The Sublethal Effects of Pesticides on Beneficial Arthropods

Nicolas Desneux,[1] Axel Decourtye,[2] and Jean-Marie Delpuech[3]

[1]Department of Entomology, University of Minnesota, St. Paul, Minnesota 55108; email: desne001@umn.edu

[2]ACTA, Institut Claude Bourgelat—ENVL, 69280 Marcy L'étoile, France; email: axel.decourtye@acta.asso.fr

[3]Laboratoire de Biométrie et Biologie Evolutive (UMR 5558); CNRS; Université Lyon 1, 69622, Villeurbanne Cedex, France; email: delpuech@biomserv.univ-lyon1.fr

Annu. Rev. Entomol. 2007. 52:81–106

First published online as a Review in Advance on July 14, 2006

The *Annual Review of Entomology* is online at ento.annualreviews.org

This article's doi: 10.1146/annurev.ento.52.110405.091440

0066-4170/07/0107-0081$20.00

Key Words

ecotoxicology, insecticide, behavior, honey bee, natural enemy

Abstract

Traditionally, measurement of the acute toxicity of pesticides to beneficial arthropods has relied largely on the determination of an acute median lethal dose or concentration. However, the estimated lethal dose during acute toxicity tests may only be a partial measure of the deleterious effects. In addition to direct mortality induced by pesticides, their sublethal effects on arthropod physiology and behavior must be considered for a complete analysis of their impact. An increasing number of studies and methods related to the identification and characterization of these effects have been published in the past 15 years. Review of sublethal effects reported in published literature, taking into account recent data, has revealed new insights into the sublethal effects of pesticides including effects on learning performance, behavior, and neurophysiology. We characterize the different types of sublethal effects on beneficial arthropods, focusing mainly on honey bees and natural enemies, and we describe the methods used in these studies. Finally, we discuss the potential for developing experimental approaches that take into account these sublethal effects in integrated pest management and the possibility of integrating their evaluation in pesticide registration procedures.

INTRODUCTION AND DEFINITIONS

Pollinator: an organism that carries pollen from one flower to another

IPM: integrated pest management

LD_{50}: lethal dose 50%

Sublethal effect: an effect (physiological or behavioral) on individuals that survive an exposure to a pesticide (the pesticide dose/concentration can be sublethal or lethal)

Sublethal dose/concentration: a dose or a concentration defined as inducing no statistically significant mortality in the experimental population

For the past 20 years, the effects of pesticides on beneficial arthropods have been the subject of an increasing number of studies, and the potential effects have been reviewed several times (24, 62, 128). Two groups of organisms, natural enemies and pollinators, have received the most attention in this regard because of their value in integrated pest management (IPM) (131) and pollination processes (105), respectively.

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 (LD_{50}) or lethal concentration (LC_{50}) 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 nontarget activity (24). However, estimation of selectivity was based on LD_{50} 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 (80), 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 sublethal 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.

PHYSIOLOGICAL EFFECTS

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 (12). 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 (97) 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. When associated with prochloraz, deltamethrin elicited a joint hypothermia at doses that did not induce a significant effect on thermoregulation when used alone (133). 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 (98). However, the results of sublethal toxicity suggest other mechanisms for synergistic toxic effects, such as combined action on a common target (97).

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. (109) demonstrated that acute toxicity tests (LD_{50} 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 (74), 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 (132). Thus, pesticide effects on important enzyme systems cannot be extrapolated or deduced from LD_{50} values.

CO: cytochrome oxidase

Effects on neurophysiology have also been described. The metabolic activity in the honey bee brain was investigated using cytochrome oxidase (CO) histochemistry. Because CO is the terminal enzyme in the electron transport chain of the mitochondrial respiratory processes, histochemistry is used as an endogenous metabolic marker for neuronal activity (145). Using CO histochemistry to carry out metabolic mapping of discrete brain regions, Armengaud et al. (7) showed that CO histochemistry could be used to identify the target structures of cholinergic ligands in the honey bee brain, particularly in the case of the neonicotinoid imidacloprid. In a behavioral and histochemical analysis of the effect of imidacloprid on olfactory learning in the honey bee, oxidative metabolism in the calyces of the mushroom body was increased after treatment (27). In parallel, the impairment of olfactory memory by imidacloprid was observed. The structure-specific increase of CO activity in the brain observed after treatment suggests that imidacloprid impairs olfactory memory by a physiological effect at the level of the mushroom body, which is reported to have an essential role in olfactory memory (51a).

Results demonstrating negative effects of pesticide at the biochemical and neurophysiological levels are difficult to interpret because their consequences at individual or population levels are often unknown. However, this is not the case with studies concerning communication between insects or the development of beneficial arthropod larvae.

Development

IGR: insect growth regulator

Parasitoid: an insect that completes its larval development within the body of another insect eventually killing it and is free-living as an adult

JHA: juvenile hormone analog

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 (117), 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 (49). 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 (75, 110, 114). Adult emergence has also been studied for the lacewing predator *Mallada signatus* exposed to the botanical insecticide azadirachtin A (AzaA) in the pupal stage (101). 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. (115) 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 (13).

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 (r_m) 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 (81). Cônsoli et al. (21) reported that *Trichogramma pretiosum* pupae displayed a higher sensitivity to pesticides in terms of development time than did either eggs, larvae, or prepupae. Increases in development time have also been reported in other predators exposed to neurotoxic insecticides (53–55) and on parasitoids exposed to botanical insecticides (18). The impact of pesticides on development time may also be a function of gender. In the pentatomid predator *Supputius cincticeps*, exposure to permethrin decreased development time for females, whereas this time increased for males (146). Malformations also occur in natural enemies after exposure to pesticides and may lead to reduction in predator or parasitoid efficiency and fitness. In a study describing sublethal effects in the predators *Coccinella septempunctata* and *Chrysoperla carnae* exposed to AzaA-treated aphids, Ahmad et al. (3) reported morphological deformities and thus calculated a rate of deformity to express all the visible deformations in adult individuals exposed to pesticides during the larval stage. Although this statistic did not provide a qualitative measure of deformation, it could be useful for estimating the potential developmental effects of pesticides. In another study, the hind tibia length was shorter in males of the parasitoid *Cotesia plutellae* emerging from *Plutella xylostella* larvae that fed on cabbage treated with botanical insecticide (18). Such a malformation in males may lead to a strong reduction in their fitness because their capacity to mate is correlated with overall body size (69). In the reduviid predator *Rhynocoris kumarii*, adults developed severe abnormalities in the alimentary canal, testis, and ovary when treated with the organophosphorus insecticides monocrotophos, dimethoate, methyl parathion, or quinalphos at sublethal doses (57). The authors determined that abnormalities in the alimentary canal were due to lysis of intercellular cementing material, pycnotic

nuclei, vacuolated cells, obliteration of the peritrophic membrane, and exfoliation of cells. In the same study, pesticides caused predator size reduction, sperm cell distortion, vacuolated spermatocytes in the testis, and crumpled follicular epithelium and vacuolization of the germarium in the ovaries. Malformation of ovaries can also occur in parasitoids exposed to IGRs (115).

For hymenopterous social pollinators, perturbations in larval development must be seen as a major threat for colonies. Reductions in brood and numbers of emerging *Apis mellifera* may be more damaging to colony health than the loss of foragers, because flexibility in the division of labor can replace foragers if there are sufficient brood and nurse bees (128). IGRs can interfere with development, particularly when exposure occurs during the larval stage. Most observations on the effects of IGRs rely on measurements of brood quantity taken under field or semifield conditions. One common approach uses the number of hive cells containing different bee brood stages to detect possible adverse effects, because disturbances to larval development could be accompanied by failure to emerge. Oral exposure of worker bees with diflubenzuron (IGR) reduced brood surface area (17). The impact of fenoxycarb on brood was manifested by the presence of malformed larvae or pupae, which were ultimately found dead in front of the hive (42). However, opposite effects were reported on *Bombus terrestris* exposed to imidacloprid (126), and these authors assumed that the reduced larval ejection rate in treated groups was due to reduction of brood size (due to mortality). Because it is impossible to dissociate effects on brood size from direct effects on larvae using these measures, an accurate assessment of the effects of pesticide exposure on larvae cannot be obtained.

