural enemies provide may be strongly affected as well (Haynes 1988, Desneux et al. 2006a, 2006b, 2007, Cabral et al. 2011, He et al. 2012, Planes et al. 2012). This is particularly important because potential side effects of pesticides on these arthropods, even subtle ones, can greatly reduce their capacity to provide crucial ecosystem services. In addition, new pesticides are often slower acting and consequently cause no short-term effects (e.g. lethal effect), leaving many survivors after pesticide applications. However, they may induce multiple physiological and behavioural sublethal effects, which may lead to a drastic decrease in population levels (Haynes 1998, Stark and Banks 2003, Desneux et al. 2007). Furthermore, susceptibility and recovery capacity to toxic products are specific for each given species; therefore the demographic levels and the demographical growth parameters can vary according to the function of specific-species life-history variables (Stark et al. 2004).

Although some pesticides have negligible direct effects (i.e. lethal and sublethal), the indirect ones, even though experimental evidences are rather rare, can be strong. Indeed, insecticides reduce overall insect abundance and thus limit prey/host availability in the treated environment. This can directly influence the body size and fecundity of natural enemies or induce their migration and postpone their recovery (Desneux et al. 2007, Pekar 2012). Therefore, the habitat disruption induced by pesticide application could lead to shifts in habitat preference and consequently to lower abundance of naturally occurring natural enemies in agroecosystems. However, it has proven that only wide scale and long term field studies can provide robust evidences on the impact of the use of pesticides on natural enemy abundance and on the ecological services (Lu et al. 2012).

1.5. Aim of the research

The aim of my work is providing key bases for including indigenous biocontrol agents of *T. absoluta* in IPM programmes on tomato in Europe. This was performed studying the mechanisms of the combination of fortuitous (indigenous) natural enemies (generalist predators and parasitoids) attacking the new host can be combined with the other tools, namely selective pesticides, employed in the integrated control programmes against this pest. Therefore, after reviewing the bibliography relying with the main characteristics of *T. absoluta* biology, distribution and damage on tomato (**chapter II**), a parasitoid survey was carried out to identify its parasitoid complex in Southern Italy (**chapter III**). Subsequently, in order to select a potential key indigenous parasitoid of *T. absoluta* of the braconid wasp, *Bracon nigricans* Szépligeti, was selected among the parasitic wasps that are getting adapted to the new host. By means of laboratory bioassays, its basic biological and behavioural features when parasitizing the exotic host in the laboratory were studied (**chapter IV**).

The following step was to assess the impact on natural enemies of commonly used pesticides in organic and/or conventional tomato cropping systems, including insecticides that have been increasingly used to control the new pest. Therefore, in a preliminary assessment of possible compatibility of biocontrol agents with pesticides used for IPM in tomato crops, the impact of a mineral fungicide/acaricide, the sulphur, on the predator *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) was assessed (**chapter V**). This species was selected, since it has been already identified as a key natural enemy of this pest in the newly invaded areas (Urbaneja et al. 2009, Desneux et al. 2010, Urbaneja et al. 2012). Giving the results of this study (mainly the harmfulness of the fungicide/acaricide on the predator vitality and fertility) a lethal and sublethal toxicity assessment of 14 pesticides (biopesticides, synthetic insecticides, fungicides and adjuvants) was carried out on the generalist predator *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) (**chapter VI**). This species was chosen as predator model because it is widely distributed in the Palaearctic region, it is a key natural enemy of various pests in agricultural ecosystems (Chambers et al.1993, Weintraub et al. 2011) and some species of the *Orius* genus showed to have some potential in preying on *T. absoluta* eggs (Desneux et al. 2010, Bueno et al. 2012). The obtained results, based on the combination of assessment of acute mortality, predator reproductive capacity and pesticides residual persistence, stressed the need of assessing the impact of these biopesticides (erroneously thought safer than synthetic ones) also on the other functional group of natural enemies, i.e. the parasitoid one. For this reason I investigated the following crucial steps in the re-colonization process after treatment by *B. nigricans*, in the laboratory: wasp development and emergence from treated cocooned pupae, adult survival after being in contact with insecticide residues, and multiple sublethal effects on the emerged/surviving adults (**chapter VII**). After, with the aim to deepen the knowledge of the non target effects on beneficial arthropods of the widely used (in IPM as well as in organic systems) biopesticides family, the spinosyns one, the recent literature on the side effects of these compounds on three functional groups; pollinators, predators and parasitoids was reviewed (**chapter VIII**). Finally, a discussion about the implications of the obtained results on the development of IPM strategies against *T. absoluta* in the newly invaded areas is presented in the **chapter IX**.

Chapter II

Tuta absoluta, an exotic invasive pest from South America now in the EPPO region; biology, distribution and damage

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Tuta absoluta, a South American pest of tomato now in the EPPO region: biology, distribution and damage

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Tuta absoluta (Meyrick) is an invasive pest of tomato native to South America, where it is responsible for extensive damage. It rapidly spread into several European countries, becoming a key pest. Knowledge of its main biological traits can be used to develop effective plant protection management strategies. It is a multivoltine species with a homodynamous behaviour, and the length of its life cycle depends on environmental conditions, particularly temperature. The larvae feed and develop inside tomato leaves, stems and fruits throughout the entire growing cycle. The adults have crepuscular habits, and in Mediterranean conditions they can be easily detected throughout the year. Damage is directly related to the reduction of plants' photosynthetic capacity and of production levels in both protected and open-field tomato crops; indirect damage can be also caused by secondary infections, with pathogens developing on the infested plant and fruit tissues.

Introduction

The tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is one of the most harmful pests of solanaceous crops. In its native area (South America), as well as in newly invaded regions, the moth preferentially attacks tomato under both field and greenhouse conditions.

The Food and Agriculture Organization of the United Nations (FAO) states that about 145 million tonnes of tomato are produced worldwide (FAOSTAT, 2011), 90% of which are obtained in the Northern Hemisphere. Tomato has become one of the most widely cultivated crops (more than 4.5 million hectares) and is of great economic importance worldwide. In 2004, *T. absoluta* was added by the European and Mediterranean Plant Protection Organization (EPPO) to the A1 List of pests recommended for regulation (pests absent from the EPPO region), and in 2009 was transferred to the A2 list (pests locally present in the EPPO region), 3 years after its arrival in Spain (Urbaneja *et al.*, 2007). During 2006 2012, the pest spread rapidly throughout the Mediterranean basin.

T. absoluta is considered a typical invasive species, due to its capacity to develop very quickly in suitable agro-ecological conditions, spreading rapidly in new areas and causing economically relevant damage (Desneux *et al.*, 2010).

Here the main characteristics of the species are briefly reviewed, with notes on biology, distribution and damage.

Biology

Tuta absoluta was described in 1917 by Meyrick as *Phthorimaea absoluta*, from a single male collected in Peruvian Andes (Meyrick, 1917). It was placed in the genus *Tuta* as *T. absoluta* by Povolny many years later (1994), after having been previously reported as *Gnorimoschema absoluta* (Clarke), *Scrobipalpula absoluta* (Povolny) and *Scrobipalpuloides absoluta* (Povolny) (EPPO, 2005; Desneux *et al.*, 2010).

The moth is a multivoltine species, showing a high reproductive potential that allows the pest population to increase very quickly (Barrientos *et al.*, 1998; Pereyra & Sánchez, 2006). Females lay eggs on the plant canopy; larvae normally hatch in the morning and wander about for 5 40 min before they start mining. Young larvae (1st 2nd instar) bore into the plant and, once mature (3rd 4th instar), they leave their mines and move to new locations for feeding. If food is available and climatic conditions are favourable, larvae feed almost continuously and generally do not enter diapause. Pupation occurs mainly on the leaves and in the soil, in function of environmental and growing conditions, whereas a small proportion may take place in other sheltered sites such as stems and fruits (Fernandez & Montagne, 1990; Uchôa-Fernandes *et al.*, 1995; Viggiani *et al.*, 2009). The pupae are normally protected by a thin, silky cocoon, which is covered by earth particles and debris for those located in the soil (Uchôa-Fernandes *et al.*, 1995). After about 5 days at 30°C the pupa becomes dark brown in colour and the adult is ready to emerge (Monserrat Delgado, 2008).

Adults have been reported to have crepuscular to nocturnal habits while remaining mostly hidden among vegetation during the day (Fernandez & Montagne, 1990), as well as auroral twilight dispersal, courtship and mating behaviour with more frequent ovipositions during the daytime (Uchôa-Fernandes *et al.*, 1995). Once stimulated, *T. absoluta* adults generally perform short flights near ground level. As is the case for other moths, adults are highly attracted by light sources, particularly light frequencies close to blue. The time period elapsed from emergence to mating is generally very short for the male (a few hours) and about 20 22 h for the female. Females mate only once a day and are able to mate up to six times during their lifespan, with a single mating lasting about 4 5 h. Adult lifespan ranges between 10 and 15 days for females and 6 7 days for males (Estay, 2000). Females live longer than males, allowing them to be sexually mature when the males emerge (Fernandez & Montagne, 1990). The majority of oviposition occurs 7 days after the first mating, and during this period a single female lays more than 70% of its eggs during the afternoon and twilight hours. During its lifetime, a single female may produce up to 260 eggs (Uchôa-Fernandes *et al.*, 1995). The eggs are laid singly or in small groups, mainly on young leaves (73%) and secondly on stems (21%), sepals (5%) and green fruits (1%) (Estay, 2000).

Distribution of the pest within the plant canopy depends on the insect development stage and the plant phenological stage (Gomide *et al.*, 2001; Torres *et al.*, 2001). This issue is important in order to define a more reliable sampling method, to place the traps correctly in the fields, and to evaluate the aggregation behaviour of predators and parasitoids. Gomide *et al.* (2001) studied this aspect in Brazil, and observed that larvae and mines were associated with expanded leaves in the medium part of the canopy, while high egg counts tended to be associated with expanded leaves in the apical part of the plant, with a significant correlation between the two data sets. Therefore they stated that damage can be estimated on the base of egg counting.

Torres *et al.* (2001) found that preferred oviposition sites in pre-flowering plants are located on the undersides of leaves in the apical portion of the plant. After flowering, no differences were found between the upper and the lower sides of the leaves, with the middle portion of the plant being affected as well as the apical part. The first three larval instars are distributed equally between the apical and the middle parts of the plant in all phenological stages. Mature larvae, however, are distributed evenly over the entire plant, including the basal portion.

The tomato borer shows a high capacity to adapt to a wide range of temperatures, and under optimal conditions it develops in about 30 days (EPPO, 2005). Barrientos *et al.* (1998) calculated an average development time of 76.3 days at 14°C, 39.8 days at 19.7°C and 23.8 days at 27.1°C. They also determined the temperature thresholds for *T. absoluta* eggs, larvae and pupae at 6.9 ± 0.5, 7.6 ± 0.1 and 9.2 ± 1.0°C, respectively. Considering all the stages, the thermal threshold is 8.1 ± 0.2°C. On the basis of this data, the corresponding thermal constants for eggs, larvae and pupae are 103.8 ± 1.4, 238.5 ± 0.5 and 117.3 ± 5.3 degree days (DD), therefore the pest requires 453.6 ± 3.9 DD to complete its development cycle.

Adults are attracted by tomato leaf volatiles to find hosts and for oviposition (Proffit *et al.*, 2011). The species has a particular preference for tomato (*Solanum lycopersicum* L.) and it is also able to attack and cause damage on different genera and species of Solanaceae family. These hosts include cultivated plants such as *Solanum tuberosum* L. (potato), *Solanum melongena* L. (eggplant) and *Solanum muricatum* Aiton (pepino), and wild solanaceous species such as *Solanum nigrum* L., *Solanum lyratum* Thumberg, *Lycopersicon hirsutum* L., *Datura stramonium* L. and *Lycium chilense* Mill. (EPPO, 2005; Urbaneja *et al.*, 2007; Viggiani *et al.*, 2009). Its presence has also been reported on French bean (*Phaseoulus vulgaris* L.) and cape gooseberry (*Physalis peruviana* L.) (EPPO, 2009; Speranza, 2009; Tropea Garzia, 2009). An exhaustive list of South American and European hosts is reported by the US Animal and Plant Health Inspection Service (APHIS) (USDA, 2011). The availability of alternative host plants is an important factor that allows the constant presence of the pest in the absence of tomato crops; this capacity must be carefully considered when using integrated control strategies.

The number of generations per year of *T. absoluta* can be more than 10 in South America although no more than five generations per year were observed in Argentina (EPPO, 2005; Korycinska & Moran, 2009). In Italy up to nine generations per year have been observed (Sannino & Espinosa, 2010), while in Spain it has been estimated the pest could complete up to 13 generations per year (Vercher *et al.*, 2010). *Tuta absoluta* can overwinter as an egg, pupa or adult (the latter as long as food is available), with a prevalence of pupa in colder periods (Sannino & Espinosa, 2010). However, under Mediterranean conditions *T. absoluta* adults can be detected all year round (Tropea Garzia, unpublished data; Vercher *et al.*, 2010).

Distribution

Tuta absoluta is native to South America, where it is considered one of the key pests of tomato. Following its first detection in Eastern Spain in late 2006 (Urbaneja *et al.*, 2007), the pest has spread into Europe, North Africa and the Middle East at exceptional speed (Table 1). The first report of the pest in Russia is noteworthy because of its potential spread into China (currently the world's leading tomato producer, followed by the USA; FAOSTAT, 2011). Additionally, some other records, such as that reporting one specimen of the pest being caught in a light trap in Denmark (near Copenhagen), which is not a tomato-growing area (Buhl *et al.*, 2010), highlight the strict connection between this species' spread and human activities such as trading.

Colonization of the Mediterranean countries has been rapid, with a geographical spread of approximately 4000 km in about 5 years. Such a speed may be explained principally by trading of tomato fruits, which appears to be the current primary pathway of long-range distribution of this pest.

Table 1 *Tuta absoluta* current distribution outside the area of origin

Country	Year	Reference
Spain [including Balearic (2006) and Canary (2008) islands]	2006	Urbaneja *et al.* (2007); EPPO (2009)
Albania	2008	EPPO (2009)
Algeria	2008	Guenaoui (2008)
France (including Corsica)	2008	EPPO (2009)
Italy (including Sicily and Sardinia)	2008	Viggiani *et al.* (2009); Tropea Garzia *et al.* (2009)
Morocco	2008	EPPO (2008)
Tunisia	2008	EPPO (2009)
Bulgaria	2009	EPPO (2010)
Croatia	2009	EPPO (2011)
Cyprus	2009	EPPO (2010)
Germany	2009	EPPO (2010)
Greece (including Crete)	2009	Roditakis *et al.* (2010)
Israel	2009	EPPO (2010); Seplyarsky *et al.* (2010)
Libya	2009	EPPO (2011)
Lithuania	2009	Ostraukas & Ivinskis (2010)
Malta	2009	EPPO (2009)
Portugal (including Azores)	2009	EPPO (2009)
Switzerland	2009	EPPO (2009)
The Netherlands	2009	EPPO (2009)
United Kingdom [including Guernsey (2010)]	2009	EPPO (2009, 2010)
Bosnia	2010	Uric & Hrncic (2010)
Egypt	2010	EPPO (2011)
Hungary	2010	EPPO (2010)
Iraq	2010	Abdul Razzak *et al.* (2010)
Kosovo	2010	EPPO (2010)
Kuwait	2010	EPPO (2011)
Montenegro	2010	Hrnčić & Radonjić (2011)
Romania	2010	Keresi *et al.* (2010)
Russia	2010	Izhevsky *et al.* (2011)
Saudi Arabia	2010	Altmani (2010); EPPO (2011)
Serbia	2010	EPPO (2010)
Sudan	2010	EPPO (2011)
Syria	2010	Altmani (2010); EPPO (2011)
Turkey	2010	Kılıç (2010)
Bahrain	2011	EPPO (2011)
Iran	2011	Banamieri & Cheraghian (2011)
Jordan	2011	EPPO (2011)
Lebanon	2011	EPPO (2011)
Qatar	2012	EPPO (2012)

However, active (through flight) and passive (through wind) spread are also probable (Desneux *et al.*, 2011).

Genetic studies have been conducted on the pest in order to describe more precisely its routes and process of invasion, as well as to propose a likely explanation for the diffusion dynamics (Desneux *et al.*, 2011).

Data obtained so far on the sampled populations, and in particular on the populations' history and origin, and on the historical, demographic and genetic features of the introduced populations, established that invasive populations are characterized by a high genetic homogeneity (Cifuentes *et al.*, 2011). This feature is not unusual in recently introduced species in a new habitat, and may be attributed to founder effects which greatly or totally reduce the genetic variability. Introduced populations often contain only a subset of the genetic variability of the native populations. However, this is not the case for *T. absoluta* in the Mediterranean region, which has a gene pool similar to that of populations in South America. The high genetic similarity between the two continents' populations suggests that the invasive Mediterranean populations are derived from a South American population, and maybe also that the South American population is itself invasive (Cifuentes *et al.*, 2011). Another possible explanation for this genetic similarity may be the replacement of more susceptible populations, both in South America and in the Mediterranean basin, by those with higher insecticide tolerance. Historical records suggest that Southern Europe was the first and maybe the sole introduction point from the native area; in fact, before its arrival in Europe *T. absoluta* was exclusively reported from South America and Easter Island (Desneux *et al.*, 2010).

Description of the invasion routes, including the identification of the precise population origin, facilitates the design of strategies for preventing new introductions and for controlling invasive populations (Estoup & Guillemaud, 2010). In particular, in the case of classical biological control, choosing strains of the predator or parasitoid with the same geographical origin as the invasive population may help in obtaining more efficient natural enemies that co-evolved with the target species (Estoup & Guillemaud, 2010). Secondly, identification of the invasion route provides information about the original environment and the genetic properties of the source population of the invading pest. Knowledge of the biotic and abiotic environment to which the pest is adapted may be helpful in defining an effective control strategy. This is particularly true when selecting pesticides, as this choice must take into account the potential resistance of the source population (Haddi *et al.*, 2012).

Damage

During the past few years, in all newly infested countries, the cost of tomato production has increased greatly due to the necessity of developing and applying new pest control and monitoring strategies, both in the field and in post-harvest processes. This increased cost has been especially difficult for producers due to the lower quantity of marketable production (Sannino & Espinosa, 2010; USDA, 2011).

Tomato is the preferred host plant for *T. absoluta* oviposition and growth, and all the epigeal plant parts are suitable for the moth development. Larval feeding activity reduces the plants' photosynthetically active surface, and consequently growth and yields (Bogorni *et al.*, 2003). The plant can be attacked in all its developmental stages, and severe injuries to seedlings can occur, leading to the death of young plants when larvae develop inside the main stem (Pereyra & Sánchez, 2006). In addition, multiple wounds on the tissues caused by larvae make the plants more vulnerable to secondary diseases, especially those caused by bacteria, which can actively penetrate damaged tissues.

Another kind of damage is due to infestation of the fruits, and these losses are generally dependent on pest control efficacy and, in some cases, can cause total crop failure (Desneux *et al.*, 2010). The larvae move into the fruits via a small entrance hole generally made under the sepals, making the first detection of infested fruits in the field very difficult. For this reason, post-harvest processes are also affected by the pest, and commercial downgrading of entire tomato lots during storage and shipment can occur. This phase represents a crucial point for the tomato industry and market chain process (USDA, 2011). Furthermore, trade in accidentally infested tomato lots is presumably the main pathway for the international spread of this pest (Desneux *et al.*, 2011).

Discussion

All the biological features of the tomato borer lead it to be considered as an *r*-selected species with high economic importance (Desneux *et al.*, 2010). Current *T. absoluta* distribution trends, quickly moving from Europe to the closer areas of Africa and Eurasia, suggest that the invasive pest is a serious threat to tomato crops at the world level (Desneux *et al.*, 2011).

Due to ecological and biological strategies, the moth shows rapid adaptation to new environments. Newly infested areas are generally very susceptible to its infestations, especially where unsuitable or untimely control strategies are applied. However, in the Mediterranean areas, where the species has initially spread, the pest populations and damages are significantly limited where appropriate control strategies, renewed attention to preventive methods, and correct use of selected insecticides occur (Biondi *et al.*, 2012). Moreover, although prompt adaptation of native natural enemies to the new invasive host has been observed (Zappalà *et al.*, 2012), antagonists need time to achieve effective biocontrol of the pest.

In this general framework, it is very important to continue to improve our knowledge on the various aspects of *T. absoluta* biology in order to find effective and environmentally friendly pest control strategies. Sharing of scientific and technical data within the international community is of high relevance to build up a network of information to limit the spread of the pest in new areas through the development of integrated pest management programmes.

Tuta absoluta, un ravageur de la tomate d'Amérique du Sud présent dans la région OEPP: biologie, répartition et dégâts

Tuta absoluta (Meyrick) est un ravageur de la tomate, originaire d'Amérique du Sud, où il est responsable de dommages considérables. Il s'est rapidement propagé dans plusieurs pays européens où il est devenu un ravageur important en raison de plusieurs facteurs. La connaissance des principaux traits biologiques du ravageur est essentielle pour développer des stratégies efficaces de protection des végétaux. *T. absoluta* est une espèce multivoltine avec un comportement homodynamique et la durée de son cycle de vie dépend des conditions environnementales, en particulier de la température. Les larves se nourrissent et se développent à l'intérieur des feuilles, des tiges et des fruits de tomate pendant toute la durée du cycle végétatif. Les adultes ont des habitudes nocturnes et dans les conditions méditerranéennes, ils peuvent être facilement détectés pendant toute l'année. Les dommages sont directement liés à la réduction de la capacité photosynthétique et des niveaux de production, tant dans les cultures de tomates en serre qu'en plein champ; des dommages indirects peuvent aussi être liés à des infections secondaires par des agents pathogènes qui se développent sur les tissus végétaux et sur les fruits infestés.

Tuta absoluta, южноамериканский вредитель томатов, присутствующий теперь в регионе ЕОКЗР: биология, распространение и вред

Tuta absoluta (Meyrick) является инвазивным вредным организмом для томатов, родом из Южной Америки, где он наносит значительный ущерб. Он быстро распространился в нескольких европейских странах и стал ключевым вредным организмом. Знание его основных биологических особенностей может быть использовано для разработки эффективных стратегий защиты растений по управлению этим вредным организмом. Это поливольтинный вид с гомодинамичным поведением, продолжительность его жизненного цикла зависит от условий окружающей среды, в особенности, от температуры. Личинки питаются и развиваются внутри томатных листьев, стеблей и плодов в ходе всего цикла произрастания растения. У взрослых особей наблюдается сумеречная активность, и в средиземноморских условиях они могут быть легко обнаружены в течение всего года. Наносимый ущерб непосредственно связан со снижением фотосинтетической способности растений и уровня производства культуры как в защищенном, так и в открытом грунте; косвенный ущерб может быть также нанесен вторичными заражениями патогенами, развивающимися на пораженных тканях растений и плодов.

References

Abdul Razzak AS, Al-YasiriII & Fadhil HQ (2010) First record of tomato borer (tomato moth) *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomato crop in Iraq. *Arab and Near East Plant Protection Newsletter* **51**, 31.

Altmani W (2010) Tomato leaf miner *Tuta absoluta* invades East Mediterranean countries. *Arab and Near East Plant Protection Newsletter* **50**, 29.

Banamieri V & Cheraghian A (2011) First report of *Tuta absoluta* in Iran and initial control strategies.EPPO/IOBC/FAO/NEPPO Joint International Symposium on management of Tuta absoluta (Tomato borer), Agadir, Morocco, November 16 18, 2011.

Barrientos ZR, Apablaza HJ, Norero SA & Estay PP (1998) [Threshold temperature and thermal constant for development of the South American tomato moth, *Tuta absoluta* (Lepidoptera: Gelechiidae)]. *Ciencia e Investigacion Agraria* **25**, 133 137 (in Spanish).

Biondi A, Desneux N, Siscaro G & Zappalà L (2012) Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. *Chemosphere* **87**, 803 812.

Bogorni PC, Silva RA & Carvalho SG (2003) [Leaf mesophyll consumption by *Tuta absoluta* (Meyrick, 1971) (Lepidoptera: Gelechidae) in three cultivars of *Lycopersicon esculentum* Mill.]. *Ciencia rural* **33**, 7 11 (in Spanish).

Buhl O, Falck P, Karsholt O, Larsen K & Vilhelmsen F (2010) [Records of microlepidoptera from Denmark in 2009 (Lepidoptera)]. *Entomologiske Meddelelser* **78**, 101 116 (in Danish).

Cifuentes D, Chynoweth R & Bielza P (2011) Genetic study of Mediterranean and South American populations of tomato leafminer *Tuta absoluta* (Povolny, 1994) (Lepidoptera: Gelechiidae) using ribosomal and mitochondrial markers. *Pest Management Science* **67**, 1155 1162.

Desneux N, Luna MG, Guillemaud T & Urbaneja A (2011) The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond the new threat to tomato world production. *Journal of Pest Science* **84**, 403 408.

Desneux N, Wajnberg E, Burgio G, Arpaia S, Wyckhuys Kris AG, Narváez-Vasquez CA, González-Cabrera J, Tabone E, Frandon J, Pizzol J, Poncet C & Urbaneja A (2010) Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *Journal of Pest Science* **83**, 197 215.

EPPO (2005) Data sheets on quarantine pests: *Tuta absoluta*. *Bulletin OEPP/EPPO Bulletin* **35**, 434 435.

EPPO (2008) EPPO Reporting Service n. 1, 9. http://www.eppo.org/PUBLICATIONS/reporting/reporting_service.htm [accessed on 10 February 2012].

EPPO (2009) EPPO Reporting Service n. 1, 2, 3, 6, 8, 9, 10, 11. http://www.eppo.org/PUBLICATIONS/reporting/reporting_service.htm [accessed on 10 February 2012].

EPPO (2010) EPPO Reporting Service n. 1, 2, 3, 6, 8. http://www.eppo.org/PUBLICATIONS/reporting/reporting_service.htm [accessed on 10 February 2012].

EPPO (2011) EPPO Reporting Service n. 4, 11. http://www.eppo.org/PUBLICATIONS/reporting/reporting_service.htm [accessed on 10 February 2012].

EPPO (2010) EPPO Reporting Service n. 5, 12. http://www.eppo.org/PUBLICATIONS/reporting/reporting_service.htm [accessed on 21 June 2012].

Estay P (2000) [The South American tomato pinworm *Tuta absoluta* (Meyrick)]. http://www.inia.cl/link.cgi/Platina/Documentos/DPlatina/Informativos/1367 [accessed on 1 October 2011] (in Spanish).

Estoup A & Guillemaud T (2010) Reconstructing the routes of invasion: what for, how and so what?*Molecular Ecology* **19**, 4113 4130.

FAOSTAT (2011). Production. Crops. http://faostat.fao.org/site/567/default.aspx#ancor [accessed on 20 October 2011]

Fernandez S & Montagne A (1990) [Biology of the tomato borer, *Scrobipalpula absoluta* (Meyrick) (Lepidoptera: Gelechiidae)]. *Boletin de Entomologia Venezolana* **5**, 89 99 (in Spanish).

Gomide EVA, Vilela EF & Picanço M (2001) [Comparison of sampling procedures for *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in tomato crop]. *Neotropical Entomology* **30**, 697 705 (in Portuguese).

Guenaoui Y (2008) Nouveau ravageur de la tomate en Algérie. Première observation de *Tuta absoluta*, Mineuse de la Tomate invasive, dans la région de Mostaganem, au printemps 2008. *Phytoma, La Défense des Végétaux* **617**, 18 19.

Haddi K, Berger M, Bielza P, Cifuentes D, Field LM, Gorman K, Rapisarda C, Williamson MS & Bass C (2012) Identification of mutations associated with pyrethroid resistance in the voltage-gated sodium channel of the tomato leaf miner (*Tuta absoluta*). *Insect Biochem Mol Biol* Doi: 10.1016/j.ibmb.2012.03.008.

Hrnčić S & Radonjić S (2011) *Tuta absoluta* Meyrick (Lepidoptera, Gelechiidae), a new pest in Montenegro. *IOBC/WPRS Bulletin* **68**, 71 74.

Izhevsky SS, Akhatov AK & Sinyov SY (2011) *Tuta absoluta* has been detected in Russia. *Zashchita i Karantin Rastenii* **3**, 40 44.

Keresi T, Ivanovic M & Tolic D (2010) [Tomato moth (*Tuta absoluta* Povolny) new potential threat to tomato production in Serbia]. *Biljni Lekar* **38**, 474 484 (in Serbian).

Kılıç T (2010) First record of *Tuta absoluta* in Turkey. *Phytoparasitica* **38**, 243 244.

Korycinska A & Moran H (2009) Plant Pest Notice: South American tomato moth. *Tuta absoluta Department for Environment, Food and Rural Affairs, Food and Environment Research Agency* **56**, 1 4.

Meyrick E (1917) Descriptions of South American Micro-Lepidoptera. *Transactions of the Royal Entomological Society of London* **65**, 1 52.

Monserrat Delgado A (2008) [The South American tomato pinworm (*Tuta absoluta*) in the Murcia Region: bases for its control]. Consejería de Agricultura y Agua, Región de Murcia (in Spanish).

Ostraukas H & Ivinskis P (2010) Records of the tomato pinworm (*Tuta absoluta* (Meyrick, 1917)) Lepidoptera: Gelechiidae in Lithuania. *Acta Zoologica Lithuanica* **20**, 151 155.

Pereyra PC & Sánchez NE (2006) Effect of two solanaceous plants on developmental and population parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology* **35**, 671 676.

Proffit M, Birgesson G, Bengtsson M, ReisJrR, Witzgall P & Lima E (2011) Attraction and oviposition of *Tuta absoluta* females in response to tomato leaf volatiles. *Journal of Chemical Ecology* **37**, 565 574.

Roditakis E, Papachristos D & Roditakis NE (2010) Current status of the tomato leafminer *Tuta absoluta* in Greece. *Bulletin OEPP/EPPO Bulletin* **40**, 163 166.

Sannino L & Espinosa B (2010) [*Tuta absoluta*, guide to knowledge and recent acquisitions for a suitable control]. *L'Informatore Agrario* **46**(suppl 1),1 113 (in Italian).

Seplyarsky V, Weiss M & Haberman A (2010) *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae), a new invasive species in Israel. *Phytoparasitica* **38**, 445 446.

Speranza S (2009) [First infestations of *Tuta absoluta* on French bean in Lazio region]. *Terra e Vita* **46**, 14 15 (in Italian).

Torres JB, Faria CA, EvangelistaJrWS & Pratissoli D (2001) Within-plant distribution of the leaf miner *Tuta absoluta* (Meyrick) immatures in processing tomatoes, with notes on plant phenology. *International Journal of Pest Management* **47**, 173 178.

Tropea Garzia G (2009) *Physalis peruviana* L. (Solanaceae), a host plant of *Tuta absoluta* (Meyrick) in Italy. *IOBC/WPRS Bulletin* **49**, 231 232.

Tropea Garzia G, Siscaro G, Colombo A & Campo G (2009) [*Tuta absoluta* recovered in Sicily]. *L'Informatore Agrario* **4**, 71. (in Italian).

Uchôa-Fernandes MA, Della Lucia TMC & Vilela EF (1995) Mating, oviposition and pupation of *Scrobipalpula absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Anais da Sociedade Entomologica do Brasil* **24**, 159 164.

Urbaneja A, Vercher R, Navarro V, Porcuna JL & Garcia-Marí F (2007) [The tomato moth, *Tuta absoluta*]. *Phytoma España* **194**, 16 24 (in Spanish).

Uric Z & Hrncic S (2010) [Tomato leaf miner *Tuta absoluta* Meyrick in the Republic of Srpska]. *Agro Znanje Agro knowledge Journal* **11**, 17 23 (in Serbian).

USDA (2011) New Pest Response Guidelines: Tomato Leafminer (*Tuta absoluta*). http://www.aphis.usda.gov/import_export/plants/manuals/emergency/downloads/Tuta-absoluta.pdf [accessed on 20 October 2011]

Vercher R, Calabuig A & Felipe C (2010) [Ecology, sampling and economic threshold of *Tuta absoluta* (Meyrick)]. *Phytoma España* **217**, 23 26 (in Spanish).

Viggiani G, Filella F, Delrio G, Ramassini W & Foxi C (2009) [*Tuta absoluta*, new moth reported also in Italy]. *L'Informatore Agrario* **2**, 66 68 (in Italian).

Zappalà L, Bernardo U, Biondi A, Cocco A, Deliperi S, Delrio G, Giorgini M, Pedata P, Rapisarda C, Tropea Garzia G & Siscaro G (2012) Recruitment of native parasitoids by the exotic pest Tuta absoluta (Meyrick) in Italy Southern. *Bulletin of Insectology*, **65**, 51 61.

Chapter III

Recruitment of native parasitoids by the exotic pest *Tuta absoluta* (Meyrick) in Southern Italy

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Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in Southern Italy

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Abstract

The tomato borer *Tuta absoluta* (Meyrick) is an invasive pest native to South America and since its arrival in Europe the tomato production has faced severe yield loss. The complex of indigenous parasitoids that colonized this new host species was monitored in Southern Italy during 2009-2011, in some of the regions where *T. absoluta* was initially detected (Campania, Sardinia and Sicily) with the aim of identifying the parasitoid complex of the tomato borer as well as finding potential biocontrol agents of this invasive pest. The survey was carried out by sampling the tomato borer on open field and protected greenhouse crops, on wild secondary hosts and by exposing sentinel infested tomato plants.

A quick shift of native parasitoids to the new invasive host was observed and the parasitoid complex associated to *T. absoluta* seems to follow the typical pattern of colonization on exotic pests. The recovered species were, in fact, mainly generalist idiobiont parasitoids causing low levels of parasitism in open field. The species found belong to 13 genera and 6 families (Ichneumonidae, Braconidae, Eulophidae, Elasmidae, Pteromalidae and Trichogrammatidae). In particular, the 10 identified species were: *Diadegma pulchripes* (Kokujev), *Bracon osculator* (Nees), *Bracon* (*Habrobracon*) *nigricans* Szepligeti, *Necremnus* sp. near *tidius* (Walker), *Necremnus* sp. near *artynes* (Walker), *Neochrysocharis formosa* (Westwood), *Pnigalio soemius* s.l. (Walker), *Pnigalio cristatus* (Ratzeburg), *Pnigalio incompletus* (Boucek) and *Halticoptera aenea* (Walker). For seven of these species, the finding on *T. absoluta* is the first host-parasitoid association report. This survey highlighted that conservation of indigenous natural enemies, also by means of habitat management techniques, should be taken seriously into account when planning integrated management strategy of the tomato borer in the Mediterranean area.

Key words: Parasitoid community, tomato borer, field sampling, spontaneous flora, sentinel plant, new host-parasitoid associations.

Introduction

The tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae), is a very injurious pest affecting tomato crops during the whole cycle, both in greenhouse and open field cultivations. This neotropical species was originally widespread in South America and, starting from 2006, it rapidly invaded Southern Europe and North Africa (Urbaneja *et al.*, 2007; Desneux *et al.*, 2010). In Italy it was first detected in 2008 (Tropea Garzia *et al.*, 2009; Viggiani *et al.*, 2009) and spread throughout the major tomato-producing regions infesting both fresh market and processing tomato. More recently, the pest was reported in most European and North African countries and in several Middle East countries as well (Desneux *et al.*, 2011).

T. absoluta is a multivoltine pest that shows high reproductive potential and short life cycle (Pereyra and Sanchez, 2006). The female lays eggs, mostly singly, on leaves and stems, the young larvae bore and develop inside the plant, continuously searching for new feeding locations and pupation occurs mainly in the soil. In the Mediterranean basin, *T. absoluta* infests other Solanaceous crops (eggplant, sweet pepper, potato and tobacco) as well as spontaneous plants, such as the black nightshade, *Solanum nigrum* L.; occasional damages have been reported on green bean (Desneux *et al.*, 2010).

The species is considered a key pest of tomato in its native area causing high yield losses (Silva *et al.*, 2011). The primary *T. absoluta* management strategy in most South American countries is chemical control (Siqueira *et al.*, 2000). However, pesticides are only partially successful because of the general endophytic behaviour of the larval instars and the rapid selection of resistant populations (Siqueira *et al.*, 2000; Lietti *et al.*, 2005; Silva *et al.*, 2011). Occurrence of *T. absoluta* at increasing population levels led growers to extensively use insecticides, which could cause many side-effects on natural enemies in tomato crops (Desneux *et al.*, 2007; Biondi *et al.*, 2012). Several eco-sustainable control methods and integrated pest management (IPM) programs have been recently evaluated (Batalla-Carrera *et al.*, 2010; Mollá *et al.*, 2011; Vacas *et al.*, 2011; Zappalà *et al.*, 2012). In this framework a key role could be played by biological control agents (Desneux *et al.*, 2010). In Italy, like in most invaded countries, the control of *T. absoluta* is still largely based on chemical applications (Sannino and Espinosa, 2010). This approach may disrupt previous successful IPM procedures adopted during the last decades all over European countries for other pests in the field. For this reason the knowledge of the indigenous antagonists and the strategies to conserve them, have to be considered as a priority in the implementation of exotic pest management (van Lenteren and Woets, 1988). In fact, a successful establishment of non-native species is theoretically related to their higher competitiveness compared to native species as well as to the reduced control by natural enemies (Grabenweger *et al.*, 2010). In newly invaded areas there can be few natural enemy species and/or their effect on the exotic species can be weak. Another point of interest is that indigenous natural enemies need time to colonize, get adapted and effectively control the exotic species. Several examples of invading pests with poor parasitoid complexes, mainly represented by idiobiont species performing low levels of parasitism, are known. Idiobiont parasitoids do not need to adapt to their host physiology and therefore they may switch more easily to new hosts (Askew and Shaw, 1986), providing, in some cases, a substantial control of insect pests, especially of leafminers (Godfray *et al.*, 1995; Urbaneja *et al.*, 2000). Most of these examples are represented by endophytic species which are among the most heavily parasitised insects (Girardoz *et al.*, 2006).

Various indigenous species of parasitoids and predators feed on *T. absoluta* in the Mediterranean basin (Desneux *et al.*, 2010; Gabarra and Arnó, 2010; Mollá *et al.*, 2010; Loni *et al.*, 2011). They are gradually getting adapted to the new pest and will probably play a central role as limiting factors in the near future. This study aims to identify the parasitoid complex of *T. absoluta* in Southern Italy as well as to find potential biocontrol agents of this invasive pest. These goals fall in the recent European IPM guidelines of pest control favouring the application of conservation biocontrol strategies and the use of indigenous species as biocontrol agents.

Materials and methods

Tomato borer laboratory rearing

T. absoluta colonies were started from vegetal material, mainly infested by tomato borer larvae, collected in Sicily and Campania and maintained in the laboratory on cherry-type tomato plants (cv. Shiren). The host plants were grown outdoor into 16 cm diameter pots and protected by pest infestations in screened cages (300 × 80 × 120 cm) under natural climatic and photoperiodic conditions until they had reached a height of 60-70 cm. In the laboratory the moth was reared inside cages (50 × 60 × 80 cm) covered with fine polyester mesh, at 25 ± 2 °C temperature, 50 ± 10% relative humidity and a L14:D10 photoperiod. Forty *T. absoluta* adults (1:1 sex ratio) were released on 12 tomato plants per cage and provided with a protein and sugary diet (Protonectar®, Lega Italia, Italy) in a water solution using a spongy dispenser. After 20 ± 2 days, when the majority of the larvae had reached the 4th instar, the infested material was collected and reared inside 5-liter screened plastic containers until adult emergence. Adults were collected using a mechanical aspirator to be inoculated in the rearing cages. To avoid high levels of endogamy, colonies were refreshed by introducing specimens collected from the field into the rearing system at least once every two months.

Sentinel tomato plants, 60-70 cm high and with 15 expanded leaves on average, bearing *T. absoluta* eggs or larvae were selected and exposed in the field to collect parasitoids. In order to obtain plants infested by coetaneous pre-imaginal stages, the adults were removed three days after their release, therefore getting contemporary ovipositions, and the infested plants were then moved in a separate cage. Sentinel plants infested with eggs and young larvae were obtained 2-4 days from the removal of inoculated adults, those with 3rd and 4th instar larvae after 8-10 days from the removal of inoculated adults.

Parasitoid sampling

The survey was conducted in some of the Italian regions where *T. absoluta* was first detected, namely Sicily, Sardinia and Campania (figure 1), by sampling in different growing and ecological environments (table 1). Two sampling methods were used to collect tomato borer parasitoids: a) infested sentinel tomato plants, and b) field sampling of infested cultivated plants and weeds.

a. Sentinel plants

From August 2009 to May 2011, sentinel tomato plants were placed in 7 different field sites (table 1). In order to expose all pre-imaginal *T. absoluta* instars to parasitoid attack, sentinel plants infested with eggs, young and mature larvae were used. Each plant was infested with an average of 50 pre-adult instars.

In sites 1 to 6, two sentinel plants were placed every week. One plant was infested with 50% eggs and 50% young larvae, the other one with 50% 3rd and 50% 4th instar larvae. The plants were removed from the field after one week, the infested material was observed in the laboratory under a stereomicroscope and then the parasitized instars were isolated until the adult wasp emergence. A total number of 140 sentinel plants were exposed and 6,484 *T. absoluta* instars were observed. The stages showing clear parasitization activity were isolated and reared until the emergence of adult parasitoids; besides, the apparently healthy instars were also reared in order to detect endoparasitoids.

In site 7, twice a week six sentinel plants, two bearing eggs, two young larvae and two mature larvae, were exposed to parasitoid attack. Plants were replaced every 3-4 days and the foliage of each plant stored in aerated cages to detect parasitoid emergences.

b. Field sampling

Infested material was also collected by direct inspection of cultivated solanaceous plants (open field tomato, potato and protected tomato crops) and *S. nigrum* spontaneously growing close to the cultivated sites as well as in urban areas (table 1). In sites 1-6 the samples were collected from August 2009 to February 2010, while in sites 8 and 9 sampling was performed from May to October 2010 on protected tomato crops. Infested leaves were collected weekly during the entire cultivation period; each sample consisted of 100 infested leaves. To avoid collecting empty old mines, since *T. absoluta* attack moves towards apical leaves in the plant, as the crop season advances, higher leaves bearing active infestation were progressively selected. Leaves were observed under a stereomicroscope to register the number

Figure 1. Geographical distribution of the survey sites (2009-11).

of *T. absoluta* and parasitoid larvae and pupae. Foliage with apparently healthy *T. absoluta* larvae was stored in aerated boxes to isolate possible emerging adult parasitoids. In addition, tomato apical shoots were collected to search for *T. absoluta* eggs and assess the egg parasitoid activity. For this purpose, 100 eggs per sampling date were examined under a stereomicroscope and then stored in aerated tubes until parasitoid emergence.

Finally, sporadic field samplings were performed from September to October 2010 in sites 10-16 where both processing and fresh market tomato crops were grown. Only one sample was collected for each site and consisted of 100 leaves infested by *T. absoluta* larvae. Collected leaves were stored in aerated boxes to isolate emerging adult parasitoids.

In site 17, samples were collected from June to July 2010 on a cherry-type tomato crop (cv. Minuetto) grown in a plastic greenhouse without insect-proof screens and transplanted in February 2010. The crop was sprayed with abamectin and spinosad at the recommended label rates on 23 April and 27 May, respectively. A total of 160, 100 and 50 infested leaves were collected on 23 June, 30 June and 7 July 2010, respectively and maintained in ventilated plexiglass cages (30 × 30 × 30 cm) in the laboratory until adult parasitoids emergence. Moreover, dead adults and larvae of *T. absoluta* were recorded in order to assess the parasitism rate, calculated as: emerged wasps / (emerged wasps + *T. absoluta* larvae + *T. absoluta* adults).

A second sampling was carried out on a tomato crop (cv. Minuetto) grown in a glasshouse with insect-proof screens and transplanted in October 2010 (table 1, site 18). Tomato plants were sprayed on 30 November with flonicamid and abamectin and on 21 December with emamectin benzoate at the recommended label rates. From November 2010 to May 2011, *T. absoluta* leaf damage, active infestation and natural parasitism were monitored weekly. The leaf damage was assessed by counting the number of mines on three randomly-chosen leaves per plant (basal, median, and apical) over 150 plants. To assess the active infestation of *T. absoluta* larvae (as the percentage of alive larvae on sampled mines) and the parasitism rate (calculated as: parasitised *T. absoluta* larvae / dead + alive + parasitised *T. absoluta* larvae), an additional sample of infested leaflets from basal, median, apical layers was collected and 70 mines from each layer (a total of 210 mines) were observed under a stereomicroscope. The number of dead and alive *T. absoluta* larvae and pre-imaginal stages of parasitoids was recorded. Parasitised larvae were iso-

Table 1. Description of *T. absoluta* parasitoid survey sites (2009-2011).

Site Location		Ecological features	Sampling method
	Sicily		
1	Catania (CT) 37°31'8"N 15°4'18"E	Urban horticultural crops (vegetables) with scattered citrus and olive trees, abundant spontaneous flora, no pesticide treatments	Sentinel plant Field sampling from: - spontaneous flora - open field solanaceous crops
2	Catania (CT) 37°32'6"N 15°4'10"E	Uncultivated citrus orchards, scattered Mediterranean trees and shrubs (black locust tree, privet, European mettle tree, laurel), abundant spontaneous flora, no pesticide treatments	Sentinel plant Field sampling from: - spontaneous flora
3	Catania (CT) 37°28'40"N 15°3'32"E	Open field vegetables crops, scarce spontaneous flora, frequent pesticide treatments	Sentinel plant Field sampling from: - spontaneous flora - open field solanaceous crops
4	Fiumefreddo (CT) 37°46'53"N 15°12'24"E	Citrus and olive orchards, vineyards and vegetables cultivation in greenhouses, abundant spontaneous flora, organic farming	Sentinel plant Field sampling from: - spontaneous flora - greenhouse solanaceous crops
5	Siracusa (SR) 37°1'27"N 15°16'21"E	Citrus orchards and open field (potato) and protected (tomato) vegetable crops, abundant spontaneous flora, occasional pesticide application	Field sampling from: - spontaneous flora - open field solanaceous crops
6	Scicli (RG) 36°45'36"N 14°43'60"E	Protected vegetable crops, scattered olive and carob trees, abundant spontaneous flora, no pesticide applications, organic farming	Sentinel plant Field sampling from: - spontaneous flora - greenhouse solanaceous crops
	Campania		
7	Portici (NA) 40°48'54"N 14°21'05"E	Urban vegetables and ornamental crops, many species of fruit tree (citrus, olive, grapevine, apricot, etc.), holm oak (*Quercus ilex*) forest and abundant Mediterranean flora, no pesticide treatments nearby the sampling site	Continuous sampling by sentinel plants: - tomato SP infested with *T. absoluta* eggs - tomato SP infested with *T. absoluta* larvae
8	Pagani (SA) 40°46'10"N 14°36'55"E	Intensive greenhouse and field vegetable crops, frequent pesticide treatments	Continuous field sampling from: - greenhouse solanaceous crops open field tomato crop - spontaneous flora
9	Carinaro (CE) 41°0'06"N 14°16'36" E	Intensive greenhouse and field vegetable crops, orchards, IPM, spontaneous flora	Continuous field from: - greenhouse solanaceous crops spontaneous flora
10	Marigliano (NA) 40°56'54" N 14°27' 06"E	Intensive greenhouse and field vegetable crops, frequent pesticide treatments	Occasional field sampling from: - open field processing tomato crop
11	Poggiomarino (NA) 40°48'07"N 14°33'35"E	Intensive greenhouse and field vegetable crops, frequent pesticide treatments	Occasional field sampling from: - greenhouse solanaceous crops
12	Gragnano (NA) 40°41'46"N 14°30'33"E	Intensive greenhouse and field vegetable crops, frequent pesticide treatments	Occasional field sampling (collection of infested material) from: - greenhouse solanaceous crops
13	Polvica (NA) 40°58'33"N 14°26'44"E	Intensive greenhouse and field vegetable crops, frequent pesticide treatments	Occasional field sampling from: - open field processing tomato crop
14	Somma Vesuviana (NA) 40°54'19"N 14°26'17"E	Intensive greenhouse and field vegetable crops, orchards, IPM	Occasional field sampling from: - greenhouse solanaceous crops
15	Acerra (NA) 40°58'02"N 14°24'19"E	Intensive greenhouse and field vegetable crops, frequent pesticide treatments	Occasional field sampling from: - greenhouse solanaceous crops
16	S. Maria la Bruna (NA) 40°45'49"N 14°24'18"E	Intensive greenhouse and field vegetable crops, ornamentals, frequent pesticide treatments	Occasional field sampling from: - greenhouse solanaceous crops
	Sardinia		
17	Pula (CA) 38°58'19"N 8°58'1"E	Vineyards and greenhouse (without insect-proof screens) tomato crops, abundant spontaneous flora. Occasional pesticide treatments	Field sampling from: - greenhouse solanaceous crops
18	Pula (CA) 38°58'19"N 8°57'55"E	Vineyards and glasshouse (with insect-proof screens) tomato crops, abundant spontaneous flora. Several pesticide treatments	Continuous field sampling from: - greenhouse solanaceous crops
19	Pula (CA) 38°57'25"N 8°57'08"E	Open field and greenhouse (with insect-proof screens) tomato crops, abundant spontaneous flora. Occasional pesticide treatments	Field sampling from: - greenhouse solanaceous crops
20	Sorso (SS) 40°48'31"N 8°29'47"E	Open field tomato and potato crops, abundant spontaneous flora. No pesticide treatments	Field sampling from: - open field solanaceous crops - spontaneous flora

lated in Petri dishes and kept under natural conditions of temperature, relative humidity and photoperiod until adult parasitoids emergence.

In addition, to detect pupal parasitoids, tomato fruits and leaves with *T. absoluta* pupae were collected from March to April 2011 in a plastic greenhouse with insect-proof screens (table 1, site 19). Pupae were placed in plexiglass cages, as described above, until emergence of either *T. absoluta* or adult parasitoids.

Finally, tomato borer infestations on an open field potato crop and *S. nigrum* weeds were monitored biweekly from November 2010 to May 2011 (table 1, site 20). Thus, 100 potato and *S. nigrum* leaves each were collected and the number of pre-imaginal stages of *T. absoluta* and parasitoids were recorded using a stereomicroscope. Since potato crops are often infested by the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera Gelechiidae), collected larvae were periodically reared until the emergence of adults and male genitalia were observed under a microscope to ensure the correct identification of the pest.

Results

Collected parasitoids

In the three surveyed regions of Southern Italy, parasitoid specimens belonging to 13 genera and 6 families (Ichneumonidae, Braconidae, Eulophidae, Elasmidae, Pteromalidae and Trichogrammatidae) were obtained and ten species were identified (table 2).

Parasitoids were collected in 14 out of 20 monitored sites, six from both Sicily and Campania and two from Sardinia. These records suggest a higher diversity of parasitoid species in sites characterized by the presence of abundant spontaneous vegetation (herbaceous, shrub and arboreal); eight species have been found in site 1, seven in site 6, four in site 7 and five in site 8 (table 2). On the other hand, parasitoids were obtained in Sardinia only from two of the three surveyed protected tomato crops (table 2). Overall, the species most abundantly recovered were *Necremnus* spp., *Bracon nigricans* Szepligeti and *Neochrysocharys formosa* (Westwood), together representing almost 70% of the specimens collected (table 2).

The essential taxonomic and biological features of the identified species are here reported.

Diadegma pulchripes (Kokujev) (= *turcator* Aubert) (Hymenoptera Ichneumonidae) is reported as endoparasitoid of mature larvae of *P. operculella* from Turkey, Cyprus, Israel, Crete and China (Pucci *et al.*, 2003; Yu and van Actherberg, 2010). In Italy, this parasitoid can constitute the major biotic mortality factor of this pest, representing up to 60% of the natural parasitism (Ortu and Floris, 1989). The wasp was introduced in India for controlling the potato tuber moth and its establishment has been proved one century later (Sankaran, 1974). In this survey, adults were obtained from *T. absoluta*-infested sentinel plants as well as from potato infested by the tomato borer in sites 1, 3 and 5 (table 2). This report is a new host-parasitoid association. The parasitoid was reared for few generations using *T. absoluta* as host, allowing laboratory observations which showed adult emergence from 4th instar larvae and pupae of the host.

Bracon osculator (Nees) (Hymenoptera Braconidae) has a Palaearctic distribution and has been recorded in almost all European countries and also in Afghanistan, Azerbaijan, Georgia, Iraq, Israel, Kazakhstan, Korea, Mongolia, Turkey and Turkmenistan. It is an idiobiont ectoparasitoid of Lepidoptera larvae belonging to the families Coleophoridae, Nepticulidae, Momphidae, Elachistidae, Choretidae, Gracillariidae, Cosmopterigidae and Tortricidae (Yu and van Actherberg, 2010). *B. osculator* has been already reported in mainland Italy on *Elachista bisulcella* (Duponchel) and *Elachista utonella* (Frey) (Lepidoptera Elachistidae) (Parenti *et al.*, 1995). This report is a new association of this species with *T. absoluta* and the first record of this braconid wasp in Sicily. During the survey, only adult females were obtained and exclusively from sentinel plants located in site 1 (table 2). Parasitoid larvae behaved as gregarious on *T. absoluta* larvae. *Bracon* species were already reported as *T. absoluta* parasitoids in the pest native areas; in Europe, an unidentified species of *Bracon* was reported from Spain (Desneux *et al.*, 2010).

Bracon (*Habrobracon*) *nigricans* Szepligeti [= *concolorans* Marshall; *concolor* Thomson; *mongolicus* (Telenga)] (Hymenoptera Braconidae) is widely distributed in the Palaearctic region and has been recorded almost all over Europe and also in Mongolia (Papp, 2009). It is a generalist larval ectoparasitoid of some Lepidoptera and one Coleoptera species. *B. nigricans* was found feeding on *P. operculella* in Sardinia (Ortu and Floris, 1989). Our report is a new association of *B. nigricans* with *T. absoluta* and the first record in Sicily. Several specimens of both sexes were collected, in the summer season, from sentinel plants located in site 1 and from open field tomato crop in site 3 (table 2). It was successfully reared for several generations with no apparent adverse effects on the parasitoid biological performances and a laboratory rearing is actually maintained to conduct studies on its main biological and behavioural traits and on its efficacy as *T. absoluta* biocontrol agent.

Necremnus sp. near *tidius* (Walker) (Hymenoptera Eulophidae) after the paper of Gibson *et al.* (2005), that reduced the limits of *N. tidius* to specimens reared on Coleoptera Curculionidae, this species is currently under revision by an integrative approach (Bernardo *et al.*, unpublished data). This parasitoid is a biparental generalist solitary ectophagous species of lepidopteran leafminers (Bernardo and Viggiani, 2003). It has been recovered on open field crops (sites 10 and 11) and in protected crops (site 17) (table 2). Another species of the same genus was detected and provisionally indicated as *Necremnus* sp. near *artynes* (Walker) (Hymenoptera Eulophidae). *N. artynes* is a biparental generalist solitary ectophagous parasitoid of lepidopteran leafminers. Its primary host is *Cosmopterix pulchrimella* Chambers (Lepidoptera Cosmopterigidae), a leafminer attacking *Parietaria diffusa* M. et K., a species very common in Italy. *N. artynes* was recently reported as a parasitoid of *T. absoluta* in Spain (Desneux *et al.*, 2010). *Necremnus* sp. near *artynes* here

Table 2. Parasitoid taxa recovered from *T. absoluta* during the survey (2009-2011). Sampling method: SP = Sentinel plant; OF = Open field collection; PC = Protected crop collection.

Parasitoid species	Sampling method	Site	Month	Host plant	N. of collected specimens (sex ratio: males/tot)	Parasitoid community composition (%)
Larval parasitoids						
Family Ichneumonidae						
Diadegma pulchripes	SP	1	Sept., Nov.	Tomato	14 (0.4)	6.1
	SP	3	September	Tomato		
	OF	5	June	Potato		
Diadegma sp.	OF	10	October	Tomato	1 (0)	0.4
Cryptinae gen. sp.	SP	2	May	Tomato	4 (0.5)	1.7
		6	April	Tomato		
Family Braconidae						
Bracon osculator	SP	1	November	Tomato	2 (0)	0.9
Bracon nigricans	SP	1	July-Sept.	Tomato	25 (0.2)	10.8
	OF	3	August	Tomato		
Family Eulophidae						
Chrysocharis sp.	SP	1	October	Tomato	5 (0)	2.2
	SP	7	May	Tomato		
	PC	8	September	Tomato		
Elachertus sp.	SP	1	October	Tomato	1 (0)	0.4
Elachertus inunctus species group	SP	7	Apr.-May	Tomato	19 (0.8)	8.2
Necremnus sp.	OF	3	August	Tomato	3 (0.7)	1.3
	OF	8	October	Tomato		
Necremnus sp. near *artynes*	SP	6	May	Tomato	89 (0.4)	38.5
	OF	3	August	Tomato		
	OF	8	September	Tomato		
	OF	11	October	Tomato		
	PC	12	October	Tomato		
	OF	10	October	Tomato		
	PC	17	June	Tomato		
	PC	18	Oct., June	Tomato		
Necremnus sp. near *tidius*	OF	10	October	Tomato	8 (0.5)	3.5
	OF	11	October	Tomato		
	PC	17	June	Tomato		
Neochrysocharis formosa	SP	1	April, Oct.	Tomato	28 (0.4)	12.1
	SP	2	Sept.-Oct.	Tomato		
	SP	4	September	Tomato		
	OF	3	August	Tomato		
	PC	6	February	Tomato		
	OF	13	February	Tomato		
Pnigalio sp. a gr. *soemius*	SP	1	May-Sept.	Tomato	6 (0.5)	2.6
		2		Tomato		
		6		Tomato		
Pnigalio sp. b gr. *soemius*	PC	8	June	Tomato	1 (0)	0.4
Pnigalio cristatus	SP	1	June-Sept.	Tomato	2 (0)	0.9
Pnigalio incompletus	PC	8	June	Tomato	1 (1)	0.4
Sympiesis sp.	SP	1	June-Sept.	Tomato	4 (0.5)	1.7
		6		Tomato		
Family Elasmidae						
Elasmus sp.	SP	2	September	Tomato	4 (0.7)	1.7
	OF	3	August	Tomato		
Family Pteromalidae						
Halticoptera aenea	SP	6	May	Tomato	1 (0)	0.4
Egg parasitoids						
Family Trichogrammatidae						
Trichogramma sp. a	SP	7	October	Tomato	1 (0)	0.4
Trichogramma sp. b	SP	7	October	Tomato	1 (0)	0.4
Trichogramma sp. a and b	SP	7	October	Tomato	6 (1)	2.6
Trichogramma sp. c	OF	6	May	Tomato	4 (0.5)	1.7
Trichogramma sp. d	PC	18	March	Tomato	1 (1)	0.4

collected is the only species, among those recovered in this survey that was found in the three regions, both on sentinel plants, open field and protected crops (table 2). This species is at moment under revision (Bernardo *et al.*, unpublished data).

Neochrysocharis formosa (Westwood) [= *Closterocerus formosus* (Westwood)] (Hymenoptera Eulophidae) is a generalist parasitoid with a cosmopolitan distribution (Burks *et al.*, 2011). It develops as primary solitary or gregarious larval endoparasitoid of a wide range of leafmining or stemboring Coleoptera, Diptera and Lepidoptera associated to various cultivated and spontaneous plants (Noyes, 2003). The species is widespread in Italy on various crop pests, such as *Phyllocnistis citrella* Stainton (Lepidoptera Gracillariidae) (Massa *et al.*, 2001) and *Holocacista rivillei* Stainton (Lepidoptera Heliozelidae) (Alma, 1995). It is regarded as a valuable natural enemy in South America and recently a parasitisation up to 5% of tomato borer larvae, predominantly during the late season, was reported in Argentina (Luna *et al.*, 2011). Also this morphospecies could represent a complex of at least two cryptic species, as pointed out by preliminary results (Adachi-Hagimori *et al.*, 2011). This is the first report for this species on *T. absoluta* in the Mediterranean basin. During the survey, various specimens of both sexes of *N. formosa* were found, all year round, on tomato borer both collected in field sampling (open field and protected crops) and sentinel plants from Campania and Sicily (sites 1, 2, 3, 4, 6 and 13) (table 2).

Pnigalio soemius s.l. (Walker) (Hymenoptera Eulophidae) is a very common Palaearctic species parasitising over 130 leafminers and gall makers belonging to Coleoptera, Diptera, Lepidoptera and Hymenoptera, many of which considered of economic interest (Noyes, 2003; Bernardo *et al.*, 2008). In consideration of the high phenotypic intra-specific variation and the coexistence of cryptic species, the whole *P. soemius* "taxonomic concept" is currently under revision also by molecular and behavioural approaches (Bernardo *et al.*, 2008; Gebiola *et al.*, 2012). Preliminary data suggest that *P. soemius* s.l. is a complex of generalist and stenophagous species, with an intense predatory behaviour both as larva and adult (host feeding and host killing). This species complex includes resilient and potentially effective natural enemies in different agricultural ecosystems in Mediterranean environment (Bernardo *et al.*, 2006; Gebiola *et al.*, 2012). Also in this case, the report is a new host-parasitoid association. Specimens collected belong at least to two different cryptic species; a few couples of one species were obtained from sentinel plants exposed in sites 1, 2 and 6, while a second species emerged from infested material collected on protected tomato crop (site 8) (table 2).

