The Sub-lethal Effect of Pesticides on Beneficial Arthropods

1. Introduction

Pesticides are designed to be toxic to target noxious organisms that cause damage on crops and economic losses. However, some active ingredients can be harmful to the environment and to non-target organisms (99, 101) despite the efforts of research to develop and promote ecologically safer and more selective molecules. Among non-target organisms, arthropod natural enemies are particularly important for crop pests control and in case of disruption of this activity due to pesticides, pest outbreaks may occur. For this reason a large number of laboratory, semi-field and field studies have been carried out in the last decades evaluating the acute or chronic toxic effects of chemicals on the biology and behavior of beneficial in terms of disruption of life span, development rate, fertility, searching behavior, etc. (103, 104).

One of the major purposes of Integrated Pest Management (IPM) strategies is to combine the safe and sustainable use of pesticides with biological control agents. Therefore, the correct evaluation of side-effects of pesticides on beneficial is a crucial point for the application of current IPM strategies (103) which are widely and successfully adopted in Italian citrus groves. Estimating the compatibility of pesticides with biological control agents is a work in progress and a continuous information upgrade is needed on newly authorized agrochemicals and their side-effects, particularly in complex agroecosystems such as citrus.

A key element of pest management programs in agroecosystems is to build an understanding of the impacts on non-target and beneficial insects (106). The use of insecticides against insect-pests still prevails as one of the main pest management tools in most agricultural settings, in addition to having potential consequences for arthropod pest resurgence (108).
Insecticides may block some physiological or biochemical processes, impacting survival, growth, development, reproduction and behavior of natural enemies of insect pests (106). Even at non-lethal levels, insecticides can still influence behavior, although there have been few detailed studies concerning the potential effects of sublethal insecticide doses on the behavior of beneficial arthropods. In general, sublethal insecticides levels affect reproduction, orientation, feeding, oviposition and learning.

2. Methods to test the side effects of pesticides

Methods to test the side effects of pesticides have been developed as a function of the beneficial arthropods and pesticides studied. In each country, regulatory insect risk assessment related to agrochemical use and registration follows specific guidelines (European Council Directive 91/414 in Europe, and the Federal Insecticide Fungicide and Rodenticide Act in the United States). For a long time, the classical laboratory method for estimating the side effects of chemicals on beneficial arthropods was to determine a median lethal dose (LD50) or lethal concentration (LC50) estimate. 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 (12). However, estimation of selectivity was based on LD50 values, and side effects of pesticides on beneficial arthropods still occurred because of the lack of attention to sublethal effects. Because of the increasing economic importance of beneficial arthropods in agriculture and the recognition of limitations associated with traditional methods for studying sublethal effects of pesticides (49), a growing body of literature is aimed at addressing this issue. Now, it is important to step back and review what these studies have documented to determine the directions of future studies and applications. 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).

A sublethal dose/concentration is defined as inducing no apparent mortality in the experimental population.We review the sublethal effects of pesticides on beneficial arthropods reported in the published literature and divide these effects into two major groups: physiology and behavior. We focus on the side effects and not on the indirect effects of pesticides, such as habitat destruction and damage to nesting, oviposition, resting, and mating sites. This review aims to (a) provide a better understanding of the different types of sublethal effects associated with pesticide exposure, (b) clarify the range of methods used to address sub-lethal effects and permit new insights into the development of better experimental approaches, (c) determine if evaluation of these effects could be included in the pesticides registration process, and (d) elucidate the possible consequences of the sublethal effects of pesticides on the efficiency of beneficial arthropods (pest limitation or pollination) and community dynamics.