Few quantitative studies have assessed the impact of IGRs on larval development, possibly because of difficulties associated with rearing larvae under consistent conditions. However, a new in vitro brood test described in several reports (10, 85) may ensure a more precise assessment of the effects of pesticides on honey bee larvae. After collection of first instars, and grafting in artificial rearing cells in laboratory, the larvae are fed with a diet containing a pesticide until adult emergence. This method appears to be promising to screen out sublethal effects of pesticides on the physiology of larval development.

IGRs may also have physiological effects on honey bee adults, particularly by inhibiting the formation of imaginal organs, which may have indirect effects on larval development. Newly emerged adults of *A. mellifera* and *Apis cerana* treated with diflubenzuron showed reduced weight gain and suppressed development of hypopharyngeal glands (59). This study demonstrated the morphogenic capacity of a chitin inhibitor, an action formerly showed with juvenile hormone mimics (65). Because hypopharyngeal glands of nurse bees produce the royal jelly used to feed the first instars of worker larvae, and all instars of queen larvae, their malformations might result in undernourished larvae and so might potentially lead to a decline in colony population or to no renewal of the queen. Similarly, nurse bees use vitellogenin to produce royal jelly (6), and pyriproxyfen (JHA) impairs vitellogenin synthesis in the hemolymph (99), suggesting repercussions on brood care. Such physiological effects might also cause disturbances in longevity, immunity, or reproduction, because vitellogenin, a lipoprotein, is of fundamental importance in each of these processes (6).

Adult Longevity

Effects on longevity after exposure to lethal or sublethal doses of pesticides have been described mostly for parasitoid species (5, 43, 47, 75, 108, 110, 115, 118) 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 r_m is determined for risk assessment of pesticides, a reduction of survival (l_x) could lead to a strong reduction of the r_m 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 Cox proportional hazard model.

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 LC_{50} decreased the number of plasmatocytes in the hemolymph of the predator *R. kumarii* by 16% 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 (111a). 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 LD_{30}, 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 LD_{50} 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.

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. Cônsoli 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 and 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 life-table 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 (108). Moreover, Rumpf et al. (108) emphasized that long-term sublethal 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.

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, 115) and testes (57). However, 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 (LD_{50}) were 58% female and offspring of the control group were 73% female (107). In *Trichogramma brassicae*, the offspring of females surviving exposure to chlorpyrifos (LD_{20}) 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.

BEHAVIORAL EFFECTS

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

Kairomone: chemical substance produced by an individual that serves as a stimulus to other individuals of another species for behavioral responses

Foraging: the behavior of searching for food, host, or prey

on the mobility of beneficial arthropods have been observed, but they are mostly due to (*a*) direct intoxication by the pesticides, resulting in knock-down effect (23, 124), uncoordinated movement (5, 14, 116), trembling, tumbling, abdomen tucking, and/or rotating and cleaning of the abdomen while rubbing the hind legs together (124); (*b*) secondary consequences of behavioral modifications (111) 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, 106) or irritant effect of pesticides (144). Several authors (111, 144) 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 (144) mainly because of irritation caused by the pesticide. The grooming behavior associated with increased mobility is thought to be a reflex action initiated by irritation of chemoreceptors located on the surface of the insect body (103). 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 (124). 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.

Navigation/Orientation

In natural enemies, navigation and orientation could involve multiple sensory cues, either chemical (135) or visual (140). 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. (130) 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 example, tarsal exposure on pesticide deposits

(34, 36, 48), exposure via feeding on contaminated sources (118), 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, 73).

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 host-plant complex in a wind tunnel decreased by 71% and 62%, respectively (118). A lower residence time on the contaminated host patch was observed with females of the parasitoid *Trissolcus basalis* exposed to deltamethrin at LD_{25}, compared with unexposed females (111). 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 LD_{20} 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 $LD_{0.1}$ of chlorpyrifos were less arrested by female sexual pheromones; however, pheromones emitted by exposed females ($LD_{0.1}$) were more arresting for untreated males (37). In contrast, when *T. brassicae* males were treated with the pyrethroid deltamethrin at $LD_{0.1}$ there was an increase in arrestment, whereas when females were treated, their pheromones were less arresting for males (39). These effects offset 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 landmarks 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 (139). 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 (134). 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

ppb: part per billion

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) (123) 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 (147). 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.

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 exposure 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.

Honey bees change their behavior in response to pesticides through reduced feeding stimulation (62). For example, topical application of deltamethrin at concentrations ranging from 0.08 to 0.16 ppm increased syrup uptake in bees, although similar concentrations delivered orally decreased uptake of treated syrup (127). Despite this work, many of the reported effects on the foraging activity of bees relate to avoidance behavior and, in a few cases, feeding stimulation. In the same manner, official guidelines for assessing the impact of pesticides on foraging activity consist mainly of measuring repellency (93). These bioassays were developed in higher-tier studies, under semifield and field conditions.

In this regard, pyrethroids are probably the best-known repellent insecticides (33, 106). For a long time, repellency associated with pyrethroids has been considered a behavioral adaptation for reducing the risk of exposure. However, it is now known that pyrethroid applications during peak foraging activity (in broad daylight) result in high exposure levels (32). Therefore, a repellent effect must not be misconstrued as providing any protection against exposure to pesticides.

In the case of parasitoids, *Aphidius rhopalosiphi* responded strongly to patches of aphid honeydew on filter paper, but the addition of increasing concentrations of deltamethrin caused increasingly early departure from the honeydew/deltamethrin-treated areas because of a repellent and/or irritant effect (84). The influence of deltamethrin on the general feeding behavior of the predatory ladybeetle *C. septempunctata* was examined via visual observations (144). The movement of ladybeetles on deltamethrin-treated plants increased, although the ladybeetles tended to stay on plant parts known to receive less pesticides during treatment (72), thus demonstrating a clear repellent effect. A similar effect was observed when ladybeetles foraged on dimethoate-treated plants (116). The authors indicated that adult and larval stages ate fewer aphids when exposed to dimethoate because they avoided the treated areas. They also reported less consumption of aphids that were previously treated (in choice experiment), indicating that a combined repellent/antifeedant effect could occur. An antifeedant effect was demonstrated for the predatory carabid *Nebria brevicollis* feeding on deltamethrin-treated aphids (143). The authors reported that 53% to 80% of beetles ingesting treated aphids exhibited a regurgitation response after consumption.

The specific sensory mechanism through which repellency operates is not well understood but may depend on the mode of exposure. Fipronil (87) and AzaA (92) reduced visitation of honey bees to treated sucrose but did not have an effect when applied to flowering canola. Several observations on the repellency of pesticides after spraying indicate that it could not be attributed to the active ingredient itself, but rather to additives in commercial formulations (13a) or the physical characteristics of the spray (wetting vegetal). These observations may explain the differences in behavior observed between treated artificial food and field applications. Although the means by which foragers detect the treatment on the crop remains uncertain, the gustatory perception of active ingredients can be assumed when there is a notable decline in the level of foraging on an artificial feeder at short time intervals (28, 87, 112). The delay in the inhibition of foraging with imidacloprid varies according to concentration tested (112). The author attributed this delay to a process occurring inside the hive rather than to effects on foragers. A decrease in feeding activity may be the result of changes in communication processes that alter foraging activity. This hypothesis was reinforced by studies reporting the impact of imidacloprid on dances. Dances produced by returning foragers contain coded information about the distance and direction of food sources and are aimed at the recruitment of foragers (139). Decreases in the frequency of waggling dances were observed when foragers had previously visited a sugar solution contaminated with approximately 20 ppb of imidacloprid (25). A lower motivation to perform waggling dances can result in a reduction of recruitment activity.