Pnigalio cristatus (= *Ratzeburgiola cristata*) (Ratzeburg) (Hymenoptera Eulophidae) has been recently synonymised on the basis of molecular and comparative morphological analysis with related species (Gebiola *et al.*, 2010). It is a European species reported on various Coleoptera, Diptera and Lepidoptera leafminers feeding on spontaneous and cultivated plants, such as *P. citrella* (Massa *et al.*, 2001; Noyes, 2003; Vercher *et al.*, 2005). Based on currently available literature, this species is reported as associated to *T. absoluta* for the first time. A few specimens of both sexes were found on sentinel plants in site 1 (table 2).

Pnigalio incompletus (= *Ratzeburgiola incompleta*) (Boucek) (Hymenoptera Eulophidae) has been recently synonymized on the basis of molecular and comparative morphological analysis with related species (Gebiola *et al.*, 2010). Also in this case, preliminary results suggest that this is a complex of monophagous species. It is an ectophagous solitary (rarely gregarious) parasitoid of several leafminers (Diptera, Lepidoptera and Coleoptera) feeding on spontaneous and cultivated plants; it is often reared in association with *P. cristatus*, with which it shares many hosts (Gebiola *et al.*, 2010). Also in this case on the basis of currently available literature, this species is for the first time reported on *T. absoluta* and it was recovered from protected tomato crop in the site 7 (table 2).

Halticoptera aenea (Walker) (Hymenoptera Pteromalidae) is a cosmopolitan polyphagous species associated mainly with Diptera leafminers (Agromyzidae) infesting various plants also of economic interest, trees and shrubs; it is an endoparasitoid emerging from pupae of the host (Noyes, 2003). Only a female of this species was obtained from one *T. absoluta* larva on a sentinel plant in site 6 (table 2); the report is a new host-parasitoid association.

Parasitism rates by *Necremnus* spp. in greenhouse

Percentages of parasitism by *Necremnus* spp. in a tomato crop under greenhouse without insect-proof screen (site 17) were in general low throughout the cropping season, ranging from 5 to 14% (table 3). *T. absoluta* density (estimated as emerged parasitoids + *T. absoluta* larvae + *T. absoluta* adults) ranged from 1.6 to 6.77 individuals/leaf (table 3).

In the other monitored glasshouse, insect-proof screened (site 18), *Necremnus* sp. near *artynes* was the only larval parasitoid emerged from samples collected in 2010-2011 (table 4). However, parasitism rates never exceeded 8% across the cropping cycle (figure 2). The infestation levels (measured as percentage of mines with feeding larvae) were > 40% most of the season, although showing at least three peaks on 21 December (75%), 2 March (65%), and 15 April (66%). After two insecticide applications, flonicamid plus abamectin and emamectin benzoate respectively, at the beginning of the season, the larval infestation was partially reduced. Afterwards, the active infestation increased progressively (figure 2). The infestation ranged from 2 mines/leaf (14 December) to a minimum of 0.5 mines/leaf (2 March), increasing afterwards to 2.8 mines/leaf on 4 May. The seasonal pattern of *T. absoluta* infestation on leaves was affected by the frequent pruning of basal leaves. *T. absoluta* dead larvae by host feeding were also recorded, although this type of mortality never exceeded 6%. Beside the larval ectoparasitoid, an egg parasitoid, *Trichogramma* sp., emerged from leaf samples collected in this site (table 2).

Table 3. Density of *T. absoluta* per leaf and parasitism rate [wasps / (emerged wasps + *T. absoluta* larvae + *T. absoluta* adults)] of *Necremnus* sp. near *artynes* and *Necremnus* sp. near *tidius* in a greenhouse without insect-proof screens (Sardinia, 2010, site 17).

	T. absoluta instars/leaf (n.)	*Necremnus* sp. near *artynes* parasitism (%)	*Necremnus* sp. near *tidius* parasitism (%)
June 23, 2010	6.77	11.3	2.9
June 30, 2010	1.64	4	1
July 7, 2010	1.60	7.3	1.9

Table 4. Number of *T. absoluta* larvae (alive and parasitised) and *Necremnus* sp. near *artynes* adults emerged from parasitised larvae collected in a glasshouse with insect-proof screens (Sardinia, 2010-2011, site 18).

Date	*T. absoluta* larvae	*Necremnus* sp. near *artynes* emerged adults	
		Total number	Sex ratio (males/total)
November 23, 2010	78	1	1
December 1, 2010	100	2	0.5
December 7, 2010	121	2	1
February 23, 2011	108	5	0.6
March 9, 2011	115	6	0.5
March 17, 2011	80	1	1
March 23, 2011	63	5	0.8
March 30, 2011	122	8	0.6
April 7, 2011	176	2	0
April 15, 2011	231	10	0.3
April 21, 2011	178	2	0
May 4, 2011	191	7	0.6

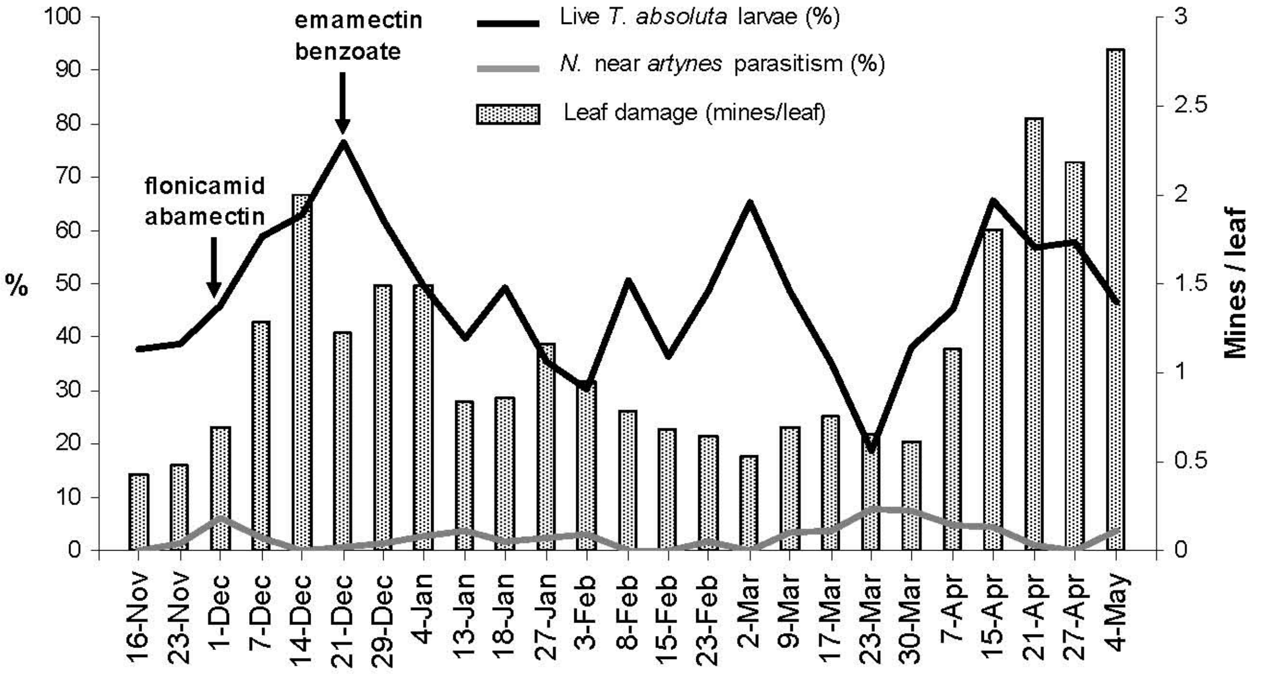

Figure 2. Seasonal pattern of active infestation of *T. absoluta* larvae, natural parasitism rate by *Necremnus* sp. near *artynes* and leaf damage in a glasshouse with insect-proof screens (Sardinia 2010-2011, site 18). Arrows indicate dates of insecticide applications.

Discussion and conclusions

The results obtained from this survey of indigenous parasitoids of *T. absoluta* conducted in a variety of different habitats (open field and protected tomato crops, other solanaceous crops and spontaneous vegetation) in Southern Italy, provided novel and relevant information contributing to the knowledge of this exotic pest in the Mediterranean basin.

Particularly, some major points are worth being mentioned. First, a prompt adaptation of native parasitoids to the new invasive host was observed, as highlighted by the natural parasitism recorded few years after the first detection of the moth. Overall six families (Ichneumonidae, Braconidae, Eulophidae, Elasmidae, Pteromalidae and Trichogrammatidae) with 13 genera and 10 identified species were recorded and in particular, the family Eulophidae was the most abundant in terms of number of species recovered. Besides, comparing *T. absoluta* parasitoid composition in Italy with that of South America, similarities arise in terms of guilds (egg, early larval, mature larval, ecto/endo, pupal, gregarious/solitary parasitoids) and families, although the number of species recorded is lower (Luna *et al.*, 2007; Desneux *et al.*, 2010). This lower species richness is typical of parasitisation pattern on exotic invasive herbivorous species, performed mainly by generalist idiobionts with relatively low levels of parasitisation in open field (Cornell and Hawkins, 1993). However, the detection of seven new associations between *T. absoluta* and the species *D. pulchripes*, *B. osculator*, *B. nigricans*, *P. soemius*, *P. cristatus*, *P. incompletus*, and *H. aenea* is noteworthy. Furthermore, *N. formosa* is the only species currently recovered on *T. absoluta* both in Europe and in South America, where it was mentioned as a potential biocontrol agent based on its previous use in other crops (Luna *et al.*, 2011). Overall, the low parasitism rate found in this survey may not support the role of the indigenous parasitoid community in effectively controlling *T. absoluta*. However, previous biological control programs of several exotic pests demonstrated the importance of indigenous natural control agents in the regulation of pest populations (Viggiani, 2000).

Species with concealed habit are among the most attractive hosts for parasitoids and one of the most recent examples of invasive leafminer species in the Mediterranean countries is *P. citrella*. Despite *T. absoluta* and *P. citrella* occupy different ecological niches, there are some interesting analogies between them. Four out of the 12 eulophids collected on *T. absoluta* were also recorded on *P. citrella* in the Mediterranean basin (Massa *et al.*, 2001; Vercher *et al.*, 2005). These parasitoid species may also develop on alternative hosts living on spontaneous flora which is very common also in the sites here surveyed. The data collected indicated that the abundance of parasitoid species was generally connected with the presence of spontaneous flora and evergreen crops as critical component of functional biodiversity. Although additional assessment of potential role of biodiversity abundance and habitat management techniques should be conducted to confirm this hypothesis, the pest populations can be reduced by enhancing the efficacy and local abundance of the existing natural enemies' community by landscape management (Gardiner *et al.*, 2009). Thus, we encourage further investigations to enhance the native parasitoid community activity through a rational habitat management within the crop as well as within the farm (Landis *et al.*, 2000). For example, evidences of negative effects of leaf pruning found in this study, which causes the removal from greenhouses of *T. absoluta* larvae suitable for parasitisation, were highlighted and a possible solution could be keeping pruned material inside greenhouses into selective mesh cages, allowing only parasitoids to emerge and move onto the crop. The increase in the abundance and diversity of the natural enemy community could be also obtained by the use of '*banker plants*', a tri-trophic system which typically consists of a non-crop plant that is deliberately infested with a non-pest herbivore (Frank, 2010). This technique was already successfully tested on tomato to control the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Rhynchota Aleyrodidae) and Diptera leafminers, *Liriomyza* spp. (van der Linden, 1992; Lambert *et al.*, 2005). However, negative interactions, such as intraguild predation, among natural enemy species may result in a long-term reduction of their ability to suppress pest populations, especially when increasing the species richness in an agricultural context (Straub *et al.*, 2008).

In this framework, improving the knowledge of endemic parasitoid host communities, still largely unknown, and of the performances of some selected key parasitoid species, both endemic and exotic, would be essential to address future studies. Besides, considering the closeness between the two moths, it could be interesting to better investigate the parasitoid complex of the potato tuber moth, *P. operculella*, for its potential role as source of parasitoids for the tomato borer. This is the case of *Copidosoma koehleri* Blanchard (Hymenoptera Encyrtidae), *B. nigricans* and *D. pulchripes*. The first species is an egg-larval parasitoid of Nearctic origin, reported in Chile on *T. absoluta* (Desneux *et al.*, 2010) and which has been recently reintroduced in Italy to control the potato tuber moth (Guerrieri and Noyes, 2005), while the two Ichneumonoidea species that were found in this survey on *T. absoluta* had already been reported as *P. operculella* natural enemies.

Necremnus sp. near *artynes*, was recovered in all the monitored regions, from May to October, and its activity could be related to the presence, in the sampled sites, of its primary hosts such as *C. pulchrimella* on *P. diffusa* (Bernardo and Viggiani, 2003). Even if this species was the most abundant and widespread, among the recovered parasitoids, its low parasitism rate does not suggest to consider this parasitoid as a key species in *T. absoluta* biological control in the Mediterranean basin. By contrast, *Necremnus* sp. near *artynes* was the only species, among those recovered, able to build up the population in treated protected tomato crops. This finding should be taken into account when applying tomato IPM programmes that should emphasize the role of natural mortality factors by selective pesticides application, by regular monitoring (both pest and its natural enemies) and by applying economic thresholds (van

Lenteren and Woets, 1988). In conclusion, information collected in this paper, supported by the theory on recruitment and accumulation of native parasitoid species on introduced herbivores (Cornell and Hawkins, 1993), may open to interesting perspective on *T. absoluta* conservation biocontrol in the Mediterranean basin.

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References

ADACHI-HAGIMORI T., MIURA K., ABE Y., 2011.- Gene flow between sexual and asexual strains of parasitic wasps: a possible case of sympatric speciation caused by a parthenogenesis-inducing bacterium.- *Journal of Evolutionary Biology*, 24: 1254-1262.

ALMA A., 1995.- Ricerche bio-etologiche ed epidemiologiche su *Holocacista rivillei* Stainton (Lepidoptera Heliozelidae).- *Redia*, 78: 373-378.

ASKEW R. R., SHAW S. R., 1986.- Parasitoid communities: their size, structure and development, pp. 225-264. In: *Insect parasitoids* (WAAGE J., GREATHEAD D., Eds).- Academic Press, London, UK.

BATALLA-CARRERA L., MORTON A., GARCÌA-DEL-PINO F., 2010.- Efficacy of entomopathogenic nematodes against the tomato leafminer *Tuta absoluta* in laboratory and greenhouse conditions.- *BioControl*, 55: 523-530.

BERNARDO U., VIGGIANI G., 2003.- Note biologiche sul *Necremnus tidius* (Walker) (Hymenoptera: Eulophidae), ectoparassitoide di *Cosmopterix pulchrimella* Chambers (Lepidoptera: Cosmopterigidae).- *Bollettino del Laboratorio di Entomologia agraria Filippo Silvestri*, 58: 87-92.

BERNARDO U., PEDATA P., VIGGIANI G., 2006.- Life history of *Pnigalio soemius* (Hymenoptera: Eulophidae) and its impact on a leafminer host trough parasitization, destructive host-feeding and host-stinging behavior.- *Biological Control*, 37: 98-107.

BERNARDO U., MONTI M. M., NAPPO A. G., GEBIOLA M., RUSSO A., PEDATA P., VIGGIANI G., 2008.- Species status of two populations of *Pnigalio soemius* (Hymenoptera: Eulophidae) reared from two different hosts: an integrative approach.- *Biological Control*, 46: 393-403.

BIONDI A., DESNEUX N., SISCARO G., ZAPPALÀ L., 2012.- Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*.- *Chemosphere*, 87: 803-812.

BURKS R. A., HERATY J. M., GEBIOLA M., HANSSON C., 2011.- Combined molecular and morphological phylogeny of Eulophidae (Hymenoptera: Chalcidoidea), with focus on the subfamily Entedoninae.- *Cladistics*, 27: 1-25.

CORNELL H. V., HAWKINS B. A., 1993.- Accumulation of native parasitoid species on introduced herbivores: a comparison of hosts as natives and hosts as invaders.- *American Naturalist*, 141: 847-65.

DESNEUX N., DECOURTYE A., DELPUECH J. M., 2007.- The sublethal effects of pesticides on beneficial arthropods.- *Annual Review of Entomology*, 52: 81-106.

DESNEUX N., WAJNBERG E., WYCKHUYS K. A. G., BURGIO G., ARPAIA S., NARVÁEZ-VASQUEZ C. A., GONZÁLEZ-CABRERA J., CATALÁN RUESCAS D., TABONE E., FRANDON J., PIZZOL J., PONCET C., CABELLO T., URBANEJA A.- 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control.- *Journal of Pest Science*, 83: 197-215.

DESNEUX N., LUNA M. G., GUILLEMAUD T., URBANEJA A., 2011.- The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production.- *Journal of Pest Science*, 84: 403-408.

FRANK S. D., 2010.- Biological control of arthropod pests using banker plant systems: past progress and future directions.- *Biological Control*, 52: 8-16.

GABARRA R., ARNÓ J., 2010.- Resultados de las experiencias de control biológico de la polilla del tomate en cultivo de invernadero y aire libre en Cataluña.- *Phytoma España*, 217: 66-68.

GARDINER M. M., LANDIS D. A., GRATTON C., DIFONZO C. D., O'NEAL M., CHACON J. M., WAYO M. T., SCHMIDT N. P., MUELLER E. E., HEIMPEL G. E., 2009.- Landscape diversity enhances biological control of an introduced crop pest in the north-central USA.- *Ecological Applications*, 19: 143-154.

GEBIOLA M., BERNARDO U., BURKS R. A., 2010.- A reevaluation of the generic limits of *Pnigalio* Schrank (Hymenoptera: Eulophidae) based on molecular and morphological evidence.- *Zootaxa*, 2484: 35-44.

GEBIOLA M., GÓMEZ-ZURITA J., MONTI M. M., NAVONE P., BERNARDO U., 2012.- Integration of molecular, ecological, morphological and endosymbiont data for species delimitation within the *Pnigalio soemius* complex (Hymenoptera: Eulophidae).- *Molecular Ecology*, 21: (5) 1190-1208.

GIBSON G. A. P., BAUR H., ULMER B., DOSDALL L., MULLER F., 2005.- On the misidentification of chalcid (Hymenoptera:Chalcidoidea) parasitoids of the cabbage seedpod weevil (Coleoptera: Curculionidae) in North America.- *Canadian Entomologist*, 137: 381-403.

GIRARDOZ S., KENIS M., QUICKE D. L. J., 2006.- Recruitment of native parasitoids by an exotic leaf miner, *Cameraria ohridiella*: host-parasitoid synchronization and influence of the environment.- *Agricultural and Forest Entomology*, 8: 49-56.

GODFRAY H. C. J., AGASSIZ D. L. J., NASH D. R., LAWTON J. H., 1995.- The recruitment of parasitoid species to two invading herbivores.- *Journal of Animal Ecology*, 64: 671-684.

GRABENWEGER G., KEHRLI P., ZWEIMÜLLER I., AUGUSTIN S., AVTZIS N., BACHER S., FREISE J., GIRARDOZ S., GUICHARD S., HEITLAND W., LETHMAYER C., STOLZ M., TOMOV R., VOLTER L., KENIS M., 2010.- Temporal and spatial variations in the parasitoid complex of the horse chestnut leafminer during its invasion of Europe.- *Biological Invasions*, 12: 2797-2813.

GUERRIERI E., NOYES J. S., 2005.- Revision of the European species of *Copidosoma* Ratzeburg (Hymenoptera: Encyrtidae), parasitoids of caterpillars (Lepidoptera).- *Systematic Entomology*, 30: 97-174.

LAMBERT L., CHOUFFOT T., TUREOTTE G., LEMIEUX M., MOREAU J., 2005.- Biological control of greenhouse whitefly (*Trialeurodes vaporariorum*) on interplanted tomato crops with and without supplemental lighting using *Dicyphus hesperus* (Quebec, Canada).- *Bulletin IOBC/wprs*, 28: 175-178.

Landis D. A., Wratten S. D., Gurr G. M., 2000.- Habitat management to conserve natural enemies of arthropod pests in agriculture.- *Annual Review of Entomology*, 45: 175-201.

Lietti M. M., Botto E., Alzogaray R. A., 2005.- Insecticide resistance in argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae).- *Neotropical Entomology*, 34: 113-119.

Loni A., Rossi E., Van Achterberg K., 2011.- First report of *Agathis fuscipennis* in Europe as parasitoid of the tomato leafminer *Tuta absoluta*.- *Bulletin of Insectology*, 64: 115-117.

Luna M. G., Sánchez N. E., Pereyra P. C., 2007.- Parasitism of *Tuta absoluta* (Lepidoptera, Gelechiidae) by *Pseudapanteles dignus* (Hymenoptera, Braconidae) under laboratory conditions.- *Environmental Entomology*, 36: 887-893.

Luna M. G., Wada V. I., La Salle J., Sánchez N. E., 2011.- *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), a newly recorded parasitoid of the Tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), in Argentina.- *Neotropical Entomology*, 40: 412-414.

Massa B., Rizzo M. C., Caleca V., 2001. - Natural alternative hosts of Eulophidae (Hymenoptera: Chalcidoidea) parasitoids of the Citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in the Mediterranean Basin.- *Journal of Hymenoptera Research*, 10: 91-100.

Mollá O., Alonso M., Monton H., Beitia F.,Verdú M. J., Gonzalez-Cabrera J., Urbaneja A., 2010.- Control biologico de *Tuta absoluta*: catalogacion de enemigos naturales y potencial de los miridos depredadores como agentes de control.- *Phytoma España*, 217: 42-47.

Mollá O., Gonzalez-Cabrera J., Urbaneja A., 2011.- The combined use of *Bacillus thuringiensis* and *Nesidiocoris tenuis* against the tomato borer *Tuta absoluta*.- *BioControl*, 56: 883-891.

Noyes J. S., 2003.- Universal Chalcidoidea Database.- World Wide Web electronic publication. [online] URL: http://www.nhm.ac.uk/entomology/chalcidoids/index.html [accessed on June 2011].

Ortu S., Floris I., 1989.- Indagine preliminare per il controllo di *Phthorimaea operculella* su coltivazioni di patata in Sardegna.- *La Difesa delle Piante*, 12: 81-88.

Papp J., 2009.- Braconidae (Hymenoptera) from Mongolia, XVII. Eleven Subfamilies.- *Acta Zoologica Academiae Scientiarum Hungaricae*, 55 (2): 139-173.

Parenti U., Bergamasco P., Scaramozzino P. L., Varalda P. G., 1995.- Limitatori naturali degli Elachistidae (Lepidoptera).- *Bollettino del Museo Regionale di Scienze Naturali di Torino*, 13 (1): 45-76.

Pereyra P. C., Sánchez N. E., 2006.- Effect of two solanaceous plants on developmental and population parameters of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae).- *Neotropical Entomology*, 35: 671-676.

Pucci C., Spanedda A. F., Minutoli E., 2003.- Field study of parasitism caused by endemic parasitoids and by the exotic parasitoid *Copidosoma koehleri* on *Phthorimaea operculella* in Central Italy.- *Bulletin of Insectology*, 56: 221-224.

Sankaran T., 1974.- Natural enemies introduced in recent years for biological control of agricultural pest in India.- *The Indian Journal Agricultural Science*, 44: 425-433.

Sannino L., Espinosa B., 2010.- *Tuta absoluta*, guida alla conoscenza e recenti acquisizioni per una corretta difesa.- *L'Informatore Agrario*, 66 (46) Supplement 1: 1-113.

Silva G. A., Picanço M. C., Bacci L., Crespo A. L., Rosado J. F., Guedes R. N. C., 2011.- Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*.- *Pest Management Science*, 67: 913-920.

Siqueira H. A. A., Guedes R. N. C., Picanço M. C., 2000.- Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae).- *Agricultural and Forest Entomology*, 2: 147-153.

Straub C. S., Finke D. L., Snyder W. E., 2008.- Are the conservation of natural enemy biodiversity and biological control compatible goals?- *Biological Control*, 45: 225-237.

Tropea Garzia G., Siscaro G., Colombo A., Campo G., 2009.- Rinvenuta in Sicilia *Tuta absoluta*.- *L'Informatore Agrario*, 65 (4): 71.

Urbaneja A., Llácer E., Tomás O., Garrido A., Jacas J. A., 2000.- Indigenous natural enemies associated with *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Eastern Spain.- *Biological Control*, 18: 199-207.

Urbaneja A., Vercher R., Navarro V., Porcuna J. L., Garcia- Marí F., 2007.- La polilla del tomate, *Tuta absoluta*.- *Phytoma España*, 194: 16-24.

Vacas S., Alfaro C., Primo J., Navarro-Llopis V., 2011.- Studies on the development of a mating disruption system to control the tomato leafminer, *Tuta absoluta* Povolny (Lepidoptera:Gelechiidae).- *Pest Management Science*, 67: 1473-1480.

van der Linden A., 1992.- *Phytomyza caulinaris* Hering, and alternative host for the development of an open rearing system for parasitoids of *Liriomyza* species.- *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society*, 3: 31-39.

van Lenteren J. C., Woets J., 1988.- Biological and integrated pest control in greenhouses.- *Annual Review of Entomology*, 33: 239-269.

Vercher R., Costa-Comelles J., Marzal C., García-Marí F., 2005.- Recruitment of native parasitoid species by the invading leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) on citrus in Spain.- *Environmental Entomology*, 34: 1129-1138.

Viggiani G., 2000.- The role of parasitic Hymenoptera in integrated pest management in fruit orchards.- *Crop Protection*, 19: 665-668.

Viggiani G., Filella F., Delrio G., Ramassini W., Foxi C., 2009.- *Tuta absoluta*, nuovo lepidottero segnalato anche in Italia.- *L'Informatore Agrario*, 65 (2): 66-68.

Yu D. S. K., van Actherberg C., 2010.- Taxapad Ichneumonoidea (May 2009 version). In: *Species 2000 & ITIS catalogue of life: 2010 annual checklist* (Bisby F. A., Roskov Y. R., Orrell T. M., Nicolson D., Paglinawan L. E., Bailly N., Kirk P. M., Bourgoin T., Baillargeon G., Eds).- DVD, Species 2000, Reading, UK.

Zappalà L., Siscaro G., Biondi A., Mollá O., González-Cabrera J., Urbaneja A., 2012.- Efficacy of sulphur on *Tuta absoluta* and its side effects on the predator *Nesidiocoris tenuis*.- *Journal of Applied Entomology*, in press (doi: 10.1111/j.1439-0418.2011.01662.x).

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Chapter IV

The biology of the Western Palaearctic parasitoid *Bracon nigricans* on the Neotropical moth *Tuta absoluta*

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Biology and Developmental Strategies of the Palaearctic Parasitoid *Bracon nigricans* on the Neotropical Moth *Tuta absoluta*

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Abstract

During the last few years following the invasion of Western Palaearctic tomato crops by the invasive South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), several indigenous generalist parasitoid species are getting adapted to the new host. Only some of them are being identified as potential biocontrol agents of this pest, and *Bracon* (*Habrobracon*) *nigricans* Szépligeti (Hymenoptera: Braconidae) is thought to be a good candidate to fill this role. This species is widely distributed in the whole Palaearctic region and most of the available data on this species relies only with taxonomic aspects and faunistic surveys. Therefore, the present study represents the first step towards establishing a scientific basis for the effective inclusion of *B. nigricans* in biological and integrated *T. absoluta* control programs in the newly invaded areas. Here, we documented the young instars development and the adult lifespan reproduction activity at constant temperature. *Bracon nigricans* was proved to be an idiobiont, synovigenic, gregarious, ectoparasitoid with a slightly male-biased progeny sex-ratio. Parasitoid females kill hosts with an injection of venom causing an irreversible paralysis of the host larva. Host killing is followed by host stinging and/or host feeding and/or oviposition. The developmental strategies of *B. nigricans* and their implications in pest biocontrol are here discussed. Although field studies would be needed, the data obtained so far in laboratory conditions suggest that this parasitoid could be considered a potential key biological control agent of *T. absoluta* in the newly invaded areas.

Keywords: Parasitoid behaviour, Host feeding, Life tables, Biological control, Tomato

1. Introduction

Invasive species represent a major threat to agronomic ecosystems (Olson 2006; Ragsdale et al. 2011) and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), an highly injurious tomato pest originating from South America, has become in the last years the most important arthropod pest of tomato cultivations in the Western Palaearctic region (Desneux et al. 2011). Integrated Pest Management (IPM) programmes have been largely implemented in many Mediterranean countries as primary control strategy since the pest invasion. This implied an increasing need of knowledge on various IPM related aspects, indeed in the last few years a growing body of literature is aimed at addressing this issue. In particular, several studies has been recently published on *T. absoluta* biology (Caparros Megido et al. 2012, Tropea Garzia et al. 2012), its susceptibility to insecticides (Roditakis et al. 2012; Tomé et al 2012); on the selectivity of the pesticides used in tomato crops (Arnò and Gabarra 2011, Biondi et al. 2012a, 2012b, Zappalà et al. 2012a); on *T. absoluta* insecticide resistance (Silva et al. 2011; Haddi et al. 2012); on the biotechnological tools for *T. absoluta* control (Cagnotti et al. 2012, Cocco et al. 2012), as well as the ecological services spontaneously provided by indigenous natural enemies, namely fortuitous biological control (Desneux et al. 2010, Zappalà et al. 2012b).

Biological control represents the most environmentally safe and economically profitable pest management options in exotic pests controlling. Recently, an increased attention has been directed to the role of generalist natural enemies as regulators of insect herbivore populations in agricultural ecosystems. Often, the use of these insect as a controller has not been implemented efficiently due to the lack of knowledge of their biology and parasitic capacity. These are obviously of value and occasionally can provide the key to success of an entire program. In this framework, the improvement of knowledge on the endemic parasitoid species attacking *T. absoluta* (still largely unknown) and of the performances of some selected key parasitoid species is essential to establish effective biological and integrated control programmes against this pest. Furthermore, new restrictive regulation on the exotic natural enemy importation in Europe has negatively affected the development of classical biological control programs (van Lenteren 2012). Therefore, before to implement classical biological control approaches, indigenous natural enemies need an extensive evaluation, since they are playing a crucial role in augmentative and conservative biological programmes.

Several generalist natural enemies of the pre-existing arthropod community in the Mediterranean basin, both predators and parasitoids, have been adapting to the new host. Some of them, such as native mirid bugs, have been successfully used in IPM programs resulting in high levels of efficacy against *T. absoluta* population (Desneux et al. 2010, Urbaneja et al. 2012, Zappalà et al. 2012a). While, the research on European effective parasitoid species is still ongoing in the whole area newly colonized by the pest (Desneux et al. 2010, Doğanlar and Yiğit 2011, Loni et al. 2011, Al-Jboory et al. 2012, Chailleux et al. 2012, Ferracini et al. 2012, Urbaneja et al. 2012, Zappalà et al. 2012b), as well as in the native area (Luna et al. 2007, 2010, 2011, 2012). Among the several natural enemy species of *T. absoluta*, some species belonging to the Braconidae family have been reported as larval parasitoids both in the host native area and in the newly invaded regions. In particular, twelve braconid endoparasitoid species belonging to seven genera have been identified in South America (Desneux et al. 2010, Luna et al. 2012), and four braconid ectoparasitoids, belonging to the *Bracon* genus and one to the *Agathis* one, were found parasitizing *T. absoluta* in the Mediterranean basin (Desneux et al. 2010, Loni et al. 2011, Al-Jboory et al. 2012, Ferracini et al. 2012, Urbaneja et al. 2012, Zappalà et al. 2012b).

Braconid wasps are, except for a few phytophagous genera, insect parasitoids and they represent one of the most diverse and rich of the parasitoid groups with more than 15,000 valid species classified in 45 subfamilies and 1,000 genera (Wharton 1993, Yu and van Achterberg 2010). Indeed, they are also extensively used as models for the study of host-parasitoid interactions, and an extraordinary amount of contributions have encompassed the broad disciplines of physiology, behaviour and evolutionary biology of this group of insects (Jervis and Kidd 1986, Hardy et al. 1992, Wharton 1993, Godfray 1994, Heimpel and Collier 1996, Jervis et al. 2001, Pennacchio and Strand 2006, Heimpel and de Boer 2008, Desneux et al. 2009, 2012). Braconids tend to attack and feed on a narrow range of hosts being specialized by biological and behavioral adaptations. For this reason, they have been of primary importance in the biological control of insect pests (Wharton 1993). Indeed, among the 219 Arthropod species commercially available worldwide for augmentative biological control programs, there are 19 braconid species belonging to 11 genera (van Lenteren 2012). These species are mass reared and commercialized to control several pests, notably Diptera and Lepidoptera, in various protected and open field crops.

Bracon (*Habrobracon*) *nigricans* Szépligeti (= *concolorans* Marshall; = *concolor* Thomson; = *mongolicus* Telenga) (Hymenoptera: Braconidae: Braconinae) is widely distributed in the whole Palaearctic region (Yu and van Achterberg 2010). Most of the available data on this species relies only with taxonomic aspects and faunistic surveys (Zappalà et al. 2012b). Its first record in association with *T. absoluta* was reported in a field survey of indigenous parasitoids attacking the new host in Italy by Zappalà et al. (2012b) and then in Jordan by Al-Jabory et al. (2012), as well as in Spain by Urbaneja et al. (2012). These data, namely related to the pest/parasitoid association discovery, are certainly insufficient to assess the role of this parasitoid as a *T. absoluta* mortality factor properly. In this framework, our study aims at gaining knowledge on the biology developmental strategies of *B. nigricans* on the new host. By means of laboratory bioassays, we developed the life-table data of *B. nigricans* at constant temperature, studying the young instars development time and survival, adult longevity, progeny sex-ratio, reproductive and non-reproductive host killing activities on *T. absoluta*.

2. Materials and methods

2.1. Study insects

Bracon nigricans colony was obtained during a survey on indigenous natural enemies attacking *T. absoluta* conducted in Italian tomato crops, by means of collection of infested material. Subsequently, the colony was maintained in the laboratory using tomato plants infested by *T. absoluta* mature larvae as hosts. *Tuta absoluta* colony was started from leaves, mainly infested by *T. absoluta* larvae, collected from commercial greenhouse tomato crops; the colony was thereafter maintained on tomato plants (cv. Marmande) in the laboratory. Seedling host plants were grown in small pots (0.3 L), watered and fertilized following the routine practices and pesticide applications were strictly avoided. Tomato plants infested by mature moth larvae (3rd and 4th instars) were obtained by releasing forty *T. absoluta* adults (1:1 sex-ratio) on 10 tomato plants inside 50 ×60 × 60 cm cages covered with a fine polyester mesh. After 14 ± 2 d of the moth adult release, when the majority of the larvae had reached the 3th instar, the plants were transferred into the parasitoid rearing cages. These measured 40 ×40 × 55 cm and were built from plastic boxes and the opening covered with a fine mesh net. *Tuta absoluta*-infested plants and honey droplets were supplied every 3 days into the wasp rearing cages.

Newly emerged 0/12-h old adults were obtained in 150mm Petri dishes ventilated by a 4cm^2 opening covered with a fine mesh net. For this, two females and four males were released into this arena, containing excised infested tomato leaves (by mature *T. absoluta* larvae) and honey droplets. After 48 hours of parasitisation activity, the wasps were removed and the infested and parasitised material was maintained inside a climatic cabinet, 10 days later newly emerged adults were collected for the bioassay, at 12-hour intervals. While, experienced, mated and 10-day old females were obtained rearing them for 10 days after their emergences, in groups of two females and four males into aerated Petri dishes with tomato leaflets, forty *T. absoluta* larvae and honey droplets. In this case, the hosts were renewed every three days.

The growing host plants and the two insect rearings were maintained in three climatic chambers (25 ± 1 °C; 60 ± 10% RH; 14:10 L.D.), while all the bioassays described below were conducted in a climatic cabinet at the same environmental conditions previously described.

2.2. Immatures survival and development

Development time and survival of *B. nigricans* young instars were studied following the growth of newly laid (≤ 6 hours) eggs until the adult emergences. A group of 40 experienced and mated were individually introduced into small (2.5 cm of diameter) Petri dishes containing honey droplets and one *T. absoluta* 4th instar larva. The parasitoid females used in this experiment were coetaneous (10/14-day old) since the mother age may affect the young instar development (see the section *3.4. Age specific oviposition activity*). The exposed host larvae were previously weighted (3.583 ± 0.092 mg on average; min: 3 mg; max 4 mg) since the host weight can significantly influence the parasitoid immature development (Hardy et al. 1992). After 6 hours of parasitization activity the females were moved into a new dish with a new host larva, and parasitized larvae bearing one and those bearing two *B. nigricans* eggs were isolated for the bioassay. This clutch sizes were chosen since pilot experiments showed that the mean number of laid eggs by *B. nigricans* females per parasitized *T. absoluta* larva is about 1.5 (see also the section *3.4. Age specific reproductive activity*). Therefore, a total of 50 one-egg-clutches and 50 two-egg-clutches , i.e. a total of 150 eggs, were isolated. The development time and survival of wasp eggs, larvae (without distinguish the different instars) and cocooned pupae were checked at six-hour intervals until *B. nigricans* adults emergences. Young instars were considered dead if no progresses in their development were noted after one week of observations or if they were totally dry. Larval stage was considered ended when the larva concluded building its cocoon and adults were considered emerged only after they completely left the cocoon.

2.3. Adults longevity

To assess whether the contact with host larvae and therefore the host feeding activity influenced the *B. nigricans* female survival, in addition to the female longevity recorded during the life-history experiment (see next section), the longevity evaluation of both sexes was conducted also in absence of host larvae. Twenty newly emerged (<12 hours) females and twenty newly emerged males were isolated individually in glass tubes (185 mm of length, 25 mm of diameter), closed with cotton lid and provided with honey droplets renewed every five days. *Bracon nigricans* survival in the tubes was daily checked and recorded. The wasps were considered dead if they remained immobile when touched with a fine paintbrush.

2.4. Age specific reproductive and host killing activity

Fifteen newly emerged (<12 hours) adult females obtained from the *Immatures survival and development* bioassay were used to assess the age specific (daily) values of reproduction and of host mortality due to non-reproductive killing (e.g. host stinging and host feeding not followed by oviposition). For this bioassay five *T. absoluta* mature larvae on five tomato leaflets were offered daily to each female, in aerated plastic dishes (130 mm diameter), until its death. This host density was used since pilot experiments showed that a single *B. nigricans* female is not able to parasitize more than five 4th instar *T. absoluta* larvae in 24 hours (Biondi A. and Zappalà L., unpublished data). To mimic the field scenario, in which parasitoid wasps use external nutrient inputs for adult feeding, such as floral and extrafloral nectar and homopteran sucking insects honeydew (Jervis et al. 2001), honey droplets were put on the internal walls of the experimental arena. To assume that mating has occurred during the whole female lifespan, each female was provided with two males that were replaced every week.

Daily checks were performed in order to assess: (i) the number of killed and not parasitized larvae, (ii) the number of parasitized larvae, (iii) the number of laid eggs, (iv) the clutch size and (iv) the parasitoid survival. Exposed larvae were defined as parasitized when at least one *B. nigricans* egg was deposited onto or near the paralyzed host. While evidences of non-reproductive *B. nigricans* killing behaviour varied from a black spot developed after insertion of the ovipositor (host stinging) to partial or complete desiccation due to feeding and for the presence of feeding tubes built up by the female (host feeding). Survived specimens were then transferred in a new box with new set of five host larvae. The boxes bearing the larvae, both alive, killed and parasitized, were reared per fifteen days in the previously described climatic cabinet and the number and the sex-ratio of the emerged progeny were recorded.

2.5. Demographic growth and kill rate parameters

Life tables were constructed using daily values of the survival and, in the case of the adult females, the progeny produced, obtained as previously described. These data were used to generate the following demographic growth parameters (Birch 1948, Bernardo et al. 2006):

Net reproductive rate or *basic reproductive rate*:

$$R_o = \sum \mathrm{l_x\, m_x}$$

where x is the pivotal age of individuals in days, l_x is the age-specific survival as proportion of individuals still alive at age x and m_x is the age-specific fecundity. This parameter corresponds to the number of times a population will multiply per generation.

Generation time:

$$T = \sum \frac{\mathrm{x\, l_x m_x}}{\mathrm{R_o}}$$

which is the average time interval separating female births of one generation from the next.

Intrinsic rate of increase:

$$r_m = \sum e^{r_m x} l_m m_x$$

which is the innate capacity of a given species to increase in numbers. Biologically this parameter is the number of times the population will multiply itself per unit of time, measured as female progeny/female/unit of time (one day in this case) and where e^{rm} is the antilog of r_m.

Finite rate of population increase

$$\lambda = e^{r_m}$$

that is the factor by which a population multiplies in each time interval step, one day in this case.

Doubling time

$$DT = \frac{log_e 2}{r_m}$$

which is the time required for a given population to grow exponentially without limit to double in size when increasing at a given r_m.

Furthermore, a parameter well representing the actual ecological services provided (i.e. host killing activity) by *B. nigricans* females in *T. absoluta* biocontrol was generated. The kill rate (K_m) was calculated using the same formula of r_m, but replacing m_x (age-specific fertility) with the age-specific killed larvae (k_x), i.e. the total larvae killed, both parasitized and not (Bernardo et al. 2006). Thus K_m, was calculated using the following formula:

$$K_m = ln \frac{K_0}{T_k}$$

where K_0 (*Net consumption rate*) is the amount of larvae killed

$$K_0 = l_x k_x$$

and T_k (*Killing generation time*) is the generation period during which the parasitoid kills hosts

$$T_k = \frac{l_x k_x}{K_0}$$

To estimate the standard error associated with r_m and K_m of *B. nigricans*, we performed the Jackknife technique on raw data, calculating the per capita r_m and K_m, each time omitting one replicate, repeating this process until pseudo-values were calculated for all the possible omission cases. Then we computed the standard error applying the Jackknife formula (Meyer et al. 1986).

2.6. Statistical analysis

To test the effects of the female age on the various life-history traits weekly age-specific data on gregariousness rate, percentages of parasitized, killed host larvae and of developed adults from laid eggs, as well as the number of laid eggs, of emerged progeny and their sex-ratio were calculated per each female. These datasets, in addition to the data on immature development, were first tested for normality and homogeneity of variance using Kolmogorov-Smirnov D test and Cochran's test respectively, and transformed if needed. Data were then analyzed by one-way ANOVA and the means were separated at the 0.05 level of significance by a multiple range test followed by LSD post hoc tests for multiple comparisons inside the different ages.

3. Results

3.1. Immatures survival and development time

Bracon nigricans adult emergences from newly laid eggs took 269.65 ± 2.70 and 270.67 ± 7.32 hours (about 11.25 days) for females and males, respectively, with no significant differences between the sexes ($F_{1,54}$ = 12.937, P=0.916) (Fig. 1). Similarly, no significant differences were highlighted when comparing males and female for their egg ($F_{1,54}$ =6.728, P=0.673), larval ($F_{1,54}$ = 12.937, P=0.523) and pupal ($F_{1,54}$ =6.728, P=0.875) development. Cocooned pupae development attained the longest duration among the three developmental stages, indeed 64.45 and 63.53 % of the whole immatures development time was spent at the cocooned pupa stage by females and males, respectively (Fig. 1). The overall survival rate of *B. nigricans* immature stages (from egg to adult) developing on *T. absoluta* fourth instar larvae was 37.4 ± 4.11%. The highest mortality rate was recorded at the larval stage, where only 42.63 ± 4.49 % of the larvae survived. By contrast, 86.4 ± 2.90 % of the eggs hatched and the 98.11± 1.88 % of adults emerged from the cocooned pupae. Overall, in this trial a total of 56 adult wasps were obtained, of which 22 female and 34 males, therefore with a sex-ratio of 0.60 mm/tot.

3.2. Adults longevity

The adult median lethal time (LT_{50}), i.e. the time needed for 50% mortality, was estimated to be 44.5 and 45 days for females in contact with host larvae in Petri dishes and for those without hosts reared in glass tubes, respectively. The average survival was 41.47 ± 2.42 days for host-feeder females and with access to honey, and 42.75 ± 2.28 for females which fed only on honey (Fig. 2). Between these two groups of females there were no statistical differences for longevity ($F_{1,33}$ =0.145, *P*=0.706). By contrast, the life expectancy was strongly sex-depended since honey feeder males lived only 27.20 ± 2.39 (min 10, max 47) days, with a LT_{50} of 27 days (Fig. 2). Indeed, when comparing the longevity of females and males reared inside the tubes significant differences were highlighted ($F_{1,38}$ =22.157, *P*<0.0001).

3.3. Age specific host killing activity

Owing to a non-reproductive host killing activity, i.e. that hosts were stunk without being parasitized, *B. nigricans* females killed in their lifespan an average of 87.40 ± 8.46 (min 55; max 123) host larvae, while the mean daily value was 2.01 ± 0.09 (min 0; max 5) killed larvae/female, i.e. the 40% of the exposed hosts. The host killing activity period was longer than the oviposition one, 39.70 ± 3.43 days. Thus, the females continued to kill hosts also after the end of the oviposition period. Analyzing the weekly values of killed and not parasitized host larvae, significant differences were highlighted between the first four weeks and the last three ($F_{6,86}$=6.728, P<0.0001), being the values of the first weeks higher than the last ones. In particular, the percentages of killed larvae ranged from 47.62 ± 4.02 % to 22.12 ± 4.95 % in the second and in the last week, respectively (Fig. 3). Whereas, when the total killed host larvae, both parasitized and not, was incorporated in the formula of the kill rate (K_m), it resulted 0.121 ± 0.071.

3.4. Age specific oviposition activity

Bracon nigricans female showed to have, as the majority of the synovigenic wasp species, a short pre-oviposition period of 1.27 ± 0.3 days, representing only a small fraction of the entire lifespan. The oviposition period lasted 37.60 ± 3.25 days with an average of 1.66 ± 0.14 (min 0, max 8) laid eggs/female/day. A total of 82.8 ± 7.81 (min 48, max 127) eggs were laid by a single female during its whole life. The mean values of daily oviposition ranged from 0 to 3.6 ± 0.55 laid eggs/female/day on the 18th day (Fig. 4), while the peak of the average progeny emergences (1.7 ± 0.43 adults produced/female/day) was recorded for 16-day old females. The progeny produced during the entire lifespan was 29.79 ± 3.01 (min 13, max 40) adults/female, with a sex-ratio of 0.63 ± 0.05 emerged males/total progeny. The number of laid eggs per parasitized host was uniform during the female lifespan, i.e. the gregariousness rate (grand mean 1.52 ± 0.07; max 5 eggs laid/parasitized host) was not significant different among the weeks ($F_{6,77}$= 1.450, P=0.207) (Fig. 5).

The weekly values of parasitized hosts reached their maximum on the 3rd week (35.83 ± 2.73 %) with significantly different values compared to those recorded in the last two weeks ($F_{6,86}$= 12.937, P<0.0001) (Fig. 3). These results are especially due to the reproductive senescence phase of the females that become egg-limited (i.e. the ovaries become exhausted of oocytes) (Mange and Heimpel, 1998). In addition, it is noteworthy that a post-oviposition period of 3.67 ± 0.6 days was recorded (Fig. 4). Also the weekly values of the number of laid eggs and emerged progeny followed undoubtedly this trend and were significantly different among the weeks (eggs laid: $F_{6,86}= 7.894$, $P<0.0001$; emerged progeny: $F_{6,86}= 8.926$, $P<0.0001$) (Fig. 6). In particular, the maximum reproductive activity was recorded in the 3rd week, with a mean of 2.50 ± 0.17 laid eggs/female/day and of 1.01 ± 0.13 emerged progeny/female/day (Fig. 6). The progeny sex-ratio was not homogeneous among the seven weeks and significant differences were found between the first and all the other weeks ($F_{6,75}= 4.096$, $P<0.001$), in particular it ranged from 0.93 ± 0.03 to 0.52 ± 0.02 emerged males/total progeny (Fig. 7). In addition, the overall progeny sex-ratio was slightly male-biased, i.e. 0.62 ± 0.05 males/total progeny.

Daily observations of the eggs laid and the following developed adults showed that, even if the grand mean of the percentage of developed adults/laid eggs in the whole parasitoid lifespan (32.11 ± 3.43 %) was similar to that obtained in the immature development bioassay, this value was strongly related to the mother age ($F_{6,83}= 5.067$, $P<0.0001$) (Fig. 8). Indeed, weekly percentages of the 1st and of the 7th weeks (13.81 ± 2.23 % and 12.77 ± 0.73 %, respectively) were significantly lower than the percentages of development observed in the 2nd, 3rd, 4th and 5th (Fig. 8).

The values of the demographic growth parameters obtained from the developed life tables were: *Net reproductive rate* (R_0) 4.444; *Generation time* (T) 29.733 days; *Intrinsic rate of increase* (r_m) 0.052 ± 0.032; *Finite rate of population increase* (λ) 1.0514 days and *Doubling time* (DT) 13.816 days.

4. Discussion

In this study, we examined the development and reproduction-related traits of *B. nigricans* feeding on the exotic host *T. absoluta*. We reported the results on the rate of successful young instars development and on its duration, on induced host mortality by both reproductive and non-reproductive behaviours of *B. nigricans* females and on the reproductive parasitoid fitness. *Bracon nigricans* proved to be an idiobiont, gregarious, ectoparasitoid with a slightly male-biased sex-ratio. Nevertheless, this species showed a behavioural plasticity in host using, since host killing is followed by host stinging and/or host feeding and/or oviposition, *B. nigricans* can perform both destructive and concurrent host feeding.

4.1. Immature development

In parasitoid waps the success of parasitism after host acceptance and therefore immature development may depend on multiple physiological and genetic factors. Phylogenetic, evolutionary and coevolutionary aspects, concering plants, pests, parasitoids and their combined interactions regulate these factors largely (Desneux et al. 2012). One of the most studied relations is the parasitoid/pest one, and usually the interaction between these actors is mediated by gene products that the adult wasp injects at oviposition or that offspring produces during its development (Pennacchio and Strand 2006). These factors usually induce complex physiological alterations in hosts that benefit the parasitoid development. They are well known for endoparasitoid/host associations and, although these mechanisms are still poorly investigated for ectoparasitoids (Pennacchio and Strand 2006), the low immature development rate found in our study suggests that the host/parasitoid interaction should be also viewed from a coevolutionary perspective. In our case, the lack of coevolution between *T. absoluta*/tomato and *B. nigricans* may explain the absence of these mechanisms and therefore the low rate of young instars development. To support this hypothesis, another factor that may have influenced the successful development of immature wasps is the potential presence of toxins within the host larvae (Desneux et al. 2009). In particular, some secondary metabolites present in solanaceous plants, such as tomato, are well-known to have insecticidal properties (Nenaah 2011, Ali et al. 2012) and some of them may be sequestered by *T. absoluta* larvae and therefore available for the immature parasitoids.

4.2. Adult longevity

The contribution of feeding regimes to survival is well documented for a diverse range of parasitoid species held under laboratory conditions (Jervis et al. 2001, Bernardo et al. 2006), and while the positive correlation between host feeding and fecundity appears to be ubiquitous, host feeding may either increase or have no effect on longevity (Heimpel and Collier 1996). Here we demonstrated that *B. nigricans* females have no advantages from host feeding in terms of longevity when another food source (i.e. honey) is also available. Whereas, to assess the actual role of host feeding on *B. nigricans* lifetime gaining further bioassay should be carried out in absence of sugary nutrients. By contrast, as already reported for other Hymenoptera parasitoids (Bernardo et al. 2006), we believe that in our experiment the sugary food provided to the wasps plays a key role in the energetic balance, and therefore in the longevity, as well as in the lifetime reproductive success of this species.

The metabolic processes of the organisms whose internal temperature variation is a consequence of variation in the ambient environmental temperature (i.e. poikilotherm species) are directly related with the environment thermal conditions, and thus parasitoid wasps longevity may be strongly correlated with the temperature. Often, small insects are longer-living in cooler environment and vice versa, as demonstrated by Andreade et al. (2011), Bernardo et al. (2006) and Spanoudis et al. (2012) for thichogrammatid, eulophid and ichneumonid species, respectively. Therefore, further studies under different temperature regimes are needed to address this point.

4.3. Reproductive strategies

The *B. nigricans* oviposition activity fluctuated along the female lifespan and, since the host availability was maintained constant, it may reflect directly the ovigeny cycle of synovigenic anhydropic species. This is true for a close species, i.e. *Bracon hebetor* (Say), whose females have a short oviducts and ovaries provided of few ovarioles that are not able to store a large number of large and yolk-rich eggs (Jervis and Kidd 1986). Owing to this reproductive mechanism the females wasp are subjected to choose the optimal clutch size decision in relation to their egg load. Gregarious parasitoids have received considerable attention by numerous researchers, since their females should lay the number of eggs that maximizes their gain in fitness from the whole clutch, i.e. eggs laid in the same event (Godfray, 1994). From egg-limitation and time-limitation models, it is theoretically expected that the optimal clutch size will vary over time in response to changes in egg load, the number of previous host encounters and host availability. Moreover, for the parasitoid it is important to optimize the ratio between time spent laying eggs and that spent on host locating (Mangel and Heimpel 1998, Jervis et al. 2001). Our results suggest that *B. nigricans* mothers, at constant host density, do not adjust their clutch size in relation to their age (i.e. the gregariousness rate was constant during the female lifespan) and therefore to their egg loads. Whereas, it appears that the higher egg load is split among a higher number of hosts, i.e. increasing the rate of parasitized larvae in the period of higher egg production.

The rate of immatures development success showed to be strongly related to the mother age and this finding may be due to the production of low-quality offspring (e.g. less viable eggs), during the 1st and the 7th weeks. *Bracon nigricans* young females, as the majority of synovigenic Hymenoptera, need to feed on hosts to allocate nutritive resources on the reproductive physiology and therefore the firstly produced progeny may be of lower quality than that of experienced females (Jervis and Kidd 1986). Besides, the production of low-quality offspring prior to mother egg limitation (i.e. reproductive senescence) is already documented for other braconid species (Mangel and Heimpel 1998). Another hypothesis to explain this result is a reproductive trade-off of the mother between host feeding and oviposition, i.e. owing to higher nutritional necessities, young and old females may practice more host feeding and concurrent host feeding/oviposition. Indeed, in the majority of the parasitoid-host systems, host feeding kills the host, making it unsuitable for oviposition (destructive host feeding), while in other systems, such as in the *B. nigricans*/*T. absoluta* one (feeding tubes were seen in the parasitized larvae), individual hosts can support both parasitism and host feeding (Jervis and Kidd 1986). By contrast, it is usually assumed that host feeding kills the host or reduces its quality as an oviposition site (Heimpel and Collier 1996). Thus, the plasticity of female parasitoid behaviour, when finding a host, makes possible the decisions of whether or not to renounce the opportunity for current reproduction (oviposition) in favour of anticipated chances for future reproduction (host feeding) (Jervis et al. 1996).

The sex-ratio of the *B.nigricans* progeny showed to be male biased and, as the majority of the Hymenoptera and in particular braconid species, this species may be arrhenotokous (i.e. virgin females produce only male progeny) (Wharton 1993, Heimpel et al. 1999, Heimpel and de Boer 2008). This hypothesis is also supported by the fact that the progeny of young female is mainly composed by males, therefore the eggs laid during the first days of oviposition may be haploid (unfertilized eggs), since females may take time to mate and to be successfully fecundated after their emergence. Indeed, many braconid parasitoids unfertilized eggs develop as haploid males, while the fertilized ones are diploid and can develop into males or females. Furthermore, haplodiploidy is often associated with a genetic mechanism, known as complementary sex determination (Heimpel and de Boer 2008). In fact, many hymenopterans that are heterozygous at the sex locus develop as females whereas homozygotes and hemizygotes at the same locus develop as diploid and haploid males, respectively (Heimpel et al. 1999). This means that high heterozygosis levels, i.e. high allele diversity, that are generally present in field and in large commercial insectary populations, bring to a female-biased sex-ratio. While in population maintained in relatively small laboratory cultures, as in the case of this study, high inbreeding levels lead to a male-biased sex-ratio (Heimpel et al. 1999). However, diploid male survival has been reported in a large number of species, and immature survival of diploid males varies among distantly related species and it may vary also among strains of the same species. For example, inter-specific variation in the viability of diploid males in closely related species of the genus *Bracon* has been also found (Holloway et al. 1999). Therefore, the survival of *B. nigricans* diploid males (i.e. eggs viability, larval survival and adult reproductive potential) should be further assessed to provide potential explanations about its actual sex-ratio, as well as on its low egg-to-adult development rate.

4.4. Host stinging

Bracon nigricans females, as idiobiont parasitoid species, kill host larvae with an injection of venom that causes after few seconds an irreversible paralysis of the host larva. After the host immobilization, females showed an intense stinging activity before effectively starting to lay eggs and/or to feed on the host haemolymph. During this pre oviposition activity, female inject venom for permanently paralyzing hosts and very likely for assessing host suitability (host acceptance), based on host size and/or host aemolymphatic kairomone recognition (La Rocca et al. 2007). After this, females may feed on the host larva directly of after building up feeding tubes built up to reach immobilized larvae inside the mines. Host tissues and hemolymph are a rich source of nutrients, such as proteins, fats, sugars and various essential vitamins and salts that are either scarce or absent in other foods. These materials are presumably digested into simpler molecules in the gut and/or haemolymph, and then absorbed, translocated and reassembled into parasitoid tissues (Heimpel and Colier 1996). It is well-known that the nutrients obtained by host feeding are used to mature eggs and, without exception, parasitoid species known to feed on host are synovigenic (Jervis and Kidd 1986; Pennacchio and Strand 2006).

The portion of killed and not parasitized hosts, besides representing a host feeding substrate may be due to a strategy to control the leafminer density present in the leaflets. This strategy aims at ensuring that a leaflet containing parasitized larvae will not be lost due to the feeding activity of survived non-parasitized larvae on the same leaflet (Patel et al. 2003). Undeniably, extreme leafmining may cause desiccation, necrosis and abscission of leaflets, thus resulting in a potential decrease of parasitoid larvae survival. Another plausible reason for this behaviour is that female parasitoids kill more host larvae to provide a food reserve for future reproduction or/and to the progeny, i.e. developing wasp larvae (Lauzière et al. 1999). Moreover, Jervis et al. (1994) previously observed a post-reproductive stinging activity in *B. hebetor*. These authors found that old female wasps continue to feed on the host larvae to maintain themselves without having the possibility to lay any eggs owing to the senescence of their reproductive system. This phenomenon has two effects: (i) the impact of the parasitoid on the host population is greater than predicted solely from the degree of parasitism (similar effect of the destructive host feeding); (ii) paralyzed larvae may be available to other female wasps that will have a reduced handling time, increasing their searching efficacy.

4.5. Demographical growth parameters

In this study we developed the population growth parameters of *B. nigricans* and, even if life history parameters are determined under artificial laboratory conditions, knowledge of the potential demographical growth is crucial for studying population dynamics and for establishing management tactics for pest control (Birch 1948, Stark et al. 2004, Bernardo et al. 2006). Furthermore, the importance of life table parameters was remarked by Stark et al. (2004) when stating that the knowledge of the demographical growth parameters of a given species is essential for pesticides risk assessment studies. Indeed, each species has its own life-history strategies (e.g. lifespan, time to first reproduction and number of offspring produced over a lifetime) and therefore its population levels recovery strategy and timing.

When comparing the intrinsic rate of natural increase of *B. nigricans* and the one of *T. absoluta*, previously developed in laboratory by Pereyra and Sanchez (2006), it is evident how the moth has an higher potential population growth capacity, having a higher r_m value of the *B. nigricans* one, i.e. 0.14 and 0.052, respectively. This result may implies that this parasitoid species be not able to control the pest populations effectively; however, in order to assess the true *B. nigricans* fitness further studies at different host density levels are still needed. Indeed, parasitoid oviposition rate may be a function of the host density, which implies different effects on the host population dynamics (Luna et al. 2007). At different host densities, the rate of parasitism, therefore the overall parasitoid fitness, may be influenced by behavioural decision, namely the time spend in host searching and handling (Walde and Murdoch 1988).

4.6. Prospects for *Tuta absoluta* biocontrol

Many efforts have been done during the last few years in the pest newly invaded areas with the aim to identify and characterize effective *T. absoluta* natural enemies. These relied mainly on faunistic surveys and host suitability assessments in laboratory conditions; whereas only generalist predators (namely mirids), preying on *T. absoluta* eggs and young larvae, have been included in effective tomato IPM programs successfully. In this study, we reported for the first time the life history strategies and the main behavioural traits of a Palaearctic parasitoid when parasitizing the mature larvae of the South American tomato pinworm.