3. PHYSIOLOGICAL EFFECTS

3.1. General Biochemistry and Neurophysiology

Studies on effects of pesticides on insect biochemistry have been conducted with both pollinator and natural enemy models. More in-depth studies have been performed using honey bees primarily because more is known about their biochemical systems. Experiments on bee physiology have been done mainly by measuring the activity of enzymes after or during exposure to pesticides. After injection of emerging honey bees in the laboratory, fenitrothion (organophosphorus) and cypermethrin (pyrethroid) led to decreases in Na+/K ATPase and acetylcholinesterase (AChE) activities (5). Related glycemic disorders were also linked to enzyme inhibition. Na+/K ATPase is a transmembrane enzyme that releases energy necessary for cell metabolism and establishes the ionic concentration balance that maintains the cell potential. Thus, the inhibition of Na+/K exchange provoked by pyrethroids might affect a wide range of cellular functions. For example, the pyrethroid deltamethrin causes marked dysfunctions in myocardial cells. Indeed, Papaefthimiou & Theophilidis (58) have demonstrated the cardiotoxicity of deltamethrin using intracellular recordings from the myocardial cells of the semi-isolated hearts of honey bee. The frequency and the force of spontaneously generated cardiac contractions were modified by deltamethrin. The imidazole fungicide prochloraz had a similar impact, but its effects were more intense. When prochloraz and deltamethrin are combined there is a synergistic interaction. The joint effects of both compounds were also investigated on honey bee thermoregulation by infrared thermography. Whenassociated with prochloraz, deltamethrin elicited a joint hypothermia at doses that did not induce a significant effect on thermoregulation when used alone. One hypothesis was that imidazoles delayed the metabolism, detoxification, and excretion of pyrethroids by inhibition of microsomal oxidation and thus enhanced the toxicity of the pyrethroid to the honey bees (59). However, the results of sublethal toxicity suggest other mechanisms for synergistic toxic effects, such as combined action on a common target (58).

In contrast to studies conducted on honey bees, few studies have investigated the effects of pesticides on the general biochemistry and enzymatic processes in natural enemies. In a study aiming to use enzyme activity as a biomarker of sublethal exposure to insecticides, Rumpf *et al.*, demonstrated that acute toxicity tests (LD50) determination could miss sublethal perturbations involving effects on enzymes. This study (on lacewings) showed that the correlation between the degree of AChE and glutathione-S-transferase inhibition and corresponding mortality caused by a given insecticide (five classes tested) was toxin specific

as well as species specific. The inhibition of AChE could lead to general perturbation in all systems because it is a major component in all synaptic transmission (43), especially when inhibition continues for a long time after exposure. For example, eight days were required after a 24 or 48-h exposure to the organophosphorus diazinon and chlorpyrifos for AChE inhibition in wolf spiders (Lycosidae) to disappear (87). Thus, pesticide effects on important enzyme systems cannot be extrapolated or deduced from LD50 values.

3.2. Development

Sublethal effects on larval development may result from perturbations in development of neural tissues by neurotoxic substances. Given the importance of the cholinergic system in insect development, many kinds of sublethal effects are possible. Insect growth regulators (IGRs) are also likely to perturb the development of beneficial arthropods. Indeed, IGRs are commercial hormone mimics that disrupt molting (juvenile hormone or ecdysone mimics) and cuticle formation (chitin inhibitors) and more generally act on endocrine systems (29). Studies reporting pesticide impacts on the development of natural enemies typically differ with the biology of the experimental subject (i.e., predators versus parasitoids). Studies using parasitoids often report effects on adult emergence from the pupal stage (44, 69, 73). Adult emergence has also been studied for the lacewing predator *Mallada signatus* exposed to the botanical insecticide azadirachtin A (AzaA) in the pupal stage (61). In most of these studies, however, it has remained unclear whether reduced adult emergence is related to the direct lethal effects of pesticides or if other perturbations such as organ malformation are primarily responsible. Other studies have further clarified this subject. Schneider *et al.* (74) reported a decrease in emergence from parasitized host after exposure to spinosad (spinosyns) in the endoparasitoid Hyposoter didymator; however, they related their findings to the apparent inability of the larvae to produce silk, a necessary material for cocooning. A similar finding has been reported for the predator Chrysoperla carnea following fenoxycarb (juvenile hormone analog, JHA) exposure (6). Another parameter often reported in association with the effects of pesticides on insect development is the developmental rate. Developmental rate can have a large impact on a natural enemy's intrinsic rate of increase (rm) and phenological synchrony with the host or prey. An increase in developmental rate could present a significant disadvantage for a parasitoid if it disrupts synchrony with a critical window of susceptibility in the host. Fenoxycarb is reported to prolong the development time of the predator *Chrysoperla rufilabris* in all stages but the pupae (51).