The process of food detection, whether the food is prey (predators and host-feeding parasitoids), nectar, or honeydew (proovigenic parasitoids or honey bees), involves sophisticated nervous activity that can be disrupted by neurotoxic pesticides (62). The reduviid predator *Acanthaspis pedestris* exhibited a decrease in excitation by prey and a decreased ability to paralyze prey after exposure to the cypermethrin (19). These perturbations were accompanied by a reduction in food intake, haphazard movement, and signs of restlessness. Reductions in the rate of aphid consumption by the predators *Coccinella*

PER: proboscis extension response

septempunctata and *Chrysoperla carnae* at the larval stage were also reported when aphids were treated with AzaA (3). The impact on food detection may also have a negative impact on parasitism capacity of parasitoids. The decrease in parasitism capacity may result from reduced energy intake, as well as from indirect effects on host detection. Indeed, it has been suggested that aphid parasitoids exploit honeydew deposits on the surfaces of leaves as cues for evaluating the numbers of aphids on the plant and therefore the amount of effort to be invested in further search (83). Thus, if the ability to detect honeydew is compromised, an effect on foraging pattern and patch time allocation may occur, resulting in a reduction in parasitism rate. In honey bees, the proboscis extension reflex (PER) can be elicited through antennal stimulation with a sucrose solution and can be used to assess the gustatory threshold to sugary foods (8). The gustatory threshold is defined as the lowest concentration of a sucrose solution, applied to the antennae, that is capable of eliciting a PER. A reduction of sensitivity for low-sucrose concentrations (55% to 60% in control versus 15% to 20% in treated bees) was observed following thoracic application of fipronil at a dose of 1 ng per bee (51), demonstrating that pesticide exposure can drastically reduce the capacity of honey bees to detect food sources.

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 reduce the chances that a natural enemy will find a suitable host or oviposition site (83, 130), 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 extrusion and consequently failed to lay eggs.

Kühner 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 LD_{20} 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 (129). 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 (136)], but this effect has not been well described.

Short-range detection of hosts may also be altered by pesticides, resulting in disruption of oviposition. When parasitoids are close to a host, they often rely on short-range chemical cues (135) or color (140). Desneux et al. (45) determined that after exposure to

lambda-cyhalothrin the parasitoid *A. ervi* exhibited less host antennal contact and antennal examination behavior than did unexposed individuals and that these observations were not associated with perturbed mobility. Similarly, the parasitoid *H. didymator* had difficulties finding hosts after exposure to AzaA (115). The parasitoid did not actively search for hosts and often walked away from hosts. This effect was independent of any repellent effect, because parasitoids were tested after their exposure to pesticides. However, sublethal effects of pesticides on host detection and oviposition may not always be unfavorable to parasitoids. Chlorpyrifos at LD_{20} caused an increase (5.1-fold) in host searching by *Leptopilina heterotoma*, a parasitoid of *Drosophila* larvae that probes substrate with its ovipositor (102). Furthermore, the authors reported that treated females found and oviposited into host larvae 46% faster than did control females.

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 (LD_{20}) (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 ($LD_{0.1}$ and LD_{20}) (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 (125). 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, 122, 142). 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).

Toxicant exposure can be carried out before (122), during (1), or after (86) PER conditioning. In an ecological context, long-term exposure to low concentrations corresponds to the case of inexperienced bees involved in foraging duties based on their learning ability after being fed a contaminated food within the hive. With this approach, reduced learning performance was observed in bees surviving 11 days of oral exposure to imidacloprid, 5-OH-imidacloprid, fipronil, deltamethrin, endosulfan, and prochloraz (29, 30). This bioassay can also help to assess how chemical treatments can interfere with the memory process and provides an indication of the ability of foragers to return to a crop where they have been exposed to a toxin while they were collecting food and memorizing the floral cues. In this regard, imidacloprid administered after trial conditioning of PER impaired medium-term olfactory memory (27). By contrast, short-term and long-term memory was unaffected. It was assumed that the consolidation process that ensures the transfer from short-term memory to medium-term memory was affected by imidacloprid. Because

flower choice in successive foraging bouts is interrupted by return to the hive (89), imidacloprid may affect this process. However, the precise consequences for foraging behavior are still unclear.

Overall, work using the PER assay has employed olfactory Pavlovian conditioning. However, pesticide exposure can affect not only associative learning task but also nonassociative learning procedures such as habituation. Habituation of the PER is a simple form of learning in which the repetition of gustatory stimulation leads to a decrease of response probability. This test sheds light on the ability of an organism to constrain a reflex response. The habituation procedure has been used to demonstrate the effect of a sublethal dose of imidacloprid on PER suppression (58, 78). These results clearly indicate task-dependent behavioral effects associated with sublethal doses of imidacloprid and can be generalized to other insecticides.

IMPACT OF SUBLETHAL EFFECTS ON COMMUNITY ECOLOGY

Because sublethal effects of pesticides interact with numerous life-history traits involved in the reproduction of beneficial arthropods (i.e., foraging, fecundity, sexual communication, and sex ratio), they likely have an impact on insect communities. However, although the effects of pesticides on insect communities have been described, sublethal effects have not specifically been analyzed. Pesticides tend to lower the abundance of both parasitoids and their hosts, and lead to the disappearance of scarce species (species that are not naturally abundant in a given agroecosystem) (J.M. Delpuech & R. Allemand, unpublished data). A potentially useful tool for evaluating these different impacts would be to integrate them in a modeling approach. However, synthetic pesticides generally had a lower impact on natural enemy populations than predicted by database analyses, and recolonization of chemically treated plots can be rapid (9). Indeed, effects of pesticides on natural enemies can be short-lived (4).

Serious losses of pollinators have been attributed to pesticides (96), and honey bees can be used effectively as bioindicators to detect environmental pollution (71). However, effects of pesticides on honey bees are poorly representative of effects on other pollinators, including other bees (68). Indeed, bees (Apoidea) constitute a highly diverse group, and bees from different taxonomic groups differ widely in their vulnerability to pesticide exposure. In honey bees, pesticides may affect social organization (reduction of food uptake or reduction of worker/brood population), but these effects may be compensated for because the queen does not take part in foraging and is probably less likely to be exposed than workers. In contrast, in other social pollinators such as bumble bees, the queen must find food during spring in order to found the colony. In this case, the potential negative effects of pesticides may substantially affect colony establishment. In summary, social pollinators having no perennial colony and no social pollinators are more likely to suffer from insecticide exposure.

IMPLICATIONS IN INTEGRATED PEST MANAGEMENT AND POLLINATION

The economic gains due to beekeeping and agricultural pollination might be reduced by intoxication of colonies with pesticides, even though there are few data to support this assertion. The best example is a long-term study conducted in eastern Canada. In that region, blueberry production, which depends largely on pollination by as many as 70 species of native insects, failed in 1970, and subsequent years, because of aerial spraying of fenitrothion (70). Although the impacts of mass mortalities of honey bees on pollination of crops are documented, less understood and often overlooked is the problem of sublethal effects that reduce agricultural production.

Studies 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 programs are established. To reduce nontarget 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 nontarget lethal toxicity (24). However, given the potential importance of sublethal effects on natural enemies (reported in this review), pesticide choice should also consider those with minimal sublethal effects on key components of beneficial efficiency. For example, a comparison of sublethal effects of two pyrethroids (lambda-cyhalothrin and deltamethrin) on key behaviors of aphid parasitoids demonstrated that lambda-cyhalothrin disrupted olfactory orientation toward host-infested plants and oviposition behavior (45) and that deltamethrin did not (43, 47, 48). These results were partially confirmed by a study in semifield conditions in which the authors showed that aphid parasitoids released on deltamethrin-treated plants significantly limited aphid population growth even when introduced one day after treatment (44). Moreover, the pesticide and parasitoid effects were additive. We expect that more thorough consideration of potential sublethal effects on natural enemies in the future will help to optimize IPM programs involving use of both natural enemies and pesticides against pests.

