7 Impact of agrochemicals on non-*Apis* bees

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Summary

Only few reports have been published on the reduction and recovery of native non-*Apis* bee populations, measured after temporary or permanent agrochemical pest control in North America. Small species were found to be the most sensitive. The assessment of pesticide toxicity and hazards to non-*Apis* bees has been practiced for about 50 years through various laboratory, semi-field, and field methods. Researches were conducted mainly on three species: *Nomia melanderi* (alkali bee), *Megachile rotundata* (alfalfa leafcutting bee), and *Bombus terrestris* (bumble bee). Toxicity tests performed in standardized conditions on adults and larvae showed that the intrinsic susceptibility of non-*Apis* bees measured by oral and topical LD_{50} or by LC_{50} varied to a great extent between species and also from *Apis mellifera*. Laboratory and semi-field tests have been used to assess the risks of sprays, field-weathered residues, or systemic compounds in nectar and pollen. The effects of several organophosphates, pyrethroids, neonicotinoids, and a carbamate, are discussed. Sublethal effects of deltamethrin, fenvalerate, trichlorfon, and imidacloprid have also been investigated. It has been shown that biochemical data from studies on detoxification in *M. rotundata* did not agree with toxicological parameters and risk assessment in the field.

Introduction

Many wild and cultivated plants are visited not only by honey bees (*Apis mellifera* in particular) but also by non-*Apis* bees which facilitate their fruit and seed setting. These Hymenoptera are represented by more than 20000 species throughout the world, belonging to nine families: *Colletidae*, *Oxaeidae*, *Halictidae*, *Andrenidae*, *Melittidae*, *Fideliidae*, *Megachilidae*, *Anthophoridae*, and *Apidae* [1]. This fauna is a natural resource which often sustains a prominent role in the pollination of crops and the maintenance of floral diversity, especially when honey bees are absent or not efficient. Many researchers have long emphasized the contribution of

non-*Apis* bees, also called wild bees, to pollen transfer in cultivated and wild plants. Several authors have surveyed the bee diversity in Europe and compared the efficacy of several species in case studies [2, 3]. Other scientists have extensively investigated the different biological traits of solitary and social non-*Apis* species which proved to be highly variable [4–6]. Some species dig burrows into the ground, while others nest in twigs, timber, soil cavities, etc., and use all sorts of materials to protect their brood cells, such as wax, mud, leaf cuttings, wool, resin, and so on.

In areas where agricultural efficiency has been increased through the destruction of hedges and adventitious flowering plants, the cutting out of waste lands, and the reduction of crop diversity, the population of native non-*Apis* bees has been depleted. Generally, in the same areas, an additional factor in this depopulation may be the misuse of insecticides on crops. The importance of non-*Apis* bees for seed setting in wild and cultivated plants and the frequent shortage of pollinators on various crops have encouraged scientists to domesticate and multiply several non-*Apis* bees. Since the Second World War, the most popular has certainly been the alfalfa leafcutting bee, *Megachile rotundata (Megachilidae)*, for which the management techniques have been constantly improved [7, 8].

This solitary bee can nest gregariously in tunnels of wooden or plastic shelters established temporarily in alfalfa fields grown for seed. For the same purpose, in the USA and New Zealand, artificial nesting beds have been created close to alfalfa seed crops, to increase the population of the ground-nesting *Halictidae* bee *Nomia melanderi* [9, 10]. Several "mason bees" (*Megachilidae*) are propagated commercially in tunneled domiciles to improve fruit production in various countries in Asia, America, and Europe: *Osmia lignaria* [11], *Osmia cornifrons* [12], and more recently, *Osmia cornuta* [13]. Since the 1990s, the social bee *Bombus terrestris* has been reared *en masse* mostly in Europe to pollinate vegetables (initially, the tomato) in greenhouses [14]. Now other bumblebee species are also being produced in Asia and North America.

Owing to the biological differences among non-*Apis* species and honey bees, one can predict that their population may not be affected similarly by agrochemicals. Thus, for example, the death of a solitary female bee in charge of a nest means the end of reproductive activity, while in social bees deficits following spraying may be compensated by workers and also by new bees emerging from the brood. Moreover, except for the species cited above, native non-*Apis* bees live in natural habitats that cannot be removed from hazardous sites. Despite technical difficulties, some researchers have investigated the impact of large-scale insecticide applications on non-*Apis* populations. In addition, the economic importance of the domesticated non-*Apis* bee has favored laboratory and field studies on the toxicity and hazards of pesticides to the three main species, *M. rotundata*, *N. melanderi*, and *B. terrestris*. The availability of individuals now produced *en masse* enables advances in methodology often inspired from

honey bee studies, and comparisons of sensitivity between *Apis* and non-*Apis* bees are now possible.

Historical background of pesticide risk assessment on non-*Apis* bees

The earliest scientific article mentioning concern about the effect of a pesticide on non-*Apis* bees appeared in 1946 in a Canadian journal [15]. The author, from the Massachusetts State College, reported very briefly on the mortality rates of unidentified solitary bees and bumble bees collected from apple flowers and introduced in cages to be exposed to DDT, "dusted lightly through the screen covering the top of the cage." He assessed mortality several times within 48 hours in a first test and 60 hours in a second experiment where honey bees were treated in the same way. The conclusions were that the number of experimental insects – 10 solitary bees, 5 to 10 bumble bees and 10 honey bees– were too low to enable definite conclusions to be made. It was also recognized that laboratory and field tests may not always agree. However, both solitary bees and honey bees seemed equally affected while the death of the bumble bees was retarded, which means they were presumably more tolerant. Further studies on DDT effects on wild pollinators were performed in the UK [16] and published in 1948 and in the USA [17, 18] in 1949 and 1950. The UK authors collected *B. terrestris*, *B. agrorum*, and solitary bees (*Andrena flavipes*), and then performed laboratory tests, using various concentrations of toxic material which was spread on a glass wall placed in boxes for contact tests or diluted in sucrose solution for feeding tests. They also used sprayed blooms in a third kind of contact laboratory test and made field observations on treated apple blossoms on which they collected several *Andrena* species and *Osmia rufa*. They concluded that the susceptibility of worker bumble bees to DDT was comparable to that of honey bees and that queens and drones of *B. agrorum* and *B. terrestris* were more resistant. The authors also insisted on the environmental impact of bumble bee queen losses which entail hundreds of workers that would not be produced. One paper from the USA reported on laboratory tests on several solitary species collected in a field, belonging to the genera *Nomia*, *Megachile*, *Melissodes*, *Anthidium*, and *Agapostemon* [18]. Experimental bees were exposed to dry DDT residues on screens which had been previously immersed in a DDT solution at different concentrations. The comparison with honey bees showed that solitary bees were more resistant than *Apis mellifera* at the same concentration and exposure duration. It was also found that females were more resistant than males. The other article by US authors [17] described an experimental procedure to evaluate the hazards to *N. melanderi* of a DDT spray on an alfalfa field in bloom. DDT was applied before the bees started foraging. Before and after the spray, the bee density on flowers was assessed by sweeping alfalfa