3.3. Adult Longevity

Effects on longevity after exposure to lethal or sublethal doses of pesticides have been described mostly for parasitoid species (2, 28, 76, 74,69, 67, 46) and to a lesser extent for predators (60, 82). Depending on the study, reduced longevity may be considered a sublethal effect or latent mortality. Extrapolation of these effects to the population level is difficult because, depending on the biology of the particular natural enemy [proovigenic or synovigenic (66), parasitoid or predator], they may be more or less likely to reproduce and/or to kill pests before their premature death. From a practical perspective, it is the resulting amount of feeding and reproduction that occurs between exposure and death that is important. The consequences of reduced longevity on population dynamics were recently emphasized by studies assessing pesticide impacts on arthropods using life table analysis (reviewed in Reference 120). When the rm is determined for risk assessment of pesticides, a reduction of survival (lx) could lead to a strong reduction of the rm and consequently a negative effect at the population level (120).

In the honey bee, the possible long-term exposure to a toxic agent by contamination of stored food has been established by studying the transfer of pesticides sprayed on crops into the hive (137). Thus, the lethal dose estimated during acute toxicity tests appears to be a partial measure of the lethal effect because of the short duration of these tests (1 to 3 days in most cases). Studies concerning long-term survival of honey bees raise the problem of statistical analysis of survival data. In chronic toxicity tests, most often only the end result of long-term poisoning (i.e., an increase of cumulative mortality) is analyzed (113). Some approaches consider how the mortality rate varied during the time of pesticide exposure by a graphic interpretation (124, 126), but not with statistical analysis. Conversely, when statistical methods are employed in survival analysis a parametric model is often used (63, 138). However, these analyses depend strongly on the validity of the assumption that the survival time has a particular probability distribution. Moreover, these statistical methods are generally based on the hypothesis of independence between bees belonging to the same group, which is not realistic. Indeed, food exchanges, contacts, and pheromonal communication occurring among workers make survival of a bee dependent on the survival of its nestmates. Dechaume-Moncharmont et al. (26) demonstrated this density dependence in pesticide effects with the use of a Coxproportional hazard model.

3.4. Immunology

Insecticides can interact with the immune capacity of insects. Depending on the type of insecticide, they can decrease or increase this capacity. Monocrotophos and methyl parathion applied at one tenth of the LC50 decreased the number of plasmatocytes in the hemolymph of the predator *R. kumarii* by16% and 13%, respectively, whereas endosulfan (organochlorine) increased this number by 15% (56). Plasmatocytes have a direct role in the immune response of insects by enabling the encapsulation of foreign bodies (71). George & Ambrose (56) reported that decreases in the number of plasmatocytes were associated with an increase in the number of granular hemocytes, which play a role in detoxification through phagocytosis. They hypothesized that plasmatocytes are transformed into granular hemocytes during the detoxification process, indicating that the tested pesticides acted on the predator's immunological response indirectly by mobilizing immunity cells for detoxification tasks. In host-parasitoid relations, pesticides may indirectly affect the parasitoids by lowering the immune reaction of the host. Dieldrin (cyclodiene) and endosulfan, applied at LD30, decreased by 25% and 23%, respectively, the immune reaction of Drosophila melanogaster against larvae of its parasitoid Leptopilina boulardi (35). However, insecticides may also increase the encapsulation of parasitoid larvae. When *L. boulardi* was exposed to an LD50 of chlorpyrifos, the encapsulation of its eggs was increased by 4.5% (41). Therefore, insecticides may have an impact on both the immune capacity of a host and the capacity of parasitoids to evade the host immune reaction.

3.5. Fecundity

Reductions in fecundity associated with pesticides may be due to both physiological and behavioral effects (the effects on behaviors are described later in the review). Many authors have reported general effects on fecundity of natural enemies regardless of the nature of perturbations (14, 22, 54 ,75) but mechanistic insights into the effects of pesticides on natural enemy fecundity have been obtained. Consoli *et al*. (21) described a reduction of fecundity of the parasitoid *T. pretiosum* when exposed to lambda-cyhalothrin (pyrethroid), teflubenzuron (IGR), or tebufenozide (ecdysone agonist) before oogenesis, but not after. They hypothesized that tebufenozide may interfere with ecdysteroid receptors, leading to a general perturbation of insect reproductive process involving ecdysteroids (vitellogenesis, ovulation of mature eggs, promotion of spermatocyte growth). A reduction in the number of hosts