METHODS PROMISING FOR INTEGRATION IN REGISTRATION PROCEDURES

Pollinators

Environmental risk assessment of pesticides on honey bees takes into account mainly the survival of adult bees exposed to pesticides over a relatively short time frame. Furthermore, sublethal effects are generally not considered. Whereas lethal effects are rather easy to observe and can lead to loss of product registration, more subtle effects on bee physiology or behavior may also affect honey bee populations. The honey bee risk assessment scheme tentatively takes into account these different aspects of exposure. U.S. EPA guidelines indicate that abnormal behavior during acute toxicity tests should be precisely recorded (i.e., kind, time of onset, duration, severity, numbers of bees affected). In Europe, when the standard procedures cannot provide clear conclusions on the harmlessness of a pesticide, the official decision-making scheme recommends the use of additional studies in order to provide adequate information (93). However, no specific protocols are outlined even though issues related to pesticides and bees are intensively discussed. In recent years, many beekeepers in European countries have complained about unusual honey bee losses and hive depletions. These losses may be due to the use of seeds dressed with newly registered pesticides (20). This issue has revealed limitations in standardized regulatory methods: underestimation of bee exposure after seed-dressing application, failure to account adequately for larval and sublethal toxicities, and absence of measures of chronic toxicity. In this context, more standardized methods to evaluate sublethal effects of pesticides may be needed.

According to guidelines, a brood feeding test is required to evaluate whether bee larvae may be at risk when exposed to a compound showing IGR activity (94). Because of environmental variation, the recommended methods (95) may not be easily reproducible. Thus, an in vitro method for rearing bee larvae has been improved and may be recommended for regulatory trials assessing pesticide toxicity to larvae (10). Sublethal effects may be investigated by measuring weight and larval developmental variation and morphology changes in adults. Further studies are necessary to determine if the method can reliably detect behavioral effects in individuals exposed during the larval stage.

The PER assay can be considered a quantifiable and reliable method to assess sublethal toxicity (31). The PER procedure enables researchers to compare responses to different chemicals and different concentrations of the same chemical, determine no-effect concentrations, and investigate the nervous circuitry underlying the olfactory learning processes when neurotoxic molecules that affect the peripheral nervous system or CNS are tested. Although the associative learning of workers investigated with PER assays may be ecologically significant, it is unknown how well they translate to the colony level under natural conditions (128). However, preliminary studies using PER assays indicate that the decrease in learning performance induced by imidacloprid at the individual level translate well to the colony level in olfactory discrimination tasks (28).

Natural Enemies

To date, no standardized methods for assessing sublethal effects on parasitoids and predators are described in the regulatory texts. The European standard characteristics of nontarget arthropod regulatory testing (ESCORT 2) workgroup has developed a method to improve the risk assessment of pesticides on natural enemies and propose the adoption of a hazard quotient (HQ) approach. HQ is calculated by dividing crop-specific application rates by the LR_{50} (lethal rate 50) derived from worst-case-scenario laboratory studies generated using two sensitive indicator species (16). This method is a welcome development, but there are still important questions to consider due to the fact that HQ is calculated from LR_{50} values, and thus potential sublethal effects are not included. This integration can be solved in part by using a demographic approach to estimate toxicity as reviewed by Stark & Banks (120). Life-table experiments are conducted by exposing individuals or groups to increasing doses of a toxicant over their life span and daily mortality and reproduction are recorded, providing data to calculate the r_m. Sublethal effects on fertility, fecundity, developmental rate, survival, and sex ratio can be detected when estimating the r_m. Life-table experiments provide a more accurate measure of toxic effect than do lethal concentration estimates (52) and have been used successfully to evaluate side effects of pesticides on several natural enemies (2, 121).

However, this method also has some limitations (120): It is expensive, time consuming, and performed under laboratory conditions that do not reflect wild conditions (such as density dependence). Several of these points have been addressed (119, 141). However, if we consider the caging conditions used during experiments, lack of detection of behavioral perturbations induced by pesticides is likely. Indeed, the ability of parasitoids to detect host-induced plant odors (synomones) is crucial because these odors are used to detect host patches at long range (135). However, in life-table experiments, parasitoids may be so close to the hosts that they detect their hosts without using long-range cues. Thus, an impairment of important aspects of foraging behavior can be missed during evaluation. It may therefore be important to add a standard behavioral test to any toxicological tests, including the r_m evaluation.

CONCLUSION AND FUTURE OUTLOOK

This review reports a wide variety of sublethal effects of pesticides on the physiological and behavioral processes in beneficial arthropods. Effects are documented according to the technical issues associated with studying various processes and also according to ecological knowledge of basic mechanisms involved in the traits that are potentially perturbed by pesticides. In most cases only one dose, punctually administered and not necessarily sublethal, was studied. Thus, misinterpretation may result from lethal concentrations mistakenly used to study sublethal effects. Acute exposure to high concentrations of a chemical can result in selection of insects that are less

sensitive to the pesticide tested. Such resistant insects may provide responses that are not representative of the population. This point should be carefully considered in future studies of the sublethal effects of pesticides because errors in choice of doses/concentrations tested may provide misleading results. Assessment of sublethal effects should be conducted by testing effects of both sublethal and lethal doses/concentrations.

Methods using the honey bee model are well defined, particularly in the field of behavior, because of its importance for studying behavioral and learning processes in insects (91). Moreover, increasing requirements related to the nontarget effects of pesticides on pollinators for new pesticide registration have motivated expansion of tests on this model (93). Thus, tests on honey bees in registration procedures are better developed than tests on natural enemies, and the development and inclusion of several promising methods into regulatory procedures is in progress (32, 128). Methods to assess nontarget effects on natural enemies are also progressing. Choice of indicator species is also being made (15, 16) and was a first step to help the development and inclusion of methods on natural enemies. However, sublethal effects are not a major concern yet, and further development of standardized methods assessing sublethal effects on key components of natural enemy efficiency must be achieved before incorporating these effects into regulatory procedures.

The link between sublethal effects of pesticides and consequences at the population and community levels are still not well understood in either pollinators or natural enemies, and the same can be said when considering how sublethal effects are taken into account for the development of IPM programs. Even though many studies have documented sublethal effects of pesticides on natural enemies, only mortality tests are considered when a choice between several pesticides must be made. To fully assess risk, it is crucial to establish a link between the toxicity of a given product in laboratory assays and the risk associated with exposure under field conditions. Although this point is often overlooked, it emphasizes the need for studies on the dynamics of exposure to pesticides. It will require the quantification of residues in different locations visited by insects and also an estimation of the degradation of pesticides under field conditions. The use of multistep bioassays to evaluate the potential effects of pesticide on beneficial insects would also help to assess risk in a more complete way by including evaluation of pesticide effects on key behavioral and physiological processes instead of considering mortality only as an endpoint. These assays, although slightly more laborious than lethal concentration estimates, will help researchers to evaluate the nontarget impacts of pesticides and promote discovery of crucial ecological side effects before pesticide registration rather than after.

SUMMARY POINTS

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.
5. Even though many studies have documented sublethal effects of pesticides on natural enemies, only mortality tests are considered when a choice between several pesticides must be made in an IPM context. To fully assess risk, it is crucial to establish a link between the toxicity of a given product in laboratory assays and the risk associated with exposure under field conditions (including lethal and sublethal effects).
6. Methods to test sublethal effects on beneficial arthropods are currently being developed, and inclusion of several promising methods into regulatory procedures is in progress (more advanced work on pollinators). However, sublethal effects are not a major concern yet, and further development of standardized methods assessing sublethal effects on key components of natural enemy efficiency will need to be achieved before incorporating these effects into regulatory procedures.

ACKNOWLEDGMENTS

We wish to thank Dr. D.S. Richmond for helpful comments on the review. N. Desneux also thanks Dr. L. Kaiser for encouraging his interest in sublethal effects of pesticides. This work was supported in part by a grant from French Ministry of Agriculture (European fund for French beekeeping).