plants with a net. Pollen loads were also collected from female hind legs to measure the rate of exposed insects. Since this species lives in aggregations close to cultivated alfalfa, dead bees could be counted at the nesting site as well as the number of active nests. Despite the inaccuracy of the method and the absence of statistical interpretation, the results evidenced a moderate repellency for a few hours and a toxicity of the residues estimated at 15 percent of nests becoming inactive.

The first calculation of LD_{50} was published in 1963 [19]. The American authors used leafcutting bees as test insects. They applied acetone dilutions of various compounds on the abdomen of anesthetized bees. They found that *M. rotundata* was more susceptible to three of the tested pesticides than honey bees, and less susceptible to two of them, including carbaryl.

The earliest research using biochemical techniques for studying the toxic action of insecticides was presented in a PhD thesis in 1972 [20]. The author estimated the effect of drugs such as the synergist piperonyl butoxide on the insecticide action of carbaryl by measuring *in vitro* the microsomal enzyme activity in *M. rotundata*. He found drug absorption enhanced this activity up to 4–5 times and reduced bee lipid content by 20–30 percent. At the same time two other American scientists [21] compared the effect of trichlorfon and carbophenothion on acetylcholinesterase in the leafcutting bee and the honey bee. In the solitary bee, enzyme inhibition was stronger with trichlorfon, and with carbophenothion enzyme recovered 10 minutes after application. Changes in enzyme activity were similar in *M. rotundata* and *A. mellifera*.

In 1975 the first estimation of the impact of an insecticide applied on a large scale was published [22]. The author compared the diversity and abundance of native pollinators of Canadian lowbush blueberries in a control area and in areas contaminated with fenitrothion sprayed on forests of New Brunswick. Pollinators were mainly *Bombus* spp., *Andrenidae*, and *Halictidae*. Data of the population census were interpreted through statistical analyses which evidenced that the lowest diversity and abundance index was in areas close to treated forests. Moreover, carcasses found in these areas showed the highest residue rates. Both results corroborated the crop failures reported by blueberry growers of the province.

If we consider that insecticide repellency, mentioned in early studies, is not a typically sublethal effect, the first report of a consequence of low doses of insecticide on non-*Apis* bees appeared in 1981 [23]. The authors, comparing two pyrethroids and organophosphates on the leafcutting bee in laboratory tests, found that a high percentage of comatose bees recovered. This was observed only with the pyrethroids fenvalerate and decamethrin (deltamethrin) which caused a strong "knock-down" effect within the first hour after application.

The earliest study on the possible effects of systemic compounds on

wild bees appeared in 1972 [24]. The American scientists tested the hazards of soil application of insecticide solutions of aldicarb, oxydemeton-methyl, and metasystox. They used sweet clover plants cultivated in pots and visited by *M. rotundata* and found no mortality at the recommended dosages, while the application of 10 times the recommended dose of aldicarb resulted in significant mortality of females, revealing that some active substance was transferred to the nectar.

The first test of an insect growth regulator (IGR) on a non-*Apis* bee was presented during a symposium in 1993 [25]. The authors observed adult mortality and brood development in bumble bee colonies (*B. terrestris*) maintained in cages. Forage plants were treated during activity hours with the IGR fenoxycarb. It was concluded that the IGR did not present a negative action on adult bumble bees but that a larval test had to be developed for an adequate assessment of the brood mortality.

Survey of testing methods

Among the various testing methods described by authors, a large number was aimed at measuring mortality rates in standardized laboratory conditions. Some procedures permitted the calculation of the LD_{50} or the LC_{50} of compounds through contact or feeding tests. They supplied data enabling comparisons of acute toxicity of pesticides between non-*Apis* bees and between honey bees and non-*Apis* bees.

Other kinds of tests were performed in a cage, tunnel, or greenhouse and their objectives were to assess the consequences of sprays or residues on bees exposed to compounds in more or less standardized conditions. In these tests, scientists did not expect to estimate the reaction of an insect to a measured substance intake or deposit but to assess risk in practice. The advantage of keeping bees in such enclosures is to ensure the permanent exposure of the insects but the counterpart is an overestimate of the hazards.

The third kind of assessment was hazard testing conducted in the field either by using domesticated non-*Apis* bees maintained in artificial domiciles or by monitoring native populations in their natural habitat. The drawback of these methods is that standardization is not possible since the exposure of experimental insects to test compounds is not controlled. In the case of native population monitoring, the main difficulty is the interpretation of data due to the number of factors involved in population changes during seasons and years.

The first category will be referred below to as "laboratory tests," the second as "semi-field tests," and the third as "field tests."