The main result of this study is that *B. nigricans* female adults showed a high rate of non-reproductive killing behavior, namely numerous host larvae were killed owing to host feeding and host stinging during the whole female lifespan. This activity shows how the biological control service of this parasitoid might be underestimated considering only the rate of parasitism or the emerged adults. Indeed, the *B. nigricans* kill rate (K_m = 0.121) is higher than its intrinsic rate of natural increase (r_m = 0.052). These results remark that despite the population dynamics theories, destructive host feeder species may be better biological control agents than other parasitoids (Jervis et al. 1996). Moreover, *B. nigricans* showed to be a typical synovigenic species and such parasitoids are long-living, are able to reproduce at lower host densities and, in the case of anhydropic species (as *B. nigricans*), to conserve reproductive material in relation to host density notably resorbing unlaid eggs. In addition, synovigenic species being not time-limited are also able to spend more time in foraging and host searching behaviours. Indeed, studies on leafminer communities indicate that generalist idiobiont parasitoids may have broad host ranges and parasitize almost all hosts of suitable size in a given search environment (Pennacchio and Strand 2006). Therefore, in order to enhance the *B. nigricans* presence and its biological control services in protected and open field tomato crops, the wasp host range (within the endemic parasitoid host communities) needs to be clarified definitively.

All the *B. nigricans* biological and behavioural traits reported in this study are potentially useful for the control of *T. absoluta* populations. Indeed, this study represents the first step towards establishing the scientific basis for the effective inclusion of *B. nigricans* in biological and integrated *T. absoluta* control programmes in the areas newly invaded by this pest. This could be achieved by the conservation of the wasp populations wildly present in the cultivated environments through the use of selective pesticides (Arnò and Gabarra 2011, Biondi et al. 2012a, 2012b) and of rational habitat management strategies (Gardiner et al. 2009) and/or by augmentative releases of mass reared wasps (van Lenteren 2012). Nevertheless, before giving overall conclusions on *B. nigricans* field efficacy, various additional factors need to be specifically tested, since they may strongly affect the biocontrol activity and population dynamics of *B. nigricans* in field conditions. These are mainly represented by pesticide non-target effects (Desneux et al. 2007, Lu et al. 2012); agronomic conditions (Desneux and Ramirez-Romero 2009, Amini et al. 2012); interactions among natural enemy species (parasitoids and predators) sharing the same host/prey (Zappalà et al. 2007, Luna et al. 2010, Bompard et al. 2013), wasp dispersal and host searching capacities (Sánchez et al. 2009, Zappalà et al. 2012c). Thus, although field studies would be needed, the data obtained so far in laboratory conditions suggest that this parasitoid could be considered a potential key biological control agent of *T. absoluta* in the newly invaded areas.

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References

Ali, A., F. Ahmad, A. Biondi, Y. Wang, and N. Desneux. 2012. Potential for using *Datura alba* leaf extracts against two major stored grain pests, the khapra beetle *Trogoderma granarium* and the rice weevil *Sitophillus oryzae*. J. Pest Sci. 85: 359-366.

Al-Jboory, I. J., A. Katbeh-Bader, and S. Al-Zaidi. 2012. First observation and identification of some natural enemies collected from heavily infested tomato by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Jordan. Middle East J. Sci. Res. 11: 435-438.

Amini, B., H. Madadi, N. Desneux, and H. A. Lotfalizadeh. 2012. Impact of irrigation systems on seasonal occurrence of *Brevicoryne brassicae* and its parasitism by *Diaeretiella rapae* on canola. J. Entomol. Res. Soc. 14: 15-26.

Andrade, G. S., D. Pratissoli, L. P. Dalvi, N. Desneux, and H. J. Gonçalves. 2011. Performance of four *Trichogramma* species (Hymenoptera: Trichogrammatidae) as biocontrol agents of *Heliothis virescens* (Lepidoptera: Noctuidae) under various temperature regimes. J. Pest Sci. 84: 313-320.

Arnó, J, and R. Gabarra. 2011. Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). J. Pest Sci. 84: 513-520.

Bernardo, U., P. A. Pedata, and G. Viggiani. 2006. Life history of *Pnigalio soemius* (Walker) (Hymenoptera : Eulophidae) and its impact on a leafminer host through parasitization, destructive host-feeding and host-stinging behavior. Biol. Control 37: 98-107.

Biondi, A., N. Desneux, G. Siscaro, and L. Zappalà. 2012a. Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. Chemosphere 87: 803-812.

Biondi, A., V. Mommaerts, G. Smagghe, E. Vinuela, L. Zappalà, and N. Desneux. 2012b. The non-target impact of spinosyns on beneficial arthropods. Pest Manag. Sci. , 68: 1523-1536.

Birch, L. C. 1948. The intrinsic rate of natural increase of an insect populaton. Jour. Animal Ecol. 17: 15-26.

Bompard, A., C. C. Jaworski, and N. Desneux. 2012. Sharing a predator: can an invasive species affect the predation pressure on a local pest? Popul. Ecol. (in press).

Cagnotti, C. L., M. M. Viscarret, M. B. Riquelme, E. N. Botto, L. Z. Carabajal, D. F. Segura, and S. N. López. 2012. Effects of X-rays on *Tuta absoluta* for use in inherited sterility programmes. J. Pest Sci. (in press) DOI: 10.1007/s10340-012-0455-9.

Caparros Megido, R., E. Haubruge, and F. J. Verheggen. 2012. First evidence of deuterotokous parthenogenesis in the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). J. Pest Sci. (in press) DOI: 10.1007/s10340-012-0458-6.

Chailleux A., N. Desneux, J. Seguret, H. Do Thi Khanh, P. Maignet, and E. Tabone. 2012. Assessing European egg parasitoids as a mean of controlling the invasive South American tomato pinworm *Tuta absoluta*. PLoS ONE 7: e48068, doi:10.1371/journal.pone.0048068

Cocco, A., S. Deliperi, and G. Delrio. 2012. Control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in greenhouse tomato crops using the mating disruption technique. J. Appl. Entomol. (in press) DOI: 10.1111/j.1439-0418.2012.01735.x.

Desneux, N., A. Decourtye, and J.M. Delpuech. 2007. The sublethal effects of pesticides on beneficial arthropods. Ann. Rev. Entomol. 52: 81-106.

Desneux, N, R. J. Barta, K. A. Hoelmer, K. R. Hopper, and G. E. Heimpel. 2009, Multifaceted determinants of host specificity in an aphid parasitoid. Oecologia 160: 387-398.

Desneux, N., E. Wajinberg, K. A. G. Wyckhuys, G. Burgio, S. Arpaia, C. A. Nàrvaez-Vasquez, J. Gonzàlez-Cabrera, D. Catàlan Ruescas, E. Tabone, J. Frandon, J. Pizzol, C. Poncet, T. Cabello, and A. Urbaneja. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. J. Pest Sci. 83: 197-215.

Desneux, N., M. G. Luna, T. Guillemaud, and A. Urbaneja. 2011. The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond. J. Pest Sci. 84: 403-408.

Desneux, N., R. Blahnik, C. J. Delebecque, and G. E. Heimpel. 2012. Host phylogeny and specialisation in parasitoids. Ecol. Lett. 15: 453-460.

Doğanlar, M., and A. Yiğit. 2011. Parasitoid Complex of the Tomato Leaf Miner, *Tuta absoluta* (Meyrick 1917), (Lepidoptera: Gelechiidae) in Hatay, Turkey. KSU J. Nat. Sci. 14: 28-37.

Ferracini, C., B. L. Ingegno, P. Navone, E. Ferrari, M. Mosti, L. Tavella, and A. Alma. 2012. Adaptation of indigenous larval parasitoids to *Tuta absoluta* (Lepidoptera: Gelechiidae) in Italy. J. Econ. Entomol. 105: 1311-1319.

Gardiner, M. M., D. A. Landis, C. Gratton, C. D. Di Fonzo, M. O'Neal, J. M. Chacon, M. T. Wayo, N. P. Schmidt, F. F. Mueller, and G. E. Heimpel. 2009. Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. Ecol. Appl. 19**:** 143-154.

Godfray, H. C. 1994. Parasitoids: Behavioural and Evolutionary Ecology. Princeton University Press, Princeton, New Jersey.

Hardy I. C. W., N. T. Griffiths, and H. C. Godfray. 1992. Clutch size in a parasitoid wasp: a manipulation experiment. J. Anim. Ecol. 61: 121-129.

Haddi, K., M. Berger, P. Bielza, D. Cifuentes , L. M. Field, K. Gorman, C. Rapisarda, M. S. Williamson, and C. Bass. 2012. Identification of mutations associated with pyrethroid resistance in the voltage-gated sodium channel of the tomato leaf miner (*Tuta absoluta*). Insect Biochem. Mol. Biol. 42: 506-513.

Heimpel, G. E., and T. R. Collier. 1996. The evolution of host-feeding behaviour in insect parasitoids. Biol. Rev. 71: 373-400.

Heimpel, G. E., M. F. Antolin, and M. R. Strand. 1999. Diversity of sex-determining alleles in *Bracon hebetor*. Heredity 82: 282-291.

Heimpel, G. E. and J. G. de Boer. 2008. Sex Determination in the Hymenoptera. Annu. Rev. Entomol. 53: 209-30.

Holloway, A. K., G. E. Heimpel, and M. R. Strand. 1999. Survival of diploid males in *Bracon* sp. near *hebetor* (Hymenoptera: Braconidae). Ann. Entomol. Soc. Am. 92: 110-116.

Jervis, M. A., and Kidd, N. A. C., 1986. Host-feeding strategies in hymenopteran parasitoids. Biol. Rev. 61:395-434.

Jervis, M. A., N. A .C. Kidd, and H. E. Almey. 1994. Post-reproductive life in the parasitoid *Bracon hebetor* (Say) (Hym., Braconidae). J. Appl. Entomol. 117: 72-77.

Jervis, M. A., B. A. Hawkins, and N. A. C. Kidd. 1996. The usefulness of destructive host feeding parasitoids in classical biological control: theory and observation conflict. Ecol. Entomol. 21: 41-46.

Jervis, M. A., G. E. Heimpel, P. N. Ferns, J. A. Harvey, and N. A. C. Kidd. 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. J. Anim. Ecol. 70: 442-458.

La Rocca A., P. Fanti, V. A. Romano, E. Marsicovetere, N. Isidoro, R. Romani, S. Rusconi, F. Pennacchio, and D. Battaglia. 2007. Functional bases of host-acceptance behaviour in the aphid parasitoid *Aphidius ervi*. Physiol. Entomol. 32: 305-312.

Lauzière, I., G. Pérez-Lachaud, and J. Brodeur. 1999. Influence of host density on the reproductive strategy of *Cephalonomia stephanoderis*, a parasitoid of the coffee berry borer. Entomol. Exp. Appl. 92: 21-28.

Loni, A., E. Rossi, and K. van Achterberg. 2011. First report of *Agathis fuscipennis* in Europe as parasitoid of the tomato leafminer *Tuta absoluta.* Bull. Insectol. 64: 115-117.

Luna, M. G., N. E. Sánchez, and P. C.Pereyra. 2007. Parasitism of *Tuta absoluta* (Lepidoptera, Gelechiidae) by *Pseudapanteles dignus* (Hymenoptera, Braconidae) under laboratory conditions. Environ. Entomol. 36: 887-893.

Luna, M. A. G., V. I. Wada, and N. E. Sánchez. 2010. Biology of *Dineulophus phtorimaeae* (Hymenoptera: Eulophidae) and field interaction with *Pseudapanteles dignus* (Hymenoptera: Braconidae), larval parasitoids of *Tuta absoluta* (Lepidoptera: Gelechiidae) in tomato. Ann. Entomol. Soc. Am. 103: 936-942.

Luna M. G., V. I. Wada, J. La Salle, and N. E. Sánchez. 2011. *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), a newly recorded parasitoid of the Tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), in Argentina.- Neotrop. Entomol. 40: 412-414.

Luna, M. G., N. E. Sánchez, P. C. Pereyra, E. Nieves, V. Savino, E. Luft, E. Virla, and S. Speranza. 2012. Biological control of *Tuta absoluta* in Argentina and Italy: evaluation of indigenous insects as natural enemies. Bulletin OEPP 42: 260-267.

Lu, Y. H., K. M. Wu, Y. Y. Jiang, Y. Y. Guo, and N. Desneux. 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nature 487: 362-365.

Mangel, M., and G. E. Heimpel. 1998. Reproductive senescence and dynamic oviposition behaviour in insects. Evol. Ecol. 12, 871-879.

Meyer, J. S., C. G. Ingersoll, L. L. McDonald, M. S. Boyce. 1986. Estimating uncertainty in population growth rates: Jackknife vs. Bootstrap techniques. Ecology 67: 1156-1166.

Nenaah, G. 2011. Individual and synergistic toxicity of solanaceous glycoalkaloids against two coleopteran stored-product insects. J. Pest Sci. 84: 77-86.

Olson, L. J. 2006. The economics of terrestrial invasive species: a review of the literature. Agric. Resour. Econ. Rev. 35 178-194.

Patel, K.J., D.J. Schuster, and G.H. Smerage. 2003. Density dependent parasitism and host-killing of *Liriomyza trifolii* (Diptera: Agromyzidae) by *Diglyphus intermedius* (Hymenoptera: Eulophidae). Fla. Entomol. 86: 8-14.

Pennacchio, F., and M. R. Strand. 2006. Evolution of developmental strategies in parasitic Hymenoptera. Ann. Rev. Entomol. 51: 233-258.

Pereyra, P. C., and N. E. Sánchez. 2006. Effect of two solanaceous plants on developmental and population parameters of the Tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Neotrop. Entomol. 35: 671-676.

Ragsdale, D. W., D. A. Landis, J. Brodeur, G. E. Heimpel, and N. Desneux. 2011. Ecology and management of the soybean aphid in North America. Ann. Rev. Entomol. 56: 375-99.

Sánchez, N. E., P. C. Pereyra, and M. G. A. Luna. 2009. Spatial patterns of parasitism of the solitary parasitoid *Pseudapanteles dignus* (Hymenoptera: Braconidae) on *Tuta absoluta* (Lepidoptera: Gelechiidae). Environ. Entomol. 38: 365-374.

Spanoudis, C. G., Andreadis S. S., and S. Stefanos. 2012. Temperature-dependent survival, development, and adult longevity of the koinobiont endoparasitoid *Venturia canescens* (Hymenoptera: Ichneumonidae) parasitizing *Plodia interpunctella* (Lepidoptera: Pyralidae). J. Pest Sci. 85: 75-80.

Stark, J. D., J. E. Banks, and R. Vargas. 2004. How risky is risk assessment: The role that life history strategies play in susceptibility of species to stress. Proc. Nat. Acad. Sci. USA 101: 732-736.

Tomé, H. V. V., E. M. G. Cordeiro, J. F.Rosado, and R. N. C.Guedes. 2012. Egg exposure to pyriproxyfen in the tomato leaf miner *Tuta absoluta*: ovicidal activity or behavioural-modulated hatching mortality? Ann. Appl. Biol. 160: 35-42.

Tropea Garzia, G., G. Siscaro, A. Biondi, and L. Zappalà. 2012. *Tuta absoluta*, an exotic invasive pest from South America now in the EPPO region: biology, distribution and damage. Bulletin OEPP 42: 205-210.

Urbaneja A., J. Gonzalez-Cabrera, J. Arno, and R. Gabarra. 2012. Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. Pest Manag. Sci. 68: 1215-1222.

van Lenteren, J. C. 2012. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl 57:1-20.

Walde, S. J., and W. M. Murdoch. 1988. Spatial density dependence in parasitoids. Annu. Rev. Entomol. 33: 441-466.

Warton, R. A.. 1993, Bionomics of the Braconidae. Ann. Rev. Entomol. 38: 121-143.

Yu D. S. K., and C. van Actherberg. 2010. Taxapad Ichneumonoidea. In: Species 2000 & IT IS catalogue of life: 2010 annual checklist (Bisby F. A., Y. R.Roskov, T. M. Orrell, D. Nicolson, L. E. Paglinawan, N. Bailly, P. M. Kirk, T. Bourgoin, G. Baillargeon,Eds). DVD, Species 2000, Reading, UK.

Zappalà, L., M. A. Hoy, and R. D. Cave. 2007. Interactions between the red imported fire ant, the citrus leafminer, and its parasitoid *Ageniaspis citricola* (Hymenoptera: Encyrtidae): Laboratory and field evaluations. Biocontrol Sci. Techn. 17: 353-363.

Zappalà, L., G. Siscaro, A. Biondi, O. Mollà, J. Gonzàlez-Cabrera, amd A. Urbeneja. 2012a. Efficacy of sulphur on *Tuta absoluta* and its side effects on the predator *Nesidiocoris tenuis*. J. Appl. Entomol. 136: 401-409.

Zappalà, L., U. Bernardo, A. Biondi, A. Cocco, S. Deliperi, G. Delrio, M. Giorgini, P. A. Pedata, C. Rapisarda, G. Tropea Garzia, and G. Siscaro. 2012b. Recruitment of native parasitoids by the exotic pest *Tuta absoluta* (Meyrick) in Southern Italy. Bull. Insectol. 65: 51-61.

Zappalà, L., O. Campolo, S. B. Grande, F. Saraceno, A. Biondi, G. Siscaro, and V. Palmeri. 2012c. Dispersal of *Aphytis melinus* (Hymenoptera: Aphelinidae) after augmentative releases in citrus orchards. Eur. J. Entomol. 109: 561-568.

Figure captions

Figure 1. Means (±SEM) of eggs, larvae, cocooned pupae and overall immatures development duration (hours) of *Bracon nigricans* males and females. No statistical differences between sexes were found (ANOVA $P > 0.05$).

Figure 2. Proportion of surviving *Bracon nigricans* adults for a given age (days). The values reported refer to: (i) the survival of males and females reared feeding only on honey and (ii) the survival of females feeding on host larvae and on honey.

Figure 3. Weekly grand means (±SEM) of daily percentages of parasitized and of killed without being parasitized host larvae by one *Bracon nigricans* female. Columns for age bearing the same lower- and upper-case letters represent means that are not statistically different among the weekly percentages of killed and parasitized host larvae, respectively (ANOVA $P < 0.05$, followed by LSD test).

Figure 4. Means (±SEM) of daily oviposition activity during *Bracon nigricans* females entire lifespan.

Figure 5. Weekly grand means (±SEM) of clutch size produced by *Bracon nigricans* females, i.e. gregariousness rate. No statistical differences among weeks were found (ANOVA $P > 0.05$).

Figure 6. Weekly grand means (±SEM) of eggs and emerged adult produced daily by one *Bracon nigricans* female. Columns for age bearing the same lower- and upper-case letters represent means that are not statistically different among the weekly numbers of laid eggs and of emerged adults, respectively (ANOVA $P < 0.05$, followed by LSD test).

Figure 7. Weekly grand means (±SEM) of sex-ratio (number of males/total progeny) of the progeny produced by one *Bracon nigricans* female. Columns for age bearing the same letters represent means that are not statistically different among the weekly values (ANOVA $P < 0.05$, followed by LSD test).

Figure 8. Weekly grand means (±SEM) of percentages of successful young instars development, i.e. number of emerged adults on eggs laid daily by one *Bracon nigricans* female. Columns for age bearing the same letters represent means that are not statistically different (ANOVA $P < 0.05$, followed by LSD test).

Fig. 1

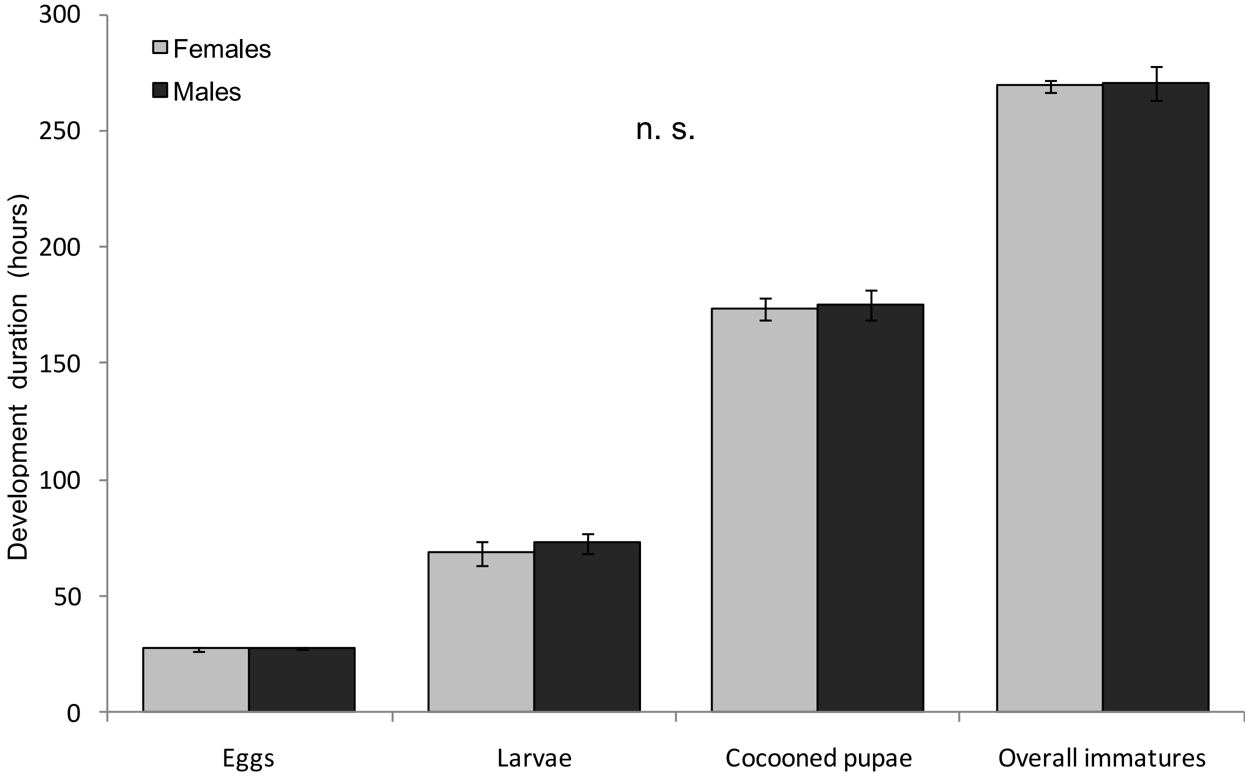

300
250
200
150
100
50
0
Development duration (hours)
Females
Males
n. s.
Eggs
Larvae
Cocooned pupae
Overall immatures

Fig. 2

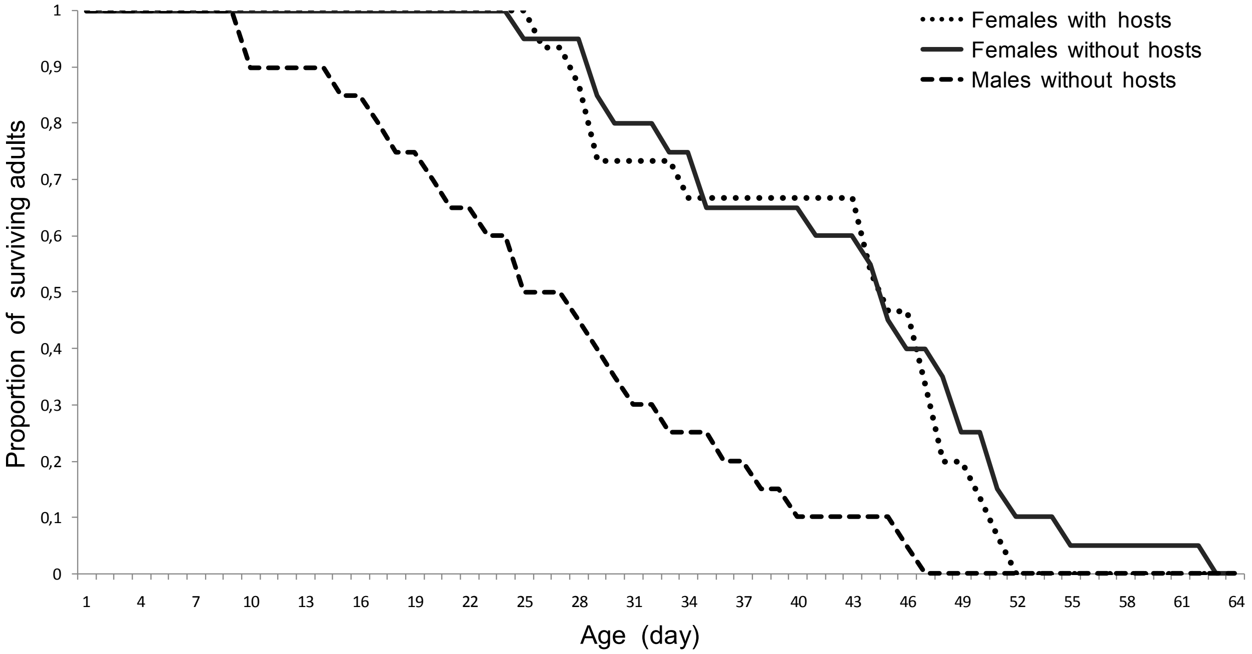

Females with hosts
Females without hosts
Males without hosts
Proportion of surviving adults
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
1
4
7
10
13
16
19
22
25
28
31
34
37
40
43
46
49
52
55
58
61
64
Age (day)

Fig. 3

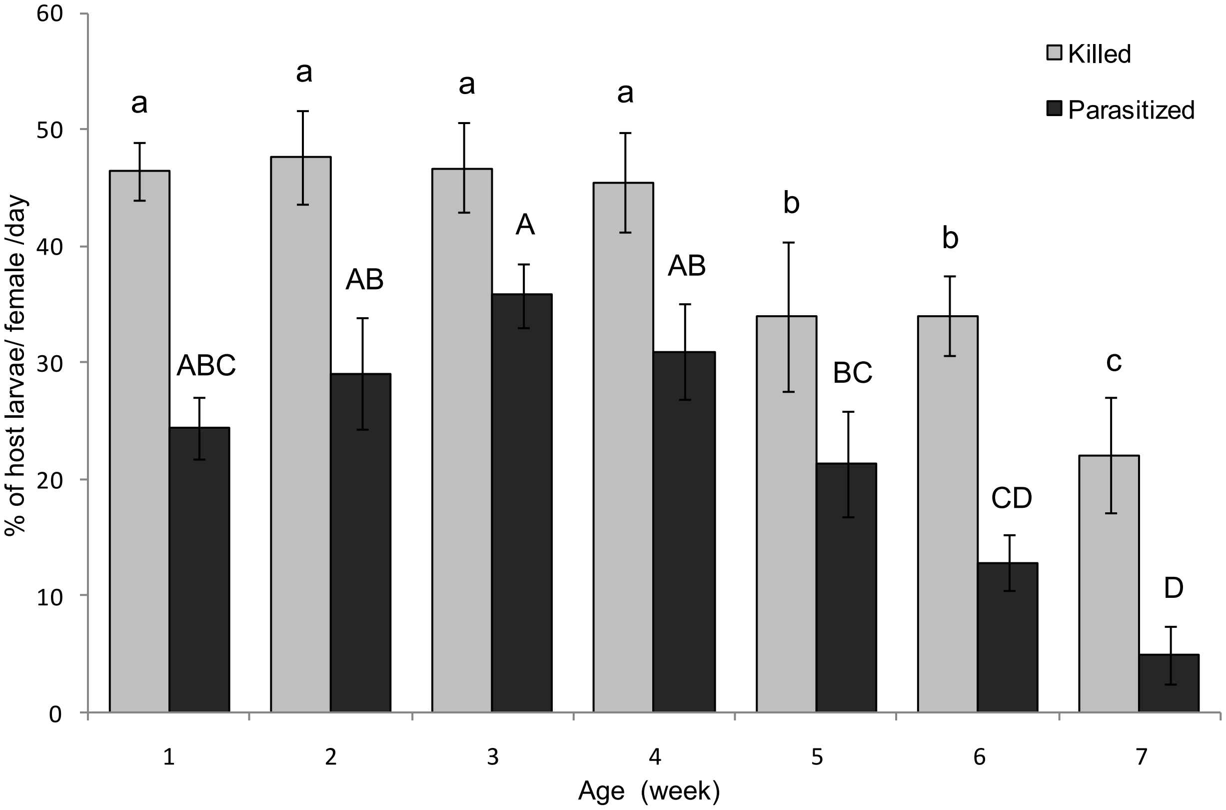

% of host larvae/ female /day
0
10
20
30
40
50
60
Age (week)
1
2
3
4
5
6
7
■ Killed
■ Parasitized
a
ABC
a
AB
a
A
a
AB
b
BC
b
CD
c
D

Fig. 4

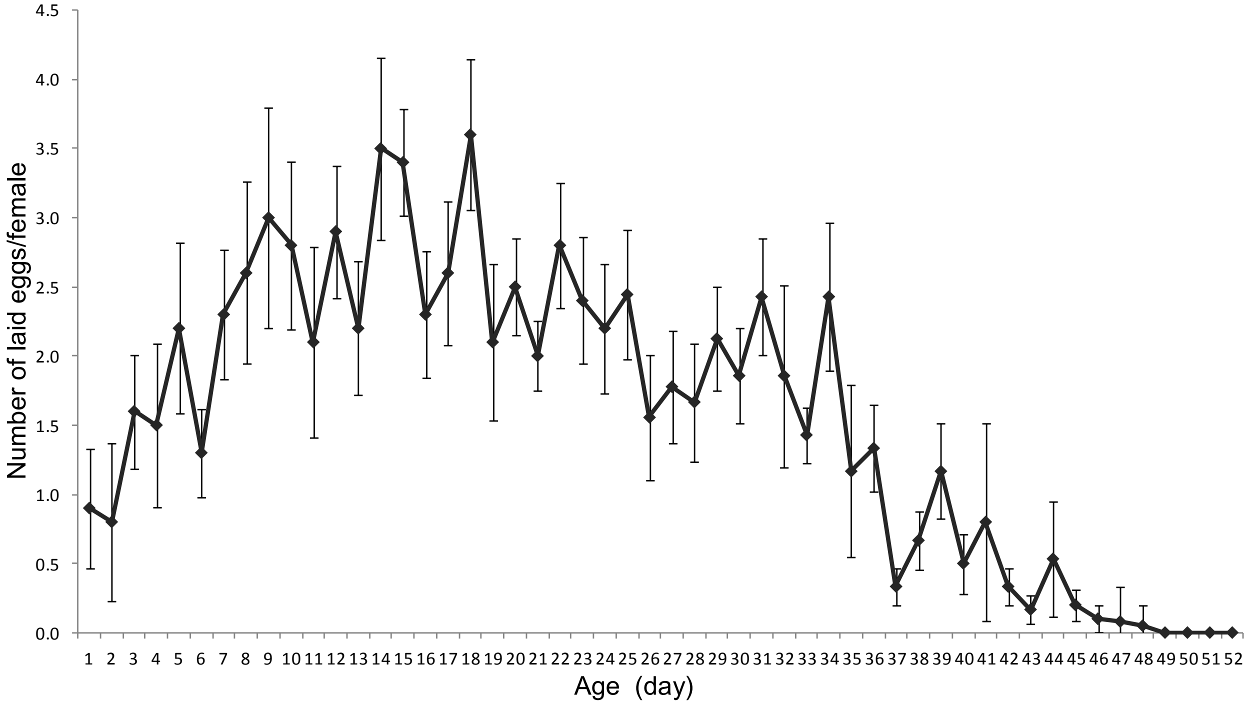

Number of laid eggs/female
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52
Age (day)

Fig. 5

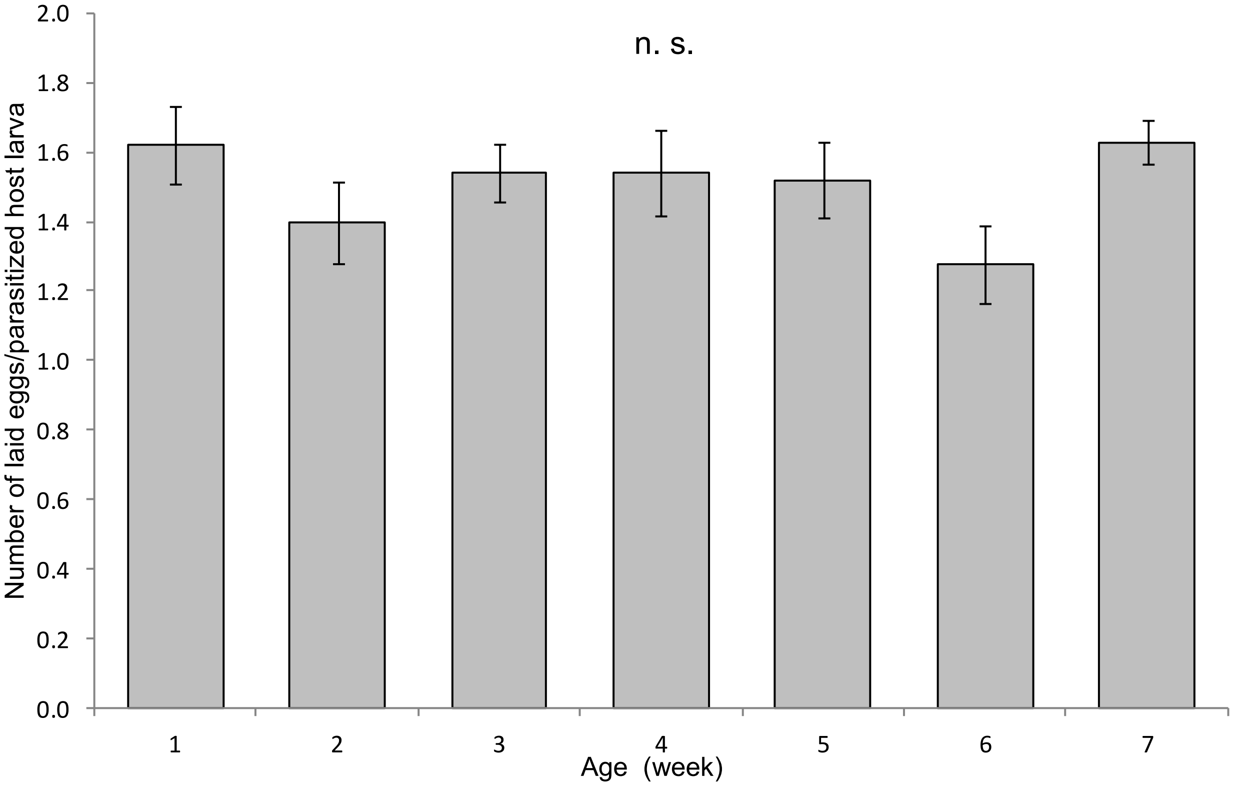

n. s.
Number of laid eggs/parasitized host larva
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
7
Age (week)

Fig. 6

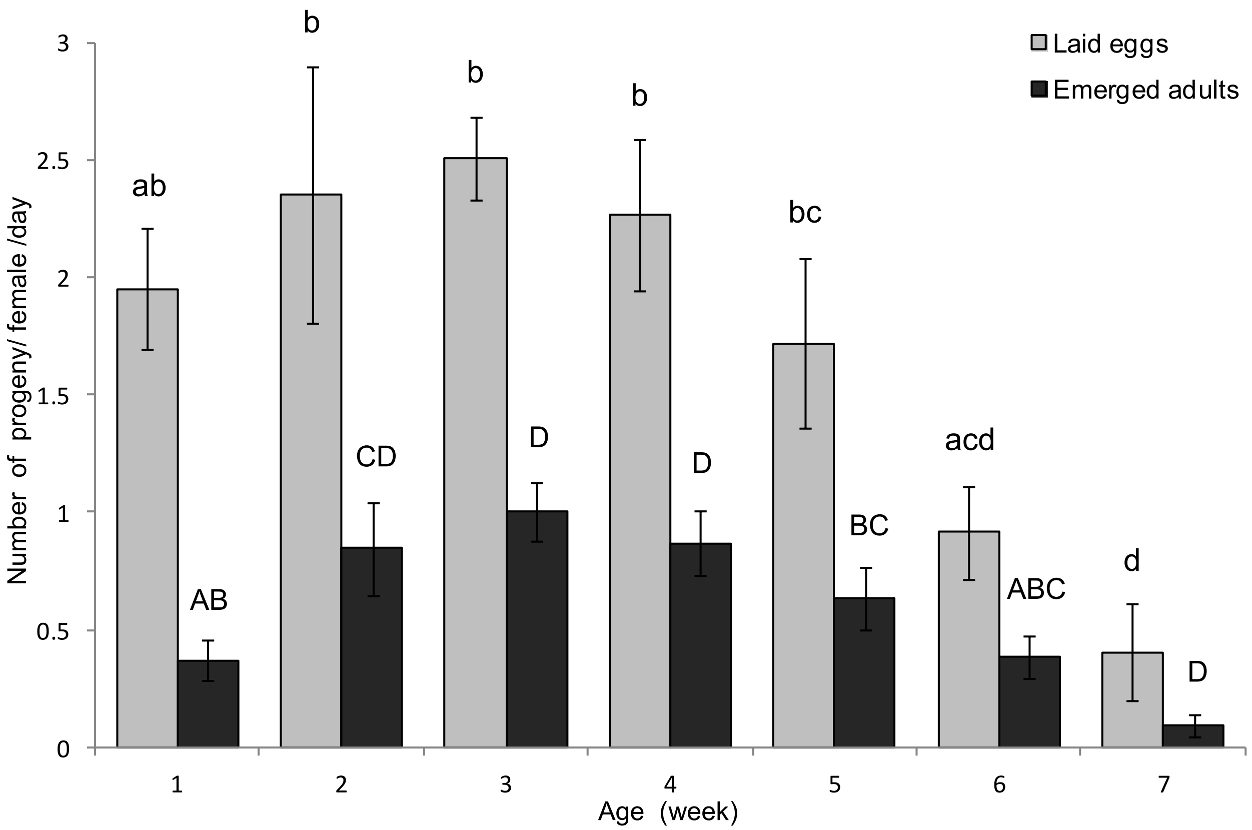
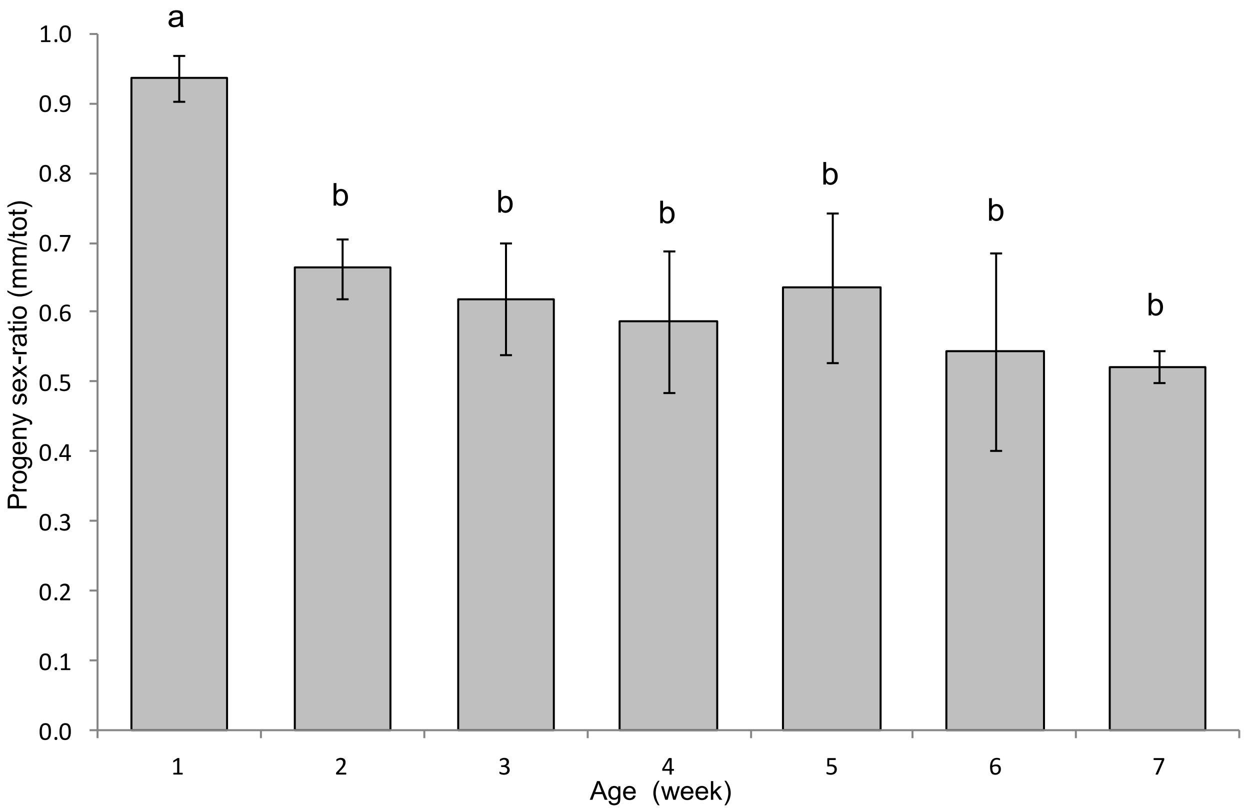
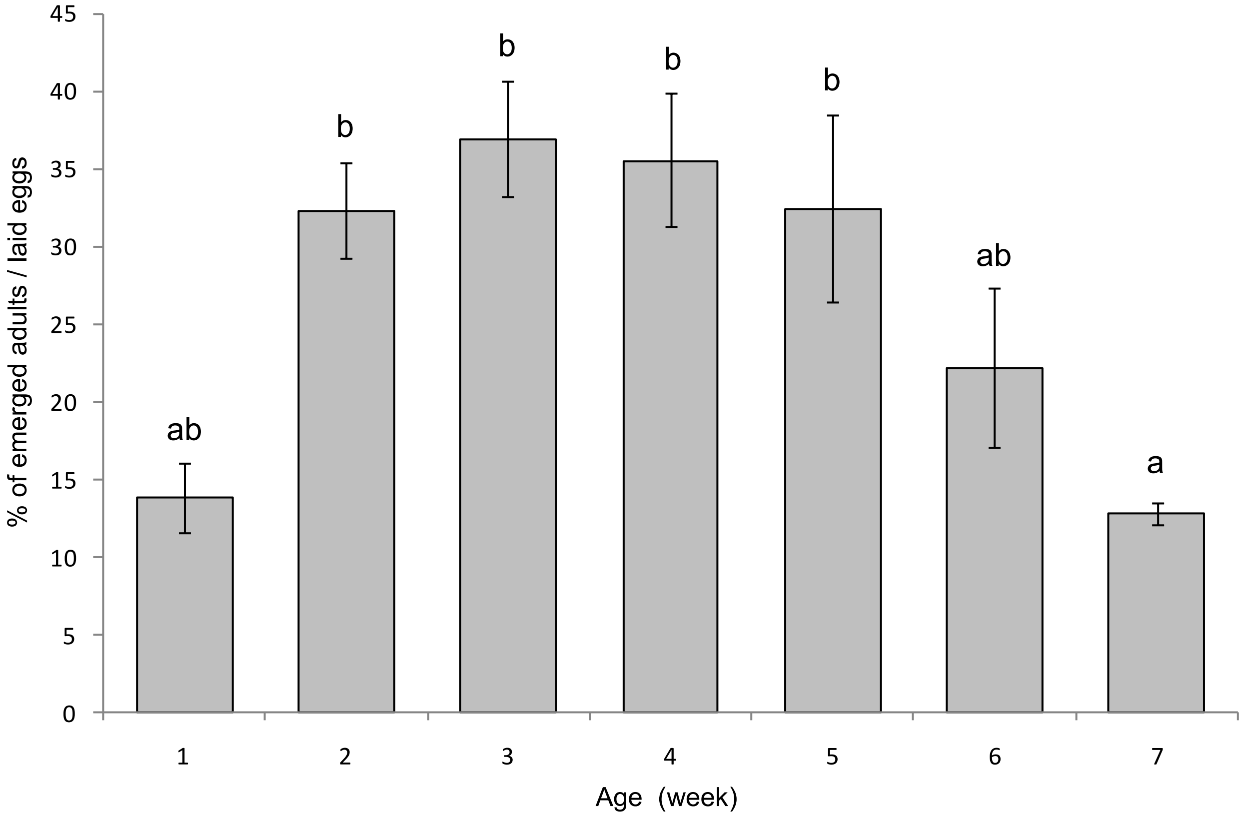

Laid eggs
Emerged adults
Number of progeny/ female /day
0
0.5
1
1.5
2
2.5
3
ab
AB
b
CD
b
D
b
D
bc
BC
acd
ABC
d
D
1
2
3
4
5
6
7
Age (week)

Fig. 7


a
b
b
b
b
b
b
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Progeny sex-ratio (mm/tot)
1
2
3
4
5
6
7
Age (week)

759 Fig. 8


% of emerged adults / laid eggs
45
40
35
30
25
20
15
10
5
0
ab
b
b
b
b
ab
a
1
2
3
4
5
6
7
Age (week)

760

Chapter V

Efficacy of sulphur on *Tuta absoluta* and its side effects on the predator *Nesidiocoris tenuis*

Zappalà Lucia, Siscaro Gaetano, **Biondi Antonio**, Mollà Oscar, Gonzàlez-Cabrera Joel, and Urbaneja Alberto, 2012, Journal of Applied Entomology, 136:401-409

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ORIGINAL CONTRIBUTION

Efficacy of sulphur on *Tuta absoluta* and its side effects on the predator *Nesidiocoris tenuis*

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Keywords

field, greenhouse, integrated pest management, Miridae, South American Tomato Pinworm

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Abstract

The South American tomato pinworm, *Tuta absoluta* (Meyrick), is one of the major pests of tomato crop. Since its detection in the Mediterranean basin, it has been commonly controlled using chemical insecticides. However, inoculation and conservation of predatory mirids, integrated with sprays of selective insecticides, has been demonstrated to be a cost-effective strategy for controlling this pest. In this work, we tested the efficacy of two sulphur formulations, dustable and wettable powder, for controlling *T. absoluta* on tomato under greenhouse and open-field conditions. In addition, the side effects of both sulphur formulations on the predator, *Nesidiocoris tenuis* (Reuter), were evaluated under laboratory conditions. Dustable sulphur, applied weekly on tomato seedlings artificially infested with *T. absoluta* in greenhouse conditions, significantly reduced the infestation levels and was demonstrated to have a repellent effect on oviposition. Wettable sulphur was not effective for controlling *T. absoluta* populations in both greenhouse and open-field experiments. In the side effect trials conducted with *N. tenuis,* only dustable sulphur resulted in being moderately harmful as a fresh residue and slightly harmful as a 7-day-old residue; no effects were recorded exposing the predator to 14-day-old sulphur residues. In contrast, wettable sulphur was classified as harmless to *N. tenuis*. Our results suggest that the use of sulphur, especially as dustable powder, could be considered as a tool in *T. absoluta* management strategies, although its side effects on *N. tenuis* should be taken into account. The implications of these results for the use of sulphur formulations in pest and disease management programmes in tomato crops are discussed.

Introduction

The South American Tomato Pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is originally from South America, where it is considered to be one of the most important pests in tomato crop (Barrientos et al. 1998; Estay 2000; EPPO 2010) In Europe, *T. absoluta* was first detected in northern Castellón de la Plana (Spain) at the end of 2006 (Urbaneja et al. 2007). Since that time, it has spread throughout the Mediterranean basin and through continental countries, including Germany, Switzerland, the Netherlands and the United Kingdom. This pest occurs throughout the entire growing cycle of tomato crops both for fresh market and processing. In the absence of control strategies, larval feeding can result in up to 100% crop losses by attacking leaves, flowers, stems and fruits (Apablaza 1992; Estay 2000).

The impact of *T. absoluta* on tomato crops has led growers to utilize insecticides extensively. In some Mediterranean areas, the use of chemical control measures is disrupting the existing Integrated Pest Management (IPM) programmes (mainly based on biological control) in tomato crop (van der Blom et al. 2009). Because of their side effects, chemicals may lower the populations of natural enemies of other tomato pests (Croft 1990; Desneux et al. 2007) and may lead to the development of insecticide resistance (Devonshire and Field 1991; Bielza 2010), as has already been detected in the pest's area of origin (Siqueira et al. 2000, 2001; Lietti et al. 2005). In the last 2 years, environmentally safer measures aimed at limiting the use of chemical insecticides have been implemented in some areas containing new infestations. The integration of inoculative releases and/or conservation of *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae), along with the use of commercial formulations based on *Bacillus thuringiensis* (Berliner) and other selective insecticides, is becoming a cost-effective strategy for controlling this pest (Desneux et al. 2010; Gabarra and Arnó 2010; González-Cabrera et al. 2011; Mollá et al. 2011).

The fungicidal activity of sulphur is largely known and has been exploited since the end of the 19th century. Furthermore, its activity as a pesticide has been widely exploited in management strategies against arthropod pests, especially mites. Recent insight into the physiological role of sulphur has prompted an increase in its application in IPM programmes (Williams and Cooper 2004; Bloem et al. 2005). Because of its reduced ecological impact, sulphur is also widely used in organic farming (Porcuna 2002; Ecologismo.com 2011).

Field observations showed that the impact of *T. absoluta* was lower in tomato plots treated with dustable powdered sulphur to prevent plant diseases in the first crop stages than in the untreated plots (authors' pers. obs.). However, several trials have shown that sulphur also has negative effects on various natural enemies, such as predatory mites, thrips (Hanna et al. 1997; James et al. 2002; Prischmann et al. 2005; Teodoro et al. 2005) and parasitoids (Flanders 1943; Williams and Gill 1996; Martinson et al. 2001; Jepsen et al. 2007a,b; Pijnakker and Ramakers 2009).

The goals of this study were to evaluate the efficacy and long-term effects of two different sulphur formulations on the control of *T. absoluta* under greenhouse and field conditions as well as their side effects on the mirid predator *N. tenuis*.

Materials and Methods

Chemicals

Sulphur was tested as dustable powder (DP) at 3.75 g/plant (Agrezufre®; Cequisa Agro, Barcelona, Spain) and as wettable powder (WP) at 300 g/hl (Lainzufre®; Lainco SA, Rubí, Spain). *Bacillus thuringiensis* var. *kurstaki* was used as treated control at 200 g/hl (Costar®; Syngenta Agro SA, Madrid, Spain) because of its demonstrated efficacy in controlling *T. absoluta* infestations (González-Cabrera et al. 2011).

Insects

Specimens of *T. absoluta* were collected in tomato fields located in the Castellón de la Plana region and reared on tomato plants in a growth chamber [25 ± 1°C, 60 ± 5% RH and 16 : 8 h (L : D) photoperiod]. Weekly, a group of six tomato plants, 30 cm in height, were placed inside a screened cage (120 × 70 × 125 cm) ($l \times w \times h$) where the rearing took place. Plants were left undisturbed for 5 weeks. This time period was sufficient to enable the moths to emerge within the cage and rendered artificial re-infestation unnecessary. When required for greenhouse assays, adults were collected using a mechanical aspirator (Hausherr's Machine Works, Toms River, NewJersy).

Nesidiocoris tenuis (NESIBUG®) was purchased from Koppert Biological Systems S.L., Águilas, Murcia, Spain; the adults were 4 days old when used in the trials.

Greenhouse trials

The experiments were conducted in a greenhouse located at IVIA in Moncada (Valencia, Spain) under controlled environmental conditions (25 ± 2°C, 65 ± 10% RH). Artificial light was supplied to provide a 14L : 10D photoperiod, simulating summer photoperiod when *T. absoluta* populations tend to increase The plants used in the trials were seedlings of tomato var. Muchamiel (Mascarell Semillas S.L., Benisoda, Spain), approximately 30 cm high. They were placed individually into screened plastic boxes (35 × 25 × 45 cm) ($l \times w \times h$).

Formulation effectiveness

Sulphur formulations were evaluated for their effectiveness against *T. absoluta* at different time intervals. The experiment was performed as a complete randomized block with six treatments and five replicates as follows: DP sulphur applied weekly, DP sulphur applied fortnightly, WP sulphur applied weekly, WP sulphur applied fortnightly, *B. thuringiensis* applied weekly (treated control) and water applied weekly (untreated control). Treatments were applied using a 1-l trigger-operated hand sprayer (Matabi®; Antzuola, Guipuzcoa, Spain). Treated plants were allowed to air-dry for 1 h.

Four *T. absoluta* adults (two females and two males) were released into the cages weekly. The trial lasted until the untreated control was completely destroyed. Weekly observations were conducted on each plant by scoring the number of total leaves, the number of infested leaves and leaflets, the percentage of infested surface area and the number of mines on four randomly selected infested leaflets.

Oviposition repellency

The potential oviposition repellency of the sulphur formulations and the subsequent effect on newly hatched larvae were evaluated in both choice and no-choice experiments. Water was used as an untreated control.

In the choice test, three treated tomato plants (one per treatment) were placed together inside a plastic box as described earlier. In the no-choice experiment, the plants were placed individually inside the boxes. Four *T. absoluta* adults (two females and two males) were released for each plant. After 4 days, the plants were examined, and the number of eggs laid by *T. absoluta* females was recorded. In addition, to evaluate the effects on the larvae, a second group of plants was utilized in an experimental design identical to the one described above. In this case, the observations of the plants were conducted 10 days after releasing the adults, when the larvae were at the second instar. The numbers of larvae on treated and untreated plants in the choice and no-choice trials were recorded. Four replicates were performed for each experiment.

Open-field trial

A field trial was conducted to verify the suitability of including sulphur treatments in an integrated management strategy for *T. absoluta*. The experiment was conducted on tomato plants (var. Muchamiel) grown in 10 l pots, from August to October 2009, in the experimental field located at IVIA in Moncada (Valencia, Spain) (N 39°35′ 13.25″, W 0°23′ 48.31″). Crop cultivation techniques typical of open-field staked tomato production in Spain were followed: the main stem of the plant was trained with plastic rings to a cane structure, secondary shoots and senescent leaves were pruned weekly, and a standard nutrient formulation for tomato was added directly to the watering solution.

Four treatments on groups of five tomato plants were compared in a randomized complete block design: *B. thuringiensis* applied weekly, DP sulphur applied weekly during the first 2 weeks followed by fortnightly treatments of *B. thuringiensis* and WP sulphur (hereafter called DP + Bt + WP), DP sulphur applied weekly during the first 2 weeks followed by weekly WP sulphur applications (hereafter called DP + WP) and untreated control (water). These treatments were chosen because it is common to sprinkle DP sulphur on many crops at the beginning of the growing season when temperatures are still below 30°C; subsequently, dust formulations are substituted with non-phytotoxic formulations such as WP sulphur.

To monitor the presence of *T. absoluta* in the tomato field, a delta-trap supplied with *T. absoluta* pheromone (PH-937-1RR; Biagro S.L., Valencia, Spain) was placed close to the experimental field. The number of captured males was recorded weekly. The sticky plate was changed once a week and the pheromone dispenser once a month.

Weekly observations were recorded for the plants including the number of total leaves, infested leaves, leaflets and fruits, the percentage of infested surface area and the number of mines on four randomly selected infested leaflets. The presence of other pests (such as whiteflies, spider mites, russet mites, stink bugs and leafminers) and of natural enemies (namely mirid predators) on one leaf from the upper third of each plant was also recorded. At the end of the trial, the harvested tomatoes were weighed.

Side effects on *Nesidiocoris tenuis*

To ascertain the potential effects of the sulphur formulations on the predator, *N. tenuis*, a toxicity trial was conducted on adults exposed to freshly dried, 7-day-old and 14-day-old DP and WP sulphur residues. An untreated control with water was also evaluated. The effect of residues on the mortality of the predator and the fertility of the survivors was evaluated. The experiment was initiated by spraying 30 tomato plants for each treatment. The plants were of the same variety, growth stage and height as those employed in the greenhouse trial described earlier. One hour after sulphur application, 10 plants per treatment were used for the fresh residue trial. The remaining plants were left undisturbed in a greenhouse at 25 ± 2°C and 65 ± 10% RH with artificial light to obtain a 14L : 10D photoperiod until their use for the 7-day-old and 14-day-old residue trials, 10 plants in each trial.

The upper half of the plants was excised and placed into a bioassay isolator consisting of two superimposed plastic glasses (Sanchez et al. 2009). The top glass had a larger volume (370 ml) and contained a hole in the bottom to allow the stem to reach water present in the bottom glass (230 ml). Five *N. tenuis* females and five *N. tenuis* males were introduced into the aforementioned arena. Fifty *Ephestia kühniella* Zeller (Lepidoptera: Pyralidae) eggs were added daily to provide food for each female. Water was offered *ad libitum* in a 1.5-ml microcentrifuge tube sealed with cotton. The arena was then closed using fine polyester mesh. Observations were conducted daily for 5 days to record the number of dead predators. After this interval, all the survivors were removed, and the number of progeny that emerged was scored to evaluate the effects on *N. tenuis* fertility.

Data analysis

Treatment effects on plant and fruit damage were analysed using a linear mixed model with repeated measurements, using time as a random factor. The measurements were expressed as a percentage of infested leaflets, a percentage of infested surface per infested leaflet and a percentage of infested fruits. Once significant differences were established, pairwise comparisons of the fixed factor level were performed using a Bonferroni's post-test ($P < 0.05$). When needed, angular transformations of percentage data were performed to fulfil the assumptions of normality and homogeneity of variance.

When significant (one-way ANOVA followed by Tukey's test at $P < 0.05$), the side effect trial data were corrected using Abbott's formula (Abbott 1925), and they were then summarized in a reduction coefficient (E) calculated separately for each pesticide residue and time interval (Urbaneja et al. 2008). The coefficient was determined by considering only the values that were significantly different after their correction. The resulting value (E) was interpreted according to IOBC standards, which include four categories: (i) harmless, (ii) slightly harmful, (iii) moderately harmful and (iv) harmful, which correspond to reductions below 30%, between 31% and 79%, between 80% and 99% and higher than 99%, respectively (Sterk et al. 1999).

Results

Greenhouse trials

Formulation effectiveness

The trial comparing the two sulphur formulations at different time intervals showed a significant effect regarding the percentage of infested leaflets per plant ($F_{5,140} = 20.970$, $P < 0.0001$) (fig. 1a). DP sulphur in weekly applications reduced the number of infested leaflets compared with the untreated control, and it was as effective as the treated control (Bt) (table 1). The percentage of infested surface area per infested leaflet was statistically different among the treatments ($F_{5,140} = 21.020$, $P < 0.0001$) (fig. 1b). In this case, the weekly application of DP sulphur resulted in a lower infested surface area compared to the untreated control but higher compared to the treatment with Bt (table 1).

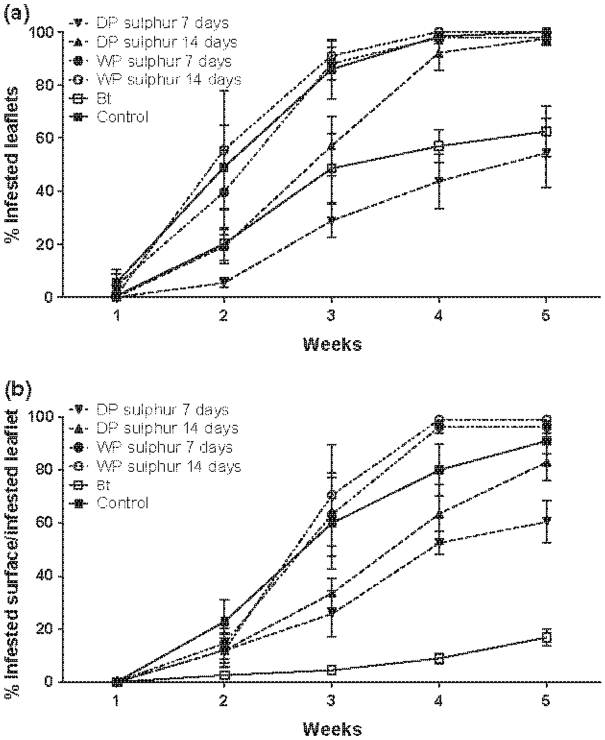

Fig. 1 Percentage of infested leaflets per tomato plant (mean ± SE) (a) and percentage of infested surface area per leaflet (mean ± SE) (b) in a greenhouse trial comparing the effectiveness of two sulphur formulations against *T. absoluta* at different time intervals.

Table 1 *P*-values for the pairwise comparison of percentage of infested leaflets per plant and the percentage of infested surface area per infested leaflet among treatments in a greenhouse trial against *Tuta absoluta* on tomatoes (T1: untreated control; T2: treated control (Bt); T3: dustable powder (DP) sulphur 7 days; T4: DP sulphur 14 days; T5: WP sulphur 7 days and T6: WP sulphur 14 days; linear mixed model with repeated measures). Values in bold correspond to statistically significant values. Bonferroni's post-test ($P < 0.05$)

	Independent variable	
	% infested leaflets/plant	% infested surface per infested leaflet
T1 vs. T2	**<0.0001**	**<0.0001**
T3 vs. T1	**<0.0001**	**0.0007**
T4 vs. T1	0.085	0.537
T5 vs. T1	1.000	1.000
T6 vs. T1	1.000	1.000
T3 vs. T2	1.000	**0.006**
T4 vs. T2	**0.045**	**<0.0001**
T5 vs. T2	**<0.0001**	**<0.0001**
T6 vs. T2	**<0.0001**	**<0.0001**

Oviposition repellency

The choice and no-choice tests conducted to evaluate the potential oviposition repellency of sulphur and the subsequent effect on larvae showed a significant effect in both experimental conditions. In the choice test, there was a significant reduction in the number of eggs laid ($F_{2,9} = 5.92$, $P = 0.023$) and in the number of larvae recorded after 10 days ($F_{2,9} = 11.94$, $P = 0.003$) (fig. 2a, c). A similar effect was recorded in the no-choice test, in which *T. absoluta* adults were placed (individually) in contact with either treated or untreated plants. There was a significant reduction both in the number of eggs laid ($F_{2,9} = 5.95$, $P = 0.023$) and in the number of larvae ($F_{2,9} = 10.08$, $P = 0.005$) (fig. 2b, d). In all cases, the highest reduction was recorded for the treatment with DP sulphur (fig. 2).