LITERATURE CITED

1. Abramson CI, Squire J, Sheridan A, Mulder PG. 2004. The effect of insecticides considered harmless to honey bees (*Apis mellifera*): proboscis conditioning studies by using the insect growth regulators tebufenozide and diflubenzuron. *Environ. Entomol.* 33:378–88
2. Acheampong S, Stark JD. 2004. Effects of the agricultural adjuvant Sylgard 309 and the insecticide pymetrozine on demographic parameters of the aphid parasitoid, *Diaeretiella rapae*. *Biol. Control* 31:133–37
3. Ahmad M, Ossiewatsch HR, Basedow T. 2003. Effects of neem-treated aphids as food/hosts on their predators and parasitoids. *J. Appl. Entomol.* 127:458–64
4. Al-Deeb MA, Wilde GE, Zhu KY. 2001. Effects of insecticides used in corn, sorghum and alfalfa on the predator *Orius insidiosus* (Hemiptera: Anthocoridae). *J. Econ. Entomol.* 94:1353–60
5. Alix A, Cortesero AM, Nénon JP, Anger JP. 2001. Selectivity assessment of chlorfenvinphos reevaluated by including physiological and behavioral effects on an important beneficial insect. *Environ. Toxicol. Chem*. 20:2530–36
6. Amdam GV, Norberg K, Hagen A, Omholt SW. 2003. Social exploitation of vitellogenin. *Proc. Natl. Acad. Sci. USA* 100:1799–802

7. Armengaud C, Causse N, Ait-Oubah J, Ginolhac A, Gauthier M. 2000. Functional cytochrome oxidase histochemistry in the honeybee brain. *Brain Res.* 859:390–93
8. Armengaud C, Lambin M, Gauthier M. 2002. Effects of imidacloprid on the neural processes of memory in honey bees. See Ref. 48a, pp. 85–100
9. Armenta R, Martinez AM, Chapman JW, Magallanes R, Goulson D, et al. 2003. Impact of a nucleopolyhedrovirus bioinsecticide and selected synthetic insecticides on the abundance of insect natural enemies on maize in southern Mexico. *J. Econ. Entomol.* 96:649–61
10. Aupinel P, Fortini D, Dufour H, Taséi JN, Michaud B, et al. 2005. Improvement of artificial feeding in a standard in vitro method for rearing *Apis mellifera* larvae. *Bull. Insectol.* 58:107–11
11. Banken JAO, Stark JD. 1998. Multiple routes of pesticide exposure and the risk of pesticides to biological controls: a study of neem and the sevenspotted lady beetle (Coleoptera: Coccinellidae). *J. Econ. Entomol.* 91:1–6
12. Bendahou N, Bounias M, Fléché C. 1999. Toxicity of cypermethrin and fenitrothion on the hemolymph carbohydrates, head acetylcholinesterase, and thoracic muscle Na^+, K^+-ATPase of emerging honeybees (*Apis mellifera mellifera*. L). *Ecotoxicol. Environ. Safety* 44:139–46
13. Bortolotti L, Sbrenna AM, Sbrenna G. 2005. Action of fenoxycarb on metamorphosis and cocoon spinning in *Chrysoperla carnea* (Neuroptera: Chrysopidae): identification of the JHA-sensitive period. *Eur. J. Entomol.* 102:27–32

13a. Bos C, Masson C. 1983. Repellent effect of deltamethrin on honey bees. *Agronomie* 3:545–53

14. Brunner JF, Dunley JE, Doerr MD, Beers EH. 2001. Effects of pesticides on *Colpoclypeus florus* (Hymenoptera: Eulophidae) and *Trichogramma platneri* (Hymenoptera: Trichogrammatidae), parasitoids of leafrollers in Washington. *J. Econ. Entomol.* 94:1075–84
15. Candolfi MP, Bakker F, Cañez V, Miles M, Neumann C, et al. 1999. Sensitivity of non-target arthropods to plant protection products: Could *Typhlodromus pyri* and *Aphidius* spp. be used as indicator species? *Chemosphere* 39:1357–70
16. Candolfi MP, Barrett KL, Campbell P, Forster R, Grandy N, et al. 2001. Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. In SETAC/ESCORT 2 Workshop Rep. 21–23, March 2000, Wageningen
17. Chandel RS, Gupta PR. 1992. Toxicity of diflubenzuron and penfluron to immature stages of *Apis cerana indica* F and *Apis mellifera* L. *Apidologie* 23:465–73
18. Charleston DS, Kfir R, Dicke M, Vet LEM. 2005. Impact of botanical pesticides derived from *Melia azedarach* and *Azadirachta indica* on the biology of two parasitoid species of the diamondback moth. *Biol. Control* 33:131–42
19. Claver MA, Ravichandran B, Khan MM, Ambrose DP. 2003. Impact of cypermethrin on the functional response, predatory and mating behaviour of a non-target potential biological control agent *Acanthaspis pedestris* (Stål) (Het., Reduviidae). *J. Appl. Entomol.* 127:18–22
20. Colin ME, Bonmatin JM, Moineau I, Gaimon C, Brun S, Vermandere JP. 2004. A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. *Arch. Environ. Contam. Toxicol.* 47:387–95
21. Cônsoli FL, Parra JRP, Hassan SA. 1998. Side effects of insecticides used in tomato fields on the egg parasitoid *Trichogramma pretiosum* Riley (Hym., Trichogrammatidae), a natural enemy of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae). *J. Appl. Entomol.* 122:43–47

22. Corrales N, Campos M. 2004. Population, longevity, mortality and fecundity of *Chrysoperla carnea* (Neuroptera, Chrysopidae) from olive orchards with different agricultural management systems. *Chemosphere* 57:1613–19
23. Cox RL, Wilson WT. 1987. The behavior of insecticide-exposed honey bees. *Am. Bee J.* 127:118–19
24. Croft BA. 1990. *Arthropod Biological Control Agents and Pesticides*. New York: Wiley. 723 pp.
25. Dechaume-Moncharmont FX. 2003. *Butinage collectif chez l'abeille* Apis mellifera *L.: etude théorique et expérimentale*. PhD thesis. Univ. Pierre-et-Marie-Curie, Paris. 309 pp.
26. Dechaume-Moncharmont FX, Decourtye A, Hennequet-Hantier C, Pons O, Pham-Delègue MH. 2003. Statistical analysis of honeybee survival after chronic exposure to insecticides. *Environ. Toxicol. Chem.* 22:3088–94
27. **Decourtye A, Armengaud C, Renou M, Devillers J, Cluzeau S, et al. 2004. Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). *Pestic. Biochem. Phys.* 78:83–92**
28. Decourtye A, Devillers J, Cluzeau S, Charreton M, Pham-Delègue MH. 2004. Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. *Ecotoxicol. Environ. Safety* 57:410–19
29. Decourtye A, Devillers J, Genecque E, Le Menach K, Budzinski H, et al. 2005. Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. *Arch. Environ. Contam. Toxicol.* 48:242–50
30. Decourtye A, Lacassie E, Pham-Delègue MH. 2003. Learning performances of honeybees (*Apis mellifera* L.) are differentially affected by imidacloprid according to the season. *Pest Manag. Sci.* 59:269–78
31. Decourtye A, Pham-Delègue MH. 2002. The proboscis extension response: assessing the sublethal effects of pesticides on the honey bee. See Ref. 48a, pp. 67–84
32. Decourtye A, Tisseur M, Taséi JN, Pham-Delègue MH. 2005. Toxicité et risques liés à l'emploi de pesticides chez les pollinisateurs: cas de l'abeille domestique. In *Enjeux Phytosanitaires pour L'agriculture et L'environnement au XXI Siècle*, ed. C Regnault-Roger, pp. 283–99. Paris: Lavoisier. 1013 pp.
33. Delabie J, Bos C, Fonta C, Masson C. 1985. Toxic and repellent effects of cypermethrin on the honeybee: laboratory, glasshouse and field experiments. *Pestic. Sci.* 16:409–15
34. Delpuech JM, Bardon C, Boulétreau M. 2005. Increase of the behavioral response to kairomones by the parasitoid wasp *Leptopilina heterotoma* surviving insecticides. *Arch. Environ. Contam. Toxicol.* 49:186–91
35. Delpuech JM, Frey F, Carton Y. 1996. Action of insecticides on the cellular immune reaction of *Drosophila melanogaster* against the parasitoid *Leptopilina boulardi*. *Environ. Toxicol. Chem.* 15:2267–71
36. Delpuech JM, Froment B, Fouillet P, Pompanon F, Janillon S, Boulétreau M. 1998. Inhibition of sex pheromone communications of *Trichogramma brassicae* (Hymenoptera) by the insecticide chlorpyrifos. *Environ. Toxicol. Chem.* 17:1107–13
37. **Delpuech JM, Gareau E, Terrier O, Fouillet P. 1998. Sublethal effects of the insecticide chlorpyrifos on the sex pheromonal communication of *Trichogramma brassicae*. *Chemosphere* 36:1775–85**
38. Delpuech JM, Legallet B, Fouillet P. 2001. Partial compensation of the sublethal effect of delthamethrin on the sex pheromonal communication of *Trichogramma brassicae*. *Chemosphere* 42:985–91

27. Demonstrates that oxidative metabolism (cytochrome oxidase) in the calyces of the mushroom body can be increased after a pesticide exposure, while the olfactory memory is affected.