Laboratory tests

Median lethal dose assessment

Laboratory procedures for estimating LD_{50} were first applied to *M. rotundata* and *N. melanderi* in the USA because of their commercial importance in areas where alfalfa seed was produced. The detailed description of topical tests among the early articles [26] indicated that test leafcutting bees were obtained from incubated cells and then immobilized at 10°C. The treatment was a drop of 1.7 µl applied to the thoracic scutum with a micro-injector. Twenty leafcutting bees were necessary for each dosage–mortality test. After treatment, bees were placed into screened boxes with feeders containing a 20 percent honey solution. The boxes were maintained in a microbiotron illuminated at 27°C. Mortality was recorded every 24 hours for 4 days following the treatment. The LD_{50} was established at 72 hours for leafcutting bees but at 48 hours for *N. melanderi*. Other scientists who studied the intrinsic susceptibility of solitary bees used similar techniques [21] or adopted variations such as the reduction to 48 hours of the duration of the mortality check in leafcutting tests [27]. This duration dropped to 24 hours for *N. melanderi* and *M. rotundata* [28]. These authors used six concentrations of the test solution falling between the limits of the expected value, and their data were analyzed with the probit analysis method. A device designed for accurate measurement of the consumption of pesticide solutions by any bee species was described in 1973 [29] but it seems it was not used in practice by other authors who established the oral LD_{50} of aldicarb in *N. melanderi* and *M. rotundata* with a more simple feeding system [30].

The first approach to studying acute contact toxicity of pesticides to bumble bees was derived from the method described for honey bees [31]. After a gap of 29 years, a detailed method to determine acute contact and oral toxicity in bumble bees was presented in a symposium [32] and completed later [33]. For the contact test the authors recommended collecting workers of average size and age then using five concentrations per replicate and performing two replicates. The 1-µl drop of pesticide solution in acetone was deposited on the ventral part of the thorax and the mortality recorded every 24 hours for 3 days. A negative control with acetone and a positive one with either dimethoate or parathion were also recommended. The method for oral toxicity derived from the European guidelines for honey bees was modified in order to be adapted to bumble bees which have no trophallaxis. The principle of the test was to cage individually 30 bumble bees per concentration, maintained in the dark at 25°C. The test substance was dissolved in a sucrose solution and the mean weight of the bees determined. Mortality checks and controls were similar to those of the contact test. Some variations in these guidelines appeared in other articles [33], in particular the use of water as solvent and the duration of 10 days for mortality recording.

The LD_{50} of several compounds was established in the social bee *Trigona spinipes* using a topical test protocol similar to the one for bumble bees [34].

Tests on residues

Even when no LD_{50} was calculated, other kinds of laboratory procedures have been used to test pesticide toxicity comparatively. The first comparisons of the susceptibility of bumble bees, solitary bees, and honey bees to DDT were performed in cages with glass or screened walls previously sprayed or immersed and dried [16, 18]. A standardized method was proposed to test pesticide effects on *M. rotundata*, using filter papers soaked in test solutions, dried, and placed on the bottom of screened boxes containing 15 individuals which emerged within 48 hours. The boxes were kept at 27°C under constant light. No food was supplied and mortality was recorded every 1 or 2 hours for 1 day. Identical boxes were used for testing the effect of sprays. In this case, after the treatment, leafcutting bees were introduced into clean boxes and placed in standardized conditions [35]. In further articles [23, 36] it was suggested that each filter paper should absorb the amount of solution corresponding to the field recommended dosage.

Several Canadian and American scientists have developed laboratory bioassays for risk assessment when non-*Apis* bees were exposed to treated plants. In the earliest studies, foliage was sampled from alfalfa plots previously treated with the test insecticide and placed in cages where 10 leafcutting bees or *N. melanderi* could walk and feed on a sugar solution. They recorded mortality after 24 or 48 hours [37, 38] and tested various ages of residues to establish the RT 25, that is the residual time required to obtain a bee mortality of 25 percent after a test exposure to field-weathered spray deposits [39]. This method was extended to the bumble bees *B. centralis* and *B. rufocinctus* and to honey bees [40]. In further research on field-weathered residual exposure tests, the authors, who followed the main guidelines, standardized the method. They sampled the upper 15-cm portion of test plants and placed about 500 cm^2 of foliage in screened cages 45 cm long with plastic Petri dishes as top and bottom. Twelve to 30 test bees were introduced into each cage maintained at 26–29°C. Mortality was recorded after 24 hours and each treatment was replicated four times [41–43]. A Canadian scientist used a "tube chamber" constructed of clear plastic sheets forming a tube 14.5 cm in diameter and 49 cm high. This exposure chamber was separated by a screen partition into a top and a bottom section to test vapor and residue hazards, respectively. Potted test plants were sprayed at the field rate then dried and moved to a climate room at 28°C and a 16 L:8 D photoperiod. A tube chamber was positioned over each plant and 10 test bees were introduced into each of the sections. Mortality was recorded after 24 hours [44].

Tests on sublethal effects

Laboratory tests were used to assess the effects of low doses or concentrations on bumble bees. To estimate whether a 0.01–0.02 μg topical application of deltamethrin affected the longevity of bumble bees, 32 workers per treatment were kept in disposable boxes each containing eight insects, maintained in the dark at 20°C. Mortality was recorded daily [45]. A more recent article reported on a feeding test conducted on queenless microcolonies of three workers of *B. terrestris* to study sublethal effects of low concentrations of imidacloprid in the food on worker survival, brood size and larval development [46].

Testing insect growth regulators

Queen right *B. terrestris* colonies were used in the laboratory for testing the toxicity of IGRs on brood when the substance was ingested by workers for 24 hours. The technique proposed was to photograph the brood every day during the week before the 24-day feeding period and over the next 5 weeks [47]. A standardized larval test was described to evaluate IGR hazards to *B. terrestris* brood. Brood cells with 10 young larvae each had to be placed in small rearing boxes at 28°C and 50 percent HR. They were attributed to groups of three nurse workers and fed with sucrose syrup and pollen dough. After pupation, workers were removed and the brood was reared until the adults emerged. The test substance had to be dissolved in the food and fed to the test groups for 24 hours. It was to be applied to larvae of different ages. For each larval age and each test substance three replications were necessary [48].

Semi-field tests

All these tests were conducted in field or greenhouse cages and also in greenhouse compartments, i.e. under nearly natural climatic conditions and permanent exposure. Sometimes, parallel experiments were conducted in greenhouse and in field cages to determine whether both situations gave similar results.