Open-field trial

The pheromone trap captures during the entire trial period averaged 34.6 males/week (max: 56; min: 10).

The results showed that the treatments had a significant effect on the infestation levels when they were expressed as a percentage of infested leaflets per plant ($F_{3,188.97} = 65.212$, $P < 0.0001$) (fig. 3a). Both control strategies based on sulphur applications (DP + WP and DP + Bt + WP) significantly reduced the percentage of infested leaflets when compared with the untreated control; the percentages were significantly lower for the combination DP + Bt + WP (table 2). However, these values were statistically higher in comparison with the treated control (Bt), which showed a percentage of infested leaflets of approximately 2% throughout the experiment.

The percentage of infested surface area per infested leaflet was also different among the treatments ($F_{5,188.971} = 64.736$, $P < 0.0001$) (fig. 3b).

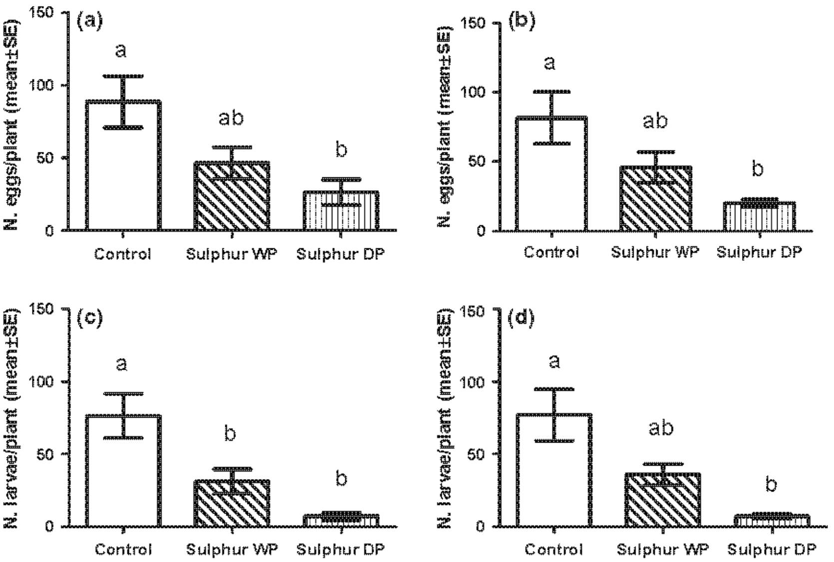

Fig. 2 Number of eggs laid and larvae developed per tomato plant (mean ± SE) in a choice (a, c) and a no-choice test (b, d) comparing plants treated with sulphur WP and sulphur dustable powder and untreated plants.

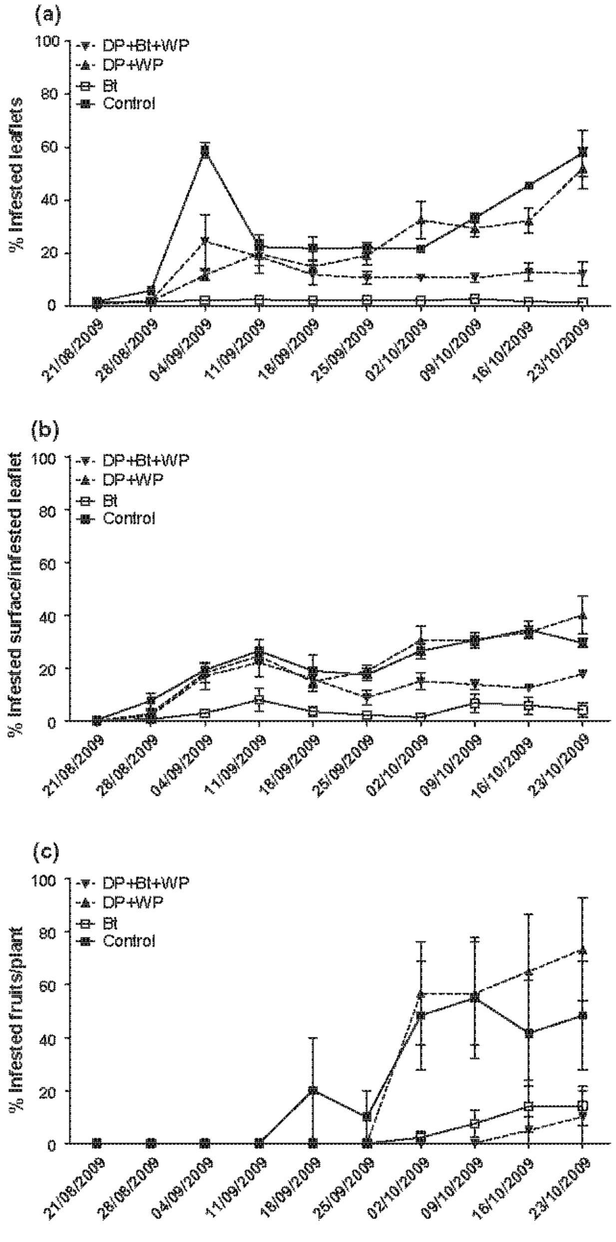

Fig. 3 Percentage of infested leaflets per tomato plant (mean ± SE) (a), percentage of infested surface area per infested leaflet (mean SE) (b) and percentage of infested fruits per plant (mean ± SE) (c) in a field trial comparing various control strategies based on the use of sulphur against *T. absoluta*.

Only the combined use of DP + Bt + WP significantly reduced the percentage of infested surface area when compared to the untreated control; however, these values were higher than those recorded for the treated control (table 2).

Table 2 P-values for the pairwise comparison of percentage of infested leaflets per plant, the percentage of infested surface area per infested leaflet and the percentage of infested fruits per plant among treatments in a field assay against *Tuta absoluta* (T1: untreated control; T2: treated control (Bt); T3: DP + WP and T4: DP + Bt + WP; linear mixed model with repeated measures). Values in bold correspond to statistically significant values. Bonferroni's post-test ($P < 0.05$)

	Independent variable		
	% infested leaflets/plant	% infested surface per infested leaflet	% infested fruits per plant
T1 vs.T2	**<0.0001**	**<0.0001**	**0.0007**
T3 vs. T1	**0.014**	1.000	1.000
T4 vs.T1	**<0.0001**	**<0.0001**	**0.001**
T3 vs.T2	**<0.0001**	**<0.0001**	**<0.0001**
T4 vs.T2	**<0.0001**	**<0.0001**	1.000

The yield per plant (untreated control: 183.5 ± 64.3 g (mean ± SE); Bt: 296.1 ± 35.4 g; DP + WP: 137.7 ± 38.1 g; DP + Bt + WP: 156.2 ± 51.7 g) was not significantly influenced by the treatment ($F_{3,56} = 0.36$, $P = 0.78$). However, the percentage of infested fruits exhibited a significant effect from the treatment ($F_{3,189.172} = 11.230$, $P < 0.0001$) (fig. 3c). The plants treated with alternating sulphur and *B. thuringiensis* (DP + Bt + WP) produced a significantly lower percentage of infested fruits than the untreated control, and this value was similar to that obtained for the treated control (Bt) (table 2, fig. 3c).

Side effects on *Nesidiocoris tenuis*

Fresh DP sulphur treatment significantly lowered the survival of *N. tenuis* adults ($F_{2,27} = 4.75$, $P < 0.05$), while no significant effects were recorded after exposing the mirids to 7-day-old ($F_{2,27} = 0.78$, $P = 0.47$) or 14-day-old residues ($F_{2,27} = 1.45$, $P = 0.35$) (table 3). Survival of *N. tenuis* adults in the other treatments, even the fresh residue treatments, was not significantly different from the untreated control.

Predator fertility was significantly lowered only by the fresh residue of DP sulphur ($F_{2,27} = 8.24$, $P = 0.002$) (table 3). Seven- and 14-day-old residues had no significant effect (7 days: $F_{2,27} = 1.87$, $P = 0.17$; 14 days: $F_{2,27} = 3.06$, $P = 0.06$).

Based on these results, the reduction coefficient (E) was calculated only for DP sulphur. The reduction coefficient for DP sulphur was 83.1%; therefore, it was classified as harmful (category 3) immediately after treatment but harmless (category 1) after 7 and 14 days. In contrast, the WP sulphur was classified as harmless (category 1) for all the time intervals (table 3).

Table 3 Percentage mortality and levels of fertility of *Nesidiocoris tenuis* exposed to fresh, 7- or 14-day-old residues of WP sulphur and dustable powder (DP) sulphur, and a water-treated control; IOBC category corresponding to the reduction coefficient *E* (%)

Residue	Product	Mortality (%) (mean ± SE)	Fertility (mean n progeny/female ± SE)	*E* (%) (IOBC category)
Fresh	WP Sulphur	36.89 ± 1.92ab	10.99 ± 0.71a	0.0 (1)
	DP Sulphur	50.89 ± 1.48b	3.19 ± 0.22b	83.1 (3)
	Control	25.80 ± 2.02a	12.43 ± 0.59a	–
7-days-old	WP Sulphur	44.25 ± 1.94a	7.38 ± 0.61a	0.0 (1)
	DP Sulphur	44.98 ± 1.86a	4.27 ± 0.39a	0.0 (1)
	Control	35.27 ± 1.98a	3.56 ± 0.37a	–
14-days-old	WP Sulphur	44.84 ± 2.07a	7.03 ± 0.51a	0.0 (1)
	DP Sulphur	35.47 ± 2.11a	1.08 ± 0.14a	0.0 (1)
	Control	30.73 ± 1.38a	4.32 ± 0.77a	–

Means within the same column followed by the same letter are not significantly different (Tukey's test, $P < 0.05$).

Discussion

The potential of sulphur formulations to reduce the impact of economically important pests and diseases is well known (Williams and Cooper 2004). However, to our knowledge, this is the first report demonstrating the efficacy of sulphur formulations against *T. absoluta*. According to our results, the effect of sulphur is likely due to the reduction in the number of eggs laid on treated plants, especially with DP formulation. This repellency appears to be the most important effect; however, we cannot ignore the toxicity of the sulphur formulations on *T. absoluta* adults. Further studies should be conducted to clarify the contribution of this effect.

Effectiveness of sulphur DP against *T. absoluta* was comparable to control of the pest by the Bt formulation in terms of number of infested leaflets per plant in the greenhouse. However, the percentage of infested surface area per leaf was higher for weekly sulphur DP treatments compared to the *B. thuringiensis*-treated plants. This suggests that once the eggs are laid, they continue their development until the larvae hatch. From this moment, there is a strong difference in the performance of the products; the toxins from Bt kill the majority of the larvae (González-Cabrera et al. 2011), while the DP sulphur does not.

The effectiveness of sulphur was also tested under field conditions. Combining two applications of sulphur DP (once a week for the first 2 weeks) with alternative weekly treatments, using *B. thuringiensis* or WP sulphur significantly reduced *T. absoluta* infestation, even though not as efficiently as Bt formulation. In these experiments, WP sulphur showed no efficacy against *T. absoluta;* therefore, the reduction in the infestation levels was entirely because of *B. thuringiensis* and DP sulphur.

Based on this work, we can recommend the preventive use of sulphur DP in combination with other selective active ingredients to control *T. absoluta*. In some tomato-growing areas of the Mediterranean basin, it is common to sprinkle the plants with sulphur DP 2–3 weeks immediately after transplantation to prevent plant diseases and mite infestations. Therefore, plants can also be protected from *T. absoluta* to a certain extent, especially when the population density is low. However, if pest control strategies are based on inoculative releases of *N. tenuis* (Calvo et al. 2009; Mollá et al. 2011), our results on the side effects suggest that sprinkling sulphur DP must be terminated at least 7 days before releasing the predator. From that point, pesticide treatments should be applied only with selected active ingredients compatible with mirid establishment. *Bacillus thuringiensis,* for example, showed no effect on mirid establishment either in the greenhouse or in open-field experiments (González-Cabrera et al. 2011; Mollá et al. 2011). During the last tomato-growing season in Almería (Spain), the inoculation of *N. tenuis* in the nursery was demonstrated to be an effective strategy, and it was successfully implemented in several commercial greenhouses (Calvo et al. 2010). This strategy involves transplanting greenhouse plants on which *N. tenuis* has already laid eggs into the nursery. Because of the side effects described in this work, it appears that the use of sulphur DP in combination with this strategy would not be possible, at least during the first weeks of *N. tenuis* establishment.

Sulphur has shown different levels of toxicity to beneficials depending on the formulation used and on the species tested (Hassan 1994; Hanna et al. 1997; Pijnakker and Ramakers 2009); therefore, its application in combination with natural enemies is a very contentious topic. However, it is generally believed that the use of DP sulphur is highly toxic to natural enemies, whereas the WP sulphur is considered, in some cases, to be less harmful (Biobest 2011; Koppert 2011).

The use of sulphur formulations is also common in tomatoes for the control of mites (Strand 1998), particularly in recent years when both formulations (DP and WP) have been widely used to control the tomato russet mite, *Aculops lycopersici* (Massee) (Acari: Eriophyidae) (Duso et al. 2010). Therefore, in those tomato fields where *N. tenuis* is being used, the application of DP sulphur should be restricted to localized treatments (hot spots) or only applied when populations of *N. tenuis* are well established. In contrast, the use of sulphur WP to control *A. lycopersici* does not pose any risk for *N. tenuis* populations, as was observed by González-Cabrera et al. (2011) in an open-field tomato experiment, with three different mirid species present (*Dicyphus marrocannus* Wagner, *N. tenuis*, and *M. pygmaeus*).

In summary, the results obtained in this study suggest that the preventive use of sulphur DP could be considered a valuable tool in strategies intended to manage *T. absoluta*, especially in organic farming, where the use of sulphur is common because of its multiple targets. In these cases, the use of DP sulphur, in combination with inoculative releases of *N. tenuis*, should be limited to applications prior to the release of the predator or until after the mirid populations are well established. Moreover, the combination of DP sulphur treatments with other active ingredients that are safe for natural enemies increases the possibility of reducing chemical residues in fruits.

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References

Abbott WS, 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Apablaza J, 1992. La polilla del tomate y su manejo. Tattersal 79, 12–13.

Barrientos ZR, Apablaza HJ, Norero SA, Estay PP, 1998. Temperatura base y constante térmica de desarrollo de la polilla del tomate, *Tuta absoluta* (Lepidoptera: Gelechiidae). Ciencia e Investigación Agraria 25, 133–137.

Bielza P, 2010. La resistencia a insecticidas en *Tuta absoluta*. Phytoma España 217, 103–106.

Biobest NV, 2011. Side-effects manual. [WWW document]. URL http://www.biobest.be/neveneffecten/3/none/ [accessed on April 2011].

Bloem E, Haneklaus S, Schnug E, 2005. Significance of sulfur compounds in the protection of plants against pests and diseases. J. Plant Nutr. 28, 763–784.

van der Blom J, Robledo A, Torres S, Sánchez JA, 2009. Consequences of the wide scale implementation of biological control in greenhouse horticulture in Almeria, Spain. IOBC/WPRS Bull. 49, 9–13.

Calvo J, Bolckmans K, Stansly PA, Urbaneja A, 2009. Predation by *Nesidiocoris tenuis* on *Bemisia tabaci* and injury to tomato. Biocontrol 54, 237–246.

Calvo J, Belda JE, Giménez A, 2010. Una nueva estrategia para el control biológico de mosca blanca y *Tuta absoluta* en tomate. Phytoma España 216, 46–52.

Croft BA, 1990. Arthropod Biological Control Agents and Pesticides. Wiley and Sons, New York.

Desneux N, Decourtye A, Delpuech JM, 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81–106.

Desneux N, Wajnberg E, Wyckhuys K, Burgio G, Arpaia S, Narváez-Vasquez C, González-Cabrera J, Catalán-Ruescas D, Tabone E, Frandon J, Pizzol J, Poncet C, Cabello T, Urbaneja A, 2010. Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, geographic expansion and prospects for biological control. J. Pest. Sci. 83, 197–215.

Devonshire AL, Field LM, 1991. Gene amplification and insecticide resistance. Annu. Rev. Entomol. 36, 1–23.

Duso C, Castagnoli M, Simoni S, Angeli G, 2010. The impact of eriophyoids on crops: recent issues on *Aculus schlechtendali, Calepitrimerus vitis* and *Aculops lycopersici*. Exp. Appl. Acarol. 51, 151–168.

Ecologismo.com, 2011. El azufre, fungicida ecológico. [WWW document]. URL http://www.ecologismo.com/2011/04/08/el-azufre-fungicida-ecologico [accessed April 2011].

EPPO, 2010. Data sheets on quarentine pests. Tuta absoluta. [WWW document]. URL http://www.eppo.org/QUARANTINE/insects/Tuta_absoluta/DS_Tuta_absoluta.pdf [accessed April 2011].

Estay P, 2000. Polilla del Tomate *Tuta absoluta* (Meyrick). [WWW document]. URL http://alerce.inia.cl/docs/Informativos/Informativo09.pdf [accessed February 2011].

Flanders SE, 1943. The susceptibility of parasitic Hymenoptera to sulfur. J. Econ. Entomol. 36, 469.

Gabarra R, Arnó J, 2010. Resultados de las experiencias de control biológico de la polilla del tomate en cultivo de invernadero y aire libre en Cataluña. Phytoma España 217, 65–68.

González-Cabrera J, Mollá O, Montón H, Urbaneja A, 2011. Efficacy of *Bacillus thuringiensis* (Berliner) for controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Biocontrol 56, 71–80.

Hanna R, Zalom FG, Wilson LT, Leavitt GM, 1997. Sulfur can suppress mite predators in vineyards. Calif. Agr. 51, 19–21.

Hassan SA, 1994. Activities of the IOBC/wprs working group Pesticides and Beneficial Organisms. IOBC/WPRS Bull. 17, 1–5.

James DG, Price TS, Wright LC, Perez J, 2002. Abundance and phenology of mites, leafhoppers, and thrips on pesticide-treated and untreated wine grapes in southcentral Washington. J. Agric. Urban Entomol. 19, 45–54.

Jepsen SJ, Rosenheim JA, Bench ME, 2007a. The effect of sulfur on biological control of the grape leafhopper, *Erythroneura elegantula*, by the egg parasitoid *Anagrus erythroneurae*. Biocontrol 52, 721–732.

Jepsen SJ, Rosenheim JA, Matthews CE, 2007b. The impact of sulfur on the reproductive success of *Anagrus* spp. parasitoids in the field. Biocontrol 52, 599–612.

Koppert BS, 2011. Side effects. [WWW document]. URL http://side-effects.koppert.nl/ [accessed April 2011].

Lietti MMM, Botto E, Alzogaray RA, 2005. Insecticide resistance in Argentine populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Neotrop. Entomol. 34, 113–119.

Martinson T, Williams L, English-Loeb G, 2001. Compatibility of chemical disease and insect management practices used in New York vineyards with biological control by *Anagrus* spp. (Hymenoptera : Mymaridae), parasitoids of Erythroneura leafhoppers. Biol. Control 22, 227–234.

Mollá O, González-Cabrera J, Urbaneja A, 2011. The combined use of *Bacillus thuringiensis* and *Nesidiocoris tenuis* against the tomato borer *Tuta absoluta*. Biocontrol. (in press).

Pijnakker J, Ramakers P, 2009. Effect of vaporizing sulphur on pest predators in greenhouses. IOBC/WPRS Bull. 49, 341–345.

Porcuna JL, 2002. Horticultura ecológica. In: Manual de Agricultura y Ganadería Ecológica. Eds by Labrador J, Porcuna JL, Bello A, Eumedia, Madrid, 73–85.

Prischmann DA, James DG, Wright LC, Teneyck RD, Snyder WE, 2005. Effects of chlorpyrifos and sulfur on spider mites (Acari: Tetranychidae) and their natural enemies. Biol. Control 33, 324–334.

Sanchez JA, Lacasa A, Arno J, Castane C, Alomar O, 2009. Life history parameters for *Nesidiocoris tenuis* (Reuter) (Het., Miridae) under different temperature regimes. J. Appl. Entomol. 133, 125–132.

Siqueira HAA, Guedes RNC, Picanco MC, 2000. Cartap resistance and synergism in populations of *Tuta absoluta* (Lep., Gelechiidae). J. App. Entomol. 124, 233–238.

Siqueira HAA, Guedes RNC, Fragoso DB, Magalhaes LC, 2001. Abamectin resistance and synergism in Brazilian populations of *Tuta absoluta* (Meyrick) (Lepidoptera : Gelechiidae). Int. J. Pest Manage. 47, 247–251.

Sterk G, Hassan SA, Baillod M, Bakker F, Bigler F, Blümel S, Bogenschütz H, Boller E, Bromand B, Brun J, Calis JNM, Coremans-Pelseneer J, Duso C, Garrido A, Grove A, Heimbach U, Hokkanen H, Jacas J, Lewis G, Moreth L, Polgar L, Rovesti L, Samsøe-Petersen L, Sauphanor B, Schaub L, Stäubli A, Tuset JJ, Vainio A, Van DeVeire M, Viggiani G, Vinuela E, Vogt H, 1999. Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group ''Pesticides and Beneficial Organisms''. Biocontrol 44, 99–117.

Strand LR, 1998. Integrated Pest Management for Tomatoes. [Publication 3274]. University of California, Division of Agriculture and Natural Resources.

Teodoro AV, Fadini MAM, Lemos WP, Guedes RNC, Pallini A, 2005. Lethal and sub-lethal selectivity of fenbutatin oxide and sulfur to the predator *Iphiseiodes zuluagai* (Acari: Phytoseiidae) and its prey, *Oligonychus ilicis* (Acari: Tetranychidae), in Brazilian coffee plantations. Exp. Appl. Acarol. 36, 61–70.

Urbaneja A, Vercher R, Navarro V, García Marí F, Porcuna JL, 2007. La polilla del tomate, *Tuta absoluta*. Phytoma España 194, 16–23.

Urbaneja A, Pascual-Ruiz S, Pina T, Abad-Moyano R, Vanaclocha P, Montón H, Castañera P, Jacas JA, 2008. Efficacy of some acaricides against *Tetranychus urticae* (Acari: Tetranychidae) and their side-effects on selected natural enemies occurring in citrus orchards. Pest Manag. Sci. 64, 834–842.

Williams JS, Cooper RM, 2004. The oldest fungicide and newest phytoalexin - a reappraisal of the fungitoxicity of elemental sulphur. Plant. Pathol. 53, 263–279.

Williams MD, Gill G, 1996. Evaluation of pesticides for side effects on the leafhopper parasitoid *Anagrus atomus* with particular reference to protected crops. Test Agrochem. Cultivar 17, 98–99.

Chapter VI

Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*

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Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*

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ABSTRACT

The generalist predator *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) is a key natural enemy of various arthropods in agricultural and natural ecosystems. Releases of this predator are frequently carried out, and it is included in the Integrated Pest Management (IPM) programs of several crops. The accurate assessment of the compatibility of various pesticides with predator activity is key for the success of this strategy. We assessed acute and sublethal toxicity of 14 pesticides on *O. laevigatus* adults under laboratory conditions. Pesticides commonly used in either conventional or organic farming were selected for the study, including six biopesticides, three synthetic insecticides, two sulfur compounds and three adjuvants. To assess the pesticides' residual persistence, the predator was exposed for 3 d to pesticide residues on tomato sprouts that had been treated 1 h, 7 d or 14 d prior to the assay. The percentage of mortality and the sublethal effects on predator reproductive capacity were summarized in a reduction coefficient (E_x) and the pesticides were classified according to the IOBC (International Organization for Biological Control) toxicity categories. The results showed that the pesticides greatly differed in their toxicity, both in terms of lethal and sub lethal effects, as well as in their persistence. In particular, abamectin was the most noxious and persistent, and was classified as harmful up to 14 d after the treatment, causing almost 100% mortality. Spinosad, emamectin, metaflumizone were moderately harmful until 7 d after the treatment, while the other pesticides were slightly harmful or harmless. The results, based on the combination of assessment of acute mortality, predator reproductive capacity pesticides residual and pesticides residual persistence, stress the need of using complementary bioassays (e.g. assessment of lethal and sublethal effects) to carefully select the pesticides to be used in IPM programs and appropriately time the pesticides application (as function of natural enemies present in crops) and potential releases of natural enemies like *O. laevigatus*.

1. Introduction

Generalist arthropod predators are worldwide known for their ability to control phytophagous insects and mites in many cultivated crops (Symondson et al., 2002). For example, most Anthocoridae are polyphagous predators which play a key role in management of various pests such as aphids, mites, whiteflies and moths (Chambers et al., 1993; Desneux et al., 2006b; Bosco et al., 2008; Fathi and Nouri-Ganbalani, 2010; Ragsdale et al., 2011; Weintraub et al., 2011) in both greenhouses and fields. These predators are able to build up their populations before pests arrive using alternative prey (Harwood et al., 2007; Desneux and O'Neil, 2008) and host plants as alternative food sources (Lattin, 1999; Lundgren et al., 2009). In addition, predators of the genus *Orius* (Heteroptera: Anthocoridae) are mass-produced and released mainly to control thrips pest *Frankliniella occidentalis* (Pergande) in various horticultural crops in Eurasia and North America (Bosco et al., 2008; Weintraub et al., 2011).

Despite the potential effectiveness of biological control, many crop protection practices are primarily based on broad spectrum chemical pesticides which are noxious to beneficial arthropods (Desneux et al., 2007) and that affect agricultural sustainability (Wilson and Tisdell, 2001). An alternative to conventional pest control is Integrated Pest Management (IPM), an approach that aims to reduce pest status to tolerable levels by using effective, ecologically-sound and economically-sustainable management methods (Van Lenteren and Woets, 1988). IPM involves using

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Table 1
Tested pesticides.[b,c]

Active ingredient (a.i.)	Trade name	Field rate (a.i.%)	Chemical family	Mode of action	Crops	Target
Biopesticides						
Abamectin	Cal-EX EW®	75 mL hL^{-1} (1.8)	Avermectin	Ingestion. Chloride channel activator	Tomato, eggplant, sweet pepper, strawberry, lettuce, cucumber, melon, cabbages, citrus, grape, ornamental plants and flowers, forest trees	Mites. thrips, psyllids, aphids, leafminers, moths
Azadirachtin[a]	Oikos®	150 mL hL^{-1} (3.2)	Botanical	Ingestion. molting disruptor	Tomato, eggplant, sweet pepper, strawberry, carrot, fennel, beans, cabbages, cucurbit crops, garlic, onion, leek, leafy vegetables, celery, stone fruits, pome fruits, actinidia, walnut, chestnut	Thrips, Hemiptera, Lepidoptera
Bacillus thuringiensis var. *kurstaki* strain SA12[a]	Costar® WG	200 g hL^{-1} (90 000 IU μg^{-1})	Cry proteins	Ingestion. Disruptor of insect midgut epithelium	Tomato, eggplant, sweet pepper, strawberry, artichoke, corn, cotton, tobacco, potato, leafy vegetables, cucurbit crops, sugar beet, cabbages, sugar beet, beans, soybean, sunflower, citrus, grape, olive, actinidia, chestnut, ornamental plants, forest trees	Lepidoptera
Emamectin benzoate	Affirm®	150 g hL^{-1} (0.95)	Avermectin	Ingestion. Chloride channel activator	Tomato, eggplant, sweet pepper, strawberry, beans, artichoke, lettuce, stone fruits, pome fruits, grape, cole crops	Lepidoptera
Borax and citrus oil[a]	PreVam®	400 mL hL^{-1} (6)	Borates tetra sodium salts and Oil - essential	Contact. Miscellaneous non-specific inhibitor	Tomato, strawberry, grape	Mites, whiteflies, mealybugs, Tomato borer Fungicide
Spinosad[a]	Laser®	25 mL hL^{-1} 75 mL hL^{-1} (48)	Spinosyn	Ingestion and contact. Nicotinic acetylcholine receptor agonist	Tomato, eggplant, sweet pepper, strawberry, potato, fennel, legumes, garlic, onion, leek, stone fruits, cucurbit crops, artichoke, leafy vegetables, caper, pome fruits, stone fruits, grape, small fruits, tree nuts, ornamental plants, grass	Thrips, Planthoppers, Lepidoptera, Coleoptera, Diptera
Synthetic insecticides						
Chlorantraniliprole	Altacor®	11.5 g hL^{-1} (20)	Anthranilic diamide	Ingestion. Ryanodine receptor modulator	Tomato, eggplant, sweet pepper, cucurbit crops, cole crops, leafy vegetables	Lepidoptera
Indoxacarb	Steward 30 WG®	12.5 g hL^{-1} (30)	Oxadiazine	Ingestion and contact. Voltage-dependent sodium channel blocker	Tomato, eggplant, sweet pepper, cucurbit crops, cole crops, leafy vegetables, artichoke, corn, pome fruits, stone fruits, grape	Lepidoptera
Metaflumizone	Alverde®	100 mL hl^{-1} (24)	Semicarbazone	Ingestion. Sodium channel modulator	Tomato, eggplant, sweet pepper, cabbages, potato, leafy vegetables	Lepidoptera, Colorado potato beetle
Fungicides						
Dust sulfur[a]	Zolfo Ventilato Stella®	30 kg ha^{-1} (94.5)	Inorganic	Contact. Repellent	Tomato, melon, pea, artichoke, leafy vegetables, cucumber, grape, ornamental flowers	Fungal disease
Wettable sulfur[a]	Zolfo Bagnabile Bayer®	200 g hL^{-1} (90)	Inorganic	Contact. Repellent	Tomato, cucumber, melon, peas, apple, peach, grape, ornamental flowers	Fungal disease
Adjuvants						
Parafinic mineral oil[a]	Ufo®	2000 mL hL^{-1} (98.8)	Petroleum derivative	Contact. Asphyxiant	Vegetable crops, citrus, pome fruits, stone fruits, grape, olive, fig, ornamental plants	–
Para-menthene[a]	Nu-film-P®	40 mL hL^{-1} (96)	Unclassified	Contact. Repellent	Vegetable and fruit crops	–
Rapeseed oil[a]	Codacide®	2500 mL hL^{-1} (86.4)	Botanical	Contact. Asphyxiant	Tomato, wheat, rice, corn, sugar beet	–

[a] Indicate pesticides that are authorized for organic farming.
[b] Italian Ministry of Health, phytosanitary products database 2011, http://www.salute.gov.it/fitosanitariwsWeb new/FitosanitariServlet.
[c] Pesticide Action Network pesticide database 2011.

pesticides when required, though their harmful effects on natural enemies should be mitigated. Accurate assessment of potential side effects of pesticides on natural enemies is critical for developing effective IPM strategies (Desneux et al., 2006a; Stark et al., 2007) and is increasingly important because the recent European Union directive on sustainable use of pesticides, i.e. 2009/128/EC, stated that IPM should be implemented in all member states by January 1st 2014 (EEC/CEE, 2009). Many laboratory assays rely almost exclusively on the assessment of lethal effects. However, pesticides could induce multiple sublethal effects in individuals that survive an exposure to a given pesticide (Desneux et al., 2007), and these effects could have important impact on natural enemies population dynamics (Stark and Banks, 2003). Sublethal effects could impair the physiology (e.g. neurophysiology, development, longevity, fecundity and sex-ratio) and the behavior (e.g. mobility, orientation, feeding, host searching, oviposition and mating) of natural enemies (Desneux et al., 2004a,b; Suma et al., 2009; Evans et al., 2010; Arnó and Gabarra, 2011; Saber, 2011; Stara et al., 2011; He et al., in press and see Desneux et al. (2007) for a thorough review). Studying more subtle endpoints (e.g. behaviors, Desneux et al., 2004c) and using multistep bioassays to evaluate the potential effects of pesticides on natural enemies is therefore required to assess risk in a more complete way (Desneux et al., 2006a, 2006c, 2007; Stark et al., 2007).

Previous laboratory studies on the effects of pesticides on predators have focused mostly on direct contact toxicity by topical application (James, 2004; Mahdian et al., 2007; Rimoldi et al., 2008) or residual toxicity (Giolo et al., 2009; Gradish et al., 2011). By contrast, fewer studies have documented effects of pesticides on predators when exposed through feeding on contaminated prey (Banken and Stark, 1998; Urbaneja et al., 2008; Cabral et al., 2011; He et al., in press) and very few have tested relationship between age of pesticide residues and lethal and sublethal effects on predators and other natural enemies of a given pest (Van de Veire et al., 2002b; Desneux et al., 2005; Gradish et al., 2011).

In this context, the aim of the present work was to provide lethal and sublethal toxicity assessment of various pesticides on the generalist predator *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae). This species was chosen as predator model because it is widely distributed in the Palaearctic region, it is a key natural enemy of various pests in agricultural ecosystems (Chambers et al., 1993; Weintraub et al., 2011) and had shown some potential for biological control of the invasive South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Desneux et al., 2010; Lins et al., 2011). We evaluated the effects of pesticides that are commonly used in organic and/or conventional cropping systems (see Table 1 for detailed information), including pesticides that have been increasingly used on tomato owing to the recent invasion of Afro-Eurasian countries by *T. absoluta* (Desneux et al., 2010,2011). In laboratory conditions, we assessed acute toxicity on predator adults and sublethal effects on their reproductive capacity (progeny size) when exposed to pesticide residues on tomato leaves. To test for potential relationship between age of pesticide residues and effects in exposed predators, we tested three different ages of pesticides residues: 1-h old, 7-d old and 14-d old.

2. Materials and methods

2.1. Insects

Orius laevigatus individuals used in the trials were provided by Bioplanet (Cesena, Italy) in commercial bottles containing 500 individuals dispersed in inert material (LeviPAK500®). Before being used in experiments, the predators were fed using UV sterilized *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs (Entofood®, Koppert Biological Systems, The Netherlands) and stored in growth chambers (26 ± 2 °C, 60 ± 10% RH, 14: 10 L.D.). Predator adults belonged to the same cohort and the individuals were 4-d old when used for the experiments.

2.2. Pesticides

The pesticides tested varied in their mode of action, chemical families and pesticide types (detailed information on active ingredients [a.i.] are provided in Table 1). We tested six biopesticides, three synthetic insecticides, two fungicides (sulfur compounds), and three adjuvant compounds. These plant protection products are currently used in various crops (including tomato) and some of them are also authorized for organic farming. The highest recommended rates for tomato crops were used for our experiments, except for spinosad, which was tested at two different recommended rates (both on tomato); one for management of *T. absoluta* and of Thrips (lowest one, hereafter named spinosad 25) and one for management of agromizid (Diptera) leafminers (highest one, hereafter named spinosad 75). All the pesticides were stored and applied following their label guidelines. For a given pesticide, plants were sprayed with the formulated product which was diluted with water (to obtain 0.5 L of solution) and applied at the rate of 1000 L ha^{-1} of solution (3 plants per square meter). The treatment was applied using a 2L power-pack aerosol hand sprayer (Matabi®, Antzuola, Guipuzcoa, Spain) and the nozzle of the sprayer was directed toward the plants from a distance of 0.5 m (this resulted in a complete and uniform wetting of the young tomato plants). An acid fertilizer (Fertacid®, Biointrachem Italia) was added to adjust pH to 4.5 in case of *Bacillus thuringiensis* (*Bt*) and Azadirachtin (neem oil) solutions as recommended by the companies.

2.3. Toxicity trials

2.3.1. Exposure to pesticides and lethal effect

The experiments were conducted at the Department of Agrifood and Environmental Systems Management of the University of Catania (Italy) under controlled environmental conditions in growth chambers (26 ± 2 °C, 60 ± 10% RH, 14:10 L.D.). The trials were performed by exposing *O. laevigatus* adults for 3 d to dried pesticide residues on tomato leaves. Plants were used as a substrate for the pesticides to avoid overestimation of toxicity that usually occurs when using pesticide residues on inert material like glass (Desneux et al., 2005, 2006a). The plants used were 40 cm high, 40-d old tomato plants (cv Missouri), grown from seeds in 2 L pots. The treatments were performed following regular agricultural practices, and control plants (untreated) were sprayed with tap water. Five plants for each trial were sprayed per pesticide tested. The plants were left to allow pesticides to dry for 1 h, 7 d or 14 d (see Section 2.3.2. *Assessment of pesticides persistence* below) and then the upper plant part (about 17 cm) was cut and placed into a bioassay isolator made up of two superposed plastic glasses. The top glass (600 mL, length: 13 cm) had a central hole on its bottom to allow tomato plant stem to reach the water present in a second (bottom) glass (350 mL, length: 11 cm). A fine mesh net was fixed on the upper opening of the largest top glass to allow ventilation (the design is presented in Fig. S1). Five females and five males of *O. laevigatus* were introduced in the described arena. Untreated *E. kuehniella* eggs were provided daily as food at the rate of 50 eggs per predator and a water source was offered *ad libitum* in a 1.5 mL Eppendorf tube sealed with cotton. Five replicates (5 × 10 = 50 predators) were performed per pesticide and per each residual trial (see Section 2.3.2). Mortality (number of dead predators) was recorded daily for 3 d. Predators were considered dead when they remained immobile after being touched with a fine paintbrush.

2.3.2. Assessment of pesticides persistence

Persistence of pesticides toxicity was studied by exposing *O. laevigatus* adults to pesticides applied at three different times prior to the assay: 1 h, 7 d and 14 d (see Section 2.3.1. for description of the bioassay). For a given pesticide, all the plants (for the three different delays after application of pesticide) were sprayed at the same time and they were maintained in insect proof cages in a greenhouse (min < *mean temperature* < max: 19.5 °C < 26.2°C < 38 °C; min < *mean RH* < max, 29% < 61.9% < 92%; natural ambient light: September 2010) to allow aging of pesticides in regular cropping conditions. Plants sprayed with tap water were used as control plants for each of the treatments.

2.3.3. Side effects on offspring production

Various plants are suitable substrate for oviposition of *Orius* spp. and various results on reproductive performances have been reported (Coll, 1996; Lundgren and Fergen, 2006; Butler and O'Neil, 2007; Bonte and De Clercq, 2009; Lundgren et al., 2009). Pilot studies showed that *O. laevigatus* effectively laid eggs and produced offspring on tomato plants in our experimental conditions (Biondi, Desneux and Zappalà, unpublished data). In order to allow the individuals to oviposit from the first day of exposure to the pesticides (most realistic scenario), potential effects of pesticides on reproductive capacity of predator females were assessed using the treated tomato leaves that also served as substrates for exposure to pesticides (described in Sections 2.3.1 and 2.3.2.). After having checked mortality for the lethal assessment assay (3 d), the survivors were removed from the experimental arena. Then the number of emerged progeny (nymphs) was scored daily for 10 d after the first day of pesticide exposure. To avoid cannibalism, the emerged nymphs were removed daily using a mouth aspirator.

2.4. Data analysis

Datasets were first tested for normality and homogeneity of variance using Kolmogorov–Smirnov D test and Cochran's test respectively, and transformed if needed. For the lethal effect and persistence assessment results, we tested the effects of pesticides (factor *pesticide*) and of application timing (factor *application time*) and potential interactions between these two factors on the number of predator found dead after 3 d of exposure to the various pesticides. For this, we used a factorial ANOVA. Subsequently, additional one-way ANOVA followed by LSD post hoc tests for multiple comparisons inside the different application time sub datasets were carried out.

Data recorded on side effects of pesticides and of application time on production of predator nymphs produced were used to calculate two different estimates which provided information on two different effects: (i) *Offspring production*: effect of pesticides on total offspring production. In such case, we analyzed the exact numbers of offspring produced per glass (5 females per each glass were tested per each replicate) and any early death of predator females in a given glass was therefore included in the overall reduction of offspring production estimate (i.e. realistic to general effect of pesticides on predator populations), (ii) *Predator reproductive capacity*: offspring production was corrected by daily survival of predator females in each glass. In this case, the number of nymphs produced was corrected by early death of a predator female per day (if any) and therefore this value provided a more accurate estimate of actual sublethal effects of pesticides on reproductive capacity of predator females. We statistically tested the effect of the two factors; pesticides and application time (and their interaction) on the two estimates, *Offspring production* and *Predator reproductive capacity*, and for this we used a factorial ANOVA. Subsequently, additional one-way ANOVA followed by LSD post hoc tests for multiple comparisons inside pesticide age sub datasets were carried out for each estimate.

Finally, to provide a single value summarizing potential deleterious effects of pesticide tested, the toxic effects (both lethal and sublethal effects) of each pesticide were also expressed as the *Reduction coefficient* E_x for pesticide *x* (Urbaneja et al., 2008) using the formula:

$$E_x = 100\left\{1 - \left[\left(1 - \frac{E_{mx}}{100}\right)\left(1 - \frac{E_{fx}}{100}\right)\right]\right\}$$

where E_{mx} is the corrected mortality (Abbott, 1925) and E_{fx} is the corrected *Predator reproductive capacity* estimated using the formula:

$$E_{fx} = 100 - \frac{F_x 100}{F_c}$$

where F_x is the mean *Predator reproductive capacity* for pesticide *x* and F_c is the *Predator reproductive capacity* recorded in the control group (untreated group). The values (E_x) were then classified and interpreted accordingly to the standards of the International Organization for Biological Control (IOBC) which include four categories: (1) harmless: $E_x < 30\%$, (2): slightly harmful: $30\% < E_x < 80\%$, (3): moderately harmful: $80\% < E_x < 99\%$, and (4): harmful: $E_x > 99\%$.

3. Results

3.1. Lethal effect and persistency

The statistical results are summarized in Table 2A. The mortality of the predators during the 3-dexposure period varied significantly among pesticides tested (significant pesticide factor) and delay between exposure to pesticides and application time (significant application time factor). There was a significant interaction between the pesticide and the application time factors, i.e. aging of residues did not affect pesticide toxicity to *O. laevigatus* in the same way among pesticides tested.

Six pesticides significantly increased *O. laevigatus* mortality ($F_{15,68} = 5.67$; $P < 0.001$) with rates of mortality ranging from 75% for spinosad 25 to 98% for abamectin. The other pesticides caused mortality levels always lower than 44% (Fig. 1a). The 7-d-old residues significantly affected *O. laevigatus* survival ($F_{15,69} = 12.14$; $P < 0.001$) in case of abamectin, emamectin benzoate (emamectin), spinosad 75 and metaflumizone, causing almost the same mortality rates (98%, 92%, 85% and 87% respectively) as those observed in case of the 1-h old residues (Fig. 1b). Finally, in case of 14-d old pesticide residues, only abamectin and spinosad 75 still induced

Table 2

Statistics from the factorial ANOVA used to analyze (A) the numbers of predator found dead after 3 d of exposure to pesticides (mortality), (B) the total numbers of offspring produced per replicate, i.e. *offspring production*, and (C) total numbers of emerged nymphs per live *O. laevigatus* females (checked and corrected per day) during 3 d of exposure (i.e. *predator reproductive capacity*) among pesticides tested (pesticide factor) and as function of the age of pesticide residues (application time factor).

Source of variation	Degrees of freedom	*F*	*p*-value
A: Mortality			
Pesticide	15	30.77	<0.001
Application time	2	28.25	<0.001
Pesticide *x* application time	30	4.64	<0.001
B: Offspring production			
Pesticide	15	11.04	<0.001
Application time	2	17.65	<0.001
Pesticide *x* application time	30	2.72	<0.001
C: Predator reproductive capacity			
Pesticide	15	4.85	<0.001
Application time	2	7.41	<0.001
Pesticide *x* application time	30	3.07	<0.001

significant mortality in exposed predators when compared to the untreated control group ($F_{15,63} = 9.09$; $P < 0.001$; Fig. 1c).

3.2. Side effects on O. laevigatus reproduction

The statistical results are summarized in Table 2B and C. Offspring production varied significantly as a function of pesticide tested (significant pesticide factor) and the delay between exposure to pesticides and application time (significant application time factor) (Table 2B). In addition, the persistence of pesticide

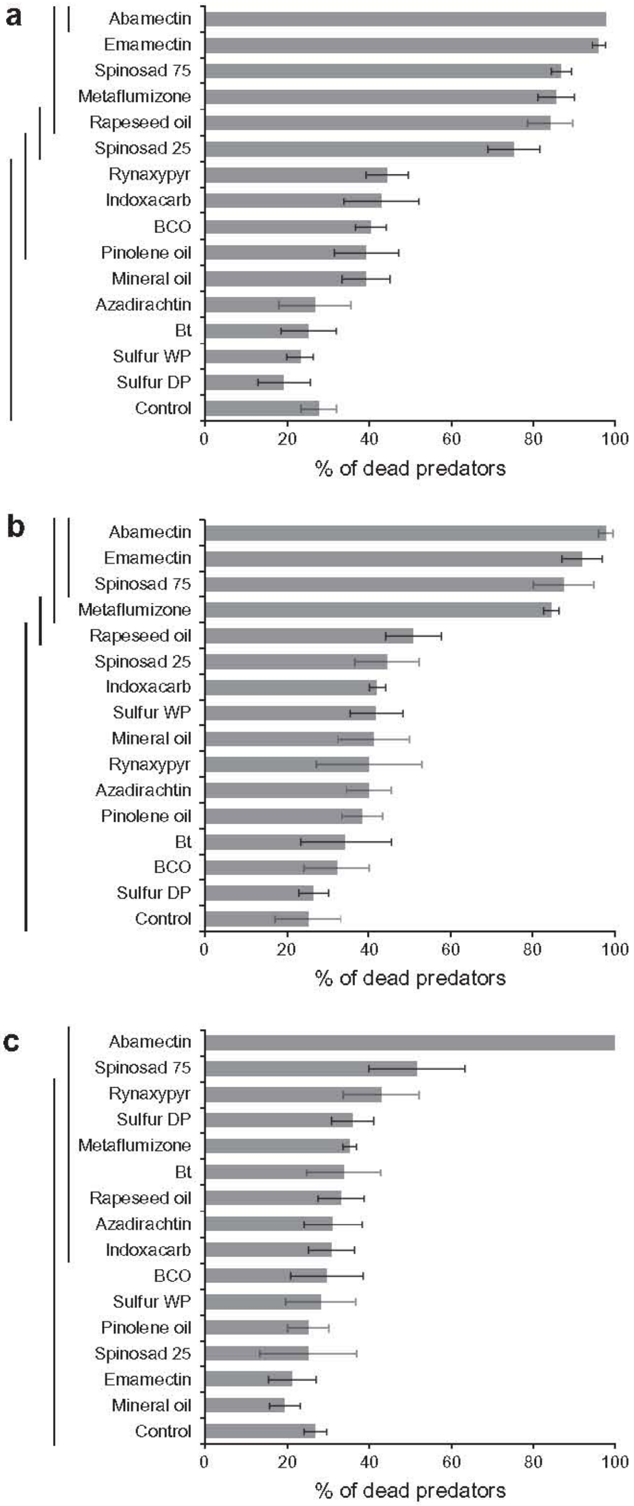

Fig. 1. Lethal effect. Means (±SEM) of mortality percentages of *Orius laevigatus* after 3 d of exposure to (a) 1-h old, (b) 7-d old and (c) 14-d old pesticide residues. Means for treatment subtended by lines do not differ at $P < 0.05$ (one-way ANOVA followed by LSD post hoc test).

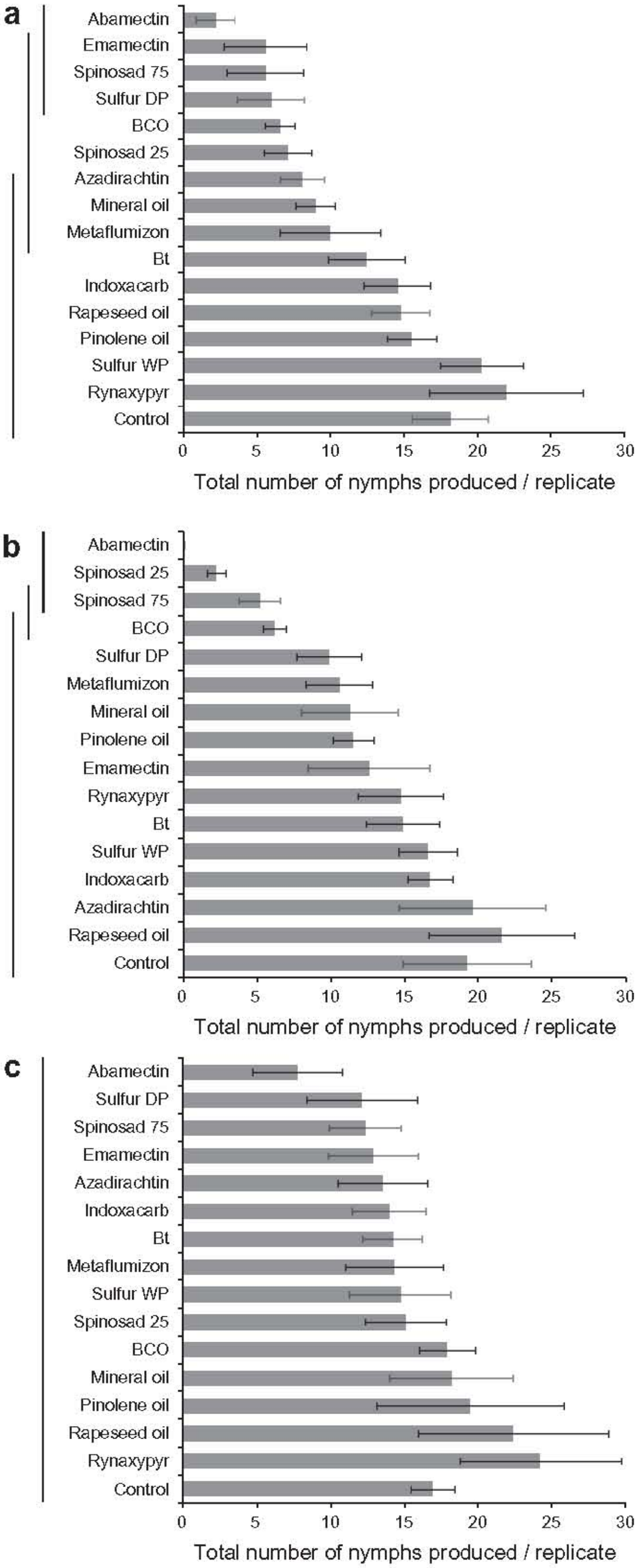

Fig. 2. Side effects of pesticides on *offspring production* by *Orius laevigatus*. Means (±SEM) of total emerged nymphs of *O. laevigatus* in each replicate during 3 d of exposure to (a) 1-h old, (b) 7-d old and (c) 14-d old pesticide residues. Means for treatment subtended by lines do not differ at $P < 0.05$ (one-way ANOVA followed by LSD post hoc test).

toxicity (i.e. effects of pesticide residues when aging) varied among the pesticides (significant interaction between pesticide and application time factors) (Table 2B). Mean number of offspring produced varied significantly among pesticides in case of 1-h old pesticide residues (one-way ANOVA: $F_{15,83} = 5.58$; $P < 0.001$). Abamectin, emamectin, borax plus citrus oil (BCO), dust sulfur (sulfur DP), spinosad 75 and spinosad 25 significantly reduced offspring production when compare to untreated control (Fig. 2a). Offspring production was also significantly reduced when the adults were exposed to the 7-d old pesticide residues ($F_{15,84} = 9.98$; $P < 0.001$) with abamectin, spinosad 75 and 25 causing significant decrease (Fig. 2b). No significant decrease in offspring production were observed when individuals were exposed to 14-d old pesticide residues ($F_{15,78} = 1.49$; $P = 0.135$; Fig. 2c).

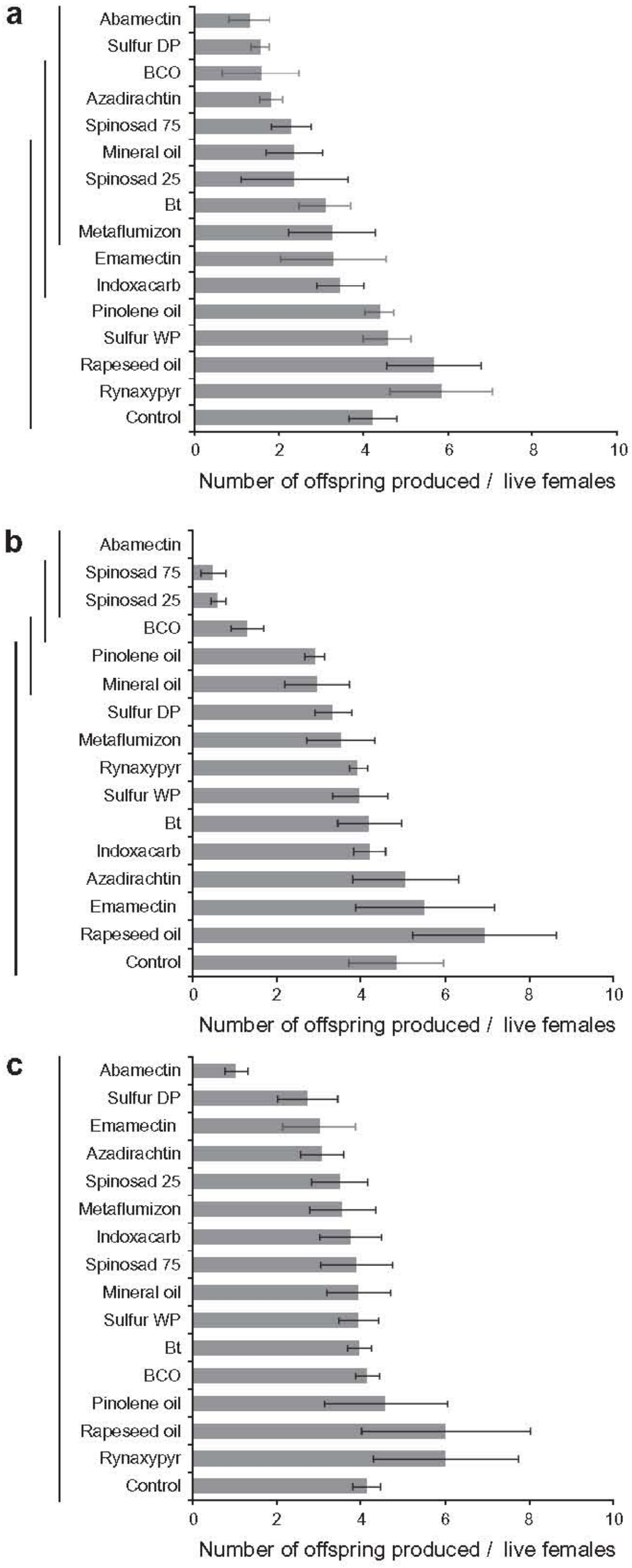

Fig. 3. Sublethal effects of pesticides on *predator reproductive capacity*. Means (±SEM) of total number of emerged nymphs per live *O. laevigatus* females (checked and corrected per day) during 3 d of exposure to (a) 1-h old, (b) 7-d old and (c) 14-d old pesticide residues. Means for treatment subtended by lines do not differ at $P < 0.05$ (one-way ANOVA followed by LSD post hoc test).

Predator reproductive capacity varied also significantly as function of pesticides (significant pesticide factor) and age of pesticide residues (significant application time factor) (Table 2C). As observed for mortality and offspring production, the persistence of pesticide toxicity varied among the pesticides (significant interaction between the two factors) (Table 2C). Predator reproductive capacity varied among pesticides tested in case of exposure to 1-h old residues ($F_{15,83} = 2.85$; $P = 0.002$) with abamectin, sulfur DP, BCO, azadiractin and spinosad 75 significantly decreasing *O. laevigatus* reproductive capacity of predator females when compared to untreated control group (Fig. 3a). In case of exposure to 7-d old residues, abamectin, BCO, Spinosad 25 and Spinosad 75 significantly reduced the number of nymphs produced per *O. laevigatus* female ($F_{15,84} = 7.7$; $P < 0.001$; Fig. 3b). No significant effect was observed when predators were exposed to 14-d old residues ($F_{15,78} = 1.26$; $P = 0.225$; Fig. 3c).

3.3. Reduction coefficient (E_x)

Abamectin was the only pesticide with a reduction coefficient $E_x > 99\%$ and it was classified as harmful when using IOBC toxicity categories (class 4; Table 3). Its harmfulness was high for all application time of residues tested (i.e. 1 h, 7-d and 14-d old). For others, four pesticides (emamectin, spinosad 25 and 75, and metaflumizone) were classified as moderately harmful (class 3), i.e. $80\% < E_x < 99\%$, and they remained classified as such when pesticide residues were 7-d old. When 14-d old, pesticide residues were harmless with $E_x < 30\%$, except for spinosad 75 that was categorized as slightly harmful ($E_x = 42.9\%$). Azadiractin, BCO, indoxacarb, sulfur DP, paraffinic mineral oil (mineral oil) and rapeseed oil were classified as slightly harmful (class 2) in the 1-h old residue trial. Then, toxicity of azadiractin, indoxacarb and sulfur DP was reduced when residues were 7-d old but BCO, mineral oil and rapeseed oil. Other pesticides tested had E_x lower than 30% were classified as harmless (class 1; Table 3).

4. Discussion

The present study demonstrated that lethal and sublethal effects of different types of pesticide residues (both in term of chemical and of toxicity persistence) on the generalist predator *O. laevigatus* varied widely, including among pesticides within the same product group (e.g. biopesticides). *Orius laevigatus* was very susceptible to abamectin ($E_x > 99\%$, IOBC class 4) even 14 d after pesticide application, notably because of high mortality induced in adults. Abamectin proved to be not compatible with this predator for IPM. Emamectin, metaflumizone and spinosad were less toxic but still induced high levels of mortality and reduced offspring produced (IOBC class 3), even in case of exposure to 7-d old residues. Finally, chlorantraniliprole (rynaxypyr), *Bt*, indoxacarb, wettable sulfur (sulfur WP), mineral oil and para-menthene (pinolene oil) proved to be harmless ($E_x < 30\%$, IOBC class 1) with mortality and reproductive capacity levels similar to what was recorded in untreated control group. Taken as a whole, the results

Table 3
Reduction coefficient E_x and IOBC toxicity classes. Pesticides, per pesticide type (i.e. biopesticide, synthetic insecticide, fungicide and adjuvant), that are the most harmful to *Orius laevigatus* are indicated in bold.

	Pesticide	1-h old residue		7-d old residue		14-d old residue	
		E_x	IOBC class	E_x	IOBC class	E_x	IOBC class
Biopesticides	**Abamectin**	**99.4**	**4**	**100**	**4**	**100**	**4**
	Azadirachtin	57.1	2	20.9	1	26.4	1
	Bacillus thuringiensis	26.8	1	0	1	0	1
	Emamectin benzoate	95.7	3	88.7	3	27.1	1
	Borax and citrus oil	69.4	2	74.5	2	0	1
	Spinosad 25	82.2	3	90.2	3	15.5	1
	Spinosad 75	91.6	3	97.0	3	42.9	2
Synthetic insecticides							
	Chlorantraniliprole	22.9	1	26.6	1	22.0	1
	Indoxacarb	35.6	2	28.9	1	0	1
	Metaflumizone	**84.8**	**3**	**87.2**	**3**	**24.1**	**1**
Fungicides							
	Dust sulfur	**69.2**	**2**	**17.1**	**1**	**25.4**	**1**
	Wettable sulfur	0	1	20.0	1	0	1
Adjuvants							
	Mineral oil	48.8	2	35.6	2	0	1
	Para-menthene	16.0	1	28.1	1	0	1
	Rapeseed oil	**77.9**	**2**	**39.1**	**2**	**0**	**1**

show that side effects of pesticides can vary largely depending upon various factors studied, like endpoint considered (lethal vs. sublethal), pesticide chemical family and pesticide type. Consequently, comprehensive and specific risk assessment should be undergone before implementing any IPM programs.

4.1. Differential effects and persistence among pesticides

The synthetic insecticides rynaxypyr and indoxacarb proved to be safe for *O. laevigatus*, at least for the traits assessed during our study. These results contrast with those obtained in a study by Studebaker and Kring (2003) in which 100% of mortality was observed when exposing *O. insidiosus* to indoxacarb dried residues on glass plates for 24 h. This is consistent with the finding that pesticides are more toxic on intert material than on plant substrate (Desneux et al., 2005,2006a), mainly because plant enzymes can affect pesticide toxicity (Schuler, 1996) and because pesticides may be adsorbed into the waxy layer of the plant leaf cuticle, making them less available to natural enemies (Desneux et al., 2005). The other synthetic insecticide tested, metaflumizone, proved to be relatively toxic with high acute mortality observed (>80%) and persistence of toxicity for at least 7 d. However, no sublethal effects on reproductive capacity were observed which means that this product might allow a rapid recolonization of treated areas from untreated surroundings or new predators releases by the process of "horizontal recruitment" (Desneux et al., 2006a,2006c), thought additional assessment of potential behavioral sublethal effects (e.g. orientation behavior and repellency) should be assessed to confirm this hypothesis.