37. Highlights that even insecticide doses inducing no apparent mortality ($LD_{0.1}$) induce perturbations in behaviors involved in the reproduction of natural enemies.

39. Delpuech JM, Legallet B, Terrier O, Fouillet P. 1999. Modifications of the sex pheromonal communication of *Trichogramma brassicae* by a sublethal dose of deltamethrin. *Chemosphere* 38:729–39
40. Delpuech JM, Meyet J. 2003. Reduction in the sex ratio of the progeny of a parasitoid wasp (*Trichogramma brassicae*) surviving the insecticide chlorpyrifos. *Arch. Environ. Contam. Toxicol.* 45:203–8
41. Delpuech JM, Tekinel-Ozalp P. 1991. Epigenetic influences of insecticide on host-parasitoid relations. *Redia* 74:417–24
42. DeRuijter A, VanderSteen J. 1987. A field study on the effect on honeybee brood of insegar (fenoxycarb) applied on blooming apple orchards. *Apidologie* 18:356–57
43. Desneux N, Denoyelle R, Kaiser L. 2006. A multi-step bioassay to assess the effect of the deltamethrin on the parasitic wasp *Aphidius ervi*. *Chemosphere* 65:1697–706
44. Desneux N, Fauvergue X, Dechaume-Moncharmont FX, Kerhoas L, Ballanger Y, Kaiser L. 2005. *Diaeretiella rapae* limits *Myzus persicae* populations following applications of deltamethrin in oilseed rape. *J. Econ. Entomol.* 98:9–17
45. Desneux N, Pham-Delègue MH, Kaiser L. 2004. 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.* 60:381–89
46. Desneux N, Rafalimanana H, Kaiser L. 2004. Dose-response relationship in lethal and behavioural effects of different insecticides on the parasitic wasp *Aphidius ervi*. *Chemosphere* 54:619–27
47. Desneux N, Ramirez-Romero R, Kaiser L. 2006. Multi-step bioassay to predict recolonization potential of emerging parasitoids after a pesticide treatment. *Environ. Toxicol. Chem.* 25:2675–82
48. Desneux N, Wajnberg E, Fauvergue X, Privet S, Kaiser L. 2004. 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.* 112:227–35
48a. Devillers J, Pham-Delègue MH, eds. 2002. *Honey Bees: Estimating the Environmental Impact of Chemicals*. London/New York: Taylor & Francis. 336 pp.
49. Dhadialla TS, Carlson GR, Le DP. 1998. New insecticides with ecdysteroidal and juvenile hormone activity. *Annu. Rev. Entomol.* 43:545–69
50. Dinter A, Poehling HM. 1995. Side effects of insecticides on two erigonid spider species. *Entomol. Exp. Appl.* 74:151–63
51. El Hassani AK, Dacher M, Gauthier M, Armengaud C. 2005. Effects of sublethal doses of fipronil on the behavior of the honeybee (*Apis mellifera*). *Pharmacol. Biochem. Behav.* 82:30–39
51a. Fahrbach SE. 2006. Structure of the mushroom bodies of the insect brain. *Annu. Rev. Entomol.* 51:209–32
52. Forbes VE, Calow P. 1999. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology? *Environ. Toxicol. Chem.* 18:1544–56
53. Galvan TL, Koch RL, Hutchison WD. 2005. Effects of spinosad and indoxacarb on survival, development and reproduction of the multicolored Asian lady beetle (Coleoptera: Coccinellidae). *Biol. Control* 34:108–14
54. George PJE, Ambrose DP. 1999. Insecticidal impact on the post-embryonic development of *Rhynocoris kumarii* Ambrose and Livingstone (Het., Reduviidae). *J. Appl. Entomol.* 123:509–12

45. Shows that orientation and oviposition behaviors of parasitoids may be impaired by low doses of lambda-cyhalothrin depending on the dose, parasitoid experience, and type of behavior.

55. George PJE, Ambrose DP. 1999. Post-embryonic developmental changes in non-target *Rhynocoris fuscipes* (Fabricius) (Insecta: Heteroptera: Reduviidae) by insecticides in cotton agroecosystem. *J. Adv. Zool.* 20:12–16

56. George PJE, Ambrose DP. 2004. Impact of insecticides on the haemogram of *Rhynocoris kumarii* Ambrose and Livingstone (Hem., Reduviidae). *J. Appl. Entomol.* 128:600–4

57. George PJE, Ambrose DP. 2004. Toxic effects of insecticides in the histomorphology of alimentary canal, testis and ovary in a reduviid *Rhynocoris kumarii* Ambrose and Livingstone (Hemiptera: Reduviidae). *J. Adv. Zool.* 25:46–50

58. Guez D, Suchail S, Gauthier M, Maleszka R, Belzunces LP. 2001. Contrasting effects of imidacloprid on habituation in 7- and 8-day-old honeybees (*Apis mellifera*). *Neurobiol. Learn. Mem.* 76:183–91

59. Gupta PR, Chandel RS. 1995. Effects of diflubenzuron and penfluron on workers of *Apis cerana indica* F. and *Apis mellifera* L. *Apidologie* 26:3–10

60. Hamilton GC, Lashomb JH. 1997. Effect of insecticides on two predators of the Colorado potato beetle (Coleoptera: Chrysomelidae). *Fla. Entomol.* 80:10–23

61. Haseeb M, Amano H. 2002. Effects of contact, oral and persistent toxicity of selected pesticides on *Cotesia plutellae* (Hym., Braconidae), a potential parasitoid of *Plutella xylostella* (Lep., Plutellidae). *J. Appl. Entomol.* 126:8–13

62. Haynes KF. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. *Annu. Rev. Entomol.* 33:149–68

63. Hutchinson TP. 2000. Graphing the survivorship of bees. *Insectes Soc.* 47:292–96

64. Idris AB, Grafius E. 1993. Pesticides affect immature stages of *Diadegma insulare* (Hymenoptera, Ichneumonidae) and its host, the diamondback moth (Lepidoptera, Plutellidae). *J. Econ. Entomol.* 86:1203–12

65. Jaycox ER, Skowrone W, Guynn G. 1974. Behavioral changes in worker honey bees (*Apis mellifera*) induced by injections of a juvenile hormone mimic. *Ann. Entomol. Soc. Am.* 67:529–35

66. Jervis MA, Copland MJW. 1996. The life cycle. In *Insect Natural Enemies: Practical Approaches to Their Study and Evaluation*, ed. MA Jervis, N Kidd, pp. 63–102. London: Chapman & Hall

67. Jewess PJ, Lee PW, Nicholls PH, Plimmer JR. 1999. Benzoylureas. In *Metabolic Pathways of Agrochemicals. Part 2: Insecticides and Fungicides*, ed. TR Roberts, DH Hutson, pp. 795–816. Cambridge, UK: R. Soc. Chem.

68. Johansen CA, Mayer DF. 1990. *Pollinator Protection. A Bee and Pesticide Handbook*. New Haven, CT: Wicwas. 212 pp.

69. Jones WT. 1982. Sex-ratio and host size in a parasitoid wasp. *Behav. Ecol. Sociobiol.* 10:207–10

70. Kevan PG. 1991. Pollination: keystone process in sustainable global productivity. *Acta Hortic.* 288:103–10

71. Kevan PG. 1999. Pollinators as bioindicators of the state of the environment: species, activity and diversity. *Agric. Ecosyst. Environ.* 74:373–93

72. Kjaer C, Jepson PC. 1995. The toxic effects of direct pesticide exposure for a nontarget weed-dwelling chrysomelid beetle (*Gastrophysa polygoni*) in cereals. *Environ. Toxicol. Chem.* 14:993–99

73. Komeza N, Fouillet P, Boulétreau M, Delpuech JM. 2001. Modification, by the insecticide chlorpyrifos, of the behavioral response to kairomones of a *Drosophila* parasitoid, *Leptopilina boulardi*. *Arch. Environ. Contam. Toxicol.* 41:436–42

56. Studies the impacts of commonly used insecticides on the hemocyte composition of a beneficial arthropod.

58. Indicates that task-dependent behavioral effects are associated with sublethal concentrations of imidacloprid and that results may be generalized to others insecticides.