parasitized by *C. plutellae* (during a 10-h period) after ingestion of the IGRs chlorfluazuron, flufenoxuron, and teflubenzuron has been reported (61). The effect was linked to a reduction in viable eggs because of the known effect of flufenoxuron an teflubenzuron on female fertility (67). Considering both neurotoxic and IGR pesticides, the IGRs may induce more long-term effects on fecundity than neurotoxics. Indeed, the lifetable parameters (which include fecundity) of the lacewing predator Micromus tasmaniae after exposure to several IGR and neurotoxic pesticides were more seriously affected by the IGRs than by the neurotoxic insecticides (87). Moreover, Rumpf *et al.* (56) emphasized that long-term sub-lethal effects described in their study may interfere with the phenological synchrony between pest species and natural enemies, leading to a global decrease in their ability to regulate pest populations.

3.6. Sex Ratio

Physiological effects of pesticides include alteration of the sex ratio of beneficial insects via differential survival as a function of sex (5, 24), but additional effects are expected because pesticides can induce deformations of ovaries (57, 88, 98) and testes (57). How- ever, very few studies have documented potential mechanisms of sex ratio alteration by pesticides for beneficial arthropods. Overall, two major causes are thought to alter the sex ratio of the offspring when adults are exposed to pesticides: (a) an effect on the fertilization of ova, especially in haplodiploid species in which the fertilization of ova is a voluntary act by females when they are laying eggs, and (b) differential survival of sexes when exposure is before the adult stage (64).

Chlorpyrifos modifies the sex ratio of hymenopteran parasitoids by decreasing the number of females in the offspring when only parental females are exposed. This phenomenon has been observed for *Aphytis melinus*. The offspring of females that survived the insecticide (LD50) were 58% female and offspring of the control group were 73% female (56). In *Trichogramma brassicae*, the offspring of females surviving exposure to chlorpyrifos (LD20) were 61% female and progeny of the control group were 73% female (40). Similar results were obtained with two pyrethroids (deltamethrin and lambda-cyhalothrin) that decreased the number of female offspring of *Aphidius uzbekistanicus* when adults were exposed to insecticides (75). This decrease in the number of female offspring may be related to the fact that hymenopteran females result from fertilized eggs, whereas males result from unfertilized

eggs. Egg fertilization is a voluntary act by females. Therefore, this behavior of fertilizing eggs may be altered through the impacts of insecticides on nerve transmission in exposed females.

4. BEHAVIORAL EFFECTS

4.1. Mobility

The mobility of beneficial arthropods after exposure to pesticides is often not directly studied. Moreover, studies are usually not accompanied by precise measures with quantitative data or statistical analysis. Effects on the mobility of beneficial arthropods have been observed, but they are mostly due to (a) direct intoxication by the pesticides, resulting in knockdown effect (23, 90), uncoordinated movement (5, 14, 98), trembling, tumbling, abdomen tucking, and/or rotating and cleaning of the abdomen while rubbing the hind legs together (78); (b) secondary consequences of behavioral modifications (70) such as disruption in the detection of kairomones that result in an increase of angular speed due to higher arrestment by kairomone patches and hydrous stress (34); and (c) a repellent (72, 84, 79) or irritant effect of pesticides (95). Several authors (70, 95) reported increases in mobility of natural enemies with the assumption that these increases would result in greater activity against pests. The predator *C. septempunctata* walked and groomed more frequently when released in a plot sprayed with deltamethrin (95) mainly because of irritation caused by the pesticide. The grooming behavior associated with increased mobility is thought to be a re- flex action initiated by irritation of chemoreceptors located on the surface of the insect body (49). This irritant effect may induce movement of the insects away from the treated areas. Consequently, increased mobility cannot be associated with increased natural enemy efficiency. In contrast, perturbations of mobility can increase natural enemy vulnerability to predation in the field (77).