In contrast to synthetic pesticides, the biopesticides largely differed in their lethal and sublethal effects on *O. laevigatus*. Azadirachtin was harmless in terms of mortality, most likely because it mainly acts on insects as molting disruptor (Sieber and Rembold, 1983) and therefore could not negatively affect adult insects. However, it showed slight negative effects on overall offspring production in case of exposure to fresh residues (which is consistent with marginally significant reduction of predator reproductive capacity in case of 1-h old residues). The effect probably resulted from lower survival of young nymphs at the time they hatched and got exposed to azadirachtin residues still present on the tomato leaves. In the same way, BCO did not affect predator survival but had negative sublethal effects, reducing both predator reproductive capacity and offspring production, and this occurred even when residues were 7-d old. This is consistent with known properties of this type of botanical insecticide, notably their repellent effect (which obviously reduced time spent by adults on leaves and consequently reduced number of eggs potentially laid) and their negative effect on egg-hatching in insects (Isman, 2000; Cordeiro et al., 2010).

However, several biopesticides, namely abamectin, emamectin and spinosad, proved to be highly toxic to *O. laevigatus*. Emamectin and abamectin showed relatively similar pattern of effects and persistence which is consistent because they are from the same chemical family: Avermectin. Among these two products, and more broadly among all tested pesticides, abamectin was the most noxious and was classified harmful, killing almost all predators, even in case of 14-d old residues, and inducing major sublethal effects on reproduction of rare survivors. These results are in agreement with previous studies which reported negative side effects of abamectin on *Orius* spp. (Van de Veire et al., 2002a,b; Studebaker and Kring, 2003; Bostanian and Akalach, 2004). The high persistence of abamectin in our study may relate to low degradation by UV, low foliar uptake and/or low translaminar activity on/in tomato plants. However, this persistence should be associated with climatic conditions occurring in Mediterranean basin greenhouses and thus abamectin degradation and subsequent effects might be different under different climatic conditions. For example, abamectin is known to be subjected to photolysis when exposed to sunlight Tomlin, (2011) and Gradish et al. (2011) found low persistence of this insecticide when plants were exposed directly to sunlight in Canadian greenhouses. By contrast, plants in Mediterranean basin greenhouses are usually protected from direct sunlight through the use greenhouse shade nets that actually reduce the exposure of plants to UV.

Spinosad was also very toxic with persistence proving to be relatively high; most of effects were still observed when predators were exposed to 7-d old residues. These results matched those of previous studies (Van de Veire et al., 2002a; Studebaker and Kring, 2003) which assessed side effects of spinosad on *O. insidiosus* and *O. laevigatus* when exposed to spinosad residues on glass plates. In our study, in most cases the highest concentration of spinosad tested (75) was twice as toxic and persistent as the lower one (25). Effects may result from side effects of spinosad on reproduction-related behaviors because spinosad is based on spinosyns (produced by the bacteria *Saccharopolyspora spinosa*) which are neurotoxic compounds primarily targeting post-synaptic nicotinic acetylcholine and GABA receptors, i.e. which are likely to impact predator behaviors (Desneux et al., 2007). Williams et al. (2003) reported that predators generally suffered very few sublethal effects following exposure to spinosad, but that parasitoids often showed multiple sublethal effects (notably a decrease in reproductive capacity). It is unclear why the predator *O. laevigatus* was negatively affected by spinosad in our study, but effects may relate to its omnivory behavior (it feeds also on plants and therefore could receive more toxins than if consuming only spinosad-contaminated prey). Finally, *Bt* showed high selectivity (i.e. harmless to *O. laevigatus*) likely owing of the inability to the *Bt* toxins to reach the insect gut in sucking insects (Gill et al., 1992). In addition, results suggest that adjuvant compounds in *Bt* formulations are also harmless to *O. laevigatus*.

During the trials, the adjuvant compounds showed low toxicity to *O. laevigatus*. Only rapeseed oil reduced predator survival in case of exposure to 1-h old residues, but no sublethal effects were observed on predator reproductive capacity and overall offspring production was never reduced. The lack of negative residual effects of adjuvant residues on *O. laevigatus* is likely because adverse effects of adjuvant compounds on insects are thought to occur mostly through suffocation at the time the insects are actually directly sprayed with the product (Acheampong and Stark, 2004; Desneux et al., 2006c). This result supports the idea that adjuvant compounds are not primarily involved in adverse effects that predators (like those from *Orius* genus) can suffer when they are exposed to residues of commercial pesticidal products. It may also mean that potential effects of adjuvant compounds on predators should be considered only when pesticides are going to be used subsequently to releases of predators in crops.

In case of fungicides, both sulfur compounds did not induce mortality in predators, but predator reproductive capacity was reduced by sulfur DP in case of exposure to 1-h old residues. This result may be related to the activity of the compound as an oviposition repellent or as an egg dryer. The establishment of *O. laevigatus* in a treated crop may be possible only if untreated refuge areas (without dust sulfur residues) are available to the predators, as reported for the same fungicides in case of the predator *Nesidiocoris tenuis* on tomato plants (Zappalà et al., in press). However, side effects of sulfur compounds should be also tested in relation to the temperature, because insecticidal activity of these compounds (notably on parasitoids) is positively correlated with temperature (Flanders, 1943).

4.2. Importance of results for IPM and organic farming

Our study provides information that could be useful for IPM programs in identifying (and avoiding) products that may prevent predator population to build up because of side effects on predator reproductive capacity and survival. These products may, in addition to compromising the efficacy of IPM programs, prevent efficient colonization or recolonization of treated crops, particularly when pesticides are highly persistent. Interestingly, Avermectin-based biopesticides, the biopesticides based on Spinosad and BCO fall in this last category. These results indicate that pesticidal products that are classified as biopesticides can easily be of major concerns when they are supposed to be used in combination with natural enemies. Given the results obtained, the use of abamectin together with natural enemies like *O. laevigatus* in IPM programs should not be considered for effective and sustainable pest management programs. Unfortunately, a similar situation applies to pesticides that are recommended for organic cropping systems and therefore that are usually thought to be harmless for non-target arthropods. Spinosad induced both lethal and sublethal effects in *O. laevigatus* and its effects were persistent for at least 7 d after initial application. This biopesticide has been classified as an environmentally-safe product and has been embraced by IPM practitioners as a biorational pesticide. However, present results suggest that other pesticides with higher selectivity should be preferred, at least for management of pests that require usage of the highest application rates authorized (like Diptera leaf miners). For this reason, spinosad application should be avoided for at least one week before predators such as *O. laevigatus* are released in crops (which would prevent sublethal effects on the reproductive capacity of predators). Overall, the safest insecticides for *O. laevigatus* appeared to be azadirachtin, *Bt*, rynaxypyr and indoxacarb. Also, sulfur WP compounds should be preferred over sulfur DP compounds as fungicide. For example, when dealing with Lepidopteran pests, *Bt* should be prioritized because (i) it proved to be safe for *O. laevigatus* (especially true because we tested one of the highest concentrations recommended in crops), (ii) it induces no lethal and sublethal effects on predators that fed on prey which fed *Bt*-treated plants (Angeli et al., 2005; Tian et al., 2010), and (iii) it is highly efficient even against leaf miners (Gonzalez-Cabrera et al., 2011). Finally, in case of azadirachtin, negative effects on the development of predator nymphs (present study and high susceptibility reported in young insect instars; Schmutterer, 1990) could be avoided by delaying predator release after insecticide application.

4.3. Interest of the approach and model for risk assessment

The experimental design used enabled us to demonstrate contrasting effects between lethal and sublethal effects among various pesticides, notably because predators had the possibility to avoid being permanently in contact with pesticide-treated plant by walking on refuge areas (untreated areas like the glass surface). Because individuals also had to oviposit on plant substrate (for laying eggs), it could lead to chronic exposure which is comparable what usually happens in field conditions (Desneux et al., 2007). Alternatively, a strong repellent effect can prevent individuals from being in contact with pesticides (Desneux et al., 2005,2007; Cordeiro et al., 2010), and it could ultimately lead to reduced number of eggs laid and subsequent offspring production (Umoru et al., 1996; Desneux et al., 2005; Urbaneja et al., 2008). For example, sulfur DP and BCO induced only sublethal effects on reproductive capacity and no lethal effect. An alternative hypothesis would be that sublethal effects of products reduced female fecundity and/or impaired female oviposition behaviors (Banken and Stark, 1998; Desneux et al., 2004a,2007) but this should be specifically tested to confirm this hypothesis. The tomato–*O. laevigatus* model proved to be a valid experimental system; we observed normal reproduction levels (i.e. 1.82 ± 0.24 nymphs produced daily per female) and survivorship levels >72% in the control groups. These offspring production and longevity values are in concordance with those recorded on other host plants for various other *Orius* spp. (Lundgren and Fergen, 2006; Butler and O'Neil, 2007) and our results match those from Coll (1996) in which *O. insidiosus* showed preference toward tomato over other plant species as oviposition substrate in laboratory trials.

5. Conclusion

One of the goals of IPM is to preserve and/or increase pests natural mortality factors by combining various compatible control measures. Better knowledge on risks associated with specific pesticides toward natural enemies is of primary importance when incorporating them in IPM programs. The results obtained in the present study could improve IPM programs in which *O. laevigatus* is involved and more broadly may be useful for implementing IPM programs involving the use of *Orius* spp. as natural enemies.

These results were obtained in laboratory conditions, which represent a high exposure scenario, and it would be ideally completed with semi-field and field experiments (Sterk et al., 1999; Desneux et al., 2005; Suma et al., 2009). The results also stress the urgent need to clarify and re-organize how pesticides are labeled and classified, e.g. several biopesticides could be more toxic than synthetic ones, and pesticides that are recommended for organic farming may be more toxic than conventional pesticides.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2011.12.082.

References

Abbott, W.S., 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Acheampong, S., Stark, J.D., 2004. Effects of the agricultural adjuvant Sylgard 309 and the insecticide pymetrozine on demographic parameters of the aphid parasitoid, *Diaeretiella rapae*. Biol. Control 31, 133–137.

Angeli, G., Baldessari, M., Maines, R., Duso, C., 2005. Side-effects of pesticides on the predatory bug *Orius laevigatus* (Heteroptera: Anthocoridae) in the laboratory. Biocontrol Sci. Technol. 15, 745–754.

Arnó, J., Gabarra, R., 2011. Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). J. Pest. Sci. 84, 513–520.

Banken, J.A.O., Stark, J.D., 1998. Multiple routes of pesticide exposure and the risk of pesticides to biological controls: a study of Neem and the seven spotted lady beetle (Coleoptera: Coccinellidae). J. Econ. Entomol. 91, 1–6.

Bonte, M., De Clercq, P., 2009. Impact of artificial rearing systems on the developmental and reproductive fitness of the predatory bug *Orius laevigatus*. J. Insect Sci. 10, 1–11.

Bosco, L., Giacometto, E., Tavella, L., 2008. Colonization and predation of thrips (Thysanoptera: Thripidae) by *Orius* spp. (Heteroptera: Anthocoridae) in sweet pepper greenhouses in Northwest Italy. Biol. Control 44, 331–340.

Bostanian, N.J., Akalach, M., 2004. The contact toxicity of indoxacarb and five other insecticides to *Orius insidiosus* (Hemiptera: Anthocoridae) and *Aphidius colemani* (Hymenoptera: Braconidae), beneficial used in the greenhouse industry. Pest Manag. Sci. 60, 1231–1236.

Butler, C.D., O'Neil, R.J., 2007. Life history characteristics of *Orius insidiosus* (Say) fed diets of soybean aphid, *Aphis glycines* Matsumura and soybean thrips, *Neohydatothrips variabilis* (Beach). Biol. Control 40, 339–346.

Cabral, S., Soares, A.O., Garcia, P., 2011. Voracity of *Coccinella undecimpunctata*: effects of insecticides when foraging in a prey/plant system. J. Pest. Sci. 84, 373–379.

Chambers, R.J., Long, S., Heyler, N.L., 1993. Effectiveness of *Orius laevigatus* (Hem.: Anthocoridae) for the control of *Frankliniella occidentalis* on cucumber and pepper in the UK. Biocontrol Sci. Technol. 3, 295–307.

Coll, M., 1996. Feeding and ovipositing on plants by an omnivorous insect predator. Oecologia 105, 214–220.

Cordeiro, E.M.G., Corrêa, A.S., Venzon, M., Guedes, R.N.C., 2010. Insecticide survival and behavioral avoidance in the lacewings *Chrysoperla externa* and *Ceraeochrysa cubana*. Chemosphere 81, 1352–1357.

Desneux, N., O'Neil, R.J., 2008. Potential of an alternative prey to disrupt predation of the generalist predator, *Orius insidiosus*, on the pest aphid, *Aphis glycines*, via short-term indirect interactions. B. Entomol. Res. 98, 631–639.

Desneux, N., Pham-Delègue, M.H., Kaiser, L., 2004a. Effects of sublethal and lethal doses of lambda-cyhalothrin on oviposition experience and host searching behaviour of a parasitic wasp *Aphidius ervi*. Pest Manag. Sci. 60, 381–389.

Desneux, N., Rafalimanana, H., Kaiser, L., 2004b. Dose-response relationship in lethal and behavioural effects of different insecticides on the parasitic wasp *Aphidius ervi*. Chemosphere 54, 619–627.

Desneux, N., Wajnberg, E., Fauvergue, X., Privet, S., Kaiser, L., 2004c. Sublethal effects of a neurotoxic insecticide on the oviposition behaviour and the patch-time allocation in two aphid parasitoids, *Diaeretiella rapae* and *Aphidius matricariae*. Entomol. Exp. Appl. 112, 227–235.

Desneux, N., Fauvergue, X., Dechaume-Moncharmont, F.X., Kerhoas, L., Ballanger, Y., Kaiser, L., 2005. *Diaeretiella rapae* limits *Myzus persicae* populations after applications of deltamethrin in oilseed rape. J. Econ. Entomol. 98, 9–17.

Desneux, N., Denoyelle, R., Kaiser, L., 2006a. A multi-step bioassay to assess the effect of the deltamethrin on the parasitic wasp *Aphidius ervi*. Chemosphere 65, 1697–1706.

Desneux, N., O'Neil, R.J., Yoo, H.J.S., 2006b. Suppression of population growth of the soybean aphid, *Aphis glycines* Matsumura, by predators: the identification of a key predator, and the effects of prey dispersion, predator density and temperature. Environ. Entomol. 35, 1342–1349.

Desneux, N., Ramirez-Romero, R., Kaiser, L., 2006c. Multi-step bioassay to predict recolonization potential of emerging parasitoids after a pesticide treatment. Environ. Toxicol. Chem. 25, 2675–2682.

Desneux, N., Decourtye, A., Delpuech, J.M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81–106.

Desneux, N., Wajnberg, E., Wyckhuys, K.A.G., Burgio, G., Arpaia, S., Narvàez-Vasquez, C.A., Gonzàlez-Cabrera, J., Catalàn Ruescas, D., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T., Urbaneja, A., 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. J. Pest. Sci. 83, 197–215.

Desneux, N., Luna, M.G., Guillemaud, T., Urbaneja, A., 2011. The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. J. Pest. Sci. 84, 403–408.

EEC/CEE, 2009. Commission Directive 2009/128/EC. J. Eur. Commun. 309, pp. 71–86.

Evans, S.C., Shaw, E.M., Rypstra, A.L., 2010. Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival. Ecotoxicology 19, 1249–1257.

Fathi, S.A.A., Nouri-Ganbalani, G., 2010. Assessing the potential for biological control of potato field pests in Ardabil, Iran: functional responses of *Orius niger* (Wolf.) and *O. minutus* (L.) (Hemiptera: Anthocoridae). J. Pest. Sci. 83, 47–52.

Flanders, S.E., 1943. The susceptibility of parasitic Hymenoptera to sulfur. J. Econ. Entomol. 36, 469.

Gill, S.S., Cowles, E.A., Pietrantonio, P.V., 1992. The mode of action of *Bacillus thuringiensis* endotoxins. Annu. Rev. Entomol. 37, 615–634.

Giolo, F.P., Medina, P., Grützmacher, A.D., Viñuela, E., 2009. Effects of pesticides commonly used in peach orchards in Brazil on predatory lacewing *Chrysoperla carnea* under laboratory conditions. Biocontrol 54, 625–635.

Gonzalez-Cabrera, J., Molla, O., Monton, H., Urbaneja, A., 2011. Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Biocontrol 56, 71–80.

Gradish, A.E., Scott-Dupree, C.D., Shipp, L., Ron Harris, C., Ferguson, G., 2011. Effect of reduced risk pesticides on greenhouse vegetable arthropod biological control agents. Pest Manag. Sci. 67, 82–86.

He, Y.X., Zhao, J., Zheng, Y., Zhan, Z., Desneux, N., Wu, K.M., in press. Lethal effect of imidacloprid on the coccinellid predator *Serangium japonicum* and sublethal effects on predator voracity and on functional response to the whitefly *Bemisia tabaci*. Ecotoxicology.

Harwood, J.D., Desneux, N., Yoo, H.J.S., Rowley, D.L., Greenstone, M.H., Obrycki, J.J., O'neil, R.J., 2007. Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: a molecular approach. Mol. Ecol. 16, 4390–4400.

Isman, M.B., 2000. Plant essential oils for pest and disease management. Crop. Prot. 19, 603–608.

James, D.G., 2004. Effect of Buprofezin on survival of immature stage of *Harmonia axyridis*, *Stethorus punctum picipes* (Coleoptera: Coccinellidae), *Orius tristicolor* (Hemiptera: Antocoridae), and *Geocoris* spp. (Hemiptera: Geocoridae). J. Econ. Entomol. 97, 900–904.

Lattin, J.D., 1999. Bionomics of the anthocoridae. Annu. Rev. Entomol. 44, 207–231.

Lins, J.C., Bueno, V.H.P., Silva, D.B., van Lenteren, J.C., Calixto, A.M., Livia, A.S., 2011. *Tuta absoluta* egg predation by *Orius insidiosus*. Bull. IOBC/WPRS 68, 55–64.

Lundgren, G.J., Fergen, J.K., 2006. The oviposition behavior of the predator *Orius insidiosus*: acceptability and preference for different plants. Biocontrol 51, 217–227.

Lundgren, G.J., Wyckhuys, K.A.G., Desneux, N., 2009. Population responses by *Orius insidiosus* to vegetational diversity. Biocontrol 54, 135–142.

Mahdian, K., Van Leeuwen, T., Tirry, L., De Clercq, P., 2007. Susceptibility of the predatory stinkbug *Picromerus bidens* to selected insecticides. Biocontrol 52, 765–774.

Ragsdale, D.W., Landis, D.A., Brodeur, J., Heimpel, G.E., Desneux, N., 2011. Ecology and management of the Soybean Aphid in North America. Annu. Rev. Entomol. 56, 375–399.

Rimoldi, F., Schneider, M.I., Ronco, A.E., 2008. Susceptibility of *Chrysoperla externa* eggs (Neuroptera: Chrisopidae) to conventional and biorational insecticides. Environ. Entomol. 37, 1252–1257.

Saber, M., 2011. Acute and population level toxicity of imidacloprid and fenpyroximate on an important egg parasitoid, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). Ecotoxicology 20, 1476–1484.

Schmutterer, H., 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annu. Rev. Entomol. 35, 271–297.

Schuler, M.A., 1996. Plant cytochrome P450 monooxygenases. Crit. Rev. Plant Sci. 15, 235–284.

Sieber, K.P., Rembold, H., 1983. The effects of Azadirachtin on the endocrine control of molting in *Locusta migratoria*. J. Insect Physiol. 29, 523–527.

Stara, J., Ourednickova, J., Kocourek, F., 2011. Laboratory evaluation of the side effects of insecticides on *Aphidius colemani* (Hymenoptera: Aphidiidae), *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), and *Neoseiulus cucumeris* (Acari: Phytoseidae). J. Pest. Sci. 84, 25–31.

Stark, J.D., Banks, J.E., 2003. Population-level effects of pesticides and other toxicants on arthropods. Annu. Rev. Entomol. 48, 505–519.

Stark, J.D., Vargas, R., Banks, J.E., 2007. Incorporating ecologically relevant measures of pesticide effect for estimating the compatibility of pesticides and biocontrol agents. J. Econ. Entomol. 100, 1027–1032.

Sterk, G., Hassan, S.A., Baillod, M., Bakker, F., Bigler, F., Blumel, S., Bogenschutz, H., Boller, E., Bromand, B., Brun, J., Calis, J.N.M., Coremans-Pelseneer, J., Duso, C., Garrido, A., Grove, A., Heimbach, U., Hokkanen, H., Jacas, J., Lewis, G., Moreth, L., Polgar, L., Roversti, L., Samsoe-Petersen, L., Sauphanor, B., Schaub, L., Staubli, A., Tuset, J.J., Vainio, A., Van De Veire, M., Viggiani, G., Vinuela, Vogt H., 1999. Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-working group "pesticides and beneficial organisms". Biocontrol 44, 99–117.

Studebaker, G.E., Kring, T.J., 2003. Effects of insecticides on *Orius insidiosus* (Hemiptera: Anthocoridae), misured by field, greenhouse and Petri dish bioassays. Fla. Entomol. 86, 178–185.

Suma, P., Zappalà, L., Mazzeo, G., Siscaro, G., 2009. Lethal and sub-lethal effects of insecticides on natural enemies of citrus scale pests. Biocontrol 54, 651–661.

Symondson, W.O.C., Sunderland, K.D., Greenstone, M.H., 2002. Can generalist predators be effective biocontrol agents? Annu. Rev. Entomol. 47, 561–594.

Tian, J.C., Liu, Z.C., Chen, M., Chen, Y., Chen, X.X., Peng, Y.F., Hu, C., Ye, G.Y., 2010. Laboratory and field assessments of prey-mediated effects of transgenic Bt rice on *Ummeliata insecticeps* (Araneida: Linyphiidae). Environ. Entomol. 39, 1369–1377.

Tomlin, C.D.S., 2011. The Pesticide Manual: A world compendium, 15th ed. BCPC, Alton, Hampshire, UK.

Umoru, P.A., Powell, W., Clark, S.J., 1996. Effect of pirimicarb on the foraging behaviour of *Diaeretiella rapae* (Hymenoptera: Braconidae) on host-free and infested oilseed rape plants. B. Entomol. Res. 86, 193–201.

Urbaneja, A., Pascual-Ruiz, S., Pina, T., Abad-Moyano, R., Vanaclocha, P., Montòn, H., Dembilio, O., Castañera, P., Jacas, J.A., 2008. Efficacy of five selected acaricides against *Tetranychus urticae* (Acari: Tetranychidae) and their side effects on relevant natural enemies occurring in citrus orchards. Pest Manag. Sci. 64, 834–842.

Van de Veire, M., Klein, M., Tirry, L., 2002a. Residual activity of abamectin and spinosad against the predatory bug *Orius laevigatus*. Phytoparasitica 30, 525–528.

Van de Veire, M., Sterk, G., Van der Staaij, M., Ramakers, P.M.J., Tirry, L., 2002b. Sequential testing scheme for the assessment of side-effects of plant protection products on the predatory bug *Orius laevigatus*. Biocontrol 47, 101–113.

Van Lenteren, J.C., Woets, J., 1988. Biological and integrated pest control in greenhouses. Annu. Rev. Entomol. 33, 239–269.

Weintraub, P.G., Pivonia, S., Steinberg, S., 2011. How many *Orius laevigatus* are needed for effective western flower thrips, *Frankliniella occidentalis*, management in sweet pepper? Crop Prot. 30, 1443–1448.

Williams, T., Valle, J., Vinuela, E., 2003. Is the naturally derived insecticide Spinosad (R) compatible with insect natural enemies? Biocontrol. Sci. Technol. 13, 459–475.

Wilson, C., Tisdell, C., 2001. Why farmers continue to use pesticides despite environmental, health and sustainability costs. Ecol. Econ. 39, 449–462.

Zappalà, L., Siscaro, G., Biondi, A., Mollá, O., González-Cabrera, J., Urbaneja, A., in press. Efficacy of sulphur on *Tuta absoluta* and its side effects on the predator *Nesidiocoris tenuis*. J. Appl. Ent. doi: 10.1111/j.1439-0418.2011.01662.x.

Chapter VII

Sublethal effects of biopesticides can affect strongly the demographic parameters of a beneficial arthropod

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Sublethal effects of biopesticides can strongly affect the demographic parameters of a beneficial wasp

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Abstract

Assessing the compatibility of biocontrol agents with pesticides is a central concern in Integrated Pest Management (IPM) programs. The most common assessments consist of simple comparisons of acute toxicity among pest species and selected biocontrol agents. A more sophisticated approach, developed by the International Organisation of Biological Control (IOBC), is based on a tiered hierarchy made up of threshold values for mortality and sublethal effects. Nevertheless, this method is unable to capture longer term population dynamics, which is often critical to the success of biocontrol and pest suppression. This is increasingly important in the case of new biopesticides that frequently cause no short-term effects inducing multiple long-term physiological and behavioural sublethal effects which may lead to important decrease in population growth. In this study, we assessed the lethal and sublethal effects of six bioinsecticides, widely used in tomato crops to control the invasive pest, *Tuta absoluta* (Lep: Gelechiidae), on one of its parasitoid *Bracon nigricans* (Hym: Braconidae). The percentage of mortality and the sublethal effects on parasitoid reproduction were summarized in a reduction coefficient (E_x) and the pesticides were classified according to the IOBC toxicity categories. The female survival and production of female offspring reductions, jointly with le life table data previously studied for a control population, were used to calculate some population growth indexes as a measure of population recovery after the pesticide exposure. Although the most of the biopesticides had low levels of acute toxicity, a greater level of sublethal effects occurred in the tested parasitoids. Furthermore, very low *B. nigricans* demographical growth indexes resulted for some pesticides that have been classified, accordingly to the IOBC classification, as slightly and moderately harmful. These findings suggest that the susceptibility of *B. nigricans* to pollutants is more complicated than previously thought and that life-history variables must be considered in assessing pesticide risk, as well as when incorporating new pesticides in IPM programmes.

Keywords: IPM, Risk assessment, Life table, Population growth delay, *Bracon nigricans*, *Tuta absoluta*

1. Introduction

Traditionally, the effects of pesticides and other toxicants on organisms have been determined using simplistic measures of effect such as the LD_{50} or LC_{50}, i.e. lethal dose or concentration that kills 50% of a the tested population (Croft, 1990), or just the assessment of the acute toxicity induced by label doses (Desneux et al., 2007; Vanaclocha et al., 2012). Lethal concentration estimates are a straightforward approach that enables quick evaluation and comparison of several toxicants with regard to their effect on individuals of a particular species (Hassan, 1992). It also brings the advantage of being relatively cheap. The underlying assumption is that exposure of a given population to LC_{50} will reduce the number of individuals by half and there is no concern on the outcome of the 50% of individuals that survive exposure (Wennergren and Stark, 2000).

In a second step, the effects of pesticides on beneficial arthropods were examined further by running selectivity tests (pest/beneficial arthropods) to identify products with the lowest non-target activity (Forbes and Calow, 1999; Stark and Banks, 2003; Urbaneja et al., 2008). However, if after exposure, some individuals survive various outcomes may occur. Indeed, the estimation of toxicant effects on populations is complicated by the fact that exposures can result in part of a population dying while surviving individuals may be impaired due to sublethal effects (Stark et al., 2007). Therefore, sublethal effects are defined as effects (either physiological or behavioral) on individuals that survive exposure to a pesticide (the pesticide dose/concentration can be sublethal or lethal) (see Desneux et al., 2007 for a seminal review). A sublethal dose/concentration is defined as inducing no apparent mortality in the experimental population, therefore even lower than LC_{50}. Sublethal effects may impair individuals to such a degree that reproduction is not successful (Biddinger and Hull, 1999; Myers and Hull, 2003; Biondi et al. 2012a, 2012b), lifespan is affected (Butter et al., 2003; Planes et al. 2012), orientation and response to semiochemical is influenced (Salerno et al. 2002; Holscher and Barrett, 2003; Desneux et al. 2004a, 2004b, 2006; Cordeiro et al. 2010) and/or the ability to provide ecological services, such as predation and parasitization is compromised

(Desneux et al. 2007; Cabral et al., 2011; Biondi et al. 2012b, He et al., 2012). Furthermore, some species can withstand high levels of mortality and recover quickly because they have high population growth rates, short generation times, early onset of reproductive activity or a combination of these attributes (Laskowski, 2000; Stark et al., 2004; Castro et al., 2012). By contrast, other species may become extinct after exposure to a toxicant at a concentration that does not kill all individuals because sublethal effects severely impact individuals. Therefore, populations of different species do not react the same to equal levels of stress and thus the LC_{50} cannot be used to compare effects of toxicants on populations over longer time intervals than a few days.

Population effects will largely depend on life history traits life-history strategies and variables, such as lifespan, time to first reproduction, and number of offspring produced over a lifetime (Stark et al., 2004; Schneider et al. 2009; Castro et al., 2012). Demography, or life tables, have been used in a small number of entomological studies to evaluate the total effects (lethal and sublethal) of toxicants on populations (see Stark and Banks, 2003 for a review of this subject). Life table data developed in demographic studies can be used in models to predict (ordinary or partial differential equation models) or project (matrix models) populations (Starks et al., 2004, 2007). Demographic toxicological analysis estimating the total effect of insecticides on populations is increasingly important when choosing new pesticides for Integrated Pest Management (IPM) purposes. Indeed, recently developed insecticides are considered *slower acting* since they have lo acute toxicity levels, while producing a greater degree of sublethal effects than conventional pesticides (Banks et al., 2005). Additionally, when pesticide exposure does not cause sublethal effects or show high acute toxicity, life tables are probably not very beneficial.

In this framework, this study aimed at providing, starting from laboratory a bioassay, a demographic toxicological analysis of some newly developed bioinsecticides that are used in tomato crops. These include agrochemicals that have been increasingly used on tomato (Biondi et al. 2012a) owing to the recent invasion of the Western Palaearctic region by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Desneux et al., 2010, 2011, Tropea Garzia et al., 2012). We chose as non-target organism the generalist ectoparasitoid *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae) since it is a generalist parasitoid model because it is widely distributed in the Palaearctic region, attacking several Lepidoptera species (Yu and Van Actherberg, 2010), and it is one of the first indigenous species that moved to attack the new exotic tomato pest *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Al-Jboory et al., 2012; Urbaneja et al., 2012; Zappalà et al., 2012a). Furthermore, species of the Braconidae family are considered as indicator organisms being selected for the evaluation of chemical product toxicity on non-target arthropods as part of the registration requirement in Europe (EEC/CEE, 1991; Candolfi et al., 1999).

We investigated the following crucial steps in the re-colonization process after treatment: wasp development and emergence from topically treated cocooned pupae, adult survival after the exposure to dried insecticide residues (fresh and 10-day old), and various sublethal effects on the emerged/surviving adults, such as the longevity, the reproductive capacity, the sex-ratio of the progeny and the biocontrol activity. Finally, using the *B. nigricans* life history variables already studied by Biondi et al. (2012c), and in order to predict the dynamics of the treated populations in the long run, various demographical indexes were calculated as an alternative endpoint of the study.

2. Materials and methods

2.1. Insects

The *B. nigricans* colony was obtained during a survey of indigenous natural enemies attacking *T. absoluta* conducted in Southern Italian tomato crops, collecting cultivated infested samples in various sites (Zappalà et al., 2012a). Subsequently, the colony was maintained in the laboratory for several generations using tomato plants infested by *T. absoluta* mature larvae as hosts. The host colony was started from leaves, mainly infested by *T. absoluta* larvae, collected from greenhouse tomato crops; the colony was thereafter maintained on tomato plants (cv. Marmande) in the laboratory. Seedling host plants were grown in small pots (0.3 L), watered and fertilized following the routine practices and pesticide applications were strictly avoided. Tomato plants infested by mature moth larvae (3rd and 4th instars) were obtained by releasing forty *T. absoluta* adults (1:1 sex ratio) on 10 tomato plants inside 50 × 60 × 60 cm cages covered with a fine polyester mesh. After 14 ± 2 d of the moth adult release, when the majority of the larvae had reached the 3th instar, the plants were transferred into the parasitoid rearing cages. These measured 40 ×40 × 55 cm and were built from plastic boxes and the opening covered with a fine mesh net. *T. absoluta*-infested plants and honey droplets were supplied every 3 d into the wasp rearing cages.

Newly emerged 0/48-h old adults and 4/5 d old cocooned pupae were obtained in 150mm Petri dishes ventilated by a 4cm^2 opening covered with a fine mesh net. Two females and four males were released into this arena, containing excised infested tomato leaves (by mature *T.absoluta* larvae) and honey droplets. After 2 d of parasitisation activity, the wasps were removed and the infested and parasitised material was maintained inside a climatic cabinet, then leaves bearing cocooned pupae and newly emerged adults were collected for the bioassay 8 and 10 d later, respectively.

The growing host plants and the two insect rearings were in three climatic chambers (26 ± 1 °C; 60 ± 10% RH; 14:10 L.D.), while the bioassays were conducted in a climatic cabinet in the same environmental conditions as the growing chambers.

2.2. Insecticides and treatments

The pesticides tested were six biopesticides available on the market authorized in several cropping systems (both organic and conventional), for the control of various pests, among them *T. absoluta*.

They were: four insecticides acting when ingested, such as the microbial *Bacillus thuringiensis* Berliner var. Kurstaki (*Bt*), the plant-derived azadirachtin (neem oil extract) and two semi-synthetic microbial-derived products, abamectin and emamectin benzoate; one non-conventional pesticide active when in contact with the pest, a mix of citrus oil and borax salt (hereafter named BCO). Finally, a biopesticides active both when in contact and when ingested, i.e. the bacteria metabolite-derivate spinosad, considering its acute toxicity towards Hymenoptera parasitoids (Biondi et al., 2012c), has been used as treated control for lethal exposure to toxicants to ensure that our exposure bioassay was reliable, i.e. wasps exposed to dried insecticide residues were effectively exposed to toxicants. These plant protection products are currently used in several crops to control various pests and some of them are also authorized for organic farming in several countries worldwide. The highest recommended rates for tomato crops were used for our experiments, detailed information on active ingredients (a.i.) are provided in Table 1.

All the pesticides were stored and applied according to their label guidelines. For a given pesticide, plants and cocooned pupae were sprayed with the formulated product which was diluted with water to obtain 0.5 L of solution and applied at the rate of 1000 L ha^{-1} of solution, i.e. 3 plants per square meter. The treatments were applied using a 2 L power-pack aerosol hand sprayer (Matabi®, Antzuola, Guipuzcoa, Spain) and the nozzle of the sprayer was directed 0.5 m away towards the plants and the cocooned pupae, thus wetting them uniformly all over. An acid fertilizer (Fertacid®, Bio-intrachem Italia) was added to modify the pH to 4.5 in the case of *Bt* and Azadirachtin solutions as recommended by the companies.

2.3. Toxicity trials

2.3.1. Insecticide exposure of adults

The bioassays were performed by exposing *B. nigricans* newly emerged adults to dried pesticide residues on tomato leaves for three days. Plants were used as a substrate for the pesticides to avoid overestimation of toxicity that usually occurs when using pesticide residues on inert material, such as glass (Desneux et al., 2006a; Arnò and Gabarra 2011). The treated plants were 40 cm high, 40-day old tomato plants (cv Marmande), grown from seeds in 2-L pots. Five plants for each trial were sprayed per each tested pesticide. The plants were left to allow pesticides to dry for 1 hour or 10 days (see section *2.3.2. Assessment of pesticides persistence* below) and then the upper plant part (about 17 cm) was cut and placed into a bioassay isolator made up of two superposed plastic glasses, following the experimental design of Zappalà et al. (2012b). The top glass (600 mL, length: 13 cm) had a central hole on its bottom to allow tomato plant stem to reach the water present in a second (bottom) glass (350 mL, length: 11 cm). A fine mesh net was fixed on the upper opening of the largest top glass to allow ventilation. Four females and four males of *B. nigricans* young adults were introduced in the described arena. Untreated droplets of honey were provided, at the beginning of the experiment, as food in the walls of the experimental arena. Five replicates (5 x 10 = 50 wasps) were performed per pesticide and per each residual trial. Insect survival, number of dead parasitoids, was recorded daily during the toxicity trial; parasitoids were considered dead when they remained immobile after being touched with a fine paintbrush. Then the survived adults where collected for the sublethal effects assessment.

2.3.2. Assessment of pesticides persistence

Persistence of pesticides toxicity was studied by exposing *B. nigricans* adults to pesticides applied at two different times prior to the assay: 1 hour and 10 days (see section 2.3.1. for description of the bioassay). To allow aging of pesticides in ordinary protected cropping conditions, all the plants (for the two different delays after application of a given pesticide) were sprayed at the same time and they were maintained in insect proof cages in greenhouse conditions (min < *mean temperature* < max: 15.6°C < *25.07°C* < 40°C; min < *mean RH* < max, 21% < *62.5%* < 89%; natural ambient light: June-July 2011). Plants sprayed with tap water were used as control plants for each of the treatments.

2.3.3. Cocooned pupae treatments

When the impact of the various tested pesticides on cocooned pupae was studied, a realistic spray exposure method was used. Tomato leaves bearing exteriorly 4/5-d old *B. nigricans* cocooned pupae (see section 2.1. Insects) were carefully attached, in group of three specimens, on rectangular glass plates with double-sided sticky tape accordingly to Desneux et al., 2006. The glass plates with leaves were thus treated with the pesticides solution with the same modalities described for the adult insecticide exposure and tap water has been used to spray the untreated control. For each tested pesticide, treated and untreated control fifteen to twenty-two groups of cocooned pupae (i.e. 45 to 66 cocooned pupae) were exposed resulting in a total of 423 were used in the experiment. Two hours after the insecticide application, treated cocooned pupae were removed from the leaves and placed individually in glass tubes kept in a climatic chambers (26 ± 1 °C; 60 ± 10% RH; 14:10 L.D.). To assess the emergence rates of each treated group of cocooned pupae, the tubes were observed twice per day for the following four days after the treatment, and then the rate of emergence was calculated for each group. The emerging adults were used for the sublethal effects assessment the day after their emergence.

2.3.4. Sublethal effects assessment

In order to assess the potential sublethal effects of the tested biopesticides (except spinosad that caused very high levels of mortality, see the *3.1.* section) on both the adults survived after the three-day exposure on leaves and the adults emerged after the topical treatment of cocooned pupae, five *T. absoluta* mature larvae and honey droplets were offered daily per each tested couple (both survived to the residual pesticide contact and emerging form treated pupae), in aerated plastic boxes (130 mm diameter), for three days (i.e. a total of fifty-teen hosts and three boxes were used per each tested female). Daily checks were performed in order to assess: (i) the number of killed larvae, (ii) the number of parasitized larvae, (iii) the number of laid eggs and (iv) the parasitoid survival. Survived specimens were then transferred in a new box with new hosts and after the last check (third day) they were placed individually in glass tubes (length: 18 cm; diameter: 1.2 cm), with access to food (honey, renewed weekly), under controlled environmental conditions (26 ± 1 °C; 60 ± 10% RH; 14:10 L.D.). The parasitoid mortality in the tubes was observed daily and the longevity was recorded. The boxes bearing the larvae, both alive and parasitized, were reared per fifteen days in the previous described climatic chamber; then, in order to assess the fitness and the progeny sex-ratio of the tested females after being exposed to the pesticide, the number and the sex of the emerged progeny were recorded.

2.4. Data analyses

All the datasets were first tested for normality and homogeneity of variance using the Kolmogorov-Smirnov D test and the Cochran test respectively and were transformed if necessary. One-way ANOVA followed by LSD post hoc was carried out to analyze the % of the survival of males and females exposed to the pesticides residues, the percentages of emerged adults from treated cocooned pupae, the number of killed larvae (paralyzed and/or parasitized hosts) and the progeny produced by the survived/emerged females during the three days of sublethal effects evaluation, as well as the progeny sex-ratio. The latter was expressed as the ratio between the females produced ant total progeny produced per each tested female during the three days of fertility evaluation.

In addition, to provide a single value summarizing the deleterious effects of the tested pesticide, the toxic effects (both lethal and sublethal effects on the fertility) of each pesticide (1-h old, 10-d old and when applied towards the cocooned pupae) were also expressed as the *Reduction coefficient* E_x for pesticide *x* (Biondi et al., 2012a) using the formula:

$$E_x = 100 \left\{ 1 - \left[\left(1 - \frac{E_{mx}}{100} \right) \left(1 - \frac{E_{fx}}{100} \right) \right] \right\}$$

where E_{mx} is the corrected mortality (Abbott 1925) and E_{fx} is the corrected fertility based on the following formula:

$$E_{fx} = 100 - \frac{F_x\,100}{F_c}$$

where F_x is the mean fertility for pesticide *x* and F_c is the fertility recorded in the control group (untreated group). These values (E_x) were then classified and interpreted accordingly to the standards of the International Organization for Biological Control (IOBC) for laboratory pesticide risk assessments which include four categories: (1) harmless, (2) slightly harmful, (3) moderately harmful and (4) harmful; corresponding to reductions below 30%, between 31 and 79%, between 80 and 99% and higher than 99%, respectively (Sterk et al. 1999).

Finally, some demographical growth indexes were calculated for the female wasps exposed to 1-h old and 10-d old pesticide residues and for those emerged from topically treated cocooned pupae. In particular, per each tested pesticide and trial performed, three parameters were calculated: the Net reproductive rate (R_o) that corresponds to the number of times a population will multiply per generation; the Doubling time (*DT*) which is the time required for a given population to grow exponentially without limit to double in size and the Intrinsic rate of increase (r_m) representing the number of times the population will multiply itself per unit of time, measured as female progeny/female/unit of time, one day in this case (Stark and Banks, 2003). To obtain these values we used the life table data (age-specific survival and fertility values), previously developed by Biondi et al. (2012c), in an *equation model*, accordingly to Stark et al. (2004). The female survival (% of mortality after the exposure to the pesticides residues and % emergences from treated cocooned pupae) and the percentages of reduction of offspring female (calculated on the number of females produced per female during the three days of sublethal effects evaluation) were included in the model.

3. Results

3.1. Lethal effect

The mortality of the adults during the 3 d-exposure period varied significantly among pesticides tested in all the three trials. In particular, after exposing the wasps to 1-h old spinosad residue no specimens, of both sexes, survived. Whereas, in the untreated control all the tested females and the 95 ± 5 % of the males survived. Of all the other pesticides, tested as 1-h old residue, only BCO and emamectin, although of only the 15 ± 5 %, caused significant female mortality levels ($F_{6,34}$=56.513, P<0.0001); this last pesticide together with abamectin significantly reduced the male survival ($F_{6,34}$=20.542, P<0.0001) (Fig. 1a). While, when exposing the adults to 10-d old residues, only spinosad caused significant mortality rates killing all the tested females ($F_{6,34}$=194.117, P<0.0001) and males ($F_{6,34}$=34.000, P<0.0001) (Fig. 1b). Finally, the same trend was proved in the rate of emerged of treated cocooned pupae, indeed only the 17.77 ± 7.18 % of adults emerged from pupae treated with spinosad and BCO and abamectin, with 65 ± 7.44 % and 71.17 ± 6.26 % of the adults emerged respectively, caused also a significant reduction on the rate of emergences ($F_{6,122}$=14.877, P<0.0001) (Fig. 1c).

3.2. Sublethal effects

The tested biopesticides caused a wide variety of sublethal effects in all the performed bioassays. Significant lower longevity levels were found on the females ($F_{5,44}$= 34.523, P< 0.001) and males ($F_{5,69}$=15.241, P< 0.001) that were exposed to 1-h old residues of azadiractin, emamectin and abamectin (Fig. 2a). However, these effects were not consistent when the pesticide residues aged for 10 d, nor for the females ($F_{5,44}$= 18.012, P =0.081) neither for the exposed males ($F_{5,69}$=8.325, P= 0.152) (Fig. 2b). While, of the treated cocooned pupae only the females ($F_{5,47}$=39.067, P< 0.001) and males ($F_{5,84}$=17.201, P< 0.001) developed from the pupae treated with emamectin lived significantly shorter (Fig. 2c). Also the reproduction was significantly affected by emamectin in all the three trials, i.e. 1-h ($F_{5,90}$= 6.607, P< 0.0001) and 10-d ($F_{5,90}$= 3.845, P= 0.003) old residues bioassays and in topical treatment of the cocooned pupae ($\mathrm{F}_{5,90}$=2.815, P= 0.001) (Fig. 3c). The sublethal toxicity of azadirachtin on this trait was found when treating the pupae and when exposing the adults to 1-h old residues, while this effect was not persistent after 10 d (Fig. 3b). Abamectin caused the lowest progeny production (1.06 ± 0.45 offspring per female in three days) only in the 1-h old residues trial (Fig. 3a).

The progeny sex-ratio resulted significantly affected only, 0.92 ± 0.02 males/total progeny, when the wasps were exposed to 1-h old residues of emamectin ($F_{5,69}$=0.632, P= 0.012) (Fig. 4a). While no significant differences among the tested pesticides were found in the 10-d trial ($F_{5,73}$=0.632, P= 0.087) (Fig. 4b) and when treating the cocooned pupae ($F_{5,58}$=0.735, P= 0.142) (Fig. 4c). The females that were exposed to 1-h old residues of BCO, abamectin and emamectin killed significantly less host larvae during the three days of sublethal effects assessment ($F_{5,90}$=12.180, P< 0.001) (Fig. 5a). While, in the 10-d old residue ($F_{5,90}$=5.264, P< 0.001) (Fig. 5b) and in the pupae ($F_{5,90}$=7.895, P< 0.001) (Fig. 5c) trials only emamectin significantly reduced the biocontrol activity of the females.

3.3. Reduction coefficient (E_x*) and IOBC categories*

When combining the lethal effect and the effects on the reproduction, spinosad was the only biopesticide, among the tested products, causing a reduction coefficient E_x higher than 99% and it was classified as harmful (class 4) when using the IOBC toxicity categories. Furthermore, its harmfulness remains high also after the pesticides aged for 10 d (Table 2). By contrast, abamectin decreased its toxicity from moderately harmful (class 3) to slightly harmful (class 2) when pesticide residues were 10 d older. While, it resulted harmless (E_x = 21.8 %) when applied to the cocooned pupae. The class 2 was also attributed to Azadirachtin and BCO in all the three trials. By contrast, emamectin, although resulted slightly harmful (class 2) to the adults, both exposed to 1-h and 10-d old residues, was moderately harmful (E_x = 87.5 %) towards the parasitoid pupa stage. The only tested biopesticide ranked as harmless (E_x < 30%) in all the bioassays was *Bt* (Table 2).

3.4. Demographical growth parameters

All the tested biopesticides affected, namely decreased, all the estimated demographical indexes (Tab. 2). In particular, 1-h old residues of abamectin and emamectin, and when emamectin is applied towards cocooned pupae, a very high decrease in the population growth is highlighted, at the point that the population may lose its capacity to growth (i.e. R_o < 1) conducting the parasitoid population to the extinction. Azadiractin and BCO, as 1-h residues, caused 25.4 and 8.4 d, respectively, in the time needed to the population for doubling in their size (*DT*). When the cocooned pupae were treated with azadiractin, BCO and abamectin the delay in population growth were 22.84 d, 13.17 d and 2.59 d, respectively. Whereas, the delay in the *DT* resulted lower, when comparing the results obtained exposing the adults to 10-d old pesticide residues with those of the control population, of 9.24 d, 7.94 d, 5.33 d, and 4.3 d for abamectin, emamectin, BCO and azadiractin, respectively. For spinosad was not possible to generate any demographical index since the total mortality of the tested specimens during the residual contact bioassay and the very high one when treating the pupae (Fig. 1). Therefore, in this case, the extinction may occur soon after the treatment. While, the effects of *Bt* on the population growth were negligible, being the estimated values very close to those of the control population with delay in *DT* never > 1.3 d (Tab. 2).

4. Discussion

The present study demonstrated that the tested biopesticides, although having low acute toxicity on the generalist parasitoid *B. nigricans*, caused a high degree of sublethal effects, such as reduction of biocontrol activity (permanently paralyzed and parasitized hosts), fertility, longevity and increase of the male portion in the progeny sex-ratio. The demographical approach highlighted the essentiality of such effects in strongly and negatively affecting the parasitoid population levels after the treatment. *Bracon nigricans* was very susceptible to spinosad ($E_x > 99\%$, IOBC class 4) even after 10 d after pesticide application, namely because of high mortality induced in adults. Therefore, spinosad proved to be not compatible with this parasitoid for IPM and organic farming programs. Emamectin and abamectin were less toxic than spinosad, but induced high levels of sublethal effects (mostly the reduction in the production of female progeny), that interpreted with demographical growth point of view, may bring the parasitoid population to the extinction. The impact of azadiracthin and BCO 1-h residues and when these are applied to the parasitoid pupa stage are also noteworthy, causing considerable delay in the population growth. Finally, *Bt* proved to be harmless ($E_x < 30\%$, IOBC class 1) with survival, sublethal traits and estimated demographical indexes very similar to those were recorded in untreated control group. Taken as a whole, the results show that side effects of pesticides can vary largely depending upon various factors considered, like endpoint considered (lethal vs. sublethal), pesticide persistence and developmental strategies of the non-target species considered. Consequently, comprehensive and specific risk assessment should be undergone before incorporating new pesticides in IPM programmes, such as in this studied model, i.e. tomato/*T. absoluta*/biopesticides.

4.1. Importance of results for IPM and organic farming

Our study provides information that could be useful for IPM programs in identifying (and avoiding) products that may prevent parasitoid to build up because of side effects on important traits and reduced survival. These products may, in addition to compromising the efficacy of IPM programs, prevent efficient colonization or recolonization of treated crops, particularly when pesticides are highly persistent. Interestingly, Avermectin-based biopesticides (i.e. abamectin and emamectin) and the biopesticide based on spinosad fall in this last category. These results hint that pesticidal products that are classified as biopesticides can easily be of major concerns when they are supposed to be used in combination with natural enemies. Given the results obtained, the use in IPM programmes of these biopesticides together with natural enemies like *B. nigricans* should not be considered for effective and sustainable pest management programs. Unfortunately, a similar situation applies to pesticides that are recommended for organic cropping systems and therefore that are usually though to be harmless for non-target arthropods. Spinosad induced total mortality of the tested specimens and its effect was persistent for at least 10 d after initial application; furthermore, very high levels (< 80 %) of cocooned pupae mortality were registered. This biopesticide has been classified as an environmentally-safe product and has been embraced by IPM practitioners as a biorational pesticide (Duso et al., 2008; Biondi et al., 2012b). However, present results suggest that other pesticides with higher selectivity should be preferred. Overall, the safest insecticides for

B. nigricans appears to be *Bt* and for example, when dealing with Lepidopteran pests, *Bt* should be prioritized because (i) it proved to be safe for predator and parasitoid (despite testing it at one of highest recommended rates concentration I.U.), (ii) it induces no lethal and sublethal effects on predators that fed on prey which fed *Bt*-treated or *Bt*-genetically engineered plants (Angeli et al., 2005; Biondi et al., 2012a; Lu et al., 2012), and (iii) it is highly efficient even against leaf miners (Gonzalez-Cabrera et al., 2011). Finally, in case of azadirachtin and BCO, since their effects showed to be not persistent in tomato plants and that they affect the cocooned pupae when in direct contact, the negative effects on the parasitoids reproduction could be avoided by avoiding to apply this pesticide during the period of high presence of parasitoid pupae and adults on the crop.

4.2. Implications of the approach for risk assessment

Our work clearly showed how the tested pesticides induce more subtle effects rather than acute ones. Indeed, all the investigated physiological and/or behavioural traits are useful to better understand the actual impact of the tested biopesticides on the parasitoids population dynamics, as well as on the biocontrol services they can provide after the pesticide application. This is particularly true in the case of the tested Avermectins, for which the survival rate of the parasitoids exposed to their 1-h old residues was reduced of maximum the 15%, while longevity, fertility and biocontrol activity were reduced from the 25% to the 80%. Furthermore, the study of the progeny sex-ratio allowed to remark that the progeny of the female that survived to the emamectin exposure was strongly male biased (>0.9 mm/tot). This, jointly to the low fertility levels and the significant reduction in the survival of the tested females strongly affected the population growth estimation, suggesting the extinction of the treated population in the field soon after the treatment. By contrast, this important result was not identified by the synthetic IOBC toxicity classification, which does not take into account the *B. nigricans* specific life history variables, classifying this pesticide as moderately harmful.

Although the demographic approach has not been widely adopted to date, this method obviously provides more information about pesticide effects on populations than traditional risk assessment based on lethal and/or sublethal effects. In addition, matrix models are particularly valuable for comparisons of populations exposed to various concentrations of pesticides or different pesticides (Stark et al., 2004). Clearly, there are great advantages to using demography for estimating the impacts of pesticides and other toxicants on biological control agents and other non-target organisms. Because r_m incorporates both lethal and sublethal effects into a single dimensionless number easy to understand and to compare. On the other hand, there are also several disadvantages associated with the development of life tables for estimation of the effects of pesticides on populations. The primary one is that development of life tables is time consuming and expensive. Furthermore, it might be argued that this approach is not realistic and that results of matrix model simulations cannot be used to predict what is going to happen under natural conditions with a high degree of confidence, because life tables are developed in the laboratory and measurements are taken on individuals, rather than populations, a realistic measure of population growth rate is not obtained. However, the obtained endpoints, such as the intrinsic rate of increase, have been shown to be a more accurate measure of toxic effect than lethal concentration estimates (Laskowski, 2000; Stark and Banks, 2003; Stark et al., 2004; Schneider et al., 2009) or, as proved in this study, those advocated by the IOBC.

5. Conclusion

One of the goals of IPM is to preserve and/or increase the natural mortality factors of the pests by combining various compatible control measures. Better knowledge on risks associated with specific pesticides toward natural enemies is of primary importance when incorporating them in IPM programs. The results obtained in the present study could improve IPM programs in which

B. nigricans is involved and more broadly may be useful for implementing IPM programs involving the use of braconid wasps as natural enemies. These results were obtained in laboratory conditions, which represent a high exposure scenario (the worst being an exposure to pesticides on inert material like glass) and it would be ideally completed with semi-field and field experiments. Nevertheless, risk prediction models from laboratory data are a necessity in the rational selection of the insecticides to be used against the new pest, *T. absoluta*, for successful IPM programmes. On the other hand, our results indicate that more ecologically relevant measures of effect such, as delays in population growth that are based on demography, may advance our knowledge of pesticide impacts on populations of natural enemy species.

References

Abbott, W.S., 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265-267.

Al-Jboory, I.J., Katbeh-Bader, A., Al-Zaidi, S., 2012. First observation and identification of some natural enemies collected from heavily infested tomato by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Jordan. Middle East J. Sci. Res. 11, 435-438.

Angeli, G., Baldessari, M., Maines, R., Duso, C., 2005. Side-effects of pesticides on the predatory bug *Orius laevigatus* (Heteroptera: Anthocoridae) in the laboratory. Biocontrol Sci. Technol. 15, 745-754.

Arnó, J., Gabarra, R., 2011. Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). J. Pest Sci. 84, 513-520.

Banks, H.T., Banks, J.E., Dick, L.K., Stark, J.D., 2005.Estimation of dynamic rate parameters in insect populations undergoing sublethal exposure to pesticides. Bull. Math. Biol. 69, 2139-2180.

Biddinger, D.J., Hull, L.A., 1999. Sublethal effects of selected insecticides on growth and reproduction of a laboratory susceptible strain of tufted apple bud moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 92, 314-324.

Biondi, A., Desneux, N., Siscaro, G., Zappalà L., 2012a. Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: Selectivity and side effects of 14 pesticides on the predator *Orius laevigatus.* Chemosphere 87, 803-812.

Biondi, A., Mommaerts, V., Smagghe, G., Vinuela, E., Zappalà, L., Desneux, N., 2012b. The non-target impact of spinosyns on beneficial arthropods. Pest Manag. Sci. 68, 1523-1536.

Biondi A., Desneux, N., Amiens-Desneux, E., Siscaro, G., Zappalà, L., 2012c. The biology of the Western Palaearctic parasitoid *Bracon nigricans* on the Neotropical moth *Tuta absoluta*. J. Econ. Entomol. Under revision

Butter, N.S., Singh, G., Dhawan, A.K., 2003. Laboratory evaluation of the insect growth regulator lufenuron against Helicoverpa armigera in cotton. Phytoparasitica 31, 200-203.

Cabral, S., Soares, A.O, Garcia, P., 2011. Voracity of *Coccinella undecimpunctata*: effects of insecticides when foraging in a prey/plant system. J. Pest Sci. 84, 373-379.

Candolfi, M.P., Bakker, F., Canez, V., Miles, M., Neumann, C., Pilling, E., Primiani, M., Romijn, K., Schmuck, R., Storck-Weyhermuller, S., Ufer, A., Waltersdorfer, A., 1999, Sensitivity of non-target arthropods to plant protection products: Could *Typhlodromus pyri* and *Aphidius* spp. be used as indicator species. Chemosphere 39, 1357-1370.

Castro, A.A., Lacerda, M.C., Zanuncio, T.V., Ramalho, F.D., Polanczyk, R.A., Serrao, J.E., Zanuncio, J.C., 2012. Effect of the insect growth regulator diflubenzuron on the predator *Podisus nigrispinus* (Heteroptera: Pentatomidae). Ecotoxicol. 21, 96-103.

Cordeiro, E.M.G., Corrêa, A.S., Venzon, M., Guedes, R.N.C., 2010. Insecticide survival and behavioral avoidance in the lacewings *Chrysoperla externa* and *Ceraeochrysa cubana*. Chemosphere 81, 1352-1357.

Croft, B.A., 1990. Arthropod Biological Control Agents and Pesticides. New York: Wiley. 723 pp.

Desneux, N., Pham-Delègue, M.H., Kaiser, L., 2004a. Effects of sublethal and lethal doses of lambda-cyhalothrin on oviposition experience and host searching behaviour of a parasitic wasp *Aphidius ervi*. Pest Manag. Sci. 60, 381-389.

Desneux, N., Rafalimanana, H., Kaiser, L., 2004b. Dose-response relationship in lethal and behavioural effects of different insecticides on the parasitic wasp *Aphidius ervi*. Chemosphere 54, 619-627.

Desneux, N., Denoyelle, R., Kaiser, L., 2006a. A multi-step bioassay to assess the effect of the deltamethrin on the parasitic wasp *Aphidius ervi*. Chemosphere 65, 1697-1706.

Desneux, N., Decourtye, A., Delpuech, J.M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81-106.

Desneux, N., Wajnberg, E., Wyckhuys, K.A.G., Burgio, G., Arpaia, S., Narvàez-Vasquez, C.A., Gonzàlez-Cabrera, J., Catalàn Ruescas, D., Tabone, E., Frandon, J., Pizzol, J., Poncet, C., Cabello, T., Urbaneja, A., 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. J. Pest. Sci. 83, 197-215.

Desneux, N., Luna, M.G., Guillemaud, T., Urbaneja, A., 2011. The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. J. Pest. Sci. 84, 403-408.

Duso, C., Malagninia, V., Pozzebon, A., Buzzettia, F.M., Tirello, P, 2008. A method to assess the effects of pesticides on the predatory mite *Phytoseiulus persimilis* (Acari Phytoseiidae) in the laboratory. Bioc Sci Techn 18, 1027-1040.

Gonzalez-Cabrera, J., Molla, O., Monton, H., Urbaneja, A., 2011. Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Biocontrol 56, 71-80.

Holscher, J.A., Barrett B.A., 2003. Effects of methofenozide-treated surfaces on the attractiveness and responsiveness of adult codling moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 96, 623-628.

He, Y.X., Zhao, J., Zheng, Y., Zhan, Z., Desneux, N., Wu K.M., 2012. Lethal effect of imidacloprid on the coccinellid predator *Serangium japonicum* and sublethal effects on predator voracity and on functional response to the whitefly *Bemisia tabaci*. Ecotoxicol. 21, 1291-1300.

EEC/CEE, 1991, Directive 91/414/EEC of the European Parliament and of the Council of 15 July 1991, concerning the placing of plant protection products on the market. Off. J. Eur. Commun. L 230, 1-32.

Forbes, V.E., Calow, P., 1999. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology? Environ. Toxicol. Chem. 18, 1544-1556.

Laskowski, R., 2000, Why short-term bioassays are not meaningful - Effects of a pesticide imidacloprid) and a metal (cadmium) on pea aphids (*Acyrthosiphon pisum* Harris). Ecotoxicol. 10, 177-183.

Lu, Y.H., Wu, K.M., Jiang, Y.Y., Guo, Y.Y., Desneux, N., 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nature 487, 362-365.

Myers, G.A., Hull L.A., 2003. Insect growth regulator impact on fecundity and fertility of adult tufted apple bud moth, Platvnota idaeusalis Walker. J. Entomol. Sci. 38, 420-430.

Planes, L., Catalan, J., Tena, A., Porcuna, J.L., Jacas, J.A., Izquierdo J., Urbaneja, A., 2012, Lethal and sublethal effects of spirotetramat on the mealybug destroyer, *Cryptolaemus montrouzieri*. J. Pest Sci. in press DOI:10.1007/s10340-012-0440-3.