74. Kreissl S, Bicker G. 1989. Histochemistry of acetylcholinesterase and immunocytochemistry of an acetylcholine receptor-like antigen in the brain of the honeybee. *J. Comp. Neurol.* 286:71–84
75. Krespi L, Rabasse JM, Dedryver CA, Nenon JP. 1991. Effect of three insecticides on the life cycle of *Aphidius uzbekistanicus* Luz. (Hym., Aphidiidae). *J. Appl. Entomol.* 111:113–19
76. Kühner C, Klingauf F, Hassan SA. 1985. Development of laboratory and semi-field methods to test the side effect of pesticides on *Diaeretiella rapae* (Hym. Aphidiidae). *Med. Fac. Landbouww. Rijksuniv. Gent* 50:531–38
77. Kunkel BA, Held DW, Potter DA. 2001. Lethal and sublethal effects of bendiocarb, halofenozide, and imidacloprid on *Harpalus pennsylvanicus* (Coleoptera: Carabidae) following different modes of exposure in turfgrass. *J. Econ. Entomol.* 94:60–67
78. Lambin M, Armengaud C, Raymond S, Gauthier M. 2001. Imidacloprid induced facilitation of the proboscis extension reflex habituation in the honeybee. *Arch. Insect Biochem. Physiol.* 48:129–34
79. Lefort S, Tisseur M, Decourtye A. 2005. De la traçabilité même chez les butineuses! *Bull. Tech. Apicole* 32:153–64
80. Little EE. 1990. Behavioral toxicology: stimulating challenges for a growing discipline. *Environ. Toxicol. Chem.* 9:1–2
81. Liu TX, Chen TY. 2001. Effects of the insect growth regulator fenoxycarb on immature *Chrysoperla rufilabris* (Neuroptera: Chrysopidae). *Fla. Entomol.* 84:628–33
82. Liu TX, Stansly PA. 2004. Lethal and sublethal effects of two insect growth regulators on adult *Delphastus catalinae* (Coleoptera: Coccinellidae), a predator of whiteflies (Homoptera: Aleyrodidae). *Biol. Control* 30:298–305
83. Longley M, Jepson PC. 1996. Effects of honeydew and insecticide residues on the distribution of foraging aphid parasitoids under glasshouse and field conditions. *Entomol. Exp. Appl.* 81:189–98
84. Longley M, Jepson PC. 1996. The influence of insecticide residues on primary parasitoid and hyperparasitoid foraging behaviour in the laboratory. *Entomol. Exp. Appl.* 81:259–69
85. Malone LA, Tregidga EL, Todd JH, Burgess EPJ, Philip BA, et al. 2002. Effects of ingestion of a biotin-binding protein on adult and larval honey bees. *Apidologie* 33:447–58
86. Mamood A, Waller G. 1990. Recovery of learning responses by honeybees following a sublethal exposure to permethrin. *Physiol. Entomol.* 15:55–60
87. Mayer DF, Lunden JD. 1999. Field and laboratory tests of the effects of fipronil on adult female bees of *Apis mellifera*, *Megachile rotundata* and *Nomia melanderi*. *J. Apic. Res.* 38:191–97
88. Medina P, Budia F, Del Estal P, Vinuela E. 2004. Influence of azadirachtin, a botanical insecticide, on *Chrysoperla carnea* (Stephens) reproduction: toxicity and ultrastructural approach. *J. Econ. Entomol.* 97:43–50
89. Menzel R. 1999. Memory dynamics in the honeybee. *J. Comp. Physiol. A* 185:323–40
90. Menzel R, Greggers U, Hammer M. 1993. Functional organization of appetitive learning and memory in a generalist pollinator, the honey bee. In *Insect Learning: Ecological and Evolutionary Perspectives*, ed. DR Papaj, AC Lewis, pp. 79–125. New York: Chapman & Hall. 416 pp.
91. Menzel R, Müller U. 1996. Learning and memory in honeybees: from behavior to neural substrates. *Annu. Rev. Neurosci.* 19:379–404
92. Naumann K, Currie RW, Isman MB. 1994. Evaluation of the repellent effects of a neem insecticide on foraging honeybees and other pollinators. *Can. Entomol.* 126:225–30

93. OEPP/EPPO. 1992. Guideline on test methods for evaluating the side effects of plant protection products on honeybees. *Bull. OEPP/EPPO* 22:203–15
94. OEPP/EPPO. 2003. Environmental risk assessment scheme for plant protection products. *Bull. OEPP/EPPO* 33:141–45
95. Oomen PA, DeRuijter A, VanderSteen J. 1992. Method for honeybee brood feeding tests with insect growth-regulating insecticides. *Bull. OEPP/EPPO* 22:613–16
96. O'Toole C. 1996. Bee systematics in Europe: the continuing crisis and some possible cures. In *The Conservation of Bees*, ed. A Matheson, SL Buchmann, C O'Toole, P Westrich, IH Williams, pp. 227–32. London: Academic. 254 pp.
97. Papaefthimiou C, Theophilidis G. 2001. The cardiotoxic action of the pyrethroid insecticide deltamethrin, the azole fungicide prochloraz, and their synergy on the semi-isolated heart of the bee *Apis mellifera macedonica*. *Pestic. Biochem. Phys.* 69:77–91
98. Pilling ED, Bromleychallenor KAC, Walker CH, Jepson PC. 1995. Mechanism of synergism between the pyrethroid insecticide lambda-cyhalothrin and the imidazole fungicide prochloraz, in the honeybee (*Apis mellifera* L.). *Pestic. Biochem. Physiol.* 51:1–11
99. Pinto LZ, Bitondi MMG, Simoes ZLP. 2000. Inhibition of vitellogenin synthesis in *Apis mellifera* workers by a juvenile hormone analogue, pyriproxyfen. *J. Insect. Physiol.* 46:153–60
100. Polonsky J, Bhatnagar SC, Griffitsh DC, Pickett JA, Woodcock CM. 1989. Activity of quassinoids as antifeedants against aphids. *J. Chem. Ecol.* 15:993–98
101. Qi BY, Gordon G, Gimme W. 2001. Effects of neem-fed prey on the predacious insects *Harmonia conformis* (Boisduval) (Coleoptera: Coccinellidae) and *Mallada signatus* (Schneider) (Neuroptera: Chrysopidae). *Biol. Control* 22:185–90
102. Rafalimanana H, Kaiser L, Delpuech JM. 2002. Stimulating effects of the insecticide chlorpyrifos on host searching and infestation efficacy of a parasitoid wasp. *Pest Manag. Sci.* 58:321–28
103. Reingold SC, Camhi JM. 1978. Abdominal grooming in cockroach: development of an adult behavior. *J. Insect Physiol.* 24:101–10
104. Reynolds DR, Riley JR. 2002. Remote-sensing, telemetric and computer-based technologies for investigating insect movement: a survey of existing and potential techniques. *Comput. Electron. Agric.* 35:271–307
105. Richards KW. 1993. Non-*Apis* bees as crop pollinators. *Rev. Suisse Zool.* 100:807–22
106. Rieth JP, Levin MD. 1988. The repellent effect of two pyrethroid insecticides on the honey bee. *Physiol. Entomol.* 13:213–18
107. Rosenheim JA, Hoy MA. 1988. Sublethal effects of pesticides on the parasitoid *Aphytis melinus* (Hymenoptera: Aphelinidae). *J. Econ. Entomol.* 81:476–83
108. Rumpf S, Frampton C, Dietrich DR. 1998. Effects of conventional insecticides and insect growth regulators on fecundity and other life-table parameters of *Micromus tasmaniae* (Neuroptera: Hemerobiidae). *J. Econ. Entomol.* 91:34–40
109. Rumpf S, Hetzel F, Frampton C. 1997. Lacewings (Neuroptera: Hemerobiidae and Chrysopidae) and integrated pest management: enzyme activity as biomarker of sublethal insecticide exposure. *J. Econ. Entomol.* 90:102–8
110. Saber M, Hejazi MJ, Kamali K, Moharramipour S. 2005. Lethal and sublethal effects of fenitrothion and deltamethrin residues on the egg parasitoid *Trissolcus grandis* (Hymenoptera: Scelionidae). *J. Econ. Entomol.* 98:35–40
111. 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.* 58:663–68