Greenhouse cages and compartments

Potted *Melilotus alba* was often used as a test plant in greenhouses because of its abundant flowering. The effects of three systemic compounds were estimated by applying converted field dosage to sweetclover testing pots placed in cages. Ten leafcutting bee were introduced into each cage and mortality was recorded every day [24]. The relative repellent effect of two pyrethroids on nesting leafcutting bee females was assessed in greenhouse compartments where treated and control sweetclover plants

were placed together [23]. The sublethal effect of low doses of deltamethrin on the fecundity of females was studied by using marked leafcutting bees nesting in the same compartment and foraging sweet-clover [27]. Comparison of the effect of imidacloprid seed dressing on the visiting rate of sunflower heads by bumble bees (*B. terrestris*) was performed by cultivating treated and control potted sunflower in a compartment [49]. For studying the effects of IGRs on bumble bee larvae, Tornier [50] suggested rearing in greenhouse compartments queen right colonies of *B. terrestris* with 30 marked workers each and a similar amount of brood. The entrance of the hives should be equipped with a dead bee and larvae trap. A picture of the brood should be taken before and after the test period [50]. Results on IGR effects on *B. terrestris* were obtained in 5×3 m greenhouse cubicles where tomato plants had been treated [51].

Field cages

For testing residues, 6×6 m alfalfa field plots were covered with cages, each containing 50 females and 50 males of *M. rotundata*, which were released a week after application. Dead females were counted by examining the straws in which they nested and larval mortality was assessed by splitting the straws lengthwise once nesting was completed [52]. The effects of a systemic granular side dressing of alfalfa on leafcutting bee mortality were also studied in cages covering 3.6×6 m plots. *M. rotundata* could nest in straws or laminated boards in insulated shelters. These devices enabled cell extraction and residue analysis of provisions [53–55]. Similar experiments were conducted in alfalfa fields, using cages ranging from 1.2×1.2 m to 6×6 m and various leafcutting bee densities in relation to the amount of forage [30–44].

Field cages were also used to test the effects of sprays on bees. For this purpose, Heller *et al.* [56] reported a comparative trial with three replications where *M. rotundata* was reared in screened tunnels 17×6 m, partitioned into three sections where *Sinapis alba* was grown as a test plant. The spray was applied during the foraging period. A field cage method was presented for testing IGR effects on *B. terrestris*, using *Phacelia* as a test plant in 3×4 m cages [48]. Before introduction into the test cage and IGR application, test colonies containing 50–70 workers were attributed standardized egg cells and brood with larvae of known age ranging from 1 to 6 days. The cage period lasted 2–3 weeks, then colonies were returned to the laboratory until adult emergence [48].

Smaller removable screened cages containing test bees were used for a standardized exposure to experimental sprays on alfalfa [19] or fenitrothion aerial spray for forest protection [57]. The first authors removed the cages after the spray and placed them in a holding room where bees could feed on a sucrose solution and they assessed the 24-hour mortality. The other authors used $5 \times 7.5 \times 3.5$ cm individual screened compartments

where bumble bee queens were introduced before the spray and fed twice daily. The caged queens were left in place for 4 hours following the treatment, before being moved to the laboratory for a 7-day observation period. "Krome-Kote" cards placed adjacent to boxes enabled a good estimate of exposure by counting the insecticide droplets on $5 \times 1\,cm^2$ per card.

Field tests

Tests with leafcutting bees

A few articles have reported on field tests on *M. rotundata* using shelters in alfalfa fields. In the earliest one the author placed two shelters ($86 \times 50 \times 39\,cm$) in two distant parts of a crop 1 km long and 45 m wide. One half of the field was sprayed with naled, the second half with trichlorfon during a calm evening. Shelters were supplied with boards drilled with hundreds of holes each accommodating paper soda straws 0.5 cm in diameter and 6.5 cm long where leafcutting bees were nesting. Fifty nests were marked and monitored in each field before and after the treatment. Straws were extracted and examined at night. When the nesting period was completed, boards were returned to the laboratory at 26°C for 1 month, then straws containing the marked nests were hibernated for 4 months prior to an incubation period of 20 days at 26°C. When incubation was completed, marked nests were dissected to record larval mortality [58]. Later authors also comparing two compounds preferred testing sprays on six alfalfa plots ranging from 200 to $1600\,m^2$, each being at least 300 m away from others. On each plot they placed a small leafcutting bee shelter where 114 to 237 females established their nests in polystyrene grooved boards 4 cm deep. Females were counted early morning before they began to fly. Treatments were applied during foraging hours when the number of females in nesting tunnels had been stable for 3 consecutive days. Two plots were used as control, two were sprayed with phosalone, and two with deltamethrin. Female numbers were assessed seven times during the 3 weeks following treatments. The exposure of foragers was estimated by analyzing pollen samples from brood cells provisioned by female bees at $t-1$ and $t+1$. At the end of flight activity nesting boards were moved to the laboratory and left until the larval development was completed. Then cells were extracted and samples of 600–800 cells per treatment were incubated after a 2-month hibernation. When adult emergence was finished, closed cells were opened to examine their content [59]. In another experiment conducted with similar lay-out and material, the authors used coded colored marks on every leaf plug as soon as a nest was completed to assess the larval mortality in relation to the date of cell provisioning. Samples of plug leaves, pollen provisions, and live larvae enabled residue analysis for the two compounds tested, alphamethrin and phosalone [36].

Tralomethrin was tested for bee hazard in alfalfa fields pollinated by leafcutting bees and treated by airplane or helicopter. In a first test, the authors observed the fate of females nesting in shelters placed in separate plots of a large field, which received applications at different rates in the evening. In a second test they preferred to use separate fields to compare the effects of treatments. Evaluation of hazards were done by pre-application and post-application records of the number of active females per 5-second scan per nesting unit. This count was replicated 10 times. The number of females in 13 nest tunnels was also assessed (25 replications) at night before the application and 2 days after the application [28].