Salerno, G., Colazza, S., Conti, E., 2002. Sub-lethal effects of deltamethrin on walking behaviour and response to host kairomone of the egg parasitoid *Trissolcus basalis*. Pest Manag. Sci. 58, 663-668.

Schneider, M.I., Sanchez, N., Pineda, S., Chi, H., Ronco, A., 2009, Impact of glyphosate on the development, fertility and demography of *Chrysoperla externa* (Neuroptera: Chrysopidae): Ecological approach. Chemosphere 76, 1451-1455.

Stark, J.D., Banks, J.E., 2003. Population-level effects of pesticides and other toxicants on arthropods. Annu. Rev. Entomol. 48, 505-519.

Stark, J.D., Banks, J.E., Vargas, R., 2004. How risky is risk assessment: The role that life history strategies play in susceptibility of species to stress. Proc. Nati. Acad. Sci. USA 101, 732-736.

Stark, J.D., Vargas, R., Banks, J.E., 2007. Incorporating ecologically relevant measures of pesticide effect for estimating the compatibility of pesticides and biocontrol agents. J. Econ. Entomol. 100, 1027-1032.

Sterk, G., Hassan, S.A., Baillod, M., Bakker, F., Bigler, F., Blumel, S., Bogenschutz, H., Boller, E., Bromand, B., Brun, J., Calis, J.N.M., Coremans-Pelseneer, J., Duso, C., Garrido, A., Grove, A., Heimbach, U., Hokkanen, H., Jacas, J., Lewis, G., Moreth, L., Polgar, L., Roversti, L., Samsoe-

Petersen, L., Sauphanor, B., Schaub, L., Staubli, A., Tuset, J.J., Vainio, A., Van De Veire, M., Viggiani, G., Vinuela, Vogt H., 1999. Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-working group ‘‘pesticides and beneficial organisms’’. Biocontrol 44, 99-117.

Tropea Garzia, G., Siscaro, G., Biondi, A., Zappalà, L., 2012. *Tuta absoluta*, an exotic invasive pest from South America now in the EPPO region; biology, distribution and damage. EPPO Bull. 42, 205-210.

Urbaneja, A., Pascual-Ruiz, S., Pina, T., Abad-Moyano, R., Vanaclocha, P., Montòn, H., Dembilio, O., Castañera, P., Jacas, J.A., 2008. Efficacy of five selected acaricides against *Tetranychus urticae* (Acari: Tetranychidae) and their side effects on relevant natural enemies occurring in citrus orchards. Pest Manag. Sci. 64, 834-842.

Urbaneja, A., Gonzalez-Cabrera, J., Arno, J., Gabarra, R., 2012. Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. Pest Manag. Sci. 68, 1215-1222.

Vanaclocha, P., Vidal-Quist, C., Oheix, S., Montón, H., Planes, L., Catalán, J., Tena, A., Verdú, M.J., Urbaneja, A., 2012. Acute toxicity in laboratory tests of fresh and aged residues of pesticides used in citrus on the parasitoid *Aphytis melinus*. J. Pest Science In press DOI: 10.1007/s10340-012-0448-8

Wennergren, U., Stark, J.D., 2000. Modeling long-term effects of pesticides on populations: beyond just counting dead animals. Ecol. Appl. 10, 295-302.

Yu, D.S.K, Van, Actherberg C., 2010. Taxapad Ichneumonoidea (May 2009 version). In: *Species 2000 & IT IS catalogue of life: 2010 annual checklist* (Bisby F. A., Roskov Y. R., Orrell T. M., Nicolson D., Paglinawan L. E., Bailly N., Kirk P. M., Bourgoin T., Baillargeon G., Eds).-DVD, Species 2000, Reading, UK.

Zappalà, L., Bernardo, U., Biondi, A., Cocco, A., Deliperi, S., Delrio, G., Giorgini, M., Pedata, P., Rapisarda, C., Tropea Garzia, G., Siscaro, G., 2012a. Recruitment of native parasitoids by the exotic pest *Tuta absoluta* in Southern Italy. Bull. Insectol. 65, 51-61.

Zappalà, L., Siscaro, G., Biondi, A., Mollá, O., González Cabrera, J., Urbaneja, A., 2012b. Efficacy of sulphur on *Tuta absoluta* and its side effects on the predator *Nesidiocoris tenuis*. J. Appl. Entomol. 136:401-409.

Fig 1. Lethal effect. Means (± SEM) of mortality percentages of *Bracon nigricans* adults (males and females) exposed to 1-h (a) and to 10-d old pesticide residues (b). Means (± SEM) of emergence percentages of *B. nigricans* adults from treated cocooned pupae (c). Means for treatment, within the same sub-figure, subtended by the same letter do not differ at $P < 0.05$ (one-way ANOVA followed by LSD post hoc test). Capital letters are for females and small letter for males.

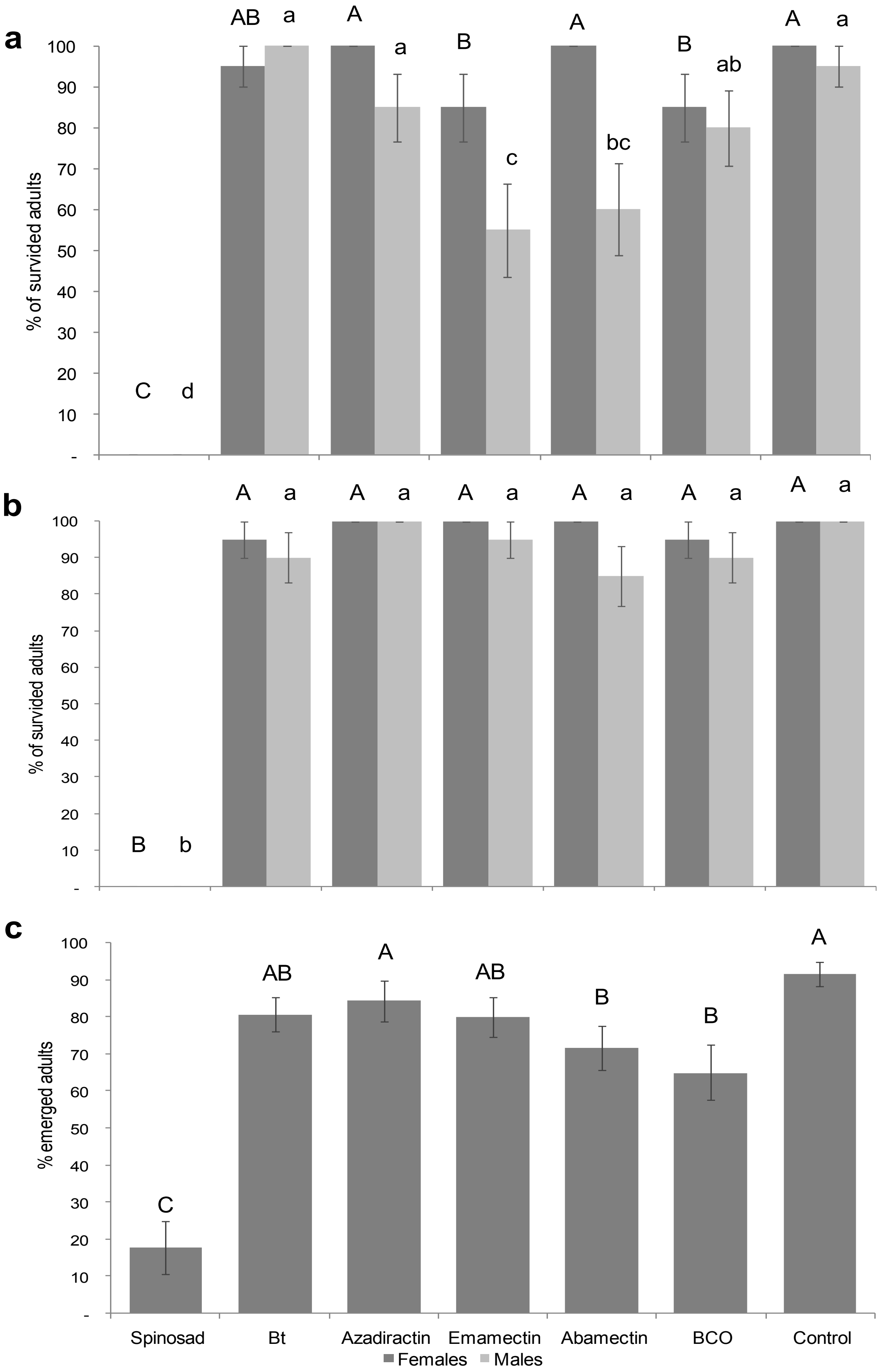
Fig 2. Sublethal effects. Longevity. Means (± SEM) of longevity (days) of *Bracon nigricans* adults (males and females) exposed to 1-h (a) and to 10-d pesticide residues (b). Means (± SEM) of emergence percentages of *B. nigricans* adults from treated cocooned pupae (c). Means for treatment, within the same sub-figure, subtended by the asterisk differ at $P < 0.05$ (one-way ANOVA followed by LSD post hoc test) to the untreated control.

Fig 3. Sublethal effects. Progeny production. Means (± SEM) of number of progeny produced in three days by each tested *Bracon nigricans* females exposed to 1-h (a) and to 10-d old pesticide residues (b) and by females emerged by the treated pupae (c). Means for treatment, within the same sub-figure, subtended by the asterisk differ at $P < 0.05$ (one-way ANOVA followed by LSD post hoc test) to the untreated control.

Fig 4. Sublethal effects. Progeny sex-ratio. Means (± SEM) of sex-ratio of the progeny produced in three days by each tested *Bracon nigricans* females exposed to 1-h (a) and to 10-d old pesticide residues (b) and by females emerged by the treated pupae (c). Means for treatment, within the same sub-figure, subtended by the asterisk differ at $P < 0.05$ (one-way ANOVA followed by LSD post hoc test) to the untreated control.

Fig 5. Sublethal effects. Killed hosts. Means (± SEM) of number killed larvae in three days by each tested *Bracon nigricans* females exposed to 1-h (a) and to 10-d old pesticide residues (b) and by females emerged by the treated pupae (c). Means for treatment, within the same sub-figure, subtended by the asterisk differ at $P < 0.05$ (one-way ANOVA followed by LSD post hoc test) to the untreated control.

Fig. 1


a
% of survided adults
100
90
80
70
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50
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% of survided adults
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a
A
a
A
a
c
% emerged adults
C
AB
A
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B
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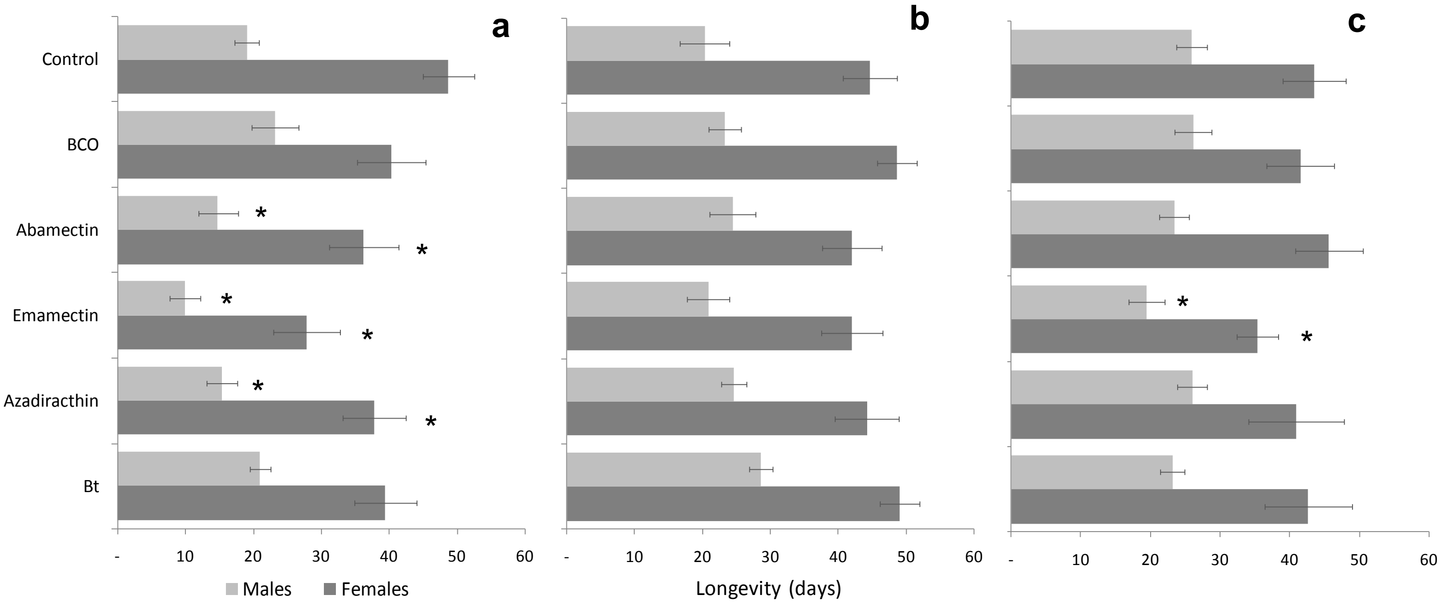
A
Spinosad
Bt
Azadiractin
Emamectin
Abamectin
BCO
Control
Females
Males

Fig. 2


a
b
c
Control
BCO
Abamectin
Emamectin
Azadiracthin
Bt
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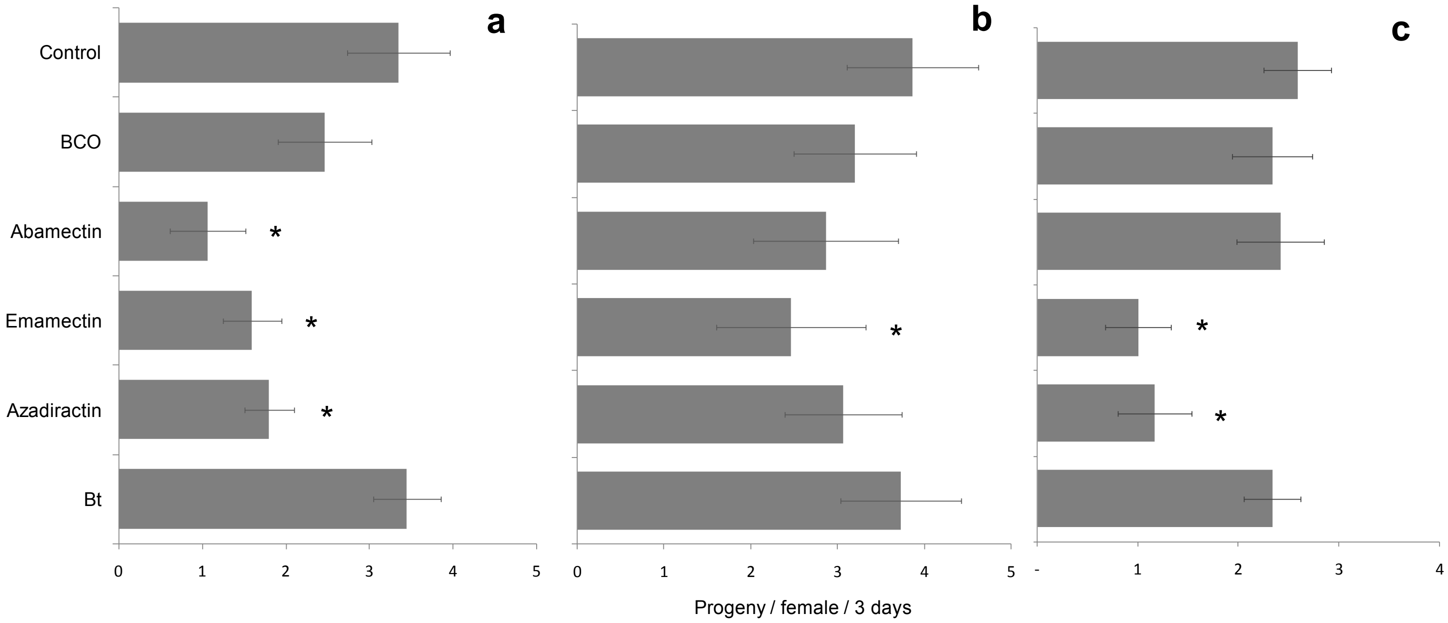
-
10
20
30
40
50
60
Males
Females
Longevity (days)

Fig. 3


a
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BCO
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Emamectin
Azadiractin
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-
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2
3
4
Progeny / female / 3 days

Fig. 4

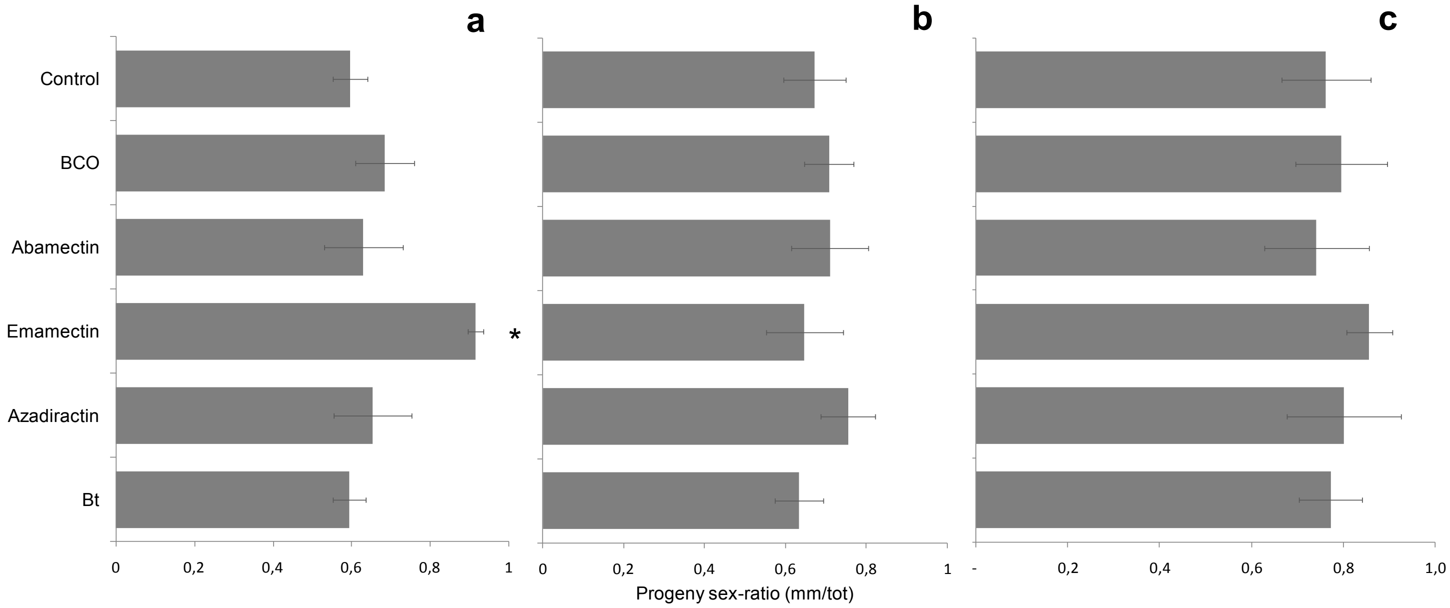
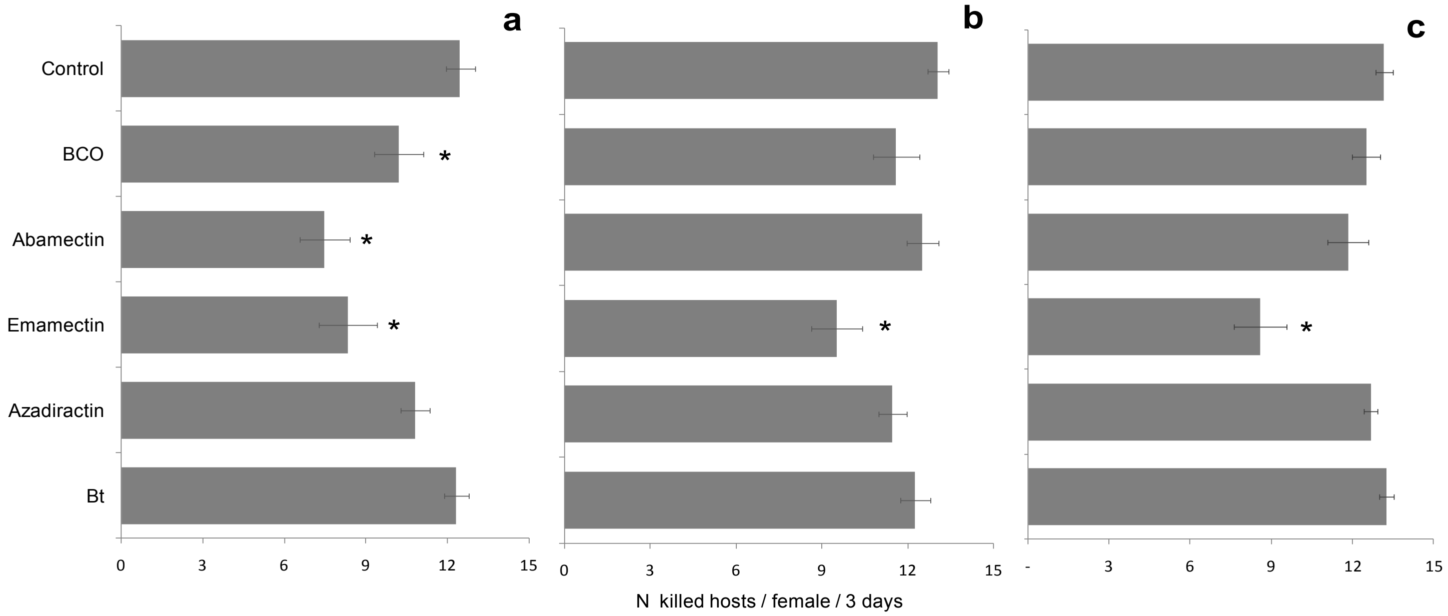

a
b
c
Control
BCO
Abamectin
Emamectin
Azadiractin
Bt
*
0
0,2
0,4
0,6
0,8
1
0
0,2
0,4
0,6
0,8
1
-
0,2
0,4
0,6
0,8
1,0
Progeny sex-ratio (mm/tot)

Fig. 5


a
b
c
Control
BCO
Abamectin
Emamectin
Azadiractin
Bt
*
*
*
*
*
0
3
6
9
12
15
0
3
6
9
12
15
-
3
6
9
12
15
N killed hosts / female / 3 days

Tab. 1

Active ingredient (a.i.)	Trade name	Field rate (a.i. %)	Chemical family	Mode of action	Crops	Target
Abamectin	Cal-EX EW®	75 ml/hl (1.8)	Avermectin	Ingestion. Chloride channel activator	Tomato, eggplant, sweet pepper, strawberry, lettuce, cucumber, melon, cabbages, citrus, grape, ornamental plants and flowers, forest trees	Mites. thrips, psyllids, aphids, leafminers, moths
Azadirachtin *	Oikos®	150 ml/hl (3.2)	Botanical	Ingestion. Moulting disruptor	Tomato, eggplant, sweet pepper, strawberry, carrot, fennel, beans, cabbages, cucurbit crops, garlic, onion, leek, leafy vegetables, celery, stone fruits, pome fruits, actinidia, walnut, chestnut	Thrips, Hemiptera, Lepidoptera
Bacillus thuringiensis var. *kurstaki* strain SA12 *	Costar® WG	200 g/hl (90000 I.U./mg)	Cry proteins	Ingestion. Disruptor of insect midgut epithelium	Tomato, eggplant, sweet pepper, strawberry, artichoke, corn, cotton, tobacco, potato, leafy vegetables, cucurbit crops, sugar beet, cabbages, sugar beet, beans, soybean, sunflower, citrus, grape, olive, actinidia, chestnut, ornamental plants, forest trees	Lepidoptera
Emamectin benzoate	Affirm®	150 g/hl (0.95)	Avermectin	Ingestion. Chloride channel activator	Tomato, eggplant, sweet pepper, strawberry, beans, artichoke, lettuce, stone fruits, pome fruits, grape, cole crops	Lepidoptera
Borax and citrus oil *	PreVam®	400 ml/hl (6)	Borates tetra sodium salts and Oil - essential	Contact. Miscellaneous non-specific inhibitor	Tomato, strawberry, grape	Mites, whiteflies, mealybugs, Tomato borer
Spinosad *	Laser®	75 ml/hl (48)	Spinosyn	Ingestion and contact. Nicotinic acetylcholine receptor agonist	Tomato, eggplant, sweet pepper, strawberry, potato, fennel, legumes, garlic, onion, leek, stone fruits, cucurbit crops, artichoke, leafy vegetables, caper, pome fruits, stone fruits, grape, small fruits, tree nuts, ornamental plants, grass	Thrips, Planthoppers, Lepidoptera, Coleoptera, Diptera

Table 1. Tested biopesticides[a, b]

* Indicate pesticides that are authorized also for organic farming

[b] Italian Ministry of Health, phytosanitary products database 2011 http://www.salute.gov.it/fitosanitariwsWeb_new/FitosanitariServlet.

[c] EU Pesticides database 2011, http://ec.europa.eu/sanco_pesticides/public/index.cfm

Tab. 2

Active ingredient	**Reduction coefficient** **(E_x)**			**IOBC toxicity class**			**Net reproductive rate** **(R_0)**			**Doubling time** **(*DT*)**			**Intrinsic rate of increase (r_m)**		
	1-h old residue	10-d old residue	on pupae	1-h old residue	10-d old residue	on pupae	1-h old residue	10-d old residue	on pupae	1-h old residue	10-d old residue	on pupae	1-h old residue	10-d old residue	on pupae
Control	-	-	-	-	-	-	4.44	4.44	4.44	13.82	13.82	13.82	0.052	0.052	0.052
Abamectin	80.5	45.8	21.8	3	2	1	**0.93**	2.44	3.51	-	23.06	16.41	-	0.030	0.042
Azadirachtin	62.5	30.3	60.5	2	2	2	1.68	3.11	1.75	39.26	18.16	36.66	0.018	0.038	0.019
Bacillus thuringiensis	7.6	5.0	12.0	1	1	1	4.11	4.22	3.99	14.59	14.31	15.11	0.047	0.048	0.046
BCO	44.2	37.5	49.3	2	2	2	2.53	2.93	2.15	22.19	19.15	26.99	0.031	0.362	0.026
Emamectin benzoate	69.2	42.1	87.5	2	2	3	**0.49**	2.58	**0.58**	-	21.76	-	-	0.032	-
Spinosad	**100**	**100**	80.6*	**4**	**4**	3*	-	-	-	-	-	-	-	-	-

Table 2. Reduction coefficient E_x, IOBC toxicity classes, Net reproductive rate R_o; Doubling time (*DT*); Intrinsic rate of increase (r_m) calculate for adults exposed to 1-h old and 10-d old pesticide residues, for adults emerged from topically treated cocooned pupae and for the control population (Biondi et al., 2012c). * Calculated only for the lethal effect.

Chapter VIII

The non-target impact of spinosyns on beneficial arthropods

Biondi Antonio, Mommaerts Veerle, Smagghe Guy, Viñuela Elisa, Zappalà Lucia, and Desneux Nicolas, 2012, Pest Management Science, 68: 1523-1536

Review

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The non-target impact of spinosyns on beneficial arthropods

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Abstract

Spinosyn-based products, mostly spinosad, have been widely recommended by extension specialists and agribusiness companies; consequently, they have been used to control various pests in many different cropping systems. Following the worldwide adoption of spinosad-based products for integrated and organic farming, an increasing number of ecotoxicological studies have been published in the past 10 years. These studies are primarily related to the risk assessment of spinosad towards beneficial arthropods. This review takes into account recent data with the aim of (i) highlighting potentially adverse effects of spinosyns on beneficial arthropods (and hence on ecosystem services that they provide in agroecosystems), (ii) clarifying the range of methods used to address spinosyn side effects on biocontrol agents and pollinators in order to provide new insights for the development of more accurate bioassays, (iii) identifying pitfalls when analysing laboratory results to assess field risks and (iv) gaining increasing knowledge on side effects when using spinosad for integrated pest management (IPM) programmes and organic farming. For the first time, a thorough review of possible risks of spinosad and novel spinosyns (such as spinetoram) to beneficial arthropods (notably natural enemies and pollinators) is provided. The acute lethal effect and multiple sublethal effects have been identified in almost all arthropod groups studied. This review will help to optimise the future use of spinosad and new spinosyns in IPM programmes and for organic farming, notably by preventing the possible side effects of spinosyns on beneficial arthropods.

Supporting information may be found in the online version of this article.

Keywords: spinosad; ecotoxicology; sublethal effects; natural enemies; pollinators; IPM; organic farming; biopesticide

1 INTRODUCTION

Biopesticides are derived from naturally occurring materials and organisms such as animals, plants, bacteria and minerals. In the past decades, this group of pesticides has been increasingly taken into consideration and has been creating interest as regards the development of environmentally sound and integrated pest management (IPM)-compatible approaches.[1] Biopesticides are used in such programmes mainly in order to diminish the negative effects that conventional pesticides may have on the environment and on human health, i.e. the health of farming operators and consumers. However, the origin of a given compound does not really relate to its toxicological properties, which result from the pesticide physicochemical properties acting on arthropod physiology and behaviour.

One of the most well-known families of naturally derived insecticides is the spinosyn family. These compounds result from the fermentation process made by the naturally occurring soil actinomycete *Saccharopolyspora spinosa* Mertz & Yao (Bacteria: Actinobacteridae). Spinosyns are highly active when ingested or through contact and cause swift death in a wide range of insect pests, e.g. caterpillars, leafminers, thrips and foliage-feeding beetles. Their main mode of action affects the nicotinic acetylcholine receptors (nAChRs) in the nervous system, specifically acting as their allosteric modulators, with some evidence suggesting additional impact on the γ-amino butyric acid (GABA) receptor physiology. Arthropods exposed to spinosad usually show compulsory muscle contractions and tremors stemming from continuous activation of motor neurons. Prolonged spinosad-induced contractions lead to paralysis, to zero feeding activity and ultimately to the death of exposed arthropods.[2]

Spinosad was the first spinosyn-based biopesticide marketed, and it contains a mixture of two tetracyclic compounds (spinosyns A and D). It is considered to be a natural product, is classified as low risk[3] and thus has been approved for use in organic farming by many regulatory agencies in several countries of the

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world.[4] Synthetic modification of natural-based pesticides is an important tool to discover new pesticidal compounds, particularly with the aim of increasing efficacy and safety. In the last decade, many semi-synthetic spinosyn analogues have been designed especially to target pests selectively.[5] Spinetoram was the first semi-synthetic spinosyn discovered owing to the synergy of microbiology, synthetic chemistry and artificial neural network technologies.[6] It is mainly composed of the new semi-synthetic spinosyns J and L. Spinetoram was registered in September 2007 by the US Environmental Protection Agency, mainly because of its reduced-risk profile during preliminary risk assessments and its toxicological properties, which proved to be similar to those of spinosad.[7] Therefore, spinetoram-based biopesticides (Delegate® WG, Radiant® SC) were first registered for use in the United States in September 2007, and they are currently on the market in Canada, New Zealand and the United States for management of various crop pests.[8]

Beneficial arthropods in agriculture, mainly natural enemies and pollinators, are increasingly important both economically and environmentally,[9,10] thus making spinosyn risk assessments on non-target arthropods crucial when these pesticides are included in IPM programmes. This is particularly important because potential side effects of pesticides on these arthropods, even subtle ones, can greatly reduce their capacity to provide crucial ecosystem services.[11,12] Overall, biopesticides are often slower acting and consequently cause no short-term effects (e.g. lethal effects), leaving many survivors after pesticide applications. However, they may induce multiple physiological and behavioural sublethal effects,[11] which may lead to a drastic decrease in population levels.[13] Furthermore, susceptibility of a given species to toxic products on the demographic level, notably intrinsic recovery capacity, can vary according to the function of specific-species life-history variables.[14]

Various methodologies have been developed for risk assessment of spinosyns on non-target arthropods according to the biological traits of the organisms studied, e.g. on arthropods' natural enemies.[15] Williams *et al.*[15] reported that spinosad can be classified as harmless to most natural enemies of arthropods tested in studies published up to 2002. They placed spinosad in class 1 in the International Organisation for Biological Control (IOBC) ranking system in 71 and 79% of laboratory and field experiments respectively. However, in the same review, when only Hymenoptera (namely parasitoids) were considered, spinosad was shown to be moderately harmful (class 3) or even harmful (class 4) in the majority of laboratory and field studies.[15] This raises the question as to the actual compatibility of spinosad and, more broadly speaking, of spinosyn-based biopesticides with hymenopteran parasitoids, which are key biological control agents in many agricultural ecosystems.[16,17] The same question arises when considering that the main pollinators belong to the Apidae family in the Hymenoptera order (e.g. honey bees *Apis mellifera* L. and bumblebees *Bombus* spp). This line of reasoning hinted at the importance of assessing possible side effects of spinosysns on beneficial arthropods belonging to the order Hymenoptera.

An increasing number of studies related to risk assessment of spinosyns on beneficial arthropods have been published in the past decade, i.e. following the seminal review by Williams *et al.*[15] Bearing this context in mind, we reviewed the present authors reviewed the lethal and sublethal effects of spinosyns (spinosad and spinetoram) on beneficial arthropods in two types of study, i.e. studies carried out in laboratory or field (semi-field, greenhouse and open-field) conditions. Experiments showing unsatisfactory experimental designs and/or lack of statistical analyses were not included in this review. The focus was on side effects in natural enemies and pollinators. Other possible beneficial arthropods, such as other terrestrial and aquatic arthropods, were not considered, although they may also be affected by spinosyns, e.g. *Daphnia* spp.[18,19] This review takes into account recent data and aims (i) at highlighting potential adverse effects of spinosyns on beneficial arthropods, and hence on the ecological services they provide in agroecosystems, (ii) at clarifying the range of methods used to address side effects of spinosyns on biocontrol agents and pollinators in order to provide new insights for the development of more accurate bioassays, (iii) at identifying pitfalls when analysing laboratory results to assess field risks and (iv) at gaining increasing knowledge on side effects when using spinosad for IPM programmes and organic farming.

2 THE SPINOSAD: HISTORY AND CURRENT USE WORLDWIDE

Spinosad was first registered and commercialised in Korea and the United States in 1996–1997. It was initially formulated as a suspension concentrate for greenhouse and open-field spray applications (e.g. Tracer®, Spintor®, Success®). In the last decade, this pesticide has also been commercialised as a prepacked sprayable bait formulation with low concentrations of spinosad mixed with a feeding attractant, mainly hydrolysed proteins, for Diptera Tephritidae adults (e.g. GF−120®, Spintor-Isco®, Spintor-Cebo®).[20,21] Spinosad is currently registered in more than 80 countries throughout the world to control several pests belonging to Lepidoptera, Diptera, Thysanoptera and Coleoptera orders, in agricultural and forestry landscapes or in veterinary environments.[3,8,22] In 2008, spinosad was included in Annex II of the EU Council Regulation 2092/91, and thus its use was authorised in organic farming in European member states.[23]

Spinosad is more effective when ingested, but it can also affect insects through contact, and it even has a small systemic action, as demonstrated by Weintraub and Mujica[24] in drench soil treatments against the Agromyzidae *Liriomyza huidobrensis* (Blanchard). Spinosad-based products are applied in many countries to control a wide range of pests in greenhouse and open-field crops. Field bait spray applications have become very common against various fruit flies and are replacing organophosphates and pyrethroids worldwide for the control of these pests (e.g. in coffee,[25] in citrus,[26,27] in olive groves,[28] in papaya[29] and in mango[30]). The high efficacy in controlling *Ceratitis capitata* (Wied.), a key pest in citrus and many other fruit crops, was discovered in 1996.[31] *Bactrocera oleae* (Rossi), the key olive pest in the Mediterranean basin, in California and in Central America, and *Rhagoletis cerasi* Loew, a key cherry pest in Europe, are also targeted by this insecticide.[32,33] Moreover, this pesticide is also effective against many other destructive fruit flies not present in Europe, included in the genera *Anastrepha* [*A. suspensa* (Loew), *A. ludens* (Loew), *A. fraterculus* Wied], *Bactrocera* [*B. cucurbitae* (Coquillet), *B. dorsalis* (Hendel), *B. tryoni* (Froggatt)] and *Rhagoletis* [*R. completa* Cresson, *R. pomonella* (Walsh)].[20] Other sensitive Diptera include the Muscidae *Stomoxys calcitrans* (L.), a pest in surburban and rural areas where horses and other livestock are stabled.[8] Other spinosad bait formulations have been developed for the control of ants of the genera *Solenopsis* (granular bait; e.g. Conserve® Professional Fire Ant Bait) and of *Musca domestica* L. (Elector®).[8]

Among lepidopteran pests, the compound is a good alternative for the control of species of the genera *Spodoptera* and *Helicoverpa* in many greenhouse and outdoor crops,[22,34–36] of the pine processionary moth [*Thaumetopoea pytiocampa* (Den. et Schiff.)][37] and of *Tuta absoluta* (Meyrick),[38,39] a major invasive pest in Afro-Eurasia.[171,172] Similarly, the insecticide is applied to control the thrips *Frankliniella* spp. in horticultural and ornamental crops[40] and *Leptinotarsa decemlineata* L. in potatoes.[41] Furthermore, even though it has not yet been registered worldwide, spinosad is active against several stored-grain pests.[42,43]

After spinosyn introduction, spinosad resistance events were documented for some insects, such as houseflies and fruit flies, Lepidoptera and thrips species.[44–49] Although spinosyns have not been proved to have a particular propensity for resistance development compared with other widely used insecticides, spinosad resistance management relies on label restrictions and use guidelines.[50] These are intended to minimise the chances of resistance development, notably limiting the number of spinosad applications and suggesting its use in alternation with pesticides of different mode of action.

3 SIDE EFFECTS OF SPINOSYNS ON NON TARGET ORGANISMS, REPORTED UNDER LABORATORY CONDITIONS

3.1 Lethal effect

3.1.1 Lethal effect on natural enemies

Based on the review by Williams *et al.*,[15] spinosad seems to have few lethal effects on predators, but it harms parasitoids. In the last decade, several laboratory studies have focused on the lethal impact of spinosad towards predators (spiders, mites and insects) and parasitoids, while only one study has dealt with the side effects of spinetoram (Table 1). Most of the studies tested field rates for one or several specific crops and for various targeted pests. In addition, a smaller number of studies also determined the median lethal dose, rate or concentration (LD/LR/LC_{50}) for various natural enemies and then assessed mortality induced by reduced concentrations (e.g. $<LC_{50}$). Detailed information regarding routes of exposure and tested concentrations/doses/rates are reported in supporting information Table S1.

Acute mortality from spinosad in laboratory assessments on arachnid predators gave diverging results. Řezáč *et al.*[51] found no significant mortality when the spider *Philodromus cespitum* (Walckenaer) was exposed to dried spinosad residues on filter paper (104 mg AI L^{-1}), whereas 100% mortality was observed for *Hibana futilis* (Banks) when exposed to very low spinosad residues (5×10^{-20} µg AI L^{-1}) on filter paper for 24 h.[52] High *Araneus pretensis* (Emerton) mortality was observed owing to the ingestion of prey treated with low spinosad rates (2.5–25% of the maximum recommended field rates).[53] Risk assessment on predatory mites revealed that the survival of some species belonging to the genera *Hypoaspis, Neoseiulus, Typhlodromus* and *Typhlodromips* was significantly affected by contact with dried spinosad residues on leaves (see supporting information Table S1).[54–57] In addition, Rahman *et al.*[57] showed, in a more realistic experimental set-up, the synergic lethal effect from the exposure to residues and ingestion of prey previously fed on treated leaves. The new, semi-synthetic spinosyn spinetoram was also very toxic when applied on eggs and larvae of the western predatory mite *Galendromus occidentalis* (Nesbitt), while 100% mortality of females was recorded after 72 h of exposure. In addition, the field rate proved to be 34-fold higher than the LC_{50} (6 mg AI L^{-1}).[58] On the other hand, spinosad has been proved by Jones *et al.*[40] to be selective for adults of the predatory mite *Amblyseius cucumeris* (Oudemans) when exposed topically and/or to spinosad residues on strawberry leaves, and by van Driesche *et al.*[55] for *Iphiseius degenerans* (Berlese) adults exposed to spinosad residues on bean leaves.

The same diverging lethal effects are caused by spinosad on insect predators and vary according to species, instars and the route of exposure considered. For example, topical treatments and exposure to dried spinosad residues at the field rate had no significant lethal effect on *Chrysoperla carnea* (Stephen),[59,61] but, when adults ingested treated prey or insecticide mixed with artificial diet (concentrations $\geq$12.5% of the field rate), 100% mortality occurred rapidly.[61] Exposure of heteropteran predators to dried residues (see supporting information Table S1) was harmless for *Nesidiocoris tenuis* (Reuter), *Macrolophus pygmaeus* (Rambur) and *Picromerus bidens* L. adults,[38,62] harmful (50% mortality) for *Geocoris punctipes* Say,[63] while it was very harmful (up to 100% mortality) for *Orius laevigatus* Fieber and *O. insidiosus* (Say) adults.[39,40,64,65] An acute lethal effect on *O. laevigatus* has also been found 14 days after the initial treatment with the rate recommended for management of Diptera leafminers in multiple crops (360 mg AI L^{-1}).[39] Furthermore, the synergic toxicity of two routes of exposure (topical and exposure to dried residues) was proved on *O. insidiosus*, with 100% mortality when exposed to the field rate.[40] When topically treated, two other Heteroptera species showed diverging responses: *Deraeocoris brevis* (Uhler) young instars and adults were resistant to the highest field rate,[66] while 100% mortality was observed for *Pilophorus typicus* Distant nymphs and adults when treated with lower spinosad concentrations ($\leq$96 mg AI L^{-1}).[67] The real implication of the effects observed after topical exposures should take into account that spinosyn effect can be magnified by the unrealistic experimental conditions of some studies. The use of organic solvents instead of water as carriers (increasing the pesticide intake inside the arthropod body) and the use of dipping methods instead of spray to apply the pesticide solution can amplify the pesticide toxicity, i.e. lead to overestimation of the possible side effects.[68]

With regard to other insect predator orders, high mortality rates were observed on the larvae of two Diptera species, *Aphidoletes aphidimyza* (Rondani) and *Episyrphus balteatus* (Deeger), exposed to spinosad dried residues (100 and 96 mg AI L^{-1} respectively), as well as on first instars of the coccinellid *Harmonia axyridis* (Pallas) and on adults of the European earwig *Forficula auricularia* L.[69–72] On the other hand, females of the coccinellids *Coccinella septempunctata* (L.) and *Adalia bipunctata* (L.) larvae and adults were not affected by spinosad.[56,69,73]

Numerous laboratory studies, conducted in the last decade, demonstrated the acute toxicity of spinosad on 22 species of hymenopteran parasitoids (Table 1 and supporting information Table S1). In contrast to predators, spinosad caused severe lethal effects towards all tested species, irrespective of the exposure route used. When testing the recommended field rates for specific crops in which a given natural enemy can occur, 100% mortality in adults was observed for *Aphidius colemani* Viereck, *Aphytis melinus* DeBach, *Coccophagus lycimnia* Walker, *Leptomastix dactylopii* (Howard) and *Diadegma insulare* (Cresson) after 24 h of exposure to dried residues of spinosad on inert materials.[72,74–76] Similar results were reported for *Trichogramma chilonis* Ishii adults when exposed to host eggs or tomato leaves previously treated with the field recommended rate.[77] Also, 100% mortality was recorded by Medina *et al.*[78] in adults of the parasitoid *Hyposoter* *didymator* (Thunberg) after topical application (2 µg AI insect^{-1}) and after the ingestion of spinosad-contaminated water source (1000 mg L^{-1}). Haseeb *et al.*[79] reported a similar lethal effect of a lower concentration of spinosad-contaminated water (53 mg AI L^{-1}) when ingested by *Cotesia plutellae* Kurdjumov adults. Significant mortality was recorded after exposing hymenopteran adults such as *C. plutellae, D. insulare, Encarsia formosa* Gahan, *Euplectrus planthypenae* Howard, *Oomyzus sokolowskii* Kurdjumov and three *Trichogramma* species to dried spinosad residues on leaves,[34,40,74,79–81] and *Chelonus insularis* Kurdjumov adults supplied with treated host eggs (see supporting information Table S1 for details).[34] Spinosad field rates proved to be 2000-, 1000- and 10-fold higher than the estimated LC$_{50}$ values for *Trichogramma pretiosum* Riley (0.23 mg AI L^{-1}), *Anaphes iole* Girault (0.50 mg AI L^{-1}) and *Habrobracon hebetor* Say (15 mg AI L^{-1}) respectively.[82,83] Studies on spinosad acute effects on natural enemies have also been conducted on young instars. In all these experiments, the authors applied the insecticide on parasitised hosts or topically on larvae and pupae, and the endpoint considered was often the rate of adult emergence. Variations in this trait may result from the acute lethal effect both on the host and on the parasitoid, as well as from the physiological effects on their development.[11] For this reason, these studies are discussed in detail in Section 3.2.1.

Various risk assessment experiments have also been conducted on spinosad-baited formulations, and diverging results were obtained owing to the experimental set-up (i.e. applied rates and route and exposure duration) and to the tested species. Acute lethal effects were reported by Urbaneja *et al.*[27] when exposing the mite *Neoseiulus californicus* (McGregor) and the parasitoid *A. colemani* to dried residues of field rate on citrus leaves. High mortality of predators, such as *C. carnea, Leucopis* sp. and *Pseudodorus clavatus* F., was recorded by Nadel *et al.*[84] and Michaud[85] when baited spinosad was offered alone at the field rate for *C. carnea* and at 1 µL per five insects for the two Diptera species, while there were no lethal effects when the predators had access to other food sources. High mortality was also found by Michaud[85] for *A. melinus* and *Lysiphlebus testaceipes* (Cresson) exposed to dried residues of baited spinosad (1 µL AI per five insects) on inert materials, and by Stark *et al.*[21] exposing two braconid wasps, *Fopius arisanus* (Sonan) and *Psyttalia fletcheri* (Silvestri), to 15 µL of dried residues in glass vials. Dose–mortality response of topical treatment was demonstrated by Ruiz *et al.*[86] for the parasitoid *Diachasmimorpha longicaudata* (Ashmead), which was also greatly affected when in contact with dried residues and when the field rate was ingested. On the other hand, this product was risk free on third-instar nymphs of *O. insidiosus*, as well as on third-instar larvae and on adults of various coccinellids.[69,85,87] Moreover, Michaud's interesting results[85] stated that *Chrysoperla rufilabris* Burmeister and *Pseudodorus clavatus* (F.) are significantly affected by the baited spinosad residues only in no-choice experiments, but not when the beneficials also had untreated food available, owing to the compulsory feeding on treated food if non-choice tests are performed (see supporting information Table S1).

3.1.2 *Lethal effect on pollinators*

Past studies conducted over several continents using different experimental methods [e.g. US EPA (United States Environmental Protection Agency) and EPPO (European and Mediterranean Plant Protection Organisation) guidelines] reported that spinosad was toxic towards bees. Indeed, exposure to spinosad (2.5–20 mg AI L^{-1}) inhibited AChE in various organs of the honey bee worker and altered the function of GABA-gated chlorine channels, causing tremors in bees, and leading to paralysis and death.[2,88] Moreover, the spinosad toxicity depended on the spinosad product used (technical grade, formulated product or fruit-fly bait), the route of exposure tested [orally, topically or via residues (wet versus dry)] and the bee genera studied (supporting information Tables S2 and S3).

For contact toxicity, technical-grade spinosad was classified as highly toxic for various bee genera.[89–95] However, based on the LC$_{50}$ values, no differences in sensitivity between bee genera were demonstrated. On the other hand, the LC$_{50}$ values of formulated spinosad (240 and 480 g AI L^{-1}) depended on the bee genera (supporting information Table S2).[95–99] Spinosad is also used as fruit-fly bait (0.2 g AI L^{-1}), but, compared with technical-grade and formulated spinosad, this was only faintly toxic for bees (48 h LC$_{50}$: >100 µg product bee^{-1}).[92,100]

For oral exposure, both spinosad (technical grade and formulated at 480 g AI L^{-1}) and the newer compound, spinetoram (formulated at 250 g AI kg^{-1}), resulted in high toxicity for worker bees.[90,93,97,98,101,102] Indeed, acute feeding tests via treated sugar water showed a 24 h LC$_{50}$ of 7.34 mg AI L^{-1} for honey bees and a 72 h LC$_{50}$ of 96 mg AI L^{-1} (formulated spinosad) and 21 mg AI L^{-1} (spinetoram) for *Bombus terrestris* (L.).[88,102] Similarly, high toxicity for bumblebees was also demonstrated after 11 weeks of chronic exposure to sugar water treated with spinosad and spinetoram (LC$_{50}$ $= 2$ mg AI L^{-1}).[102] In addition to treated sugar water, only two other experiments have been conducted so far where pollen treated with spinosad and pollen from sweet corn collected at different time points (up to 3 days) after spinosad treatment was fed to *Bombus impatiens* (Cresson) colonies during a period of 3 weeks and to newly emerged worker honey bees for 24 h respectively.[94,103] According to Morandin *et al.*,[103] no significant bee mortality was observed after acute and chronic exposure to realistic field rates.

For residual toxicity (supporting information Table S3), contact exposure to dry residues of spinosad (up to 200 g AI ha^{-1}) on leaves/flowers in crops such as alfalfa, citrus and kiwifruit or glass plates was either slightly harmful or harmless to honey bees up to 100 h after exposure.[91–94,104–107] Although no lethal effects were observed on honey bees, several authors demonstrated that other bee genera, including *Bombus* and *Megachile*, but not *Protonectarina*,[107] were more sensitive.[92,95,99] In addition, exposure might also occur via wet residues. For formulated spinosad (480 g AI L^{-1}) and spinetoram (250 g AI kg^{-1}), high toxicity was obtained after both wet and dry residue exposure, whereas for the fruit-fly bait a difference between wet and dry exposure was noted only after 24 h.[102,108] Taking into account the toxicity seen after exposure to wet residue, Mayes *et al.*[92] found that toxicity was not related to the spinosad volume applied. Furthermore, chronic oral intake of dry residue of real spinosad field rates via pollen was safe for *B. impatiens* colonies.[103]

3.2 Sublethal effects

3.2.1 *Physiological and behavioural sublethal effects on natural enemies*

Even though most spinosad risk assessment experiments were usually based on acute toxicity tests, spinosad was also shown to cause sublethal effects towards beneficial arthropods. Some key physiological and behavioural traits could be impaired by pesticides,[11] and spinosad was shown to have an influence on larval development and on longevity and reproductive capacity of adults, both treated and developed from young treated instars (Table 1 and supporting information Tables S4 and S5).

Table 1. Overview of reviewed studies on side effects of spinosyns on beneficial arthropods. Studies were considered as positive for adverse effects of spinosyns on a beneficial arthropod when a significant lethal effect or sublethal effects were demonstrated. Positive effects deriving from one single study (low significance) are indicated in italics. Papers reporting the effects of two spinosyn products or formulations have been considered twice

	Laboratory studies				Field and semi-field studies	
	Testing lethal effect		Testing sublethal effects			
Total number of studies per group	Number of studies	% Positive	Number of studies	% Positive	Number of studies	% Positive
Natural enemies						
85	54	81	30	94	40[a]	48
Predators						
48	35	71	16	88	26[a]	34
Parasitoids						
39	19	100	14	100	14[a]	64
Araneae[b]						
3	3	67	2	100	0	–
Mites[b]						
12	8	88	3	67	6	50
Dermaptera[c]						
3	1	*100*	2	100	1	*100*
Neuroptera[c]						
5	5	80	3	100	0	–
Hemiptera[c]						
10	10	60	4	75	9	34
Diptera[c]						
3	3	67	1	*100*	0	–
Coleoptera[c]						
6	5	20	1	*100*	2	0
Hymenoptera[c]						
36	20	100	14	100	11	80
Pollinators						
39	26	77	3	33	13	54
Apis mellifera[c]						
29	19	63	1	*0*	11	45
Bombus spp.[c]						
8	6	83	2	50	2	100
Other pollinators[c]						
5	5	100	0	–	0	–

[a] Studies also considering species complexes.
[b] Class: Arachnida.
[c] Class: Insecta.

Larval development perturbations were observed by some authors for both predator and parasitoid species. For predatory mites, Villanueva and Walgenbach[54] reported that spinosad significantly interfered with *Neoseiulus fallacis* (Garman) development from egg to adult when the individuals were exposed to dried residues on bean leaves (from the lowest field recommended rate on pome fruit orchards). Mandour[61] noted different degrees of toxicity of spinosad on various young instars of the lacewing *C. carnea*, i.e. first instars were more sensitive than eggs and mature larvae to a range of low spinosad concentrations (from 4 to 72 mg AI L^{-1}). No effects on the development of treated nymphs of the generalist predator *D. brevis* were highlighted. However, after exposing adults to a spinosad field rate, a transgenerational effect was observed on the development of the offspring, i.e. duration of larval development increased.[66] Many studies on the development of parasitoid larvae reported effects of spinosad on adult emergence when exposed at the pre-imaginal stages. Dose-related effects on the pupation rate of *Apophua simplicipes* (Cresson) were noted by Cossentine *et al.*[109] when second- and fourth-instar parasitised hosts fed on leaf materials previously treated with low spinosad concentrations (from 0.01 to 5 mg AI L^{-1}), and when fourth-instar parasitised hosts were topically treated with higher, more realistic, spinosad doses (from 25 to 1000 mg AI L^{-1}, the field rate being 88 mg AI L^{-1}). Schneider *et al.*[110,111] reported a decrease in the adult emergence rate from topically treated (5.6 µg AI insect^{-1}) pupae and third larvae of *H. didymator*, and they related their findings to the apparent inability of the treated larvae to produce silk needed for cocooning. When spinosad was applied on parasitised hosts (eggs and larvae), very low adult emergences were observed for *Opius chromatomyiae* Belokobylskij and Wharton, *Neochrysocharis formosa* Westwood, *Eretmocerus mundus* (Mercet) and for all the pre-imaginal stages of four *Trichogramma* species (see supporting information Table S4).[77,81,112,113]

Physiological effects might also cause disturbance of other essential biological traits of natural enemies, such as longevity, immune system and reproduction. Unfortunately, there are no studies that have associated the effects on young instar development with adult longevity, but data on the spinosad effect on adult longevity are available for various insect species. Diverging results were reported on the impact of spinosad on longevity of predatory bugs. *Deraeocoris brevis* longevity was strongly affected when the predator was directly exposed to spinosad solution (i.e. topical exposure) and ingested spinosad-contaminated prey at the same time, whereas no significant effect was found on the longevity of *P. bidens* after being in contact with dried residues of spinosad.[62,66] These diverging results likely resulted from the different exposure routes tested in these studies, and actually the study by Kim *et al.*[66] may better mimic the real exposure scenario occurring in field conditions. Indeed, predators are exposed to spinosad both through contact with dried residues and through ingestion when attacking and consuming spinosad-sprayed pests,[114] but also directly to insecticide spray droplets at the time spinosad is sprayed in the crops.[115] Studies involving parasitoids showed that adult longevity was always affected by spinosad when topically applied on hosts parasitised by *Eretmocerus warrae* Naumann and Schmidt[116] and *N. formosa*[112] and on *H. didymator* pupae,[110] or when *D. insulare* adults were fed with spinosad-treated water-honey food (for more details, see supporting information Table S4).[74]

A decrease in reproductive activity owing to exposure to pesticides can result from both physiological and behavioural effects.[11] Many authors have reported general spinosad effects on fecundity and fertility of natural enemies, in spite of documenting their biological basis. However, mechanistic insights into the effects of pesticides on natural enemy reproduction have been described in several studies. Spinosad at low rate (30 mg AI L^{-1}, 25% of the maximum field recommended concentrations) reduced the number of viable eggs of the spider *A. pretensis*[53] and the amount of laid eggs by predatory mites, such as *N. fallacis*,[54] *Neoseiulus cucumeris* (Oudemans) and *Iphiseius degenerans* (Berl.),[55] when applied at field recommended rates. Fewer offspring were produced by adults of the heteropteran predators *M. pygmaeus*, *N. tenuis*[38] and *O. laevigatus* when exposed to dried residues of spinosad (at the highest field rate recommended for tomato); furthermore, the negative effects lasted for at least 7 days after the day of spinosad application on leaves.[39] For the bug *D. brevis*, spinosad treatment at the full field rate resulted in a reduced oviposition activity, whereas adults developed from treated nymphs showed a regular reproductive activity.[66] The fertility of the lacewing *C. carnea* adults did not decrease after being in contact with treated oviposition substrate, when topically treated or when supplied with spinosad-contaminated water. Nevertheless, adults stopped ovipositing after feeding for 2 days on spinosad-treated prey and spinosad-treated artificial diet.[60,61] A similar stop in oviposition activity was also reported in *E. balteatus* when this sirfid predator had been previously exposed during its larval stage to dried residues of spinosad on glass at the maximum recommended field rate for brassica crops (96 mg AI L^{-1}). By contrast, *C. carnea* reproduction was not affected when young instars had been topically treated (see supporting information Table S4 for details).[61] The reproductive activity of egg parasitoids was also affected when treating host eggs; reductions in the parasitisation rate were reported for *C. insularis*,[34] *Telenomus remus* (Nixon)[117] and *T. chilonis*,[77] or when *T. pretiosum* was exposed to dried residues on glass (supporting information Table S4).[118] The reproduction of the larval endoectoparasitoid *H. didymator* was considerably affected after topical treatment (5.6 μg AI insect^{-1}) of mature larvae and pupae.[110,111]

Spinosad-baited formulation, when ingested at a rate of 20 mg AI L^{-1}, caused a reduction in the number of laid eggs by the lacewing *C. carnea*[84] and a 45% reduction in the net fecundity (longevity × fecundity) of *D. longicaudata* parasitoid females after feeding on baited spinosad (0.08 mg AI L^{-1}).[86] When *H. axyridis* was topically treated with a range of concentrations of 10–100% of the field recommended rate, a dose-dependent effect on the development time, rate of pupation of treated larvae, adult weight and hatchability of the eggs laid by the developed adults was observed for all the tested concentrations.[69] Hernàndez *et al.*[169] studied the effect of the field recommended rate of spinetoram on the longevity of two parasitoid species of Diptera leafminer, *Ganaspidium nigrimanus* (Kieffer) and *N. formosa*. The survivorship of these parasitoids decreased largely when individuals were exposed to spinetoram either topically, on pepper leaves, and through feeding on a spinoteram-contaminated food source.

Spinosad, like the majority of the other neurotoxic compounds, may affect various behavioural traits in predators and parasitoids.[11] It has been reported in several studies that aimed to test the side effects of various recommended field rates as well as lower rates (see supporting information Table S5 for details). Works demonstrated that natural enemies are affected mostly exclusively when they are directly exposed to spinosad (both topically treated or through contact with residues), while no behavioural effects have been found in other cases, i.e. when attacking treated prey/host and when choosing between treated and untreated substrates.

Although effects on mobility were not always directly studied, spinosad side effects on this trait were observed for the predators *C. carnea*,[60] *D. brevis*,[66] *Doru luteipes* (Scudder)[119] and *F. auricularia*[70] and for the parasitoids *Eiphosoma vitticolle* Cresson[34] and *H. didymator*.[110,111] This effect is mostly due to direct intoxication by the pesticide, which results in a knockdown effect, trembling and lack of coordination in legs, wings, mandibles and palpus. When these symptoms are not easily detectable, other neurotoxic effects may be responsible for the modification of other important behaviours, such as feeding, oviposition, navigation and orientation.[11] All these behavioural modifications may result in decreasing predatory capacity, as shown for the spider *P. cespitum* after larvae were exposed to dried residues of spinosad at a low concentration (60 mg AI L^{-1}),[51] and for *A. pratensis* and *C. carnea* when they were fed prey treated with low concentrations of spinosad (3 and 36 mg AI L^{-1} respectively).[53,61] Nonetheless, modifications of the oviposition behaviour on treated prey/host of *C. carnea*[61] and *E. vitticolle*[34] have not been observed. Based on studies by Van Driesche *et al.*,[55] Rahaman *et al.*[57] and Penagos *et al.*,[34] spinosad seems to have no repellent effects on five predatory mite species. Similar findings were reported for the parasitoid *C. insularis* when exposed to untreated and spinosad-treated leaves at the same time at concentrations ranging from 10 to 100% of the recommended field rate.[34] Nadel *et al.*[84] proved that baited spinosad had no repellent effects on *C. carnea*, and that this predator consumed the same quantity of spinosad bait (20 mg AI L^{-1}) and control bait when offered in a no-choice test.

3.2.2 *Sublethal effects on pollinators*

For honey bees, dry residues of spinosad were harmless to larvae.[92] On the other hand, when *B. impatiens* colonies were chronically fed on pollen treated with realistic field rates, the weight of emerging bees was lower, as was the foraging efficiency of adults on artificial flowers (previously fed during their larval development with spinosad-treated pollen).[103] Recently, Besard *et al.*[102] observed that oral exposure of *B. terrestris* to spinosad and spinetoram did not cause adverse effects on reproduction and on foraging behaviour when applied at 1/1000 and 1/100 of their respective field recommended rates. Therefore, the newer spinosyn (spinoteram) may pose less risk to foraging and pollination by bumblebees than spinosad. However, actual highest recommended field rates induced 100% of mortality in a worst case exposure scenario in the laboratory,[102] and field studies suggested that side effects on brood production and foraging activities of bees may occur depending on field application rate and crop considered (see section 5). However, there is currently a need in studies assessing thoroughly possible sublethal effects of spinosyns on key traits in the honey bee and wild bees.