To study chemical effects on the motor activity of beneficial arthropods, more subtle endpoints that provide quantitative data might be more useful. The amount of inactive time and the position of topically treated worker bees (in an open-field-like arena allowing observation of bee vertical displacement) were compared with those of control bees (78). Adverse effects of imidacloprid on motor activity were dependent on insecticide dose. The lowest dose (1.25 ng per bee) resulted in increased motor activity, whereas the higher doses (2.5 to 20 ng per bee) decreased displacements in the arena. The influence of imidacloprid on

mobility could also change with time (86). Therefore, we can assume that with the same dose of imidacloprid, it is possible to observe inverse effects according to the time of observation. Using the same paradigm described by Lambin *et al.* (78), a study reported that fipronil (pyrazole) had no effect on motor activity whatever the route of exposure (oral or topical) (51). Here, other endpoints were used: distance covered and time spent in each of the six levels of the arena. This test is based on negative geotaxis or positive phototaxis because honey bees tend to migrate upward against the force of gravity to the light source. This test provides an accurate assessment of motor function of walking bees, but it does not measure flying activity, which is essential in the process of foraging.

4.2. Navigation/Orientation

In natural enemies, navigation and orientation could involve multiple sensory cues, either chemical (46) or visual (35). Natural enemies spend a significant proportion of their life searching for hosts or prey. Navigation depends entirely on nervous transmissions, which are targeted by neurotoxic insecticides through different modes of action. Therefore, effects on navigation are frequently reported. Longley & Jepson (83, 84) and Umoru *et al*. (85) reported perturbations of the foraging pattern in parasitoids, but specific effects of the pesticides were not isolated and repulsive and direct behavioral effects remained unknown. In general, insects have been confined to pesticide-treated plants and the position of the natural enemies was recorded at various times. The authors described a reduction in time spent on treated plants and an inversion in leaf side preference, but direct effects on orientation behaviors remained unknown. However, other studies have more precisely described potential effects on navigation behavior by combining a controlled exposure time and dose followed by the use of a specific behavioral apparatus. Exposure methods can mimic natural exposure conditions, for ex- ample, tarsal exposure on pesticide deposits (34, 34, 48) exposure via feeding on contaminated sources (72), and direct exposure by topical application (50). Behavioral tests can assess most important steps involved in the navigation process. Results show that pesticides induce different and sometimes opposite effects on host searching by parasitoids depending on the species and insecticide used. Indeed, positive sublethal effects of pesticides on natural enemy orientation behaviors have been reported (34, 37). However, most of the studies reported negative effects on orientation behavior. When the parasitoid *Microplitis croceipes* consumed extrafloral nectar of cotton

contaminated with imidacloprid or aldicarb (carbamate), its response to odors of the hostplant complex in a wind tunnel decreased by 71% and 62%, respectively (28). A lower residence time on the contaminated host patch was observed with females of the parasitoid *Trissolcus basalis* exposed to deltamethrin at LD25, compared with unexposed females (70). In a four armed olfactometer, the capacity of aphid parasitoids to orient toward host-induced plant odors (synomones) could be decreased by exposure to a sublethal dose of lambda-cyhalothrin (45) and to increasing doses of triazamate (carbamate) (46). Desneux *et al*. (45) also emphasized that these effects could be temporary and that insects could recover after a period without exposure. With predators, studies designed to assess the effects of pesticides on navigation typically focus on relatively short-range prey detection and hunting. Cypermethrin, at recommended field rates, reduced the attack rate of *Acanthaspis pedestris* 2.4- to 6.4-fold, with the effect increasing with prey density (19).

Disruption of sexual communication and mate-finding has also been reported. Pesticides modify chemical communication between sexual partners by altering the capacity for stimulus creation by the emitter or stimulus perception by the receiver. Stimulus detection and integration by the CNS are potential targets for perturbations by pesticides (62). For example, *T. brassicae* males exposed to a LD20 of chlorpyrifos are less arrested by female sexual pheromones, and exposed females emit less of these pheromones (36). Sublethal doses may also disrupt sexual communication. *T. brassicae* males exposed to a LD0.1 of chlorpyrifos were less arrested by female sexual pheromones; however, pheromones emitted by exposed females (LD0.1) were more arresting for untreated males (37). In contrast, when *T. brassicae* males were treated with the pyrethroid deltamethrin at LD0.1 there was an increase in arrestment, whereas when females were treated, their pheromones were less arresting for males (39). These effects off- set each other when both sexes are exposed, with a mean response to sexual pheromones similar to that of the control. However, the kinetics of the response are modified (38).