Tests with bumble bees

For studying the possible effect of a systemic dressing of sunflower seeds on homing behavior and nest development of *B. terrestris*, 20 colonies of approximately 50 individuals were prepared and all the workers were marked on the thorax the day they were moved to fields, i.e. at the beginning of flowering. Ten colonies were placed in a large treated field surrounded by more than 400 ha of treated sunflower. The other 10 colonies were in a control field, 20 km away in a large nontreated zone. Exposure to residues in sunflower nectar and pollen was estimated by identifying pollen grains carried by a total of 241 nectar and pollen gatherers collected at the hive entrance. After a 9-day field period, the 20 hives were removed to the laboratory after sunset. They received identical food until new queens emerged, then marked and unmarked workers were counted [49]. An attempt to establish a standardized field test for IGRs was not satisfactory. The authors placed six small colonies of *B. terrestris* (less than 50 workers) near a 2400 m^2 *Phacelia* plot and applied triflumuron 3 days after colony introduction. They recorded the forager density on $5 \times 1\,m^2$ spots, the flight activity for 10 min every day at the hive entrance, the origin of the pollen collected by the workers, and the larval mortality by counting dead larvae inside and outside the colony and also by counting the number of larvae, egg cells, and cocoons from pictures taken every day. Counting dead larvae was almost impossible and the authors suggested that a special trap to assess larval loss should be devised. Data interpretation was difficult due to the kind of colony development which is unpredictable in bumble bees [60].

*Monitoring populations of native non-*Apis *bees*

The impact of chemical control of North American forest moths is of great concern for scientists and fruit growers close to treated areas and various methods have been used to assess the consequences of aerial sprays on native pollinators. Short-term effects were studied by observing 25 "sight units," each unit being a small blooming plot of about 0.8 m^2. The 25 units were on the same plant species. Each sighting conducted on warm hours of

the day lasted 10s. Weekly observations preceded and followed closely pesticide applications [61]. A quite different method was described for assessing bumble bee density. Twenty line transects were selected along roadsides. All these sample areas were classified in categories related to their "spray history." Each site was visited at least once by an observer walking at a constant pace. The caste, sex, and species of bumble bee were recorded and divided by the forage quantity in each transect thus giving a "bees per forage-mile" estimate. The forage quantity was calculated by measuring the length of each stand of the dominant visited plants [57]. Sampling wild bee population with a net was used for assessing the impact of fenitrothion on blueberry pollinators. One hundred sweeps were taken in selected flowering blueberry crops, during the warmest hours of 3 days of sampling [22].

Comparative toxicity and hazards of pesticides to non-*Apis* bees

Acute and chronic toxicity

Data on acute toxicity have been gathered in Tables 7.1 to 7.3. Table 7.1 shows that the median lethal dose (LD_{50}) in the leafcutting bee varied from 0.0003 to 30 µg/bee depending on the test substance [19, 26]. For *M. rotundata* the most toxic insecticides in topical tests were malathion and dicrotophos, whereas the least was carbaryl [19, 26], *N. melanderi* was also most susceptible to dicrotophos but less susceptible to fipronil than *M. rotundata* [26, 43]. Dieldrin toxicity was the highest to *T. spinipes* while that of carbaryl was the lowest [34]. The least susceptible species to carbaryl was *M. rotundata*, and the most, *T. spinipes*, with a ratio of about 41 [19, 34], the honey bee being intermediate. Deltamethrin was 76 times more toxic to *M. rotundata* females than to *B. terrestris* workers [27, 63]. This pyrethroid showed a similar toxicity to leafcutting bees and honey bees [27, 64]. With deltamethrin, trichlorfon, and carbophenothion, female leafcutting bees were about twice as tolerant as males [21, 27] Immature stages may be more susceptible to insecticides than adults. For example, after topical application, aldicarb was seven times more toxic to third instar larvae of *M. rotundata* than to adults [30]. The LD_{50} can also be expressed in µg/g of bee which is considered by some authors as a better approach to the intrinsic toxicity of a substance to bees [21, 30, 63]. According to several authors, the mean weights of *M. rotundata*, *N. melanderi*, *A. mellifera*, and *B. terrestris* are 0.036, 0.100, 0.118, and 0.190 g, respectively. In the case of deltamethrin the new LD_{50} in the female leafcutting bee, *B. terrestris*, and *A. mellifera* is 0.33, 4.8, and 0.08 µg/g (if we take 0.01 µg/bee as the LD_{50} for honey bees [64]). This means that honey bees are less tolerant to deltamethrin than leafcutting bees and bumble bees.

In Table 7.2 the feeding test with two IGRs revealed a much greater susceptibility of *B. terrestris* larvae to diflubenzuron than to fenoxycarb.

Table 7.1 Acute toxicity* of pesticides to four non-*Apis* bee species and honey bees (topical LD_{50} (μg/bee))

Year/Ref.	*Compound*	*Megachile rotundata*	*Nomia melanderi*	*Bombus terrestris*	*Trigona spinipes*	*Apis mellifera*
1988/27	Deltamethrin	0.0052 (♂)				
		0.012 (♀)				
1987/63	Deltamethrin			0.91		
1982/64	Deltamethrin					0.01
1991/28	Tralomethrin	0.011				
1984/30	Aldicarb	0.4308				
1973/21	Trichlorfon	8.975 (♂)				3.374
		18.488 (♀)				
	Carbophenothion	0.154 (♂)				1.491
		0.22 (♀)				
1973/26	Trichlorfon	0.136	0.0465			0.0240
	Dieldrin	0.0036	0.0023			0.0006
	Parathion	0.0157	0.0015			0.0030
	Oxydemeton methyl	0.133	0.0082			0.0030
	Dicrotophos	0.0003	0.0010			0.0010
	Malathion	0.0005	0.0036			0.0020
1999/43	Fipronil	0.004	1.130			0.0130
1963/19	Carbaryl	30.50				1.2700
1989/34	Parathion			0.0956		
	Carbaryl			0.7472		
	Malathion			0.2649		
	Dieldrin			0.0289		
	Dicrotophos			0.1685		
1999/33	Imidacloprid		2.5			

Note
*If no indication of sex is given, the chemical has been tested against females.