4 SIDE EFFECTS OF SPINOSYNS, REPORTED IN SEMI-FIELD, GREENHOUSE AND FIELD CONDITIONS

4.1 Side effects on natural enemies

Most trials on the side effects of pesticides have been conducted under laboratory conditions, and those on the effects of spinosad are no exception. However, a main concern in ecotoxicology is linked to problems arising from extrapolation from laboratory conditions to those in the field, and field studies may be necessary to validate the effects of pesticides on the natural enemies present in agroecosystems.[120] Furthermore, field risk assessment experiments may involve multiple routes of exposure, including topical, residual and ingestion and mixed age classes of natural enemies.[66,78,115,121,122] Spinosad concentrations are also likely to vary in treated agroecosystems, notably because the concentrations of pesticides depend on how often the products are applied and how quickly the active ingredients are degraded owing to abiotic factors, such as sunlight and rainfall.[2,123,124]

Several field trials on side effects have been conducted on predatory mites (Table 1 and supporting information Table S6). Olszak and Sekrecka[56] confirmed the results of laboratory trials, i.e. high mortality of the phytoseiid mite *Typhlodromus pyri* Scheuten after one spinosad application (144 g AI ha^{-1}) in apple orchards, even if the acute toxicity slightly decreased 5 weeks after the treatment. The same kind of negative impact on predatory mites was also recorded after a single application of spinosad at a rate of 120 mg AI L^{-1} in citrus orchards, where cumulative predatory mite-days over the total sampling period were significantly lower in the plot treated with spinosad than in the control.[125] A negative effect of spinosad (150 g AI L^{-1}) in citrus orchards was also recorded by Khan and Morse[126] on the most common predatory mite found on California citrus, *Euseius tularensis* Congdon, whose predation index (accumulated mite-days) was affected negatively. Van Driesche *et al.*[127] reported a decrease (0.78 mL AI L^{-1}) in population growth and in pest biocontrol activity of the predator *Neoseiulus cucumeris* (Oudeman) in a greenhouse treated with spinosad.[127]

By contrast, when spinosad was either sprayed in the presence of three phytoseiid and two laelapid species or applied on the plant and then exposed to the predators in semi-field trials, no significant effect at any of the tested concentrations (96–360 g AI L^{-1}) was observed.[128] Jones *et al.*[40] found no acute toxicity after exposing *A. cucumeris* for 48 h in clip-caged cucumber leaves in a greenhouse trial. Similarly, no detrimental effects on the survival, predation and reproduction rates of *Phytoseiulus persimilis* (Athias-Henriot) females were detected in a greenhouse trial with ivy geranium after spinosad application at a rate of 63 mg AI L^{-1}.[122] Spinosad effects on the population level have been monitored on the European earwig *F. auricularia* after insecticide application (150 g AI ha^{-1}) in apple orchards. One week after the treatment, the number of earwigs was significantly lower (52%) compared with the untreated control. Considering that this is a monovoltine species, in spite of decreasing toxic effects in the following weeks, such a reduction in population can lead to a significant disturbance in predation efficacy.[129]

For true predatory bugs, exposure during 24 h to spinosad dried residues (90 and 199 g AI ha^{-1}) had no acute effect on caged *O. insidiosus* adults and nymphs in cotton fields and in an experimental greenhouse.[64] Similar results were reported for *O. laevigatus* in a semi-field test.[93] In a greenhouse trial with 48 h of exposure of *O. insidiosus* adults (inside clip-caged cucumber leaves), spinosad was classified as slightly harmful, 1 day after the treatment, but its toxicity rapidly decreased 8 days after the treatment.[40] Spinosad applications (210 g AI ha^{-1}) did not have any effect on the field abundance of the predators *D. brevis* and *Anthocoris* spp. throughout the growing season.[130] No significant acute effect, 48 h after spinosad treatment (144 mg AI L^{-1}) in apple and pear orchards, was recorded for *Anthocoris nemoralis* (F.) adults and nymphs.[131] The survival of *G. punctipes* adults was assessed under greenhouse conditions following exposure to dry residues of spinosad (sprayed at 50 g AI ha^{-1}). Predator survival was reduced significantly after a 6 day exposure period, while no differences were highlighted for shorter exposure periods, thus indicating that the side effects were related to the time the predator takes to ingest and absorb the compound.[63] The same exposure-time-dependent effect has also been demonstrated by Lakshmi *et al.*[132] when the two mirid bugs *Cyrtorhinus lividipennis* Reuter and *Tytthus parviceps* (Reuter) were exposed to spinosad (56 g AI ha^{-1}) under controlled greenhouse conditions the mortality rates increased in relation to the length of exposure time (24, 48 and 72 h after treatment). The population recovery of another mirid, *M. calliginosus*, took 14 days after direct spinosad application (360 mg AI L^{-1}) on pepper plants.[128] One field risk assessment was conducted with the semi-synthetic spinosyn analogue spinetoram to evaluate its effects on *O. insidiosus* in a field trial on pepper. When spinetoram was applied at 61 g AI ha^{-1}, the predator was still sufficiently abundant to suppress thrips population growth.[133]

Coleoptera predators seem to be unaffected by spinosad treatment in field experiments. In fact, the density of *H. axyridis* larvae in plots treated 3 times with spinosad (110 g AI ha^{-1}) did not differ significantly from the untreated control.[134] Furthermore, a similar selectivity was also performed on the two aphidophagous coccinellids *Stethorus punctillum* (Weise) and *Scymnus subvillosus* (Goeze).[131] Nevertheless, spinosad spray applications may also be dangerous for soil predators. Indeed, Pereira *et al.*[135] demonstrated how soil ant communities were strongly affected, in terms of composition and frequency, by spinosad application (33 g AI ha^{-1}) in corn fields up to 70 days after the treatment.

As shown in different studies assessing the risk of spinosad to natural enemies, Hymenoptera parasitoids are often negatively affected by this insecticide also under semi-field and field conditions (Table 1 and supporting information Table S6). Dried residues of spinosad (240 mg AI L^{-1}) on young orange trees affected the survival of *L. dactylopii* for 24 h after the treatment, but no effects on the longevity, fertility and progeny sex ratio of the surviving specimens were observed.[76] Spinosad treatments on a pome fruit orchard killed an average of 70% of chalcid and braconid parasitoid adults when checked 48 h after the treatment.[131] Also, in greenhouse conditions, spinosad toxicity was highly persistent towards the parasitoid *E. formosa*, which was greatly affected (mortality higher than 97%) by the treatment up to 28 days after the application.[40] However, when comparing greenhouse conditions among Belgium, Italy and Spain (for two seasons), spinosad toxicity on *E. formosa* was slightly persistent ($<$15 days) in Italy, while in Belgium and Spain (unusual cloudy summer) it was more persistent ($<$30 days).[123] The persistence of spinosad is likely related to the exposure of treated plants to UV. Therefore, the persistence should always be associated with the climatic conditions occurring in the particular geographic areas considered, the seasons and also the cropping systems used. For example, plants in Mediterranean basin greenhouses are often protected from direct sunlight through the use of greenhouse shade nets, which actually reduce the exposure of plants to UV.

Several experiments assessed spinosad impact on various egg parasitoid species under field and semi-field conditions with parasitised host egg masses previously treated with spinosad. In particular, Koppel *et al.*[136] found 100% failures in adult emergences after spinosad application (180 g AI ha^{-1}) on the scelionid wasp *Telenomus podisi*. Garcia Nevarez *et al.*[137] found a severe effect on the adult emergence of *Trichogramma platneri* (Nagakartti), *T. exiguum* (Pinto & Platner) and *T. pretiosum* after spinosad treatment (105 g AI ha^{-1}). By contrast, the rate of emergence of *T. minutum* (Riley) adults from parasitised eggs was not negatively affected by the treatment (105 g AI ha^{-1}).[138] These diverging results may be due to the different spinosad penetration capacity in the different host eggs, i.e. stink bugs (Hemiptera: Pentatomidae), moth (Lepidoptera: Gelechiidae) and fruitworm (Lepidoptera: Pyralidae) respectively.

As spinosad has also been registered as a stored-grain protectant at the rate of 1 ppm (1 mg AI kg^{-1} grain), trials have been conducted in these specific conditions. Parker *et al.*[139,140] recorded no survival of the parasitoid *Anisopteromalus calandrae* (Howard) and only limited survival of the parasitoid *Choetospilus elegans* Westwood in treated corn after storage periods ranging from 10 to 24 months. However, in the same trial, populations of the predator *Xylocoris flavipes* (Reuter) remained relatively unaffected. In a similar trial, limited survival of parasitoids and moderately high populations of *X. flavipes* were recorded.[43]

Spinosad in an experimental granular phagostimulant formulation (100 and 300 g AI ha^{-1}) has been tested for its potential side effects on Hymenoptera parasitoids emerging from *Spodoptera frugiperda* (Smith) larvae collected in treated fields. The number of emerging parasitoids was reduced in all treatments compared with the control, with greater reduction at the highest spinosad rate; however, the percentage of parasitism was not different among treatments.[22] Ruiz *et al.*[86] showed that spinosad-based bait (200 mg AI L^{-1}) had no effect on the survival, longevity and reproductive capacity of *D. longicaudata* when applied under semi-field and field conditions on mango trees. In citrus orchards, baited spinosad (296 mg AI ha^{-1}) caused no negative effect on the total number of natural enemies captured in sticky traps, notably on *C. bifasciata*, *Aphytis* spp. and artificially released *C. capitata* sterile males.[141] Another field study has been conducted by Gonçalves *et al.*[33] with the aim of assessing the impact of spinosad bait on the natural enemy communities living in olive groves. In a 2 year field experiment, they found no harmful effects of baited spinosad treatments on the natural enemy community monitored by suction devices. The results of this study should be considered carefully, as the control plots were sprayed with water only (i.e. not with untreated baits), and some natural enemies might have moved to the plots where spinosad bait sprays were applied, looking for food sources, thereby invalidating the population monitoring among the experimental plots. Chapman *et al.*[142] proved the same low impact on natural enemy communities, except for ichneumonid and chalcidid wasps, in a small-plot and multilocation field study on bell pepper when treated with spinosad at a rate of 26 g AI ha^{-1}. By contrast, spinosad at a rate of 120 mg AI L^{-1} stopped predator and parasitoid population growth in citrus orchards.[125]

Spinosad applications can also affect natural enemy communities by multiple indirect effects. Modifications at community level are related to species interactions and to the different toxicity rates towards the community components. Therefore, an accurate assessment of spinosad community impact is difficult to achieve. Indeed, spinosad field risk assessments have mostly been based on short-term experiments carried out in small experimental plots, and the endpoint has almost exclusively been the abundance of entomophagous species after the treatments. Interestingly, Thomas and Mangan[141] investigated baited spinosad effects on secondary pests infesting treated citrus orchards, and they found that 90% of the observed species decreased their population levels. This result may be responsible for community alterations through prey/host availability reduction, which, jointly with habitat destruction, represents the main pesticide indirect side effect.[11,143]

4.2 Side effects on pollinators

The economic and ecological importance of pollinators calls for an evaluation on a higher-tier level. To date, spinosad products have been evaluated under semi-field, greenhouse and field conditions (supporting information Table S7). Semi-field tests following the EPPO guidelines showed that a single application of 144 and 540 g AI ha^{-1} of spinosad in a volume of 1500 L ha^{-1} on plants did not affect bee worker survival or hive activity.[92,93,144] However, severe sublethal effects on foraging (up to 7 days after treatment) and on the number of brood cells (post-treatment) were reported with 540 g AI ha^{-1}.[92,93,144] Similarly, considering the cumulative effects of repeated spinosad applications at different times during the day or at different intervals, Halsall[145] and Miles[93] demonstrated that concentrations of 144 g AI ha^{-1} and 216 g AI ha^{-1} (which are used in practice) were harmless, whereas 540 g AI ha^{-1} resulted in a small increase in mortality up to 24 h after application.

Besides semi-field tests, several greenhouse studies were conducted to address the effect of a foliar application (residues) of spinosad on both honey bees and bumblebees.[92,146–148] For honey bees, the application of 100 g AI ha^{-1} spinosad (250 g AI L^{-1} formulation) in a volume of 1000 L ha^{-1} on strawberry crops resulted in a delay in pupal formation for young larvae of up to 3 days after spinosad application, while the survival of older larvae was affected during a longer time period (up to 7 days).[92,148] For bumblebees, a 2 day exposure of foraging bumblebee colonies to spinosad residue (120 g AI ha^{-1}; 250 g AI L^{-1} formulation in a volume of 1200 L ha^{-1}) was also shown to be detrimental for the brood.[92,147] Eggs and early larval development were inhibited when colonies were placed in the greenhouse on days 0, 2 and 4 after the application of the insecticide. However, for a later exposure period (6 and 8 days), no effect was observed. Similarly, Aldershof[146] reported no effect on brood development (post-treatment), but found a temporal effect on bee foraging when tomato crops were sprayed with 540 g AI ha^{-1} of spinosad (480 g AI L^{-1}) in a volume of 1500 L ha^{-1} prior to bumblebee exposure.

The low degree of spinosad risk towards bees in the field was also confirmed by various field studies in which concentrations higher than the mean application rate of spinosad, namely 25–140 g AI ha^{-1}, were evaluated. Indeed, these studies did not report lethal or sublethal effects (the number of bees present in the crop, the activity of bees flying in and out of the hive and the number of capped brood cells) until 12 days after treatment.[91,92,105,149–152] Moreover, when considering the time of spraying, it was clear that a single application in the evening/night or early morning that was allowed to dry at least 3 h prior to bee foraging was not detrimental.[105,152] Furthermore, when spinosad was applied at the moment bees were flying, only a slight risk was observed.[151] In addition, fruit-fly-baited spinosad at a concentration of up to 0.25 g AI ha^{-1} was safe for honey bees up to 6 weeks after the application.[153,154] In this case, the lack of side effects may be explained by the repellent property of the product.[155]

5 SPINOSAD AUTHORISATION FOR USE IN ORGANIC CROPS

Pest management strategies for organic crops give priority to cultural practices compatible with natural processes, vegetation management to enhance biocontrol services and inundative or inoculative release of biological control agents instead of using approved pesticides.[156] When pesticide use is strictly needed, i.e. when pest infestations are seriously threatening the crop, the applied substances must be of natural origin. For example, in the EU member states, the number of pesticides is rather limited and covers different inorganic compounds (mainly as repellent),[157] botanical compounds and microorganisms such as *Bacillus thuringiensis* Berliner and some granulosis viruses.[158] Spinosad use in organic farming was initially authorised in non-European countries (e.g. the United States, USDA National Organic Program),[159] and it was not included in Annex IIB of the Council Regulation 2092/1991 regarding pesticides authorised in organic farming in the EU.[158] In May 2008, based on the report of ad hoc requested experts stating that the compound is essential for the control of particular key pests in some organic agroecosystems (i.e. *C. capitata* in citrus, *B. oleae* in olive and thrips in leek) and contributes to the sustainability of other cropping systems, spinosad was included in the annex. The fact that it was included meant that the use of the few authorised products could be alternated for the control of some pests, diminishing the risk of pesticide resistance development by the pest. In fact, in Annex II it is clearly stated that its use is allowed 'only where measures are taken to minimise the risk to key parasitoids and to minimise the risk of development of resistance'.[23]

The actual data on the impact of spinosad on beneficial arthropods as provided by the numerous laboratory and field studies described above suggest the need to clarify and, if necessary for particular agroecosystems, to revise how spinosad is labelled and advised to farmers in order to make it more compatible with organic farming. Many results acquired in the past decade about the ecotoxicological profile of spinosad support the idea that, even if the spinosad is obtained from a naturally occurring soil organism, this does not mean that it is safe for non-targeted organisms. This comment is also valid for some other biological-based pesticides; they are often erroneously considered to be less toxic. Indeed, various studies have demonstrated the toxicity of many natural substances towards warm-blooded animals and beneficial arthropods.[160–162]

6 CONCLUSION AND FUTURE OUTLOOK

Spinosyn-based products (mostly spinosad) have been widely recommended by extension specialists and agribusiness companies owing to their property as broad-spectrum insecticides; spinosad is a powerful insecticide of microbial origin that controls or suppresses many pest insects from different insect orders/families in various cropping systems. For example, in only a few years, spinosad has become a key pesticide for both conventional and organic cropping systems. Following this worldwide adoption of spinosad-based products, a growing number of ecotoxicological studies have focused on the risk assessment of spinosad towards beneficial arthropods. Studies have demonstrated that spinosad can affect various physiological and behavioural traits of beneficial arthropods, although the occurrence and intensity of observed effects depend on concentration, exposure method and beneficial considered (Table 1 and the supporting information tables). Interestingly, although the effects of low spinosyn doses were largely tested, no stimulatory effects (i.e. hormesis[163]) were found in any insects.

Among natural enemies (i.e. parasitoids and predators), those belonging to the Hymenoptera order are clearly the most susceptible group, with high levels of mortality, marked typical poisoning symptoms and reduced mobility after exposure to spinosad. In contrast to studies undertaken on natural enemies, possible sublethal effects of spinosad on pollinators have not yet been largely investigated. Further studies focusing on the side effects of spinosad on important life traits in bees (e.g. honey bees, wild bees, *Bombus* spp. and in other wild species), such as foraging behaviour, orientation and learning capacities (e.g. Ramirez-Romero *et al.*[164] and Han *et al.*[165]; see Desneux *et al.*[11] for a thorough review), which are crucial for pollinators,[166] are also needed to gain a better understanding of how spinosad can impair the activity of surviving individuals. Moreover, risk of potential exposure to spinosad in young instars of honey bee and wild bees still needs more attention. Indeed, when considering the negative effects on bee weight and on foraging efficiency, as reported by chronic exposure of *B. impatiens* larvae to pollen treated with field realistic concentrations of spinosad,[103] it is likely that such side effects would affect the overall pollination services. Interestingly, the few studies undertaken on bees seem to indicate that spinosad is less toxic to Hymenoptera in the case of pollinators (bees) than in the case of natural enemies (parasitoids/parasitic wasps). This difference may result from different biology and feeding behaviours among the two Hymenoptera groups, with the latter showing habits that may increase either the risk of exposure to spinosad or its effect on the exposed insects.

The toxicity of spinosad depended mainly on the spinosad product used, i.e. formulated product or fruit-fly bait. Laboratory, semi-field and field risk assessment experiments have proved that spinosad bait application is safer than other spinosad application methods with regard to beneficial arthropods. This harmlessness may be due to the very low quantity of active ingredient applied in the field and to the selective attraction of the baits towards pests, although further studies are needed to confirm this result for other beneficial arthropod species.[21,27,85,170] The use of bait formulation is therefore to be considered as a valid alternative to chemical insecticides in IPM programmes. The adverse effects of spinetoram have not been sufficiently investigated yet. A first comparative study on bumblebees suggests that the use of spinetoram is safer for bumblebees by direct contact (residue) and oral exposure,[102] while two other experiments confirmed that the new spinosyn is posing adverse effects towards a mite species and two Hymenoptera parasitoid species.[58,169] Therefore, documenting the possible adverse effects of newly developed spinosyn products towards beneficial arthropods should be an important step before these products are implemented in IPM programmes and in organic farming.

In spite of the fact that most studies dealing with side effects of spinosad on beneficial arthropods have been conducted under laboratory conditions, field studies have also provided concordant results (see, for example, Mayes *et al.*,[92] Jones *et al.*,[40] Garcia Nevarez *et al.*,[137] Pereira *et al.*[135] and Hertlein *et al.*,[43] and Table 1 and supporting information Tables S6 and S7). The ecotoxicological risk assessments were based on laboratory tests where organisms were exposed under standard conditions, i.e. with constant and optimal environmental parameters. By contrast, arthropods never experience worst-case exposure in field conditions, where great fluctuations in climatic factors, as well as in food availability, are the norm. Hence, other parameters occurring in the field, such as synergistic interactions among toxicants and natural stressors,[167] multiple routes of exposure to toxicants and the presence of untreated refuge areas, should be considered in further studies. To date, various experimental assays have been developed/used with the aim of thoroughly assessing the risks posed to beneficial arthropods by spinosad-based products, e.g. route of exposure, environmental condition, duration of chronic exposure, age of the tested individuals and spinosad residues, concentrations and endpoints tested/considered.[18,39,76,78,82,85,119] The present review reports a wide variety of sublethal effects of spinosad on natural enemies. In spite of not necessarily inducing an immediate decrease in natural enemy populations (i.e. no lethal effect), spinosad can drastically affect demographic and biocontrol-related traits in predators and parasitoids. Multistep studies, aimed at following the steps in which the beneficial arthropods can be exposed to pesticides in the various crop systems (e.g. Desneux *et al.*[11,115,121] and Biondi *et al.*[39]), are therefore crucial for pesticide risk assessment.

The choice of pesticides that farmers commonly use depends on the trade-off between the costs and benefits of using the various products available. The benefit is often identified as the rapid suppression of pest populations after application of the pesticide. In conventional cropping systems, application of chemical pesticides often leads to extinction of natural enemy populations, either through the direct effect of the pesticides or through the disappearance of the food/host (i.e. the pests) to sustain natural enemies. By contrast, an increase in sustainability and profitability of using a given pesticide is directly related to its selectivity, i.e. killing the targeted pest(s) and preserving the beneficial arthropods (which could provide ecological services, such as biological control and pollination). Sustaining the efficacy of natural enemies against pests is a key component in IPM programmes and organic farming, and thus pesticide applications should mitigate the possible harmful effects to these beneficial arthropods. Given the spinosyn ecotoxicological profile, these products may be of major concern when they are supposed to be used in combination with natural enemies or in crops where pollinators are needed/released. This is increasingly important because the recent European Union directive on sustainable use of pesticides, i.e. 2009/128/EC, states that IPM should be implemented in all member states by 1 January 2014.[168] Therefore, the present authors believe that spinosad and newer spinosyns can be of use, but only with caution, in organic cropping systems and in IPM programmes in general.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Copping LG and Menn JJ, Biopesticides: a review of their mode of action and efficacy. *Pest Manag Sci* **56**:651–676 (2000).
2 Salgado VL, Studies on the mode of action of spinosad: symptoms and physiological correlates. *Pestic Biochem Physiol* **60**:91–102 (1998).
3 Thompson GD, Dutton R and Sparks TC, Spinosad – a case study: an example from a natural products discovery programme. *Pest Manag Sci* **56**:696–702 (2000).
4 Cleveland CB, Environmental and health assessments for spinosad against the backdrop of organic certification, in *Certified Organic and Biologically Derived Pesticides: Environmental, Health, and Efficacy Assessment. Symposium Series*, ed. by Felsot AJ and Racke KD. American Chemical Society, Washington, DC, pp. 109–130 (2007).
5 Crouse GD, Sparks TC, Schoonover J, Gifford J, Dripps J, Bruce T, *et al*, Recent advances in the chemistry of spinosyns. *Pest Manag Sci* **57**:177–185 (2001).
6 Sparks TC, Crouse GD, Dripps JE, Anzeveno P, Martynow J, DeAmicis CV, *et al*, Neural network-based QSAR and insecticide discovery: spinetoram. *J Comput Aided Mol Des* **22**:393–401 (2008).
7 Chloridis A, Downard P, Dripps JE, Kaneshi K, Lee LC, Min YK, *et al*, Spinetoram (XDE-175): a new spinosyn. *Proc XVI Int Plant Protection Congr*, Glasgow, UK, pp. 44–49 (2007).
8 Dripps JE, Boucher RE, Chloridis A, Cleveland CB, DeAmicis CV, Gomez LE, *et al*, The spinosyn insecticides, in *Green Trends in Insect Control*, ed. by López O and Fernández-Bolaños JG. Royal Society of Chemistry, London, UK, pp. 163–212 (2011).
9 Losey JE and Vaughan M, The economic value of ecological services provided by insects. *Bioscience* **56**:311–323 (2006).
10 Wratten SD, Gillespie M, Decourtye A, Mader E and Desneux N, Pollinator habitat enhancement: benefits to other ecosystem services. *Agr Ecosyst Environ* **159**:112–122 (2012).
11 Desneux N, Decourtye A and Delpuech JM, The sublethal effects of pesticides on beneficial arthropods. *Ann Rev Entomol* **52**:81–106 (2007).
12 Lu YH, Wu KM, Jiang YY, Guo YY and Desneux N, Widespread adoption of *Bt* cotton and insecticide decrease promotes biocontrol services. *Nature* **487**:362–365 (2012).
13 Stark JD and Banks JE, Population-level effects of pesticides and other toxicants on arthropods. *Annu Rev Entomol* **48**:505–519 (2003).
14 Stark JD, Banks JE and Vargas R, How is risk assessment: the role that life history strategies play in susceptibility of species to stress. *Proc Natl Acad Sci USA* **101**:732–736 (2004).
15 Williams T, Valle J and Viñuela E, Is the naturally derived insecticide spinosad (R) compatible with insect natural enemies? *Biocontrol Sci Techn* **13**:459–475 (2003).
16 Van Driesche RG and Bellows TS, *Biological Control.* Chapman and Hall, New York, NY (1996).
17 Hawkins BA, Cornell HV and Hochberg ME, Predators, parasitoids and pathogens as mortality agents in phytophagous insect populations. *Ecology* **78**:2145–2152 (1997).

18 Stark JD and Vargas RI, Demographic changes in *Daphnia pulex* (Leydig) after exposure to the insecticides spinosad and diazinon. *Ecotoxicol Environ Saf* **56**:334–338 (2003).

19 Duchet C, Inafuku MM, Caquet T, Larroque M, Franquet E, Lagneau C, *et al*, Chitobiase activity as an indicator of altered survival, growth and reproduction in *Daphnia pulex* and *Daphnia magna* (Crustacea: Cladocera) exposed to spinosad and diflubenzuron. *Ecotoxicol Environ Saf* **74**:800–810 (2011).

20 GF-120 naturalyte fruit fly bait. Technical bulletin, Dow Agrosciences, 7 pp. (2002).

21 Stark JD, Vargas R and Miller N, Toxicity of spinosad in protein bait to three economically important tephritid fruit fly species (Diptera: Tephritidae) and their parasitoids (Hymenoptera: Braconidae). *J Econ Entomol* **97**:911–915 (2004).

22 Williams T, Cisneros J, Penagos DI, Valle J and Tamez-Guerra P, Ultralow rates of spinosad in phagostimulant granules provide control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize. *J Econ Entomol* **97**:422–428 (2004).

23 Commission regulation (EC) No. 404/2008 of 6 May 2008 amending Annex II to Council Regulation (EEC) No. 2092/91 on organic production of agricultural products as concerns the authorization of spinosad, potassium bicarbonate and copper octanoate and the use of ethylene. *Off J Eur Commun L* **120**:8–10 (2008).

24 Weintraub PG and Mujica N, Systemic effects of spinosad insecticide on *Liriomyza huidobrensis* larvae. *Phytoparasitica* **34**:21–24 (2006).

25 Peck SI and McQuate GT, Field tests of environmentally friendly malathion replacements suppress wild Mediterranean fruit fly populations. *J Econ Entomol* **93**:280–289 (2000).

26 Vargas RI, Peck SL, McQuate GT, Jackson CG, Stark JD and Amstrong JW, Potential for area wide integrated management of Mediterranean fruit fly with a braconid parasitoid and a novel bait spray. *J Econ Entomol* **94**:817–825 (2001).

27 Urbaneja A, Chueca P, Montón H, Pascual-Ruiz S, Dembilio O, Vanaclocha P, *et al*, Chemical alternatives to malathion for controlling *Ceratitis capitata* (Diptera: Tephritidae), and their side effects on natural enemies in Spanish citrus orchards. *J Econ Entomol* **102**:144–151 (2009).

28 González-Núñez M, Pascual S, Seris E, Esteban-Durán J, Medina P, Budia F, *et al*, Effects of different control measures against the olive fruit fly (*Bactrocera oleae* (Gmel)) on beneficial arthropod fauna. Methodology and first results of field assays. *IOBC/WPRS Bull* **35**:26–31 (2008).

29 Piñero JC, Mau RFL and Vargas RI, Managing oriental fruit fly (Diptera: Tephritidae) with spinosad-based protein bait sprays and sanitation in papaya orchards in Hawaii. *J Econ Entomol* **102**:1123–1132 (2009).

30 Vayssieres JF, Sinzogan A, Korie S, Ouagoussounon I and Thomas-Odjo A, Effectiveness of spinosad bait sprays (GF-120) in controlling mango-infesting fruit flies in Benin. *J Econ Entomol* **102**:515–521 (2009).

31 Adán A, Del Estal P, Budia F, González M and Viñuela E, Laboratory evaluation of the novel naturally-derived compound spinosad against *Ceratitis capitata*. *Pestic Sci* **43**:261–268 (1996).

32 Yee WL, Oviposition in sweet cherry by reproductively mature Western cherry fruit fly (Diptera: Tephritidae) fed spinosad and neonicotinoid insecticide baits. *J Econ Entomol* **103**:379–385 (2010).

33 Gonçalves MF, Santos SAP and Monteiro Torres L, Efficacy of spinosad bait sprays to control *Bactrocera oleae* and impact on non-target arthropods. *Phytoparasitica* **40**:17–28 (2012).

34 Penagos DI, Cisneros J, Hernández O and Williams T, Lethal and sublethal effects of the naturally derived insecticide spinosad on parasitoids of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Biocontrol Sci Technol* **15**:81–95 (2005).

35 Pineda S, Schneider M, Smagghe G, Martínez AI, Del Estal P, Viñuela E, *et al*, Lethal and sublethal effects of methoxyfenozide and spinosad on *Spodoptera littoralis*. *J Econ Entomol* **100**:773–780 (2007).

36 Santis EL, Hernández LA, Martínez AM, Campos J, Figueroa JI, Lobit P, *et al*, Long-term foliar persistence and efficacy of spinosad against beet armyworm under greenhouse conditions. *Pest Manag Sci* **68**:914–921 (2012).

37 Semiz G, Cetin H, Isik K and Yanikoglu A, Effectiveness of a naturally derived insecticide, spinosad, against the pine processionary moth *Thaumetopoea wilkinsoni* Tams (Lep.: Thaumetopoeidae) under laboratory conditions. *Pest Manag Sci* **62**:452–455 (2006).

38 Arnó J and Gabarra R, Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). *J Pest Sci* **84**:513–520 (2011).

39 Biondi A, Desneux N, Siscaro G and Zappalà L, Using organic-certified rather than synthetic pesticides may not be safer for biological control agents: selectivity and side effects of 14 pesticides on the predator *Orius laevigatus*. *Chemosphere* **87**:803–812 (2012).

40 Jones T, Scott-Dupree C, Harris R, Shipp L and Harris B, The efficacy of spinosad against the western flower thrips, *Frankliniella occidentalis*, and its impact on associated biological control agents on greenhouse cucumbers in southern Ontario. *Pest Manag Sci* **61**:179–185 (2005).

41 Osman MA, Biological efficacy of some biorational and conventional insecticides in the control of different stages of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). *Plant Protec Sci* **46**:123–134 (2010).

42 Athanassiou CG, Arthur FH, Kavallieratos NG and Throne JE, Efficacy of spinosad and methoprene, applied alone or in combination, against six stored-product insect species. *J Pest Sci* **84**:61–67 (2011).

43 Hertlein MB, Thompson GD, Bhadriraju Subramanyam and Athanassiou CG, Spinosad: a new natural product for stored grain protection. *J Stored Prod Res* **47**:131–146 (2011).

44 Liu NN and Yue X, Insecticide resistance and cross-resistance in the house fly (Diptera: Muscidae). *J Econ Entomol* **93**:1269–1275 (2000).

45 Sayyed AH, Omar D and Wright DJ, Genetics of spinosad resistance in a multi-resistant field-selected population of *Plutella xylostella*. *Pest Manag Sci* **60**:827–832 (2004).

46 Hsu JC and Feng HT, Development of resistance to spinosad in oriental fruit fly (Diptera: Tephritidae) in laboratory selection and cross-resistance. *J Econ Entomol* **99**:931–936 (2006).

47 Bielza P, Quinto V, Contreras J, Torne M, Martin A and Espinosa PJ, Resistance to spinosad in the western flower thrips, *Frankliniella occidentalis* (Pergande), in greenhouses of south-eastern Spain. *Pest Manag Sci* **63**:682–687 (2007).

48 Reyes M, Franck P, Charmillot PJ, Ioriatti C, Olivares J, Pasqualin E, *et al*, Diversity of insecticide resistance mechanisms and spectrum in European populations of the codling moth, *Cydia pomonella*. *Pest Manag Sci* **63**:890–902 (2007).

49 *Arthropod Pesticide Resistance Database*. [Online]. Michigan State University (2011). Available: http://www.pesticideresistance.org [15 January 2012].

50 Sparks TC, Dripps JE, Watson GB and Paroonagian D, Resistance and cross-resistance to the spinosyns – a review and analysis. *Pestic Biochem Phys* **102**:1–10 (2012).

51 Řezáč M, Pekàr S and Stara J, The negative effect of some selective insecticides on the functional response of a potential biological control agent, the spider *Philodromus cespitum*. *BioControl* **55**:503–510 (2010).

52 Elzen GW and Pfannenstiel RS, Extreme susceptibility of *Hibana futilis* Spiderlings to selected insecticides in a laboratory bioassay. *Southw Entomol* **34**:103–106 (2009).

53 Benamu MA, Schneider MI, Pineda S, Sanchez NE and Gonzalez A, Sublethal effects of two neurotoxican insecticides on *Araneus pratensis* (Araneae: Araneidae). *Commun Agric Appl Biol Sci* **72**:557–559 (2007).

54 Villanueva RT and Walgenbach JF, Development, oviposition and mortality of *Neoseiulus fallacies* (Acari: Phytoseiidae) in response to reduced-risk insecticides. *J Econ Entomol* **98**:2114–2120 (2005).

55 Van Driesche RG, Lyon S and Nunn C, Compatibility of spinosad with predacious mites (Acari: Phytoseiidae) used to control western flower thrips (Thysanoptera: Thripidae) in greenhouse crops. *Fla Entomol* **89**:396–401 (2006).

56 Olszak RW and Sekrecka M, Influence of some insecticides and acaricides on beneficial mites and on *Coccinella septempunctata* (Coleoptera: Coccinellidae) larvae. *IOBC/WPRS Bull* **35**:101–108 (2008).

57 Rahman T, Spafforda H and Broughtonc S, Compatibility of spinosad with predaceous mites (Acari) used to control *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Pest Manag Sci* **67**:993–1003 (2011).

58 Lefebvre M, Bostanian NJ, Thistlewood HMA, Mauffette Y and Racette G, A laboratory assessment of the toxic attributes of

six 'reduced risk insecticides' on *Galendromus occidentalis* (Acari: Phytoseiidae). *Chemosphere* **84**:25–30 (2011).

59 Nasreen A, Mustafa G and Ashfaq M, Selectivity of some insecticides to *Chysoperla carnea* (Stephen) (Neuroptera: Chrysopidae) in laboratory. *Pakistan J Biol Sci* **6**:563–538 (2003).

60 Medina P, Budia F, Del Estal P and Viñuela E, Effects of three modern insecticides, pyriproxyfen, spinosad and tebufenozide, on survival and reproduction of *Chrysoperla carnea* adults. *Ann Appl Biol* **142**:55–61 (2003).

61 Mandour NS, Influence of spinosad on immature and adult stages of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). *BioControl* **54**:93–102 (2009).

62 Mahdian K, Van Leeuwen T, Tirry L and De Clercq P, Susceptibility of the predatory stinkbug *Picromerus bidens* to selected insecticides. *BioControl* **52**:765–774 (2007).

63 Myers L, Liburd OE and Arevalo HA, Survival of *Geocoris punctipes* Say (Hemiptera : Lygaeidae) following exposure to selected reduced-risk insecticides. *J Entomol Sci* **41**:57–64 (2006).

64 Studebaker GL and Kring TJ, Effects of insecticides on *Orius insidiosus* (Hemiptera: Anthocoridae), measured by field, greenhouse and Petri dish bioassays. *Fla Entomol* **86**:178–185 (2003).

65 Torres FZV, Carvalho GA, Souza JR and Rocha LCD, Seletividade de inseticidas a *Orius insidiosus*. *Bargantia* **66**:433–439 (2007).

66 Kim DS, Brooks DJ and Riedel H, Lethal and sublethal effects of abamectin, spinosad, methoxyfenozide and acetamiprid on the predaceous plant bug *Deraeocoris brevis* in the laboratory. *BioControl* **51**:465–484 (2006).

67 Nakahira K, Kashitani R, Tomoda M, Kodama R, Ito K, Yamanaka S, *et al*, Side effects of vegetable pesticides on a predatory Mirid bug, *Pilophorus typicus* Distant (Heteroptera: Miridae). *Appl Entomol Zool* **45**:239–243 (2010).

68 He YX, Zhao J, Zheng Y, Zhan Z, Desneux N and Wu KM, Lethal effect of imidacloprid on the coccinellid predator *Serangium japonicum* and sublethal effects on predator voracity and on functional response to the whitefly *Bemisia tabaci*. *Ecotoxicology* **21**:1291–1300 (2012).

69 Galvan TL, Koch RL and Hutchison WD, Effects of spinosad and indoxacarb on survival, development, and reproduction of the multicolored Asian lady beetle (Coleoptera: Coccinellidae). *Biol Control* **34**:108–114 (2005).

70 Shaw PW and Wallis DR, Susceptibility of the European earwig, *Forficula auricularia*, to insecticide residues on apple leaves. *NZ Plant Prot* **63**:55–59 (2010).

71 Moens J, De Clerq P and Tirry L, Side effects of pesticides on the larvae of the hoverfly *Episyrphus balteatus* in the laboratory. *Phytoparasitica* **39**:1–9 (2011).

72 Stara J, Ourednickova J and Kocourek F, Laboratory evaluation of the side effects of insecticides on *Aphidius colemani* (Hymenoptera: Aphidiidae), *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), and *Neoseiulus cucumeris* (Acari: Phytoseidae). *J Pest Sci* **84**:25–31 (2011).

73 Jalali MA, van Leeuwen T, Tirry L and De Clerq P, Toxicity of selected insecticides to the two-spot ladybird *Adalia bipunctata*. *Phytoparasitica* **37**:323–326 (2009).

74 Xu YY, Liu TX, Leibee GL and Jones WA, Effects of selected insecticides on *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of *Plutellaxylostella* (Lepidoptera: Plutellidae). *Biocontrol Sci Technol* **14**:713–723 (2004).

75 Takahashi Y, Kojimoto T, Nagaoka H, Takagi Y and Oikawa M, Tests for evaluating the side effects of chlorothalonil (tpn) and spinosad on the parasitic wasp (*Aphidius colemani*). *J Pestic Sci* **30**:11–16 (2005).

76 Suma P, Zappalà L, Mazzeo G and Siscaro G, Lethal and sub-lethal effects of insecticides on natural enemies of citrus scale pests. *BioControl* **54**:651–661 (2009).

77 Sattar S, Ullah F, Saljoqi AUR, Arif M and Qazi JI, Toxicity of some new insecticides against *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) under laboratory and extended laboratory conditions. *Pakistan J Zool* **43**:1117–1125 (2011).

78 Medina P, Morales J, Smagghe G and Viñuela E, Toxicity and kinetics of spinosad in different developmental stages of the endoparasitoid *Hyposoter didymator* (Hymenoptera: Ichneumonidae) and its host *Spodoptera littoralis* larvae (Lepidoptera: Noctuidae). *BioControl* **53**:569–578 (2008).

79 Haseeb M, Liu TX and Jones WA, Effects of selected insecticides on *Cotesia plutellae*, endoparasitoid of *Plutella xylostella*. *BioControl* **49**:33–46 (2004).

80 Cordero RJ, Bloomquist JR and Kuhar TP, Susceptibility of two diamondback moth parasitoids, *Diadegma insulare* (Cresson) (Hymenoptera; Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera; Eulophidae), to selected commercial insecticides. *Biol Control* **42**:48–54 (2007).

81 Ksentini I, Jardak T and Zeghal N, *Bacillus thuringiensis*, deltamethrin and spinosad side-effects on three *Trichogramma* species. *Bull Insect* **63**:31–37 (2010).

82 Williams L and Price LD, A space-efficient contact toxicity bioassay for minute Hymenoptera, used to test the effects of novel and conventional insecticides on the egg parasitoids *Anaphes iole* and *Trichogramma pretiosum*. *BioControl* **49**:163–185 (2004).

83 Dastjerdi HR, Hejazi MJ, Ganbalani GN and Saber M, Toxicity of some biorational and conventional insecticides to cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae), and its ectoparasitoid, *Habrobracon hebetor* (Hymenoptera: Braconidae). *J Entomol Soc Iran* **28**:27–37 (2008).

84 Nadel H, Johnson MW, Gerik M and Daane KM, Ingestion of spinosad bait GF-120 and resulting impact on adult *Chrysoperla carnea*. *Biocontrol Sci Technol* **17**:995–1008 (2007).

85 Michaud JP, Toxicity of fruit fly baits to beneficial insects in citrus. *J Insect Sci* **3**:1–9 (2003).

86 Ruiz L, Flores S, Cancino J, Arredondo J, Valle J, Diaz-Fleischer F, *et al*, Lethal and sublethal effects of spinosad-based GF-120 bait on the tephritid parasitoid *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae). *Biol Control* **44**:296–304 (2008).

87 Medina P, Pérez I, Budia F, Adán A and Viñuela E, Development of an extended-laboratory method to test novel insecticides in bait formulation. *IOBC/WPRS Bull* **27**:59–66 (2004).

88 Rabea EI, Nasr HM and Badawy MEI, Toxic effect and biochemical study of chlorfluazuron, oxymatrine, and spinosad on honey bees (*Apis mellifera*). *Arch Environ Contam Toxicol* **58**:722–732 (2010).

89 Hoxter KA, Benard WL and Smith GJ, XDE-105 insecticide: an acute contact toxicity study with the honey bee. Report DECO-ES-2551, Dow Chemical, Indianapolis, IN (1992).

90 Halsall N and Grey AP, Spinosad technical acute toxicity to honey bees (*Apis mellifera*). Report GHE-T_849. Dow AgroSciences, Indianapolis, IN (1998).

91 Cleveland CB, Mayes MA and Cryer SA, An ecological risk assessment for spinosad use on cotton. *Pest Manag Sci* **58**:70–84 (2002).

92 Mayes MA, Thompson GD, Husband B and Mark MM, Spinosad toxicity to pollinators and associated risk. *Rev Environ Contam Toxicol* **179**:37–71 (2003).

93 Miles M, The effects of spinosad, a naturally derived insect control agent to the honey bee. *Bull Insect* **56**:119–124 (2003).

94 Bailey J, Scott-Dupree C, Harris R, Tolman J and Harris B, Contact and oral toxicity to honey bees (*Apis mellifera*) of agents registered for use for sweet corn insect control in Ontario, Canada. *Apidologie* **36**:623–633 (2005).

95 Scott-Dupree CD, Conroy L and Harris CR, Impact of currently used or potentially useful insecticides for canola agroecosystems on *Bombus impatiens* (Hymenoptera: Apidae), *Megachile rotundata* (Hymenoptera: Megachilidae), and *Osmia lignaria* (Hymenoptera: Megachilidae). *J Econ Entomol* **102**:177–182 (2009).

96 Perina VCF, Acute contact toxicity of Tracer to honey bee (*Apis mellifera* L.). Report D.4.25/96, Dow AgroSciences, Indianapolis, IN (1996).

97 Aldershof S, Determination of the acute contact LD_{50} of spinosad (formulated as the 480 G/LSC, NAF-85) for the bumble bee *Bombus terrestris* L. Report GHE-P-7875, Dow AgroSciences, Indianapolis, IN (1999).

98 Aldershof S, Determination of the acute contact LD_{50} of spinosad (formulated as the 480 G/LSC, NAF-85) for the bumble bee *Bombus terrestris* L. Report GHE-P-7874, Dow AgroSciences, Indianapolis, IN (1999).

99 Mayer DF, Kovacs G, Brett BL and Bisabri BL, The effects of spinosad insecticide to adults of *Apis mellifera*, *Megachile rotundata* and *Nomia melanderi* (Hymenoptera: Apidae). *Int J Hortic Sci* **7**:93–97 (2001).

100 Hahne R, GF-120 (spinosad fruit fly bait): acute contact toxicity test with the honey bee, *Apis mellifera*. Report 000110, Dow Chemical, Indianapolis, IN (2000).

101 Halsall N and Grey AP, NAF-85 (480 g/l SC of Spinosad): acute toxicity to honey bees. Report GHE-T-850, Dow AgroSciences, Indianapolis, IN (1998).

102 Besard L, Mommaerts V, Abdu-Allaa G and Smagghe G, Lethal and sublethal side-effect assessment supports a more benign profile of spinetoram compared with spinosad in the bumblebee *Bombus terrestris*. *Pest Manag Sci* **67**:541–547 (2011).

103 Morandin LA, Winston ML, Franklin MT and Abbott VA, Lethal and sub-lethal effects of spinosad on bumblebees (*Bombus impatiens* Cresson). *Pest Manag Sci* **61**:619–626 (2005).

104 Palmer SJ and Krueger HO, NAF-315: a foliage residue toxicity study with the honey bee. Report 103–420, Dow Chemical, Indianapolis, IN (1997).

105 Goodwin RM and Haine HM, Effect of applying spinosad to staminate kiwifruit flowers on the survival of foraging honey bees. Report GHF-R-445, Dow AgroSciences, Indianapolis, IN (1998).

106 Kranzfelder JA, Spinosad (NAF-315): a foliage residue toxicity study with the honey bee (*Apis mellifera*). Report 990123, Dow Chemical, Indianapolis, IN (1999).

107 Fernandes MED, Fernandes FL, Picano MC, Queiroz RB, da Silva RS and Huertas AAG, Physiological selectivity of insecticides to *Apis mellifera* (Hymenoptera: Apidae) and *Protonectarina sylveirae* (Hymenoptera: Vespidae) in citrus. *Sociobiology* **51**:765–774 (2008).

108 Edwards CR, Gerber C and Hunt GJ, A laboratory study to evaluate the toxicity of the Mediterranean fruit fly, *Ceratitis capitata*, bait Success 0.02 CB, to the honey bee, *Apis mellifera*. *Apidologie* **34**:171–180 (2003).

109 Cossentine JE, Zurowski CL and Smirle MJ, Impact of spinosad on ichneumonid-parasitized *Choristoneura rosaceana* larvae and subsequent parasitoid emergence. *Entomol Exp Appl* **136**:116–122 (2010).

110 Schneider MI, Smagghe G, Gobbi A and Viñuela E, Toxicity and pharmacokinetics of insect growth regulators and other novel insecticides on pupae of *Hyposoter didymator* (Thunberg 1822) (Hym., Ichneumonidae), a parasitoid of early larval instars of Noctuid pests. *J Econ Entomol* **96**:1054–1065 (2003).

111 Schneider MI, Smagghe G, Pineda S and Viñuela E, Action of insect growth regulator insecticides and spinosad on life history parameters and absorption in third-instar larvae of the endoparasitoid *Hyposoter didymator*. *Biol Control* **31**:189–198 (2004).

112 Babul Hossain M and Poehling HM, Non-target effects of three biorationale insecticides on two endolarval parasitoids of *Liriomyza sativae* (Dipt., Agromyzidae). *J Appl Entomol* **130**:360–367 (2006).

113 Fernáàndez M, Medina P, Estal P and Viñuela E, Testing side-effects of pesticides on the most protected life stage of *Eretmocerus mundus* (Mercet) (Hymenoptera, Aphelinidae), parasitoid of the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera, Aleyrodidae) in the laboratory. *IOBC/WPRS Bull* **55**:101–107 (2010).

114 Cabral S, Soares AO and Garcia P, Voracity of *Coccinella undecimpunctata*: effects of insecticides when foraging in a prey/plant system. *J Pest Sci* **84**:373–379 (2011).

115 Desneux N, Denoyelle R and Kaiser L, A multi-step bioassay to assess the effect of the deltamethrin on the parasitic wasp *Aphidius ervi*. *Chemosphere* **65**:1697–1706 (2006).

116 Kumar P, Whitten M, Thoeming G, Borgemeister C and Poehling HM, Effects of bio-pesticides on *Eretmocerus warrae* (Hym., Aphelinidae), a parasitoid of *Bemisia tabaci* (Hom., Aleyrodidae). *J Appl Entomol* **132**:605–613 (2008).

117 Carmo EL, Bueno AF and Bueno RCOF, Pesticide selectivity for the insect egg parasitoid *Telenomus remus*. *Biocontrol* **55**:455–464 (2010).

118 Stefanello Júnior GJ, Grützmacher AD, Grützmacher DD, Dalmazo GO, Paschoal MDF and Härter WR, The effect of insecticides used in corn crops on the parasitism capacity of *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae). *Arq Inst Bio São Paulo* **75**:187–194 (2008).

119 Campos MR, Picanço MC, Martins JC, Tomaz AC and Guedes RNC, Insecticide selectivity and behavioral response of the earwig *Doru luteipes*. *Crop Prot* **30**:1535–1540 (2011).

120 Stark JD, Jepson PC and Mayer DF, Limitations to use of topical toxicity data for predictions of pesticide side-effects in the field. *J Econ Entomol* **88**:1081–1088 (1995).

121 Desneux N, Ramirez-Romero R and Kaiser L, Multi-step bioassay to predict recolonization potential of emerging parasitoids after a pesticide treatment. *Environ Toxicol Chem* **25**:2675–2682 (2006).

122 Holt KM, Opit GP, Nechols JR and Margolies DC, Testing for non-target effects of spinosad on two spotted spider mites and their predator *Phytoseiulus persimilis* under greenhouse conditions. *Exp Appl Acarol* **38**:141–149 (2006).

123 Van de Veire M, Viñuela E, Bernardo U, Tirry L, Adán A and Viggiani G, Duration of the toxicity of abamectin and spinosad on the parasitic wasp *Encarsia formosa* in Northern and Southern Europe. *IOBC/WPRS Bull* **27**:21–30 (2004).

124 Desneux N, Fauvergue X, Dechaume-Moncharmont FX, Kerhoas L, Ballanger Y and Kaiser L, *Diaeretiella rapae* limits *Myzus persicae* populations after applications of deltamethrin in oilseed rape. *J Econ Entomol* **98**:9–17 (2005).

125 Tena A, Catalán J, Monzó C, Jacas JA and Urbaneja A, Minimizing the use of pesticides to control *Pezothrips kellyanus* and their side-effects on natural enemies. *IOBC/WPRS Bull* **62**:247–253 (2011).

126 Khan I and Morse JG, Impact of citrus thrips chemical treatments on the predatory mite *Euseius tularensis*. *J Appl Entomol* **130**:386–392 (2006).

127 Van Driesche RG, Lyon S, Stanek EJ, Xu B and Nunn C, Evaluation of efficacy of *Neoseiulus cucumeris* for control of western flower thrips in spring bedding crops. *Biol Control* **36**:203–215 (2006).

128 Miles M, The effects of spinosad on beneficial insects and mites used in integrated pest management systems in greenhouses. *IOBC/WPRS Bull* **29**:53–59 (2006).

129 Vogt H, Grutzmacher A and Just J, Impact of four insecticides on the European earwig, *Forficula auricularia* L., in an apple orchard. *IOBC/WPRS Bull* **54**:141–145 (2010).

130 Arthurs SP, Lacey LA and Miliczky ER, Evaluation of the codling moth granulovirus and spinosad for codling moth control and impact on non-target species in pear orchards. *Biol Control* **41**:99–109 (2007).

131 Civolani S, Previati E, Peretto R, Pasqualini E and Leis M, Preliminary investigation on the toxicity of different formulations on some groups of beneficial arthropods in Emilia-Romagna orchards. *Ital J Agron* **2**:157–160 (2007).

132 Lakshmi VJ, Krishnaiah NV and Katti GR, Potential toxicity of selected insecticides to rice leafhoppers and planthoppers and their important natural enemies. *J Biol Control* **24**:244–252 (2010).

133 Srivastava M, Bosco L, Funderburk J, Olson S and Weiss A, Spinetoram is compatible with the key natural enemy of *Frankliniella* species thrips in pepper. *Plant Health Progress* DOI:10.1094/PHP-2008-0118-02-RS (2008).

134 Galvan TL, Koch RL and Hutchinson WD, Toxicity of commonly used insecticides in sweet corn and soybean to multicolored Asian lady beetle (Coleoptera: Coccinellidae). *J Econ Entomol* **98**:780–789 (2005).

135 Pereira JL, Picanco MC, da Silva AA, de Barros EC, da Silva RS, da Silva Galdino TV, *et al*, Ants as environmental impact bioindicators from insecticide application on corn. *Sociobiology* **55**:153–164 (2010).

136 Koppel AL, Herbert DAJ, Kuhar TP, Malone S and Arrington M, Efficacy of selected insecticides against eggs of *Euschistus servus* and *Acrosternum hilare* (Hemiptera: Pentatomidae) and the egg parasitoid *Telenomus podisi* (Hymenoptera: Scelionidae). *J Econ Entomol* **104**:137–142 (2011).

137 Garcia Nevarez G, Quinones Pando FJ, Barajas Ontiveros CG and Sanchez NC, Dispersal of *Trichogramma* spp. on pecan trees and its susceptibility to selective insecticides. *Southw Entomol* **34**:319–326 (2009).

138 Wise JC, Jenkins PE, Poppen RV and Isaacs R, Activity of broad-spectrum and reduced-risk insecticides on various life stages of cranberry fruitworm (Lepidoptera: Pyralidae) in highbush blueberry. *J Econ Entomol* **103**:1720–1728 (2010).

139 Parker RD, Falconer LL and Livingston SD, *Evaluation of Insecticides for Control of Insects in Stored Sorghum through Nineteen Months: Part 1. Results of Insect Control Evaluations on Corn, Sorghum, and Cotton in Texas Coastal Bend Counties and Gulf Coast Crop Hybrid/Variety Comparisons.* [Online]. Texas Cooperative Extension, Texas A&M University (2004). Available: http://varietytesting.tamu.edu/corn/otherpublications/2004cgdhbook.pdf, pp. 54–79 Accessed [23 August 2012].

140 Parker RD, Falconer LL and Livingston SD, *Evaluation of Insecticides for Control of Insects in Stored Sorghum through Twenty-Four Months: Part 2. Results of Insect Control Evaluations on Corn,*

Sorghum, and Cotton in Texas Coastal Bend Counties and Gulf Coast Crop Hybrid/Variety Comparisons. [Online]. Texas Cooperative Extension, Texas A&M University (2004). Available: http://varietytesting.tamu.edu/corn/otherpublications/2004cgdhbook.pdf, pp. 80–95 Accessed [23 August 2012].

141 Thomas DB and Mangan RL, Nontarget impact of spinosad GF-120 bait sprays for control of the Mexican fruit fly (Diptera: Tephritidae) in Texas citrus. *J Econ Entomol* **98**:1950–1956 (2005).

142 Chapman AV, Kuhar TP, Schultz PB, Leslie TW, Fleischer SJ, Dively GP, *et al*, Integrating chemical and biological control of European corn borer in bell pepper. *J Econ Entomol* **102**:287–295 (2009).

143 Awkerman JA, Marshall MR, Williams AB, Gale GA, Cooper RJ and Raimondo S, Assessment of indirect pesticide effects on worm-eating warbler populations in a managed forest ecosystem. *Environ Toxicol Chem* **30**:1843–1851 (2011).

144 Vinall S, A semi-field test to evaluate the effects of Spinosad 480 SC (NAF-85), a suspension concentrate formulation containing 480 g/l DE-105, on the honey bee, *Apis mellifera* (Hymenoptera: Apidae). Report GHE-P-7111, Dow AgroSciences, Indianapolis, IN (2000).

145 Halsall N, A semi-field test to evaluate the effects of multiple applications of spinosad 480 SC (NAF-85), a suspension concentrate formulation containing 480 g/l DE-105, on the honey bee *Apis mellifera* (Hymenoptera: Apidae). Report EA01T6P024, Dow AgroSciences, Indianapolis, IN (2001).

146 Aldershof S, Effect of spinosad (formulated as 480 g/l SC, NAF-85) on the bumble bee *Bombus terrestris* determined under greenhouse conditions. Report GHE-P-8398, Dow AgroSciences, Indianapolis, IN (2000).

147 Kaneshi K, Toxicity and side effects of spinosad to bumblebees, *Bombus terrestris*, in lab and greenhouse conditions. Report GHF-P-2265, Dow AgroSciences, Indianapolis, IN (2000).

148 Kaneshi K, Toxicity and side effects of spinosad to honey bees, *Apis mellifera*, in lab and greenhouse conditions. Report GHF-P-2264, Dow AgroSciences, Indianapolis, IN (2000).

149 Forey D, Evaluation of Success 2SC (NAF-315) on honey bees. Report 116-99, Dow AgroSciences, Indianapolis, IN (1999).

150 Mayer DF, Honey bee field investigation of mitigation methods for the use of Success on alfalfa. Report WSU 99-003, Dow AgroSciences, Indianapolis, IN (1999).

151 Kirkland RL, Evaluation of Success 2SC on honey bees. Report 219-99, Dow AgroSciences, Indianapolis, IN (1999).

152 Taylor MA and Goodwin RM, The effect of Success Naturalyte on honey bee (*Apis mellifera*) brood when applied as a single application to avocado trees in full bloom. Report GFH-R-576, Dow AgroSciences, Indianapolis, IN (2000).

153 Burns RE, Harris DL, Moreno DS and Eger JE, Efficacy of spinosad bait sprays to control Mediterranean and Caribbean fruit flies (Diptera: Tephritidae) in commercial citrus in Florida. *Fla Entomol* **84**:672–678 (2001).

154 Spencer JP, Ibarra J and Rendon PA, Effect of spinosad on honey bees (Hymenoptera: Apidae) in Guatemala. *Southw Entomol* **28**:211–216 (2003).

155 Mangan RL and Moreno AT, Honey bee foraging preferences, effects of sugars and fruit fly toxic bait components. *J Econ Entomol* **102**:1472–1481 (2009).

156 Zehnder G, Gurr GM, Kuhne S, Wade MR, Wratten SD and Wyss E, Arthropod pest management in organic crops. *Ann Rev Entomol* **52**:57–80 (2007).

157 Zappalà L, Siscaro G, Biondi A, Mollá O, González-Cabrera J and Urbaneja A, Efficacy of sulphur on *Tuta absoluta* and its side effects on the predator *Nesidiocoris tenuis*. *J Appl Ent* **136**:401–409 (2012).

158 Commission Regulation (EEC) N 2092/91 of 24 June 1991 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs. *Off J Eur Commun L* **198**:1–15 (1991).

159 National Organic Standards Board Technical Advisory Panel Review: Spinosad. USDA National Organic Program (2002).

160 Schmutterer H, Properties and potential of natural pesticides from the Neem tree, *Azadirachta indica*. *Ann Rev Entomol* **35**:271–297 (1990).

161 Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV and Greenamyre JT, Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* **3**:1301–1306 (2000).

162 Hanley TR, Breslin WJ, Quast JF and Carney EW, Evaluation of spinosad in a two-generation dietary reproduction study using Sprague–Dawley rats. *Toxicol Sci* **67**:144–152 (2002).

163 Zanuncio TV, Serrao JE, Zanuncio JC and Guedes RNC, Permethrin-induced hormesis on the predator *Supputius cincticeps* (Stal 1860) (Heteroptera: Pentatomidae). *Crop Prot* **22**:941–947 (2003).

164 Ramirez-Romero R, Desneux N, Decourtye A, Chaffiol A and Pham-Delègue MH, Does Cry1Ab protein affect learning performance of the honey bee *Apis mellifera* L. (Hymenoptera, Apidae)? *Ecotox Environ Saf* **70**:327–333 (2008).

165 Han P, Niu CY, Lei CL, Cui JJ and Desneux N, Use of an innovative T-tube maze assay and the Proboscis Extension Response assay to assess sublethal effects of GM products and pesticides on learning capacity of the honey bee *Apis mellifera* L. *Ecotoxicology* **19**:1612–1619 (2010).

166 Menzel R, Associative learning in honey bees. *Apidologie* **24**:157–168 (1993).

167 Holmstrup M, Bindesbol AM, Oostingh GJ, Duschl A, Scheil V, Kohler HR, *et al*, Interactions between effects of environmental chemicals and natural stressors: a review. *Sci Total Environ* **408**:3746–3762 (2010).

168 Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides. *Off J Eur Commun L* **309**:71–86 (2009).

169 Hernàndez R, Guo K, Harris K and Liu TX, Effects of selected insecticides on adults of two parasitoid species of *Liriomyza trifolii*: *Ganaspidium nigrimanus* (Figitidae) and *Neochrysocharis formosa* (Eulophidae). *Insect Sci* **18**:512–520 (2011).