For pollinators, visual learning of land- marks is important in spatial orientation. Honey bees use visual landmarks to navigate to a food source as well as to communicate accurately to their nest mates the distance and direction to fly to reach it (39). A bee exposed to pesticide during a foraging trip may incorrectly acquire or integrate visual patterns, causing disorientation and loss. Aside from impairing the orientation behavior of exposed foragers, insecticides could affect the accuracy of information relayed through the dances of the returning foragers. Recently, the effects of deltamethrin on the homing ability of foragers were investigated. Honey bees were trained to forage on an artificial feeder filled with

sucrose solution and were individually marked with colored number tags. In an insect-proof tunnel with the feeder located 8 m from the hive, deltamethrin altered the homing flight in foragers treated topically at sublethal doses (34). The percentage of short-term flights back to the hive decreased in treated foragers, which flew in the direction of the sun.

Still, a relatively small number of studies have investigated the impact of pesticides on homing flight, perhaps because of the difficulty of measuring parameters such as direction of flight or the route time between the food source and the hive. Most techniques are limited by the number of individuals who might be monitored simultaneously and by the time span during which observations can be made. However, techniques of automatic tracking and identification of individuals have the potential to revolutionize the study of behavioral ecotoxicology. In this regard, several different types of transponders such as harmonic radar (104) and radio frequency identification devices (RFID) (12) may be useful for studies using the honey bee. Presently, RFID tags offer the most advantages (unlimited number of individual insects, large numbers of events recorded, rapid reading) (79) and they cause less disturbance to the insects than harmonic radar, which requires the attachment of an antenna. Given the large range of biological parameters potentially affected by pesticides, another approach measuring the orientation performance of bees in a complex maze relies on associative learning between a visual mark and a reward of sugar solution (14). Using this experimental setup, researchers examined whether foragers receiving 1 ppb (parts per billion) fipronil (administered orally) can learn to fly through a maze according to the presence or absence of a visual cue (*A. Decourtye*, unpublished data). The bees learned the maze by making correct and incorrect decisions. The maze simulates learning of complex routes under field conditions. Results for experimental controls showed that 89% of bees flew through the entire path and arrived at the goal (reward of sugar solution). However, when the bees were exposed to pesticide, the rate fell to 60%. In parallel, the percentage of bees that did not find the goal within 5 min of entering the maze increased dramatically when exposed (34% and 4% in exposed and control groups, respectively). Thus, the orientation capacity of foragers in a complex maze was highly affected by fipronil.

4.3. Feeding Behavior

Pesticides may interfere with the feeding behavior of exposed insects in three general ways. First, some pesticides are well documented to have repellent effects on beneficial insects, and this effect may conflict with feeding behavior. Second, some pesticides are used specifically

for their antifeedant properties (100) with the possibility that beneficial insects may also be discouraged from feeding when exposed. Third, disruption in the ability to locate food may occur after expo- sure to pesticides because of reduced olfactory capacity (31). However, the consequences of effects may depend on the organisms considered. For proovigenic natural enemies, reduced feeding may influence the overall parasitism/predation rate because of reduced longevity. However, this effect may be limited because these insects do not require energy for egg production (66). In contrast, reduced feeding by the adults of synovigenic species may reduce egg production, leading to reduced fitness. Moreover, perturbation of host feeding behavior exhibited by many parasitoids (66) and predation by predators may drastically reduce the efficiency of natural enemies. In the case of honey bees, impaired feeding behavior can induce a drastic decline in hive population. In large-scale farming areas, when food resources are reduced to cultivated plants, the repellent effect of pesticides may reduce pollen and nectar uptake, potentially leading to a demographic decrease of the colony.

4.4. Oviposition Behavior

Most studies concerning the effects of pesticides on oviposition behavior have been done on parasitoids because of the direct linkage between Oviposition and parasitism rate and consequently pest regulation. However, few studies in this regard have been conducted on predators (11), and to our knowledge none have been conducted on pollinators. Pesticides can disrupt the very precise coordination between the insect nervous and hormonal systems, resulting in a breakdown in the complex series of behavioral and physiological events related to oviposition. Indirect perturbations in oviposition behavior may be induced by the repellent effect of pesticides, which can re- duce the chances that a natural enemy will find a suitable host or oviposition site (83,13), and also by occurrence of uncoordinated movements after pesticide exposure (5, 46). In these two last studies, after exposure to lethal and sublethal doses of pesticides, *Aphidius ervi* and *Trybliographa rapae* females exhibited an irreversible uncoordinated ovipositor extru- sion and consequently failed to lay eggs.