Table 7.2 Oral toxicity of two IGRs to *Bombus terrestris* larvae (after Gretenkord and Drescher, 1996) [48]

Compound	*Age of larvae (days)*	*LD_{50} (ng/larva)*
Fenoxycarb	1	>650
	4	>1740
	6	>3710
Diflubenzuron	1	7.7
	4	52.9
	6	5112.0

Table 7.3 Oral toxicity of insecticides to three non-*Apis* bees and honey bees (oral LD_{50} (μg/bee))

Year/Ref.	*Compound*	*Megachile rotundata*	*Nomia melanderi*	*Bombus terrestris*	*Apis mellifera*
1999/33	Imidacloprid			0.04	
1984/30	Aldicarb	0.398 (♂)	0.41		0.071
		0.244 (♀)			
1993/62	Deltamethrin			0.6	
	Oxydemeton methyl			0.75	
	Pirimicarb			8.5	
	Phosalone			60.0	

Another striking difference was the rapid decrease of toxicity of diflubenzuron between 4-day-old and 6-day-old larvae [48].

Table 7.3 reveals that oral toxicity has not been investigated as much as topical toxicity. In *B. terrestris* the oral toxicity of phosalone was 1500 times lower than that of imidacloprid [33, 62]. The topical toxicity of imidacloprid in *B. terrestris* was 62 times lower than the oral toxicity [33]. *M. rotundata* and *N. melanderi* were less susceptible to aldicarb than honey bees. Contrary to the topical toxicity of other compounds [21, 27], the oral toxicity of aldicarb was lower in male leafcutting bees than in females [30].

A chronic feeding test was performed for 21 days with aldicarb, which showed medium lethal concentration values of 1.6, 2.0, and 3.9 mg/kg for honey bees, *N. melanderi*, and *M. rotundata*, respectively [30]. With IGR insecticides, LC_{50} estimated on young bumble bee larvae was higher for diflubenzuron than for fenoxycarb while the converse was observed for honey bees [48].

Susceptibility of bees to residues

Tests with contaminated paper

Through tests on paper it was possible to classify several pesticides used on blooming alfalfa, according to their hazards to male leafcutting bees. In

a first experiment it was shown that toxicity decreased according to the following ranking: endosulfan > trichlorfon > phosalone > oxydemeton-methyl > pirimicarb. The last substance did not affect bees whereas residues of the other pesticides were still active after 5 days [65]. The mortality curves of the residual action of deltamethrin and fenvalerate did not have the typical aspect of those related to nonpyrethroid insecticides (Figure 7.1). This was an indication of the "knock-down" effect which was more marked in fenvalerate, a larger proportion of male leafcutting bees recovering, than in deltamethrin [23]. Alphamethrin residues at the field rate of 10 g/ha were less hazardous to *M. rotundata* males than phosalone at the rate of 1000 g/ha; after a 4-hour exposure the mortality rate recorded at 24 hours was 12 and 47 percent, respectively [36].

Tests with contaminated leaves

Acidified residues of trichlorfon were more efficient against pest insects. They were tested on alfalfa-treated leaves kept in Petri dishes and proved to be no more hazardous to leafcutting bees than the non-acidified compound. Conversely, mortality in honey bees was twice as much as that with