111a. Schmid-Hempel P. 2005. Evolutionary ecology of insect immune defenses. *Annu. Rev. Entomol.* 50:529–51
112. Schmuck R. 1999. No causal relationship between Gaucho seed dressing in sunflowers and the French bee symptome. *Pflanzenschutz Nachrichten Bayer* 52:257–99
113. Schmuck R. 2004. Effects of a chronic dietary exposure of the honeybee *Apis mellifera* (Hymenoptera: Apidae) to imidacloprid. *Arch. Environ. Contam. Toxicol.* 47:471–78
114. Schneider MI, Smagghe G, Gobbi A, Vinuela E. 2003. Toxicity and pharmacokinetics of insect growth regulators and other novel insecticides on pupae of *Hyposoter didymator* (Hymenoptera: Ichneumonidae), a parasitoid of early larval instars of lepidopteran pests. *J. Econ. Entomol.* 96:1054–65
115. Schneider MI, Smagghe G, Pineda S, Vinuela E. 2004. 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* 31:189–98
116. Singh SR, Walters KFA, Port GR, Northing P. 2004. Consumption rate and predatory activity of adult and fourth instar larvae of the seven spot ladybird, *Coccinella septempunctata* (L.), following contact with dimethoate residue and contaminated prey in laboratory arenas. *Biol. Control* 30:127–33
117. Smallman BN, Mansingh A. 1969. Cholinergic system in insect development. *Annu. Rev. Entomol.* 14:387–408
118. Stapel JO, Cortesero AM, Lewis WJ. 2000. Disruptive sublethal effects of insecticides on biological control: altered foraging ability and life span of a parasitoid after feeding on extrafloral nectar of cotton treated with systemic insecticides. *Biol. Control* 17:243–49
119. Stark JD, Banken JAO. 1999. Importance of population structure at the time of toxicant exposure. *Ecotoxicol. Environ. Safety* 42:282–87
120. Stark JD, Banks JE. 2003. Population-level effects of pesticides and other toxicants on arthropods. *Annu. Rev. Entomol.* 48:505–19
121. Stark JD, Vargas R, Miller N. 2004. Toxicity of spinosad in protein bait to three economically important tephritid fruit fly species (Diptera: Tephritidae) and their parasitoids (Hymenoptera: Braconidae). *J. Econ. Entomol.* 97:911–15
122. Stone J, Abramson C, Price J. 1997. Task-dependent effects of dicofol (Kelthane) on learning in the honey bee (*Apis mellifera*). *B. Environ. Contam. Toxicol.* 58:177–83
123. Streit S, Bock F, Pirk CWW, Tautz J. 2003. Automatic life-long monitoring of individual insect behaviour now possible. *Zoology* 106:169–71
124. Suchail S, Guez D, Belzunces LP. 2001. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environ. Toxicol. Chem.* 20:2482–86
125. Takeda K. 1961. Classical conditioned response in the honey bee. *J. Insect Physiol.* 6:168–79
126. Taséi JN, Lerin J, Ripault G. 2000. Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. *Pest Manag. Sci.* 56:784–88
127. Taséi JN, Sabik H, Pirastru L, Langiu E, Blanche JM, et al. 1994. Effects of sublethal doses of deltamethrin (Decis ce) on *Bombus terrestris*. *J. Apic. Res.* 33:129–35
128. Thompson HM. 2003. Behavioural effects of pesticides in bees: their potential for use in risk assessment. *Ecotoxicology* 12:317–30
129. Tran DH, Takagi M, Takasu K. 2004. Effects of selective insecticides on host searching and oviposition behavior of *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae), a larval parasitoid of the American serpentine leafminer. *Appl. Entomol. Zool.* 39:435–41

116. Shows that predatory efficacy of larvae and adult *C. septempunctata* was reduced following dimethoate treatment and that the ladybeetle prefers to consume untreated aphids.

128. Reviews a wide variety of behavioral effects reported in bees following exposure to pesticides.

130. Umoru PA, Powell W, Clark SJ. 1996. Effect of pirimicarb on the foraging behaviour of *Diaeretiella rapae* (Hymenoptera: Braconidae) on host-free and infested oilseed rape plants. *Bull. Entomol. Res.* 86:193–201
131. Van Driesche RG, Bellows TS. 1996. *Biological Control*. New York: Chapman & Hall. 539 pp.
132. Van Erp S, Booth L, Gooneratne R, O'Halloran K. 2002. Sublethal responses of wolf spiders (Lycosidae) to organophosphorus insecticides. *Environ. Toxicol.* 17:449–56
133. Vandame R, Belzunces LP. 1998. Joint actions of deltamethrin and azole fungicides on honey bee thermoregulation. *Neurosci. Lett.* 251:57–60
134. Vandame R, Meled M, Colin ME, Belzunces LP. 1995. Alteration of the homing-flight in the honey bee *Apis mellifera* L. exposed to sublethal dose of deltamethrin. *Environ. Toxicol. Chem.* 14:855–60
135. Vet LEM, Dicke M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annu. Rev. Entomol.* 37:141–72
136. Viggiani G. 1984. Bionomics of the Aphelinidae. *Annu. Rev. Entomol.* 29:257–76
137. Villa S, Vighi M, Finizio A, Serini GB. 2000. Risk assessment for honeybees from pesticide-exposed pollen. *Ecotoxicology* 9:287–97
138. Visscher PK, Dukas R. 1997. Survivorship of foraging honey bees. *Insectes Soc.* 44:1–5
139. Von Frisch K. 1967. *The Dance Language and Orientation of Bees*. Cambridge, MA: Harvard Univ. Press. 566 pp.
140. Wackers FL, Lewis WJ. 1999. A comparison of color-, shape- and pattern-learning by the hymenopteran parasitoid *Microplitis croceipes*. *J. Comp. Physiol. A* 184:387–93
141. Walthall WK, Stark JD. 1997. Comparison of two population-level ecotoxicological endpoints: the intrinsic (rm) and instantaneous (ri) rates of increase. *Environ. Toxicol. Chem.* 16:1068–73
142. Weick J, Thorn RS. 2002. Effects of acute sublethal exposure to coumaphos or diazinon on acquisition and discrimination of odor stimuli in the honey bee (Hymenoptera: Apidae). *J. Econ. Entomol.* 95:227–36
143. Wiles JA, Jepson PC. 1993. The dietary effects of deltamethrin upon *Nebria brevicollis* (F.) (Coleoptera: Carabidae). *Pestic. Sci.* 38:329–34
144. Wiles JA, Jepson PC. 1994. Sub-lethal effects of deltamethrin residues on the within-crop behaviour and distribution of *Coccinella septempunctata*. *Entomol. Exp. Appl.* 72:33–45
145. Wong-Riley MTT. 1989. Cytochrome oxidase: an endogenous metabolic marker of neuronal activity. *Trends Neurosci.* 12:94–101
146. Zanuncio TV, Serrao JE, Zanuncio JC, Guedes RNC. 2003. Permethrin-induced hormesis on the predator *Supputius cincticeps* (Stål, 1860) (Heteroptera: Pentatomidae). *Crop Prot.* 22:941–47
147. Zhang SW, Lehrer M, Srinivasan MV. 1999. Honeybee memory: navigation by associative grouping and recall of visual stimuli. *Neurobiol. Learn. Mem.* 72:180–201

134. Highlights that sublethal doses of an insecticide induce perturbations in homing flight in foragers.

Annual Review of Entomology

Volume 52, 2007

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Entomology* chapters (if any, 1997 to the present) may be found at http://ento.annualreviews.org/errata.shtml