Kuhner *et al.* (76) described the negative effects of herbicides on the parasitic behavior of *Diaeretiella rapae*, which included a reduction in the number of attempted stings. For another aphid parasitoid, *A. ervi*, females showed significantly less oviposition activity compared with the controls after exposure to a LD20 of lambda-cyhalothrin (45). The frequency of sting attempts and related behaviors were significantly reduced. The parasitoid *Neochrysocharis formosa* exhibited a reduction in the number of ovipositor insertions into a

host, host mine drumming frequency, and the number of eggs laid when foraging on imidacloprid-treated leaves (29). Similar reduction in the number of hosts stung has been reported in the parasitoid *Colpoclypeus florus* after exposure to two commercial formulations of spinosad (14). These authors also reported that for one formulation no offspring were produced. Egg deposition may have been disrupted in these experiments, as uncontrolled egg laying associated with egg losses could occur after pesticide exposure (5). Effects were formulation dependent, which implied that adjuvants may be worthy of consideration. An effect on egg deposition may also be due to perturbation of chemoreceptors or information integration during host acceptance [occurring during ovipositor insertion into host (36)], but this effect has not been well described.

4.5. Learning Performance

Effects of pesticides on learning processes of beneficial arthropods have been studied mostly in pollinator models and, more specifically, in honey bees because of the better understanding of their learning processes and the importance of learning in the foraging process (91). In contrast, very few studies have investigated the effects of pesticides on the learning capacity of natural enemies, and impairment of specific learning traits has not been reported. Odor conditioning in the parasitoid *L. heterotoma* (probing into substrate) was not modified by tarsal exposure to dry residues of chlorpyrifos (LD20) (102). In the aphid parasitoid *A. ervi,* learning capacity for synomones and consequent olfactory orientation in an olfactometer were not modified after tarsal exposure to lambda-cyhalothrin (LD0.1 and LD20) (45).

When landing on a flower, each honey bee forager is subjected to a conditioning process in which floral cues (smell, color, and shape) are memorized after being associated with food (91). Once memorized, the odors play a prominent role in flower recognition during subsequent trips (90). Under laboratory conditions, olfactory learning can be studied using a bioassay based on conditioning of the PER in restrained individuals (25). The PER assay simulates natural honey bee–plant interactions that take place when landing on the flower; the forager extends its proboscis as a reflex when the gustatory receptor set on the tarsi, antennae, or mouthparts are stimulated with nectar. This reflex leads to the uptake of nectar and promotes memorization of concomitant floral odors. The PER assay has been used with restrained workers to investigate the behavioral effects of about 20 different pesticides (1, 29, 30, 86, 22, 14). However, in order to confirm that the effect of a pesticide on conditioned PER levels is due strictly to failure of learning or memory ability, it is necessary to consider

impacts on motor functions and gustatory and olfactory senses that underlie the endpoint (8, 31, 51).

5. Conclusions

1. Physiological sublethal effects on the development of beneficial arthropods occur at multiple levels. The parameter generally recorded is the developmental rate. However, new parameters such as malformation rates in natural enemies (when emerging from pupae) and in pollinators (in the cells inside the hive) are now used.
2. Studies have generally reported perturbations of the foraging pattern in parasitoids and honey bee. Other studies have described more precisely the potential effects on navigation behavior by combining a controlled exposure time and dose followed by the use of a specific behavioral apparatus.
3. Pesticides may interfere with the feeding behavior by repellent, antifeedant, or reduced olfactory capacity effects. A more drastic effect should be observed for synovigenic species that need feeding for egg production all life long.
4. Learning processes depend on a high functionality of sensory and integrative nervous systems, which in particular have high importance in the honey bee (floral and nest recognition, spatial orientation). Therefore, the impact of neurotoxic pesticides on these processes has been largely studied and identified in this insect.

6. References

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