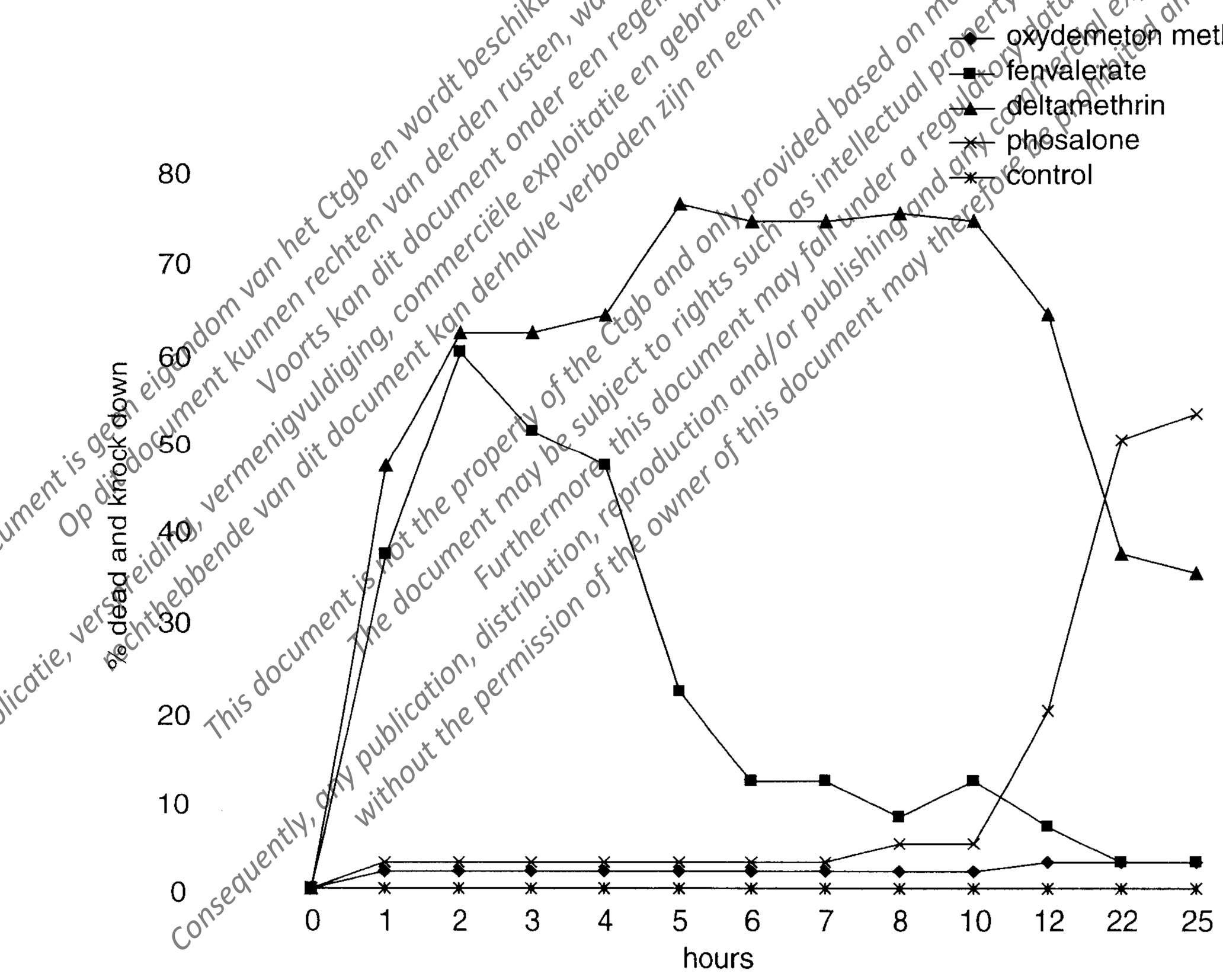

Figure 7.1 Mortality rate and knock-down effect of four insecticides against *Megachile rotundata* males exposed to residues on paper (after Tasei and Dinet, 1981) [23].

Table 7.4 Residues of naled and oxydemeton methyl recovered in alfalfa leaves, pollen, nectar and pollen ball of leafcutting bees (after George and Rincker, 1985) [54]

	Sampling interval (days following spray)	*Leaves*	*Pollen*	*Nectar*	*Pollen ball*
Naled/Dichlorvos* (mg/kg)	0	0.32/8.44	−/−	−/−	−/−
	1	0.63/1.37	nd**/3.99	0.20/nd**	−/−
	5	nd**/0.02	nd**/nd**	nd**/nd**	−/−
	13	nd**/0.34	−/−	−/−	nd**/nd**
Oxydemeton methyl (mg/kg)	0.5	56.4			–
	3	16.6			0.1
	14	1.2			0.3

Notes
*Dichlorvos is a metabolite of naled.
**not detected.

trichlorfon alone [38]. After a 24-hour contact with residues on alfalfa leaves, trichlorfon was more hazardous to *M. rotundata* than deltamethrin and methoxychlor. Males were more affected than females [44]. Residues of acephate and naled applied on alfalfa foliage with the stickers "Sur-tix®" and "Bond®" were less hazardous than residues without the stickers, whereas malathion caused 100 percent mortality even with the stickers [41]. Residues were measured in the leaf, pollen, and nectar of alfalfa treated with naled and oxydemeton methyl and pollen-nectar balls extracted from nests of leafcutting bees foraging the caged test flowers. More residues of the second insecticide were recovered in the leaves. A metabolite of naled (dichlorvos) was recovered in the pollen and leaves 1 day and 13 days after application. No residue could be detected in the pollen balls. Oxydemeton methyl residues were determined in pollen balls (Table 7.4). No adverse effect was observed on bees in the cages [54]. The residual toxicity of endosulfan, carbaryl, and trichlorfon was assessed on alfalfa foliage 3 hours after application. The mortality of the test insects with trichlorfon was 31, 5, and 17 percent, in *N. melanderi*, *M. rotundata*, and honey bees, respectively, which was considered as a low level, whereas with endosulfan the proportions were: 100, 71, and 11 percent. With carbaryl, the mortality rate of the three species was higher than 91 percent [37], which is consistent with a study reporting that female *M. rotundata* was affected when foraging alfalfa sprayed with carbaryl before bloom (Figure 7.2) [52]. The "Residual Time 25," which is the age of residues causing 25 percent mortality among the tested bees, was used by several authors to classify insecticides according to their hazards and recommend for late-evening sprays those with a RT 25 less than 8 hours. RT 25 estimation of field-weathered residues on alfalfa showed that tralomethrin was not hazardous to *M. rotundata* and