170 Zhou XW, Niu CY, Han P and Desneux N, Field evaluation of attractive lures for the fruit fly *Bactrocera minax* (Diptera: Tephritidae) and their potential in spot sprays in Hubei province (China). *J Econ Entomol* **105**:1277–1284 (2012).

171 Desneux N, Wajnberg E, Wyckhuys KAG, Burgio G, Arpaia S, *et al*, Biological invasion of European tomato crops by *Tuta absoluta*: Ecology, history of invasion and prospects for biological control. *J Pest Sci* **83**:197–215 (2010).

172 Desneux N, Luna MG, Guillemaud T and Urbaneja A, The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond – the new threat to tomato world production. *J Pest Sci* **84**:403–408 (2011).

Chapter IX

9. Discussion

Exotic species and in particular the invasive ones, represent a major threat to agronomic ecosystems and since the time of *T. absoluta* initial detection in Europe, this pest has caused serious damages to tomato cultivation in the newly invaded areas. Currently it is considered a key agricultural threat to the Western Palaearctic tomato industry (Desneux et al. 2011). The invasion of *T. absoluta* has prompted applied research in all the invaded countries to undertake IPM programmes implementation as primary control strategy since the pest invasion.

This implied an increasing need of knowledge on various IPM related aspects therefore, in the last few years a growing body of literature is aimed at addressing this issue. In particular, several studies has been recently published on *T. absoluta* biology (Caparros Megido et al. 2012) and on its distribution and infestation levels (Desneux et al. 2011, Abbes et al. 2012, Guedes and Picanço 2012, Siscaro et al. 2012, Speranza and Sannino 2012, Zlof and Suffert 2012); on its susceptibility to various insecticides (Gonzáles-Cabrera et al. 2012, Bassi et al. 2012, Braham et al. 2012, Dağl et al. 2012, Roditakis et al. 2012a, 2012b, Tomé et al. 2012, Zappalá et al. 2012); on the development of insecticide resistance (Silva et al. 2011, Gontijo et al. 2012, Haddi et al. 2012); on the biotechnical tools for its control (Cagnotti et al. 2012, Chermiti and Abbes 2012, Cocco et al. 2012), as well as on the ecological services spontaneously provided by indigenous natural enemies, namely *fortuitous biological control agents* (Urbaneja et al. 2009, Loni et al. 2011, Al-Jboory et al. 2012, Boualem et al. 2012, Bueno et al. 2012, Cabello et al. 2012, Chailleux et al. 2012, Ferracini et al. 2012, Luna et al. 2012, Nannini et al. 2012, Urbaneja et al. 2012).

However, in spite of all these efforts, there is little evidence that IPM has been implemented to significant extent in *T. absoluta* management both in the newly invaded countries and in the area of origin. Therefore, the occurrence of *T. absoluta* at increasing population levels led growers to extensively use insecticides, which could disrupt the ecological services provided by natural enemies in tomato crops, spontaneously or by means of artificial releases (Stark and Banks 2003, Desneux et al 2007, Arnó and Gabarra 2011). Moreover, the European Directive 2009/128/EC (EEC/CEE, 2009b), also known as the *Sustainable use of pesticides Directive*, aims to make European agriculture less dependent on the use of pesticides. Each European member state has to develop a national action plan for the safe use of pesticides and the application of IPM should become the compulsory crop protection method from January 1st 2014.

In this framework, a key role could be played by indigenous biological control agents and their knowledge together with the strategies to conserve them have to be considered a priority in the implementation of exotic pest management (van Lenteren and Woets 1988, Wearing 1988, Croft 1990, Kogan 1998, Myers et al. 2000, Viggiani 2000, Desneux et al. 2007, Bale et al. 2008, Desneux et al. 2010, Pyšek and Richardson 2010, Suckling and Brockerhoff 2010, Ragsdale et al. 2011, Mollà et al. 2011, Calvo et al. 2012, Urbaneja et al. 2012).

9.1. *Tuta absoluta* indigenous parasitoids

The results obtained from the survey of indigenous parasitoids of *T. absoluta* conducted in a variety of different habitats (open field and protected tomato crops, other solanaceous crops and spontaneous vegetation) in Southern Italy, provided novel and relevant information contributing to the knowledge of this exotic pest in the Mediterranean basin. Particularly, some major points are worth being mentioned. First, a prompt adaptation of native parasitoids to the new invasive host was observed, as highlighted by the natural parasitism recorded few years after the first detection of the moth. Overall six families (Ichneumonidae, Braconidae, Eulophidae, Elasmidae, Pteromalidae and Trichogrammatidae) with 13 genera and 10 identified species were recorded and in particular, the families Eulophidae and Braconidae were the most abundant in terms of number of species recovered. This parasitoid complex is typical of parasitisation pattern on exotic invasive herbivorous species, performed mainly by generalist idiobionts with relatively low levels of parasitisation in open field (Cornell and Hawkins, 1993). However, the detection of seven new associations between *T. absoluta* and the species *Diadegma pulchripes* (Kokujev), *Bracon osculator* (Nees), *B.* (Habrobracon) *nigricans* Szépligeti, *Pnigalio soemius* (Walker), *P. cristatus* (Ratzeburg), *P. incompletus* (Boucek) and *Halticoptera aenea* (Walker), is noteworthy. On the other hand, the low parasitism rate found in this survey may not support the role of the indigenous parasitoid community in sucessifully controlling *T. absoluta*. Additionally, although with low parasitism rates, *Necremnus* sp. near *artynes* was recovered in all the monitored regions and it was the only species, among those recovered, able to build up its populations in protected tomato crops where pesticides where applied. This finding should be taken into account when applying tomato IPM programmes that should emphasize the role of natural mortality factors by selective pesticides application.

Some of the recovered species were also found developing on *T. absoluta* in other infested areas by other researchers (Al-Jboory et al. 2012, Boualem et al. 2012, Ferracini et al. 2012, Urbaneja et al. 2012). In particular, *B. nigricans* is widely distributed in the whole Palaearctic region (Yu and van Achterberg 2010) and was associated to the new host in Jordan by Al-Jboory et al. (2012) and in Spain by Urbaneja et al. (2012). Most of the available data on this species rely only on taxonomic aspects and faunistic surveys. Therefore, I documented the young instars development and the adult lifespan reproduction activity at constant temperature of this species in laboratory conditions. *Bracon nigricans* was proved to be an idiobiont, synovigenic, gregarious, ectoparasitoid with a slightly male-biased progeny sex-ratio.

The main result of this study, in terms of pest biocontrol activity, is that *B. nigricans* female adults showed a high rate of non-reproductive killing behavior, namely numerous host larvae were killed owing to host feeding and host stinging during the whole female lifespan. This activity shows how the biological control service of this parasitoid might be underestimated considering only the rate of parasitism or the emerged adults. Indeed, *B. nigricans* kill rate ($K_m = 0.121$) is higher than its intrinsic rate of natural increase ($r_m = 0.052$). These results remark that despite the population dynamics theories, *destructive host feeder* species may be more effective biological control agents than other parasitoids (Jervis et al. 1996). Moreover, *B. nigricans* showed to be a typical synovigenic species and such parasitoids are long-living, able to reproduce at lower host densities and, in the case of anhydropic species (as *B. nigricans*), to conserve reproductive material in relation to host density notably readsorbing unlaid eggs. In addition, synovigenic species being not time-limited are also able to spend more time in foraging and host searching behaviours. Indeed, studies on leafminer communities indicate that generalist idiobiont parasitoids may have broad host ranges and parasitize almost all hosts of suitable size in a given search environment (Pennacchio and Strand 2006). Parasitoid females kill hosts with an injection of venom causing an irreversible paralysis of the host larva. Host killing is followed by host stinging and/or host feeding and/or oviposition. Although field studies would be needed, the data obtained so far in laboratory conditions suggest that this parasitoid could be considered a potential key biological control agent of *T. absoluta* in the newly invaded areas. In conclusion, information collected in this study, supported by the theory on recruitment and accumulation of native parasitoid species on introduced herbivores (Cornell and Hawkins, 1993), open to interesting perspective on *T. absoluta* conservation biocontrol in the Mediterranean basin.

9.2. Side effects of pesticide used in tomato crops

The choice of pesticides that farmers commonly use depends on the trade-off between costs and benefits of applying the available agrochemicals. The benefit is often identified as the rapid suppression of pest populations after application of the pesticide. In conventional cropping systems, application of chemical pesticides often leads to extinction of natural enemy populations, either through the direct effect of the pesticides or through the disappearance of the food/host (i.e. the pests) needed to sustain natural enemies. By contrast, an increase in sustainability and profitability of using a given pesticide is directly related to its selectivity, i.e. killing the targeted pest(s) and preserving beneficial arthropods, which could provide ecological services, such as biological control. Preserving the efficacy of natural enemies against pests is a key component in IPM programmes, and thus pesticide applications should mitigate the possible harmful effects to these beneficial arthropods. Indeed, better knowledge of the risks associated with specific pesticides toward natural enemies is of primary importance when incorporating them in IPM programmes.

With this purpose, I studied the impact on natural enemies of commonly used pesticides in organic and/or conventional tomato cropping systems, including insecticides that have been increasingly used to control the tomato borer.

The results of the preliminary assessment of the compatibility of sulphur compounds (mineral fungicide/acaricide) showed that dustable powder formulation significantly lowered the survival of *N. tenuis* adults and its fertility when the predator was exposed to fresh residues of the compound, while these effects disappeared 7 d after the treatment. In addition, it was effective in reducing the number of eggs laid (i.e. oviposition repellence) by the pest on treated plants. Therefore, if pest control strategies are based on inoculative releases of *N. tenuis* (Molla et al. 2011, Calvo et al. 2012, Urbaneja et al 2012), our results on the side effects suggest that sprinkling dustable sulphur must be terminated at least 7 days before releasing the predator. The same effect, i.e. lethal and sublethal effect on the reproduction was obtained testing dustable powder sulphur on *O. laevigatus.*

The results of this last study, in which 14 pesticides were tested, demonstrated that lethal and sublethal effects of different types of pesticide residues (both in terms of chemical family and of toxicity persistence) on the generalist predator *O. laevigatus* varied widely, including among pesticides belonging to the same product group (e.g. biopesticides). *Orius laevigatus* was very susceptible to abamectin even 14 d after pesticide application, notably because of high mortality induced in adults. Abamectin proved to be not compatible with this predator for IPM. Emamectin benzoate, metaflumizone and spinosad were less toxic but still induced high levels of mortality and reduced offspring produced, even in case of exposure to 7-d old residues. Finally, chlorantraniliprole (rynaxypyr), *Bt*, indoxacarb, wettable sulphur, mineral oil and para-menthene, proved to be harmless showing mortality and reproductive capacity levels similar to what was recorded in untreated control group. Taken as a whole, the results of this study show that side effects of pesticides can vary largely depending upon various factors studied, like endpoint considered (lethal vs. sublethal), pesticide chemical family and pesticide category. Consequently, comprehensive and specific risk assessment should be undergone before implementing any IPM programmes.

Besides, the experimental design used showed to be a valid tool to demonstrate contrasting effects between lethal and sublethal effects among various pesticides, notably because predators had the possibility to avoid being permanently in contact with pesticide-treated plant by walking on refuge areas (in this case untreated areas of the experimental arena). Since individuals also had to oviposit on plant substrate, this could lead to chronic exposure which is comparable to what usually happens in field conditions. Alternatively, a strong repellent effect can prevent individuals from being in contact with pesticides, and it could ultimately lead to reduced number of eggs laid and subsequent offspring production.

I used the same experimental setup to expose the *B. nigricans* adults to the residues of the biopesticides previously tested on the predator. The obtained results, jointly to those obtained treating topically the cocooned pupae of the wasp, showed that spinosad, even ten days after the treatment, has a very strong acute toxicity on the wasp making it impossible to assess the sublethal effects and proving its incompatibility with the parasitoid activity. By contrast, the results obtained testing the *Bt* preparation were very close to those of the untreated control. Whereas, all the other tested biopesticides (except spinosad) were proved to be *slower acting* because of the absence of acute toxicity and of their multiple sublethal effects that impaired all the observed physiological and behavioural traits. These results were used jointly with the *B. nigricans* life table data of a control population, previously studied, to develop a population-growth model. The demographical toxicology data highlighted how these pesticides could impact the parasitoids long term population in relation to its life history parameters. The multiple sublethal effects caused by emamectin benzoate and abamectin may result in a potential extinction of *B. nigricans* population in the treated environment (i.e. r_m values close to 0). Azadiractin, the preparation of borax plus citrus oil and *Bt* application let the parasitoid population grow after exposure to the pesticides and, considering that these pesticides are able to decrease the pest population level, this aspect could be determinant in the post treatment parasitoid/pest demographical balance. Field studies may always be necessary to validate the effects these pesticides have on the parasitoid activity in pest control, due to the natural mortality factors and species interactions that are not considered in this experiment. Nevertheless, risk prediction models from laboratory data are a necessity in the rational selection of the insecticides to be used against *T. absoluta* for successful IPM programmes. On the other hand, our results indicate that more ecologically relevant measures of effect, such as delays in population growth that are based on demography, may advance our knowledge of pesticide impacts on populations of natural enemy species.

However, spinosyn-based products (mostly spinosad) have been widely recommended by plant protection extension specialists and agribusiness companies owing to their property as broad-spectrum insecticides. Spinosad is a powerful insecticide of microbial origin that controls or suppresses many pest insects from different insect orders/families in various cropping systems. Following this worldwide adoption of spinosad-based products, a growing number of ecotoxicological studies have focused on the risk assessment of spinosad towards beneficial arthropods. Studies have demonstrated that spinosad can affect various physiological and behavioural traits of beneficial arthropods, although the occurrence and intensity of the observed effects depend on concentration, exposure method and beneficial considered. Although spinosyns have a great lethal (in 71% of the laboratory tests) and sublethal (in 88% of the laboratory tests) effects on arthropod predators, Hymenoptera parasitoids order are clearly the most susceptible group, with high levels of mortality (in 100% of the cases in laboratory tests), marked typical poisoning symptoms and reduced mobility after exposure to spinosad. In spite of the fact that most studies on side effects of spinosad on beneficial arthropods have been conducted under laboratory conditions, field studies have also provided concordant results. Given the spinosyn ecotoxicological profile, these products may be of major concern when they are supposed to be used in combination with natural enemies.

9.3. Conclusion

The main aim of the agriculture is to provide human beings with healthier and pure foodstuffs. Hence, particularly in sustainable agriculture applications, emphasis must be put on biological control methods that take part, in combination with selective pesticides, to IPM packages. The results obtained in this research fit with this concept providing key elements to establish the scientific bases of rational and suitable *T. absoluta* management strategies.

Parasitic Hymenoptera are some of the most abundant natural enemies on cultivated crops and the data collected in this research on the adaptation of indigenous parasitoids to a new host, jointly with the biological and behavioural characterization of the braconid *B. nigricans*, are useful in understanding the ecological services that they can provide in *T. absoluta* populations control. Indeed, this study represents the first step towards establishing the scientific basis for the effective inclusion of *B. nigricans* in integrated *T. absoluta* control programmes in the areas newly invaded by this pest. The role of the recovered parasitoids in the regulation of *T. absoluta* populations remains still uncertain, but much evidence for its importance as a regulatory factor comes from biological control programmes. Certainly, the importance of natural control of insect pests worldwide, in all situations, cannot be over emphasised. Biological, cultural and protective methods can be adopted to enhance the activity of parasitic Hymenoptera; among them the use of selective pesticides to control pests and disease is nowadays the more realistic option.

The ecotoxicological part of this research provides information that could be useful for IPM programmes in identifying and avoiding products that may prevent natural enemy populations to build up because of side effects on their reproductive capacity and survival. These products may, in addition to compromising the efficacy of IPM programmes, prevent efficient colonization or re-colonization of treated crops, particularly when pesticides are highly persistent. Interestingly, avermectin-based biopesticides (i.e. avemerctin and emamectin benzoate) and spinosad fall in this last category. These results indicate that agrochemicals that are classified as biopesticides can easily be of major concerns when they are supposed to be used in combination with natural enemies both spontaneously present in the field or artificially released. Unfortunately, a similar situation applies to pesticides that are recommended also for organic cropping systems and therefore that are usually thought to be harmless for non-target arthropods. This is the case of spinosad, which has been classified as an environmentally-safe product and has been embraced by IPM practitioners as a biorational pesticide. However, present results suggest that other pesticides with higher selectivity, such as the *Bt* formulation, should be preferred for a successful inclusion in IPM packages.

References

Abbes K., Harbi A., and Chermiti B., 2012, The tomato leafminer *Tuta absoluta* (Meyrick) in Tunisia: current status and management strategies. EPPO Bull. 42:226-233

Al-Jboory I.J., Katbeh-Bader A., and Al-Zaidi S., 2012, First observation and identification of some natural enemies collected from heavily infested tomato by *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Jordan. Middle East J. Sci. Res. 11: 435-438

Arnó J., and Gabarra R., 2011, Side effects of selected insecticides on the *Tuta absoluta* (Lepidoptera: Gelechiidae) predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae). J. Pest Sci. 84: 513-520

Askew R.R., and Shaw M.R., 1986, Parasitoid communities: their size, structure and development. Insect Parasitoids. Waage J.K., and Greathead D. (Eds), pp. 225-264, Academic Press, London

Bassi A., Rison J.L., Roditakis E., and Sannino L., 2012, Chlorantraniliprole (Rynaxypyr$^{®}$, Coragen$^{®}$, Altacor$^{®}$) key features for sustainable control of *Tuta absoluta*. IOBC/WPRS Bull. 80:193-198

Braham M., Glida-Gnidez H. and Hajji L., 2012, Management of the tomato borer, *Tuta absoluta* in Tunisia with novel insecticides and plant extracts. EPPO Bull. 42:291-296

Baker R.H.A., Battisti A., Bremmer J., Kenis M., Mumford J., Petter F., Schrader G., Bacher G., De Barro P., Hulme P. E., Karadjova O., Lansink A.O., Pruvost O., Py P., Roques A., Baranchikov Y., and Sun J.H., 2009, PRATIQUE: A research project to enhance pest risk analysis techniques in the European Union. EPPO/OEPP Bull. 39:87-93

Bajwa W.I., and Kogan M., 2002, Compendium of IPM Definitions (CID), What is IPM and how is it defined in the Worldwide Literature? IPPC Publication 998, 15pp. Available on the World Wide Web: http://www.ippc.orst.edu/IPMdefinitions/ [accessed on 4 December 2012]

Bale J.S, van Lenteren J. C., and Bigler F., 2008, Biological control and sustainable food production. Phil. Trans. R. Soc. B 363:761-776

Bellows T.S., and Fisher T.W. (Eds), 1999, Handbook of biological control. San Diego, CA, Academic Press

Bigsby H. and Whyte C., 2001, Quantifying phytosanitary barriers to trade. In N.Hooker and E. Murano (Eds.), Interdisciplinary Food Safety Research. New York, NY: CRC Press.

Boller E.F., Avilla J., Jörg E., Malavolta C., Wijnands F.G. and Esbjerg P.(Eds.), 2004, Integrated Production: Principles and technical Guidelines. 3rd edition. IOBC\WPRS Bulletin 27:1-49

Boualem M., Allaoui H., Hamadi R., and Medjahed M., 2012, Biologie et complexe des ennemis naturels de *Tuta absoluta* a Mostaganem (Algérie), EPPO Bull. 42 :268-274

Bueno V.H.P., van Lenteren J.C., Lins J.C., Calixto A.M., Montes F.C., Silva D.B., Santiago L.D., and Pérez L.M., 2012, New records of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) predation by Brazilian Hemipteran predatory bugs. J. Appl. Entomol. in press doi: 10.1111/jen.12017

Burton O.J., Phillips B.L., and Travis J.M.J., 2010, Trade-offs and the evolution of life-histories during range expansion. Ecol. Lett. 13:1210-1220

Cabello T., Gámez M., Varga Z., Garay J., Carreño R., Gallego J. R., Fernández F. J., and E. Vila, 2012, Selection of *Trichogramma* spp. (Hym.: Trichogrammatidae) for the biological control of *Tuta absoluta* (Lep.: Gelechiidae) in greenhouses by an entomo-ecological simulation model. IOBC/WPRS Bull. 80:171-176

Cabral S., Soares A.O., and Garcia P., 2011, Voracity of *Coccinella undecimpunctata*: effects of insecticides when foraging in a prey/plant system. J. Pest. Sci. 84:373-379

Cagnotti C. L., Viscarret M. M., Riquelme M. B., Botto E. N., Carabajal L. Z., Segura D. F., and López S. N., 2012, Effects of X-rays on *Tuta absoluta* for use in inherited sterility programmes. J. Pest Sci. 85:413-421

Caparros Megido R., Haubruge E., and Verheggen F. J., 2012, First evidence of deuterotokous parthenogenesis in the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). J. Pest Sci. 85:409-412

Candolfi M.P., Blümel S., Forster R., Bakker F.M., Grimm C., Hassan S.A., Heimbach U., Mead-Briggs M.A., Reber B., Schmuck R., and Vogt H., 2000, Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/wprs, Gent, pp. 158

Caceres S., 1992, La polilla del tomate en Corrientes. Biologıa y control. Estacion Experimental Agropecuaria Bella Vista, INTA

Carson R., 1962, Silent Spring. Boston, MA: Houghton Mifflin. 368 pp

Castle S. and Bentley W., 2009, In Focus: 50 years of the integrated control concept in arthropod IPM, Pest Manag. Sci. 65: 1265–1266

CBD, 1992, The convention on biological diversity. Available on the World Wide Web: http://www.cbd.int/. [Accessed 4 December 2012]

Calvo F.J., Lorente M.J., Stansly P.A., Belda J.E., 2012, Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisa tabaci* in greenhouse tomato. Entomol. Exp. Apll. 143:111-119

Chailleux A., Desneux N., Seguret J., Do Thi Khanh H., Maignet P., and Tabone E., 2012, Assessing European egg parasitoids as a mean of controlling the invasive South American tomato pinworm *Tuta absoluta*. PLoS ONE 7: e48068, doi:10.1371/journal.pone.0048068

Chambers R.J., Long S., and Heyler N.L., 1993, Effectiveness of *Orius laevigatus* (Hem.: Anthocoridae) for the control of *Frankliniella occidentalis* on cucumber and pepper in the UK. Biocontrol Sci. Technol. 3:295-307

Chermiti B., and Abbes K., 2012, Comparison of pheromone lures used in mass trapping to control the tomato leafminer *Tuta absoluta* (Meyrick, 1917) in industrial tomato crops in Kairouan (Tunisia). EPPO Bull. 42:241-248

Clavero M., and Garcia-Berthou, E., 2005. Invasive species are a leading cause of animal extinctions. Trends Ecol. Evol. 20:110

Cock M.J.W., van Lenteren J.C., Brodeur J., Barratt B.I.P., Bigler F., Bolckmans K., Consoli F.L., Haas F., Mason P.G., and Parra J.R.P., 2010, Do new Access and Benefit Sharing procedures under the Convention on Biological Diversity threaten the future of biological control? BioControl 55:199-218

Cocco A., Deliperi S., and Delrio G., 2012. Control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in greenhouse tomato crops using the mating disruption technique. J. Appl. Entomol. in press doi: 10.1111/j.1439-0418.2012.01735.x

Collier T., and van Steenwyk R., 2004, A critical evaluation of augmentative biological control. Biological Control 31:245-256

Cornell H.V., and Hawkins B.A., 1993, Accumulation of native parasitoid species on introduced herbivores: a comparison of hosts as natives and hosts as invaders. Amer. Natural. 141:847-65 Croft B.A., and Brown A.W.A., 1975, Responses of arthropod natural enemies to insecticides. Annu. Rev. Entomol. 20: 285-335

Croft B.A., 1990, Arthropod Biological Control Agents and Pesticides. New York: Wiley.723 pp.

Dağlı F., Ikten C., Sert E., and Bolucek. E., 2012, Susceptibility of tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) populations from Turkey to 7 different insecticides in laboratory bioassay

DeBach P., 1964, Biological control of insect pests and weeds. London, UK: Chapman and Hall

Desneux N., Denoyelle R., and Kaiser L., 2006a, A multi-step bioassay to assess the effect of the deltamethrin on the parasitic wasp *Aphidius ervi*. Chemosphere 65:1697-1706

Desneux N., Ramirez-Romero R., and Kaiser L., 2006b, Multi-step bioassay to predict recolonization potential of emerging parasitoids after a pesticide treatment. Environ. Toxicol. Chem. 25:2675-2682

Desneux N., Decourtye A., and Delpuech J.M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81-106

Desneux N., and O'Neil R.J., 2008, Potential of an alternative prey to disrupt predation of the generalist predator, *Orius insidiosus*, on the pest aphid, *Aphis glycines*, via short-term indirect interactions. Bull. Entomol. Res. 98:631-639

Desneux, N., Wajnberg, E., Wyckhuys, K.A.G., Burgio, G., Arpaia, S., Narvaez-Vasquez C.A., Gonzalez-Cabrera J., Catalan Ruescas D., Tabone E., Frandon J., Pizzol J., Poncet C., Cabello T., and Urbaneja A., 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. J. Pest Sci. 83, 197-215

Desneux N., Luna M.G., Guillemaud T., and Urbaneja A., 2011, The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. J. Pest Sci. 84:403-408

EEC/CEE, 2009a. Regulation 2009/1107/EC of the European parliament and of the council of 21 October 2009, concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Off. J. Eur. Commun. L 309:1-50

EEC/CEE, 2009b, Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009, establishing a framework for Community action to achieve the sustainable use of pesticides. Off. J. Eur. Commun. L 309:71-86

Ehler L.E., 2006, Integrated pest management (IPM): definition, historical development and implementation, and the other IPM. Pest Manag. Sci. 62:787-789

Ehlers R.U. (Ed), 2011, Regulation of Biological Control Agents, 1st Edition, Springer 417 pp

Evans, E., Spreen, T., and Knapp, J., 2002, Economic issues of invasive pests and diseases and food safety. The 2nd International Agricultural Trade and Policy Conference, Gainesville, FL

Evans E.E., 2003, Economic Dimensions of Invasive Species, Choices, Second Quarter 2003: 5-10.

FAO, 1967, Report of the first session of the FAO Panel of Experts on Integrated Pest Control, Rome (Italy), Sept. 18-22, 1967, 19 pp

FAOSTAT, 2011, Production. Crops. Available on the World Wide Web: http://faostat.fao.org/site/567/default.aspx#ancor [accessed on 4 December 2012]

Feyereisen R., 1995, Molecular biology of insecticide resistance. Toxicol. Lett. 82:83-90

Fernandez S., and Montagne A., 1990, Biologıa del minador del tomate, *Scrobipalpula absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Bol. Entomol. Venez. 5:89-99

Ferracini C., Ingegno B. L., Navone P., Ferrari E., Mosti M., Tavella L., and Alma A., 2012, Adaptation of indigenous larval parasitoids to *Tuta absoluta* (Lepidoptera: Gelechiidae) in Italy. J. Econ. Entomol. 105:1311-1319

Food and Agricultural Organization, 1967, Report of the first session of the FAO Panel of Experts on Integrated Pest Control, Rome (Italy), Sept. 18-22, 1967, 19 pp

Gardiner M.M., Landis D.A., Gratton C., Di Fonzo C.D., O'Neal M., Chacon J. M., Wayo M.T., Schmidt N.P., Mueller F.F., and Heimpel G.E., 2009, Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. Ecol. Appl. 19: 143-154

Geier, P.W. and Clark L.R., 1961, An ecological approach to pest control. In Proceedings of the eighth technical meeting. International Union of Conservation of Nature and Natural Resources, Warsaw, 1960, pp. 10-18

Girardoz S., Kenis M., and Quicke D.L.J., 2006, Recruitment of native parasitoids by an exotic leaf miner, Cameraria ohridiella: host-parasitoid synchronization and influence of the environment. Agric. Forest Entomol. 8:49-56

Gontijo P.C., M.C. Picanço, E.J.G. Pereira, J.C. Martins, M. Chediak and Guedes R.N.C., 2012. Spatial and temporal variation in the control failure likelihood of the tomato leaf miner, *Tuta absoluta*. Ann. Appl. Biol. in press: Doi: 10.1111/aab.12000

Gonzáles-Cabrera J., Mollá O., Montón H., and Urbaneja A., 2011, Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). BioControl 56: 71-80.

Guedes R.N.C., and Picanço M.C., 2012, The tomato borer *Tuta absoluta* in South America: pest status, management and insecticide resistance. EPPO Bull. 42:211-216

Gurr G.M., Wratten S.D., and Barbosa P., 2000, Success in conservation biological control of arthropods. In Measures of success in biological control. Gurr G., and Wratten S. (Eds), pp 105-132. Dordrecht, The Netherlands: Kluwer Academic Publishers

Haack R.A., Herard F., Sun J.H. and Turgeon J.J., 2010, Managing invasive populations of Asian longhorned beetle and citrus longhorned beetle: a worldwide perspective. Annu. Rev. Entomol. 55:521-46

Haddi, K., Berger M., Bielza P., Cifuentes D., Field L.M., Gorman K., Rapisarda C., Williamson M.S., and Bass C., 2012, Identification of mutations associated with pyrethroid resistance in the voltage-gated sodium channel of the tomato leaf miner (*Tuta absoluta*). Insect Biochem. Mol. Biol. 42:506-513

Hafsi A., Abbes K., Chermiti B., and Nasraoui B., 2012, Response of the tomato miner *Tuta absoluta* (Lepidoptera:Gelechiidae) to thirteen insecticides in semi-natural conditions in Tunisia. EPPO Bull. 42:312-316

Hanafi A., 2005, Invasive pests and diseases: A challenge to IPM in greenhouse crops. Phytoparasitica 33:423-426

Harwood J.D., Desneux N., Yoo H.J.S., Rowley D.L., Greenstone M.H, Obrycki J.J., and O'Neil R.J., 2007, Tracking the role of alternative prey in soybean aphid predation by Orius insidiosus: a molecular approach. Molec. Ecol. 16:4390-4400

Hassell M.P., 1980, Foraging strategies, population models and biological control: a case study. J. Anim. Ecol. 49:603-628

Hawkins B.A., and Lawton J.H., 1987, Species richness for parasitoids of British phytophagous insects. Nature 326, 788-790

Harvey J.A., Poelman E.H., and Tanaka T., 2012, Intrinsic inter and intraspecific competition in parasitoid wasps. Annu. Rev. Entomol. in press DOI: 10.1146/annurev-ento-120811-153622

Hassan S.A., 1992, Guidelines for testing the effects of pesticides on beneficial organisms: description of test methods. IOBC/WPRS Bulletin 15:186pp

Haynes K.F., 1988, Sublethal effects of neurotoxic insecticides on insect behavior. Annu. Rev. Entomol. 33:149-168

He Y.X., Zhao J., Zheng Y., Zhan Z., Desneux N., and Wu K.M., 2012. Lethal effect of imidacloprid on the coccinellid predator *Serangium japonicum* and sublethal effects on predator voracity and on functional response to the whitefly *Bemisia tabaci*. Ecotoxicology 21:1291-300

Hodek I., 1967, Bionomics and ecology of predaceous coccinellidae. Annu. Rev. Entomol. 12:79-104

Hoskins W.M., Borden A.D., and Michelbacher A.E., 1939, Recommendations for a more discriminating use of insecticides. Proc. 6th Pac. Sci. Congr. 5:119-23

Howarth F.G., 1991, Environmental impacts of classical biological control. Annu. Rev. Entomol. 36:485-509

Hulme P.E., Nentwig W., Pysek P., and Vila M. 2009. Common market, shared problems: time for a coordinated response to biological invasions in Europe? Neobiota 8:3-19

Invasive Species Program National Invasive Species Council (NISC) USA, 1999, Executive Order N° 13112, Available on the World Wide Web: http://www.invasivespecies.gov. [accessed on December 4th 2012]

Jervis M.A., Hawkins B.A., and Kidd N.A.C., 1996, The usefulness of destructive host feeding parasitoids in classical biological control: theory and observation conflict. Ecol. Entomol. 21: 41-46

Jervis M. A., Ellers J., and Harvey J. A., 2008, Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. Annu. Rev. Entomol. 53:361-385

Jones D.P., 1970, Applied biology as an evolutionary process. Annu. Appl. Biol. 66:179-91

Kogan M., 1998, Integrated pest management: Historical perspectives and contemporary development. Annu. Rev. Entomol. 43:243-70

Kolar C.S., and Lodge D.M., 2001, Progress in invasion biology: predicting invaders. Trends Ecol. Evol. 16:199-204

Landis D.A., Wratten S.D., and Gurr G.M., 2000, Habitat management to conserve natural enemies of arthropod pests in agriculture. Annu. Rev. Entomol. 45:175-201

Loni A., Rossi E., and van Achterberg K., 2011, First report of *Agathis fuscipennis* in Europe as parasitoid of the tomato leafminer *Tuta absoluta*. Bull. Insectol. 64: 115-117

Lu Y.H., Wu K.M., Jiang Y.Y., Guo Y.Y., and Desneux N., 2012, Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nature 487:362-365

Luna M.G., Sanchez N.E., Pereyra P.C., Nieves E., Savino V., Luft E., Virla E., and Speranza S., 2012, Biological control of *Tuta absoluta* in Argentina and Italy: evaluation of indigenous insects as natural enemies. EPPO Bull. 42:260-267

Lundgren J.G., Wyckhuys K.A.G., and Desneux N., 2009, Population responses by *Orius insidiosus* to vegetational diversity. Biocontrol 54:135-142

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., and Bazzaz, F.A., 2000, Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications 10, 689-710

McNeely J.A., Mooney H.A., Neville L.E., Schei P. and Waage J.K. (Eds), 2001, Global strategy on invasive alien species. Gland, Switz. Cambridge, UK

Messing R.H., and Wright M.G., 2006, Biological control of invasive species: solution or pollution? Front Ecol Environ 4:132-140

Myers J.H., Simberloff D., Kuris A.M., and Carey J.R., 2000, Eradication revisited: dealing with exotic species. Trends in Ecology and Evolution, 15:316-320

Mills N., 2005, Selecting effective parasitoids for biological control introductions: Codling moth as a case study. Biological Control 34:274-282

Mollà O., Gonzalez-Cabrera J., and Urbaneja A., 2011, The combined use of *Bacillus thuringiensis* and *Nesidiocoris tenuis* against the tomato borer *Tuta absoluta*. Biocontrol 56:883-891

Nannini M., Atzori F., Murgia G., Pisci R., and Sanna F., 2012, Use of predatory mirids for control of the tomato borer *Tuta absoluta* (Meyrick) in Sardinian greenhouse tomatoes. EPPO Bull. 42:255-259

Olson L.J., 2006, The economics of terrestrial invasive species: a review of the literature. Agric. Resour. Econ. Rev. 35:178-194

Paini D.R., and Roberts J.D., 2005, Commercial honey bees (*Apis mellifera*) reduce the fecundity of an Australian native bee (*Hylaeus alcyoneus*). Biol. Conserv. 123:103-112

Paini D. R., Funderburk J.E. and Reitz S.R., 2008, Competitive exclusion of a worldwide invasive pest by a native. Quantifying competition between two phytophagous insects on two host plant species. J. Anim. Ecol. 77:184-190

Pekàr S, 2012, Spiders (Araneae) in the pesticide world: an ecotoxicological review. Pest Manag. Sci. 68:1438-1446

Pennacchio F., and Strand M. R., 2006, Evolution of developmental strategies in parasitic Hymenoptera. Ann. Rev. Entomol. 51:233-258

Phillips B.L., Brown G.P., Webb J.K., and Shine R., 2006, Invasion and the evolution of speed in toads. Nature 439:803

Planes L., Catalán J., Tena A., Porcuna J.L., Jacas J.A., Izquierdo J., and Urbaneja A., 2012, Lethal and sublethal effects of spirotetramat on the mealybug destroyer, *Cryptolaemus montrouzieri*. J. Pest Sci. In press doi:10.1007/s10340-012-0440-3

Pyšek P., Richardson D.M., Pergl J., Jarošík V., Sixtová Z. and Weber E., 2008, Geographical and taxonomic biases in invasion ecology. Trends Ecol. Evol. 23:237-244.

Pyšek P., and Richardson D.M., 2010, Invasive species, environmental change and management, and health. Annu. Rev. Environ. Resour. 35:25-55

Pimentel D., Lach L., Zuniga R., and Morrison D., 2000, Environmental and economic costs of non indigenous species in the United States. BioScience 50:53-65

Pimentel D., Zuniga R. and Morrison D., 2005, Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52: 24 273-288.

Ragsdale D.W., Landis D.A., Brodeur J., Heimpel G.E., and Desneux N., 2011, Ecology and management of the soybean aphid in North America. Annu. Rev. Entomol. 56:375-399

Roditakis E., Skarmoutsou C., Staurakaki M., Martínez-Aguirre M.R., García-Vidal L., Bielza P., Haddi K., Rapisarda C., Rison J.L., Bassi A., and Teixeira L.A., 2012a, Determination of baseline susceptibility of European populations of *Tuta absoluta* (Meyrick) to indoxacarb and chlorantraniliprole using a novel dip bioassay method. Pest Manag. Sci. in press doi: 10.1002/ps.3404

Roditakis E., Skarmoutsou C., and Staurakaki M., 2012b, Toxicity of insecticides to populations of tomato borer *Tuta absoluta* (Meyrick) from Greece. Pest Manag. Sci. in press doi: 10.1002/ps.3442

Samways MJ (2007) Insect conservation: a synthetic management approach. Annu. Rev. Entomol. 52:465-487

Sannino L., and Espinosa B., 2010, *Tuta absoluta*, guida alla conoscenza e recenti acquisizioni per una corretta difesa. L'Informatore Agrario, 66 Supplement 1, 113 pp

Shine R., Brown G.P., and Phillips B.L., 2011, An evolutionary process that assembles phenotypes through space rather than through time. Proc Natl Acad Sci USA 108:5708-5711

Silva G.A., Picanço M.C., Bacci L., Crespo A.L., Rosado J.F., and Guedes R.N.C., 2011, Control failure likelihood and spatial dependence of insecticide resistance in the tomato pinworm, *Tuta absoluta*. Pest Manag. Sci. 67: 913-920.

Simberloff D., and Stiling P., 1996, How risky is biological control? Ecology 77:1965-1974

Simberloff D., 2012, Risks of biological control for conservation purposes. BioControl 57:263-276

Siqueira H.A.A., Guedes R.N.C. and Picanço M.C., 2000, Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae).- Agric. Forest Entomol. 2:147-153

Siscaro G., Biondi A., Buonocore E., Tropea Garzia G., and Zappalà L., 2012, Four-year flight dynamics of *Tuta absoluta* in Sicily and implications for IPM strategies. IOBC-WPRS Bulletin 80:326

Smith S.M., 1996, Biological control with Trichogramma: advances, successes, and potential of their use. Annu. Rev. Entomol. 41:375-406

Speranza S., and Sannino L., 2012, The current status of *Tuta absoluta* in Italy. EPPO Bull. 42: 328-332

Stark J.D., and Banks J.E., 2003, Population-level effects of pesticides and other toxicants on arthropods. Annu Rev Entomol 48:505-519

Stark J.D., Banks J.E., and Vargas R., 2004, How is risk assessment: the role that life history strategies play in susceptibility of species to stress. Proc. Nati. Acad. Sci. USA 101:732-736

Stark J.D., Vargas R., and Banks J.E., 2007, Incorporating ecologically relevant measures of pesticide effect for estimating the compatibility of pesticides and biocontrol agents. J. Econ. Entomol. 100: 1027-1032

Sterk G., Hassan S.A., Baillod M., Bakker F., Bigler F., Blumel S., Bogenschutz H., Boller E., Bromand B., Brun J., Calis J.N.M., Coremans-Pelseneer J., Duso C., Garrido A., Grove A., Heimbach U., Hokkanen H., Jacas J., Lewis G., Moreth L., Polgar L., Roversti L., Samsoe-Petersen L., Sauphanor B., Schaub L., Staubli A., Tuset J.J., Vainio A., van De Veire M., Viggiani G., Vinuela E., and Vogt H., 1999, Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms". Biocontrol 44:99-117

Stern V.M., Smith R.F., van den Bosch R., and Hagen K. S., 1959, The integrated control concept. Hilgardia, 29: 81-101

Suckling D.M., and Brockerhoff E.G., 2010, Invasion biology, ecology, and management of the light brown apple moth (Tortricidae). Annu. Rev. Entomol. 55:285-306

Symondson, W.O.C., Sunderland, K.D., and Greenstone, M.H., 2002. Can generalist predators be effective biocontrol agents? Annu. Rev. Entomol. 47:561-94.

Tatem A.J., and Hay S.I., 2007, Climatic similarity and biological exchange in the worldwide airline transportation network. Proc. R. Soc. Lond., Ser. B: Biol. Sci. 274:1489- 1496

Thomas MB., 1999, Ecological approaches and the development of 'truly integrated' pest management. Proc. Natl. Acad. Sci. USA 96:5944-51

Tomé H.V.V., Cordeiro E.M.G., Rosado J.F., and Guedes R.N.C., 2012, Egg exposure to pyriproxyfen in the tomato leaf miner *Tuta absoluta*: ovicidal activity or behavioural-modulated hatching mortality? Ann. Appl. Biol. 160:35-42

Turchin P., Taylor A.D., and. Reeve J.D., 1999. Dynamical role of predators in population cycles of a forest insect: an experimental test. Science 285:1068-1071

Urbaneja A., Vercher R., Navarro V., García Marí F., and Porcuna J.L., 2007, La polilla del tomate, *Tuta absoluta*. Phytoma España 194:16-23

Urbaneja A., Pascual-Ruiz S., Pina T., Abad-Moyano R., Vanaclocha P., Montòn H., Dembilio O., Castañera P., and Jacas J.A., 2008, Efficacy of five selected acaricides against *Tetranychus urticae* (Acari: Tetranychidae) and their side effects on relevant natural enemies occurring in citrus orchards. Pest Manag. Sci. 64, 834-842

Urbaneja A., Montón H., and Mollá O., 2009, Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis.* J. Appl. Entomol. 133:292-296

Urbaneja A., Gonzalez-Cabrera J., Arno J., and Gabarra R., 2012, Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. Pest Manag. Sci. 68: 1215-1222

van Lenteren J.C., 1986, Evaluation, mass production, quality control and release of entomophagous insects. In Biological Plant and Health Protection, ed. J. M . Franz, pp. 3 1-56. Stuttgart: Fischer. 341 pp.

van Lenteren J.C., and Woets J., 1988, Biological and integrated pest control in greenhouses. Annu. Rev. Entomol. 1988.33:239-269

van Lenteren J.C., 1993, Biological control of pests. In Modern crop protection: developments and perspectives. Zadoks J. C. (Ed.) pp. 179-187. Wageningen, The Netherlands, Wageningen Pressvan

van Lenteren J.C., and Godfray H.C.J., 2005, European science in the enlightenment and the discovery of the insect parasitoid life cycle in The Netherlands and Great Britain. Biol. Control 32:12-24

van Lenteren J.C., 2012, The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl 57:1-20

Vet L.E.M., and Dicke M., 1992, Ecology of infochemical use by natural enemies in a tritrophic context. Annu. Rev. Entomol. 37: 141-172.

Viggiani G., 1984, Bionomics of the Aphelinidae. Annu. Rev. Entomol. 29:257-276

Viggiani G., 2000, The role of parasitic Hymenoptera in integrated pest management in fruit orchards. Crop Prot. 19:665-668

Vitousek P.M., D'Antonio C.M., Loope L.L., and Westbrooks R., 1996, Biological invasions as global environmental change. American Scientist, 84, 468-478

Walthall W.K., and Stark J.D., 1997, A comparison of acute mortality and population growth rate as endpoints of toxicological effect. Ecotox. Environ. Saf. 37:45-52

Ward N.L. and Masters G.J., 2007, Linking climate change and species invasion: an illustration using insect herbivores. Global Change Biol. 13:1605-1615

Warton, R.A., 1993, Bionomics of the Braconidae. Ann. Rev. Entomol. 38: 121-143.

Wearing C.H., 1988, Evaluating the IPM implementation process. Ann. Rev. Enlomol. 33:17-38

Weintraub P.G., Pivonia S., and Steinberg S., 2011, How many *Orius laevigatus* are needed for effective western flower thrips, *Frankliniella occidentalis*, management in sweet pepper? Crop Prot. 30:1443-1448

Weisenburger D.D., 1993, Human health: effects of agrichemicals use. Hum. Pathol. 24:571-576

Wennergren U., and Stark J., 2000, Modeling long-term effects of pesticides on populations: Beyond just counting dead animals. Ecolog. Appl. 10:295-302

Wijnands F.G , Baur R., Malavolta C., and Gerowitt B., 2012, Integrated Pest Management: Design and application of feasible and effective strategies. IOBC / WPRS Commission "IP-Guidelines and Endorsement". IOBC/wprs

Williamson M. 1996. Biological Invasions. London: Chapman & Hall. 256 pp

Wittenberg, R. and M.J. W. Cock (Eds.), 2001, Invasive Alien Species: A toolkit of best prevention and management practices. CAB International, Wallingford, Oxon, UK, 153 pp

Yu D.S.K., and van Actherberg C., 2010, Taxapad Ichneumonoidea. In: Species 2000 & IT IS catalogue of life: 2010 annual checklist (Bisby F. A., Y. R.Roskov, T. M. Orrell, D. Nicolson, L. E. Paglinawan, N. Bailly, P. M. Kirk, T. Bourgoin, G. Baillargeon,Eds). DVD, Species 2000, Reading, UK

Zappala L., Biondi A., Tropea Garzia G., and Siscaro G., 2012, Efficacy of commercial strains of *Bacillus thuringiensis* in controlling *Tuta absoluta*: laboratory tests. IOBC/WPRS Bull. 80:283-288

Zlof V., and M. Suffert, 2012, Report of the EPPO/FAO/IOBC/NEPPO Joint international Symposium on management of *Tuta absoluta* (tomato borer). EPPO Bull. 42:203-204

Abstract

The recent invasion of the Mediterranean basin by the South American tomato pinworm, *Tuta absoluta* (Lepidoptera: Gelechiidae), has lead to a swift increase in insecticide applications in tomato crops. Such increase may compromise the tomato Integrated Pest Management (IPM) packages that were sustainable and commonly used by farmers. My work aimed at providing key bases for including indigenous biocontrol agents of *T. absoluta* in IPM programs on tomato in Europe. A survey carried out in 3 Italian regions demonstrated that *T. absoluta* was rapidly used as host by indigenous parasitoids. The recovered species were mainly generalist idiobiont parasitoids (mainly belonging to the Eulophidae and Braconidae families) which showed relatively low levels of parasitism when attacking the invasive moth. Among them, only few were identified as potential biocontrol agents of the pest. More specifically, *Bracon nigricans* (Hymenoptera: Braconidae), an idiobiont, synovigenic, gregarious ectoparasitoid, showed some potential for inclusion in tomato IPM programmes. In a preliminary assessment of possible compatibility of biocontrol agents with pesticides used for IPM in tomato crops, dustable sulphur (mineral fungicide/acaricide) was showed to be moderately harmful (as a fresh residue) for a generalist predator (*Nesidiocoris tenuis* [Hemiptera: Miridae]) commonly used on tomato for biological control purposes. In a second step, we assessed the risks of 14 pesticides, commonly used in tomato crops, on a generalist predator model: *Orius laevigatus* (Hemiptera: Anthocoridae). We demonstrated that the pesticides greatly differed in their toxicity, both in terms of lethal and sublethal effects, as well as in their persistence after initial application on plants. Among the tested biopesticides only *Bacillus thuringiensis* proved to be harmless; whereas spinosad, emamectin benzoate and metaflumizone were moderately harmful until 7d after the treatment and abamectin was the most noxious and persistent and was classified as harmful up to 14 days after the treatment. While the other tested pesticides (including the synthetic ones) were slightly harmful or harmless. Further risk assessments of biopesticides showed that most of them have low acute toxicity (i.e. lethal effect) on *B. nigricans*. By contrast, spinosad proved strong acute toxicity on the tested wasps (100% of mortality). All biopesticides, except *Bt*, caused multiple sublethal effects, notably reductions in parasitism rate on *T. absoluta*, fertility, longevity and also a male-biased sex-ratio of the progeny. In addition, demographic models developed by integrating possible sublethal effects of pesticides on *B. nigricans* life history traits, showed that biopesticides would strongly impact long-term population growth of the wasp. A thorough review of articles assessing side effects of spinosyns on beneficial arthropods further hinted the potential multiple negative sublethal effects of this type of products in IPM packages.

Taken as a whole, my research showed that indigenous biocontrol agents could play a key role in the control of *T. absoluta* in Europe and that various pesticides should be of use only with high caution in tomato IPM programmes.

Riassunto

La recente invasione del bacino del Mediterraneo da parte della tignola del pomodoro, *Tuta absoluta* (Lepidoptera: Gelechiidae), è stata all'origine di un rapido incremento nei trattamenti insetticidi sulla coltura del pomodoro. Tale aumento può compromettere le strategie di gestione integrata (*Integrated Pest Management* - IPM) comunemente impiegate dagli agricoltori nella coltura del pomodoro. Questo lavoro ha come obiettivo principale quello di fornire le basi per l'inserimento di agenti biotici indigeni di controllo di *T. absoluta* in programmi di controllo integrato su pomodoro in Europa. Un monitoraggio condotto in 3 regioni italiane ha dimostrato che *T. absoluta* è stata prontamente utilizzata come ospite da parte di diversi parassitoidi indigeni. Le specie ritrovate sono state principalmente parassitoidi idiobionti generalisti (principalmente appartenenti alle famiglie degli Eulophidae e dei Braconidae) che hanno fatto registrare livelli di parassitizzazione relativamente bassi a carico del lepidottero esotico. Tra questi parassitoidi, solo poche specie sono state identificate come potenziali agenti di controllo biologico del fitofago. Più specificamente *Bracon nigricans* (Hymenoptera: Braconidae), un ectoparassitoide idiobionte, sinovigenico, gregario, ha mostrato un buon potenziale per essere incluso all'interno di prgrammi di controllo integrato. In uno studio preliminare per la valutazione della compatibilità di agenti di controllo biologico con pesticidi utilizzati nel controllo integrato su pomodoro, é stato dimostrato che lo zolfo in formulazione polverulenta (fungicida/acaricida minerale) é moderatamente dannoso (come residuo fresco) per un predatore generalista (*Nesidiocoris tenuis* [Hemiptera: Miridae]) comunemente impiegato su pomodoro come agente di controllo biologico. In un secondo step, sono stati valutati gli effetti di 14 pesticidi, normalmente utilizzati su pomodoro, su un predatore generalista modello: *Orius laevigatus* (Hemiptera: Anthocoridae). Le prove hanno permesso di dimostrare che i pesticidi differiscono profondamente nella loro tossicità, così come nella loro persistenza dopo l'applicazione iniziale sulle piante. Tra i biopesticidi testati solo *Bacillus thuringiensis* è risultato innocuo; mentre spinosad, emamectina benzoato e metaflumizone sono stati classificati come moderatamente dannosi fino a 7 giorni dopo il trattamento e abamectina è stato il pesticida più dannoso e persistente fino a 14 giorni dopo il trattamento. Gli altri pesticidi testati (inclusi quelli sintetici) sono risultati lievemente dannosi o innocui. Ulteriori test di tossicità sui biopesticidi hanno mostrato che la maggior parte di essi ha una ridotta tossicità acuta (effetti letali) su *B. nigricans*. Al contrario, spinosad ha fatto registrare un'elevata tossicità acuta sul parassitoide testato (mortalità del 100%). Tutti i biopesticidi, eccezion fatta per *Bt*, hanno causato numerosi effetti subletali, in particolare riduzioni del tasso di parassitismo su *T. absoluta*, della fertilità, della

longevità e anche una sex-ratio della progenie sbilanciata a favore dei maschi. Inoltre, modelli demografici, sviluppati integrando possibili effetti subletali dei pesticidi sui parametri biologici di *B. nigricans*, hanno mostrato che i biopesticidi possono avere un rilevante impatto di lungo termine sull'andamento delle popolazioni del parasitoide. Un'approfondita revisione di pubblicazioni volte a valutare gli effetti secondari delle spinosine su artropodi utili, ha ulteriormente sottolineato i potenziali effetti sub letali negativi multipli di questo tipo di prodotti in programmi di controllo integrato. I risultati delle ricerche condotte hanno dimostrato nel complesso che agenti di controllo biologico indigeni potrebbero svolgere un ruolo chiave nel contenimento delle popolazioni di *T. absoluta* in Europa e che diversi pesticidi dovrebbero essere impiegati con grande attenzione all'interno di programmi di controllo integrato su pomodoro.

Résumé

L'invasion récente de l'Europe par le lépidoptère invasif *Tuta absoluta* (Lepidoptera: Gelechiidae) (originaire d'Amérique du sud) a provoqué un fort accroissement de l'utilisation des insecticides dans les cultures de tomates. Ces insecticides sont problématiques parce que leurs effets négatifs sur les auxiliaires des cultures (ennemis naturels) peuvent compromettre des méthodes de lutte intégrée (IPM) préalablement efficace. Dans ce contexte, mon travail de thèse a consisté à établir des bases solides pour développer l'utilisation d'agents de lutte biologique indigènes contre *T. absoluta* en IPM sur tomate en Europe. Un inventaire faunistique a été réalisé dans 3 régions d'Italie et les résultats ont démontré que *T. absoluta* a rapidement été utilisé comme hôte par de nombreux parasitoïdes indigènes suite à l'invasion. Les parasitoïdes retrouvés ont été majoritairement des espèces généralistes idiobiontes présentant un taux de parasitisme modéré sur *T. absoluta*. Plus précisément, l'ectoparasitoïde synovigénique *Bracon nigricans* (Hymenoptera: Braconidae) a montré des traits biologiques intéressants pour la lutte contre *T. absoluta*. Par la suite, j'ai montré lors d'une étude préliminaire, qu'un composé pesticide largement utilisé sur tomate (fongicide) pouvait avoir des effets négatifs sur un prédateur généraliste couramment utilisé en lutte biologique sur tomate (*Nesidiocoris tenuis* [Hemiptera: Miridae]). Dans une étude plus poussée visant à évaluer les risques de 14 pesticides (tous couramment utilisés sur tomate) sur un prédateur généraliste modèle: *Orius laevigatus* (Hemiptera: Antocoridae), j'ai mis en évidence que ces produits diffèrent largement dans les effets observés (létaux et sublétaux) ainsi que dans leur rémanence après traitement sur tomate. Parmi les pesticides testés, *Bacillus thuringiensis* (*Bt*) est le seul à avoir été classé (classes IOBC) comme sans danger pour le prédateur. A l'inverse, des pesticides comme le spinosad, emamectin benzoate et le metaflumizone ont été classés comme modérément toxique jusqu'à 7 jours après traitement des plantes, et l'abamectin est le composé le plus toxique et le plus rémanent (très toxique même après 14 jours). La plupart des autres pesticides (y compris des pesticides chimiques) ont été classés comme peu ou pas toxique pour le prédateur. Les travaux suivants ont permis de démontrer que la plupart des biopesticides n'ont pas d'effets létaux sur *B. nigricans*. Cependant, le spinosad a induit un fort effet létal sur le parasitoïde. De plus, tout les biopesticides (sauf le *Bt*) ont causé de multiples effets sublétaux, notamment des réductions du taux de parasitisme, de la longévité et la fécondité ainsi qu'une modification du sex-ratio (plus de males). De surcroit, des modèles démographiques basés sur ces données de laboratoire ont souligné un effet probable assez fort sur la dynamique des populations du parasitoïde. Une synthèse bibliographique des effets du spinosad sur les arthropodes bénéfiques a confirmé l'importance du risque d'effets

sublétaux sur les arthropodes bénéfiques lors de l'inclusion du spinosad dans des programmes d'IPM sur tomate. L'ensemble de mes travaux de thèse ont montré que des ennemis naturels indigènes comme *B. nigricans* peuvent jouer un rôle important dans la lutte contre *T. absoluta* en Europe mais que les pesticides (y compris biopesticides) utilisés sur tomate en IPM doivent l'être de façon mesurée pour s'assurer de ne pas compromettre l'effet bénéfique des auxiliaires des cultures.

Resumen

La reciente invasión en la cuenca Mediterránea de la polilla sudamericana del tomate, *Tuta absoluta* (Lepidoptera: Gelechiidae), ha comportado un rápido incremento de los tratamientos químicos aplicados en los cultivos de tomate. Este incremento puede comprometer las herramientas sostenibles de manejo integrado de plagas (MIP) en tomate que son ampliamente utilizadas por los agricultores. Mi trabajo tiene como objetivo proveer las bases fundamentales para incluir agentes de control biológico nativos de *T. absoluta* en los programas de manejo integrado de plagas en tomate en Europa. Un muestreo, llevado a cabo en tres regiones italianas, demostró que *T. absoluta* fue rápidamente utilizada como huésped por parasitoides nativos. Las especies recuperadas, fueron principalmente parasitoides generalistas idiobiontes que mostraron niveles de parasitismo relativamente bajos cuando atacaban a la polilla invasora. Entre ellos, solamente unos pocos fueron identificados como agentes potenciales de control biológico de la plaga. Específicamente, *Bracon nigricans*(Hymenoptera: Braconidae), un ectoparasitoide gregrario, idiobionte y sinovigénico, mostró potencial para su inclusión en los programas de MIP en tomate. En una prueba preliminar de posible compatibilidad de los agentes de control biológico con los plaguicidas utilizados en el MIP en tomate, demostramos que el azufre espolvoreado (fungicida/acaridida mineral) es moderadamente nocivo (como residuo fresco) para un depredador generalista (*Nesidiocoris tenuis* [Hemiptera: Miridae]) utilizado comúnmente en control biológico en tomate. En un segundo paso, probamos el riesgo de 14 plaguicidas, comúnmente utilizados en los cultivos de tomate, sobre un depredador generalista modelo: *Orius laevigatus* (Hemiptera: Antocoridae). Demostramos que los plaguicidas difieren ampliamente en su toxicidad, tanto en términos de efectos letales y subletales, como en su persistencia tras su aplicación inicial en las plantas. Entre los biopesticidas testados solamente el *Bacillus thuringiensis*se mostró inocuo, mientras que el spinosad, el benzoato de emamectina y la metaflumizona fueron moderadamente nocivos hasta siete días después del tratamiento. La abamenctina fue la más tóxica y persistente y fue clasificada como nociva hasta 14 días después del tratamiento. Por otra parte, el resto de plaguicidas probados (incluyendo los sintéticos) fueron ligeramente nocivos o inocuos. Posteriores análisis de riesgo de biopesticidas demostraron que la mayoría de ellos tienen una baja toxicidad aguda (efecto letal) en *B. nigricans*. En cambio, el spinosad mostró una fuerte toxicidad aguda sobre los parasitoides probados (100% mortalidad). Todos los biopesticidas, excepto *Bt*, causaron múltiples efectos subletales, notables reducciones en las tasas de parasitismo de *T. absoluta*, fertilidad, longevidad y también un mayor número de machos en la descendencia. Además, modelos demográficos desarrollados mediante la

integración de posibles efectos subletales de los plaguicidas sobre los parámetros vitales de *B. nigrans*, mostraron que los biopesticidas pueden tener un fuerte impacto a largo plazo sobre el crecimiento de las poblaciones del parasitoide. Una revisión exhaustiva de artículos que estudian los efectos colaterales de las espinosinas sobrelos enemigos naturales indicóque este tipo de productos tienen efectos negativos subletales múltiples que pueden afectar en las estrategias de MIP. En conjunto, mi estudio muestra que los agentes de control biológico nativos pueden ser una pieza clave en el control de *T. absoluta* en Europa y que varios plaguicidas deberían solamente ser utilizados con gran precaución en los programas de MIP de tomate.

Highlights

- ✓ Several indigenous parasitoid species got adapted to the new host
- ✓ *Bracon nigricans* was proved to be an idiobiont, synovigenic, gregarious, ectoparasitoids of *Tuta absoluta*
- ✓ *Bracon nigricans* showed a good potential as *T. absoluta* biocontrol agent to be included in IPM programmes
- ✓ Control of *T. absoluta* by *Nesidiocoris tenuis* can also be obtained with careful applications of powder sulphur
- ✓ Some organic-certified pesticides used to control *T. absoluta* are not safe for the predator *Orius laevigatus*, by contrast some synthetic insecticides are compatible with its activity
- ✓ Some biopesticides used to control *T. absoluta* cause several sublethal effects on *B. nigricans* leading to potential extinction of the parasitoid populations
- ✓ *Bacillus thuringiensis* showed to have no side effects on both predators and parasitoids
- ✓ Lethal and multiple sublethal effects of spinosad were identified on almost all the tested parasitoid, predator and pollinator arthropods

Keywords

Bacillus thuringiensis, Biodiversity, Biological control, Biopesticides, *Bracon nigricans*, Demography, Ecotoxicology, Globalization, Habitat management, IOBC, Invasive pest, Leafminer, Life table, Mediterranean basin, *Nesidiocoris tenuis*, Organic farming, *Orius laevgatus,* Parasitoid, Predator, Persistence, Risk assessment, Side effects, Spinosad, Sublethal effects, Sulphur, South American tomato pinworm.