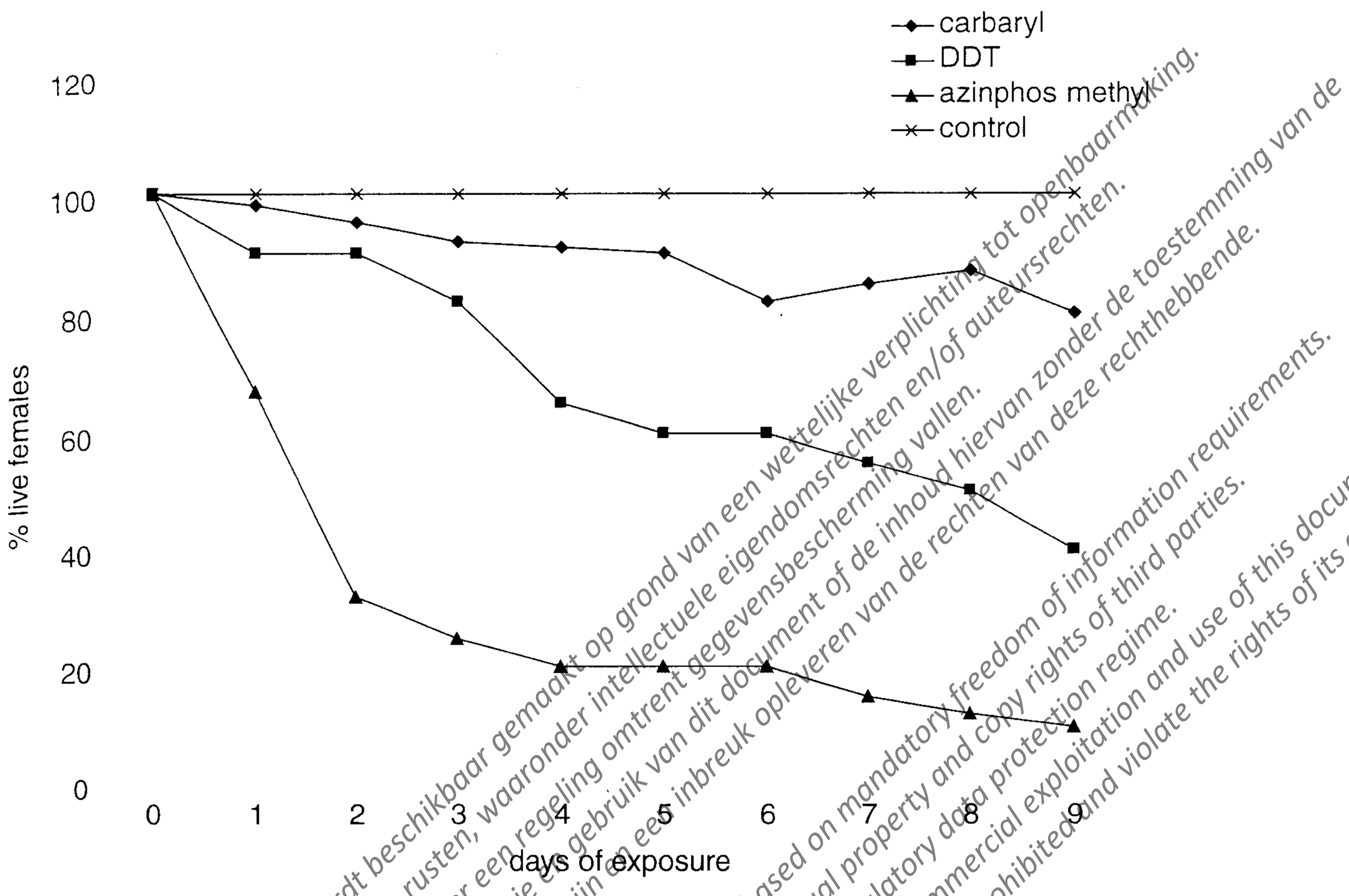

Figure 7.2 Survival of *Megachile rotundata* females nesting in greenhouse cages and exposed to *Medicago sativa* treated with three insecticides (after Waller, 1969) [52].

N. melanderi if applied late evening [28]. The same procedure testing the hazards of naled, imidacloprid, endosulfan, esfenvalerate, and oxydemeton methyl to *M. rotundata*, *N. melanderi*, and *B. occidentalis* revealed that the only insecticide with an RT 25 less than 8 hours in the three species was oxydemeton methyl [42]. It has been suggested that in contact testing and in the field practice, "insecticide pick-up" by pollinators, which is a parameter of hazards to bees, may be correlated with insect size. The pick-up, which is the ratio "weight of insecticide/weight of bee body" increases as the ratio "bee surface/bee volume." We can say that the larger the insect, the lower the pick-up since the volume and thus weight increase more rapidly than the surface [40]. According to this author, small bees such as *M. rotundata* (26 mg) and *N. melanderi* (87 mg) are more sensitive than large ones such as *B. centralis* (221 mg), honey bees being intermediate at 128 mg. The "surface/volume" ratios are: 1.0, 1.3, and 2.0 for *A. mellifera*, *N. melanderi*, and *M. rotundata*, respectively [39].

Susceptibility of larvae to contaminated food

Phosalone and alphamethrin were applied at 1000 and 10 g/ha on two experimental alfalfa fields where *M. rotundata* shelters were established.

Pollen ball samples were extracted from nests 5, 10, and 27 days following sprays, for residue analysis. No residues of the pyrethroid could be detected and phosalone concentration decreased from 1 to 0.1 mg/kg within the 3-week sampling period. Larval mortality was very stable in the four cell samples collected when the larval development was completed: before treatment 3.5 and 4.8 percent of larvae died in alphamethrin and phosalone samples versus 3.5 and 4.8 percent after treatment, respectively. No residues were detected in live larvae, which means that both molecules were metabolized [36]. Residues of deltamethrin were determined in leaf-cutting bee provisions collected in a field shelter placed in an alfalfa crop sprayed at the recommended rate. The maximum concentration was 0.01 mg/kg. A laboratory feeding test with pollen artificially contaminated with 1 mg/kg deltamethrin resulted in 55 percent larval mortality while no mortality occurred when the contamination rate was 0.1 mg/kg. It was concluded that the recommended dose of deltamethrin, 7.5 g/ha, was not hazardous to *M. rotundata* larvae [27]. Deltamethrin sprayed at 12.5 g/ha on rape, *Brassica napus oleifera*, was determined in anthers, nectar, bumble bee foragers and honey pots provisioned by a *B. terrestris* colony. In 1-day-old samples, residues were 0.2, 0.02, 0.15, and 0.005 mg/kg, respectively. A chronic feeding test using sugar solution contaminated at 0.01 and 0.2 mg/kg demonstrated that even the high-level concentration did not affect bumble bee larvae, which means that a dose twice as much as the recommended rate was nonhazardous to *B. terrestris* larvae [45]. After application at the recommended rates of aldicarb, dimethoate, carbofuran, and trichlorfon to alfalfa plots visited by leafcutting bees, pollen balls were sampled from their nests within various periods following treatment. No residues of the first three substances were detected whereas the maximum rate determined was 5 mg/kg for trichlorfon, and no larval mortality was observed in the plots treated with the three systemic insecticides whereas trichlorfon resulted in 22 percent dead larvae [53]. Two IGRs, diflubenzuron and fenoxycarb, were sprayed on caged *Phacelia* at the rate of 300 g/ha (recommended rate) and 1200 g/ha (double rate), respectively. Residues of diflubenzuron in pollen collected by *B. terrestris* ranged from 62 to 2 mg/kg within the 7-day period following application. During the same period, the figures for fenoxycarb varied from 217 to 7.5 mg/kg. Two days after application, diflubenzuron killed almost all the larvae except the old ones which was consistent with previous laboratory studies indicating an LC_{50} of 1.18 mg/kg and an LD_{50} 664 times higher in 6-day-old larvae than in 1-day-old ones [48]. In addition, during the whole period in the cage, colonies were not able to rear new brood even though queens continued to lay eggs. It was suggested that diflubenzuron had an ovicidal effect on queen ovaries. Normal eggs were laid when colonies were returned to the laboratory and fed with noncontaminated pollen. Fenoxycarb was totally nonhazardous to *B. terrestris* whereas it is harmful to honey bees, and diflubenzuron which was safe to bumble bees is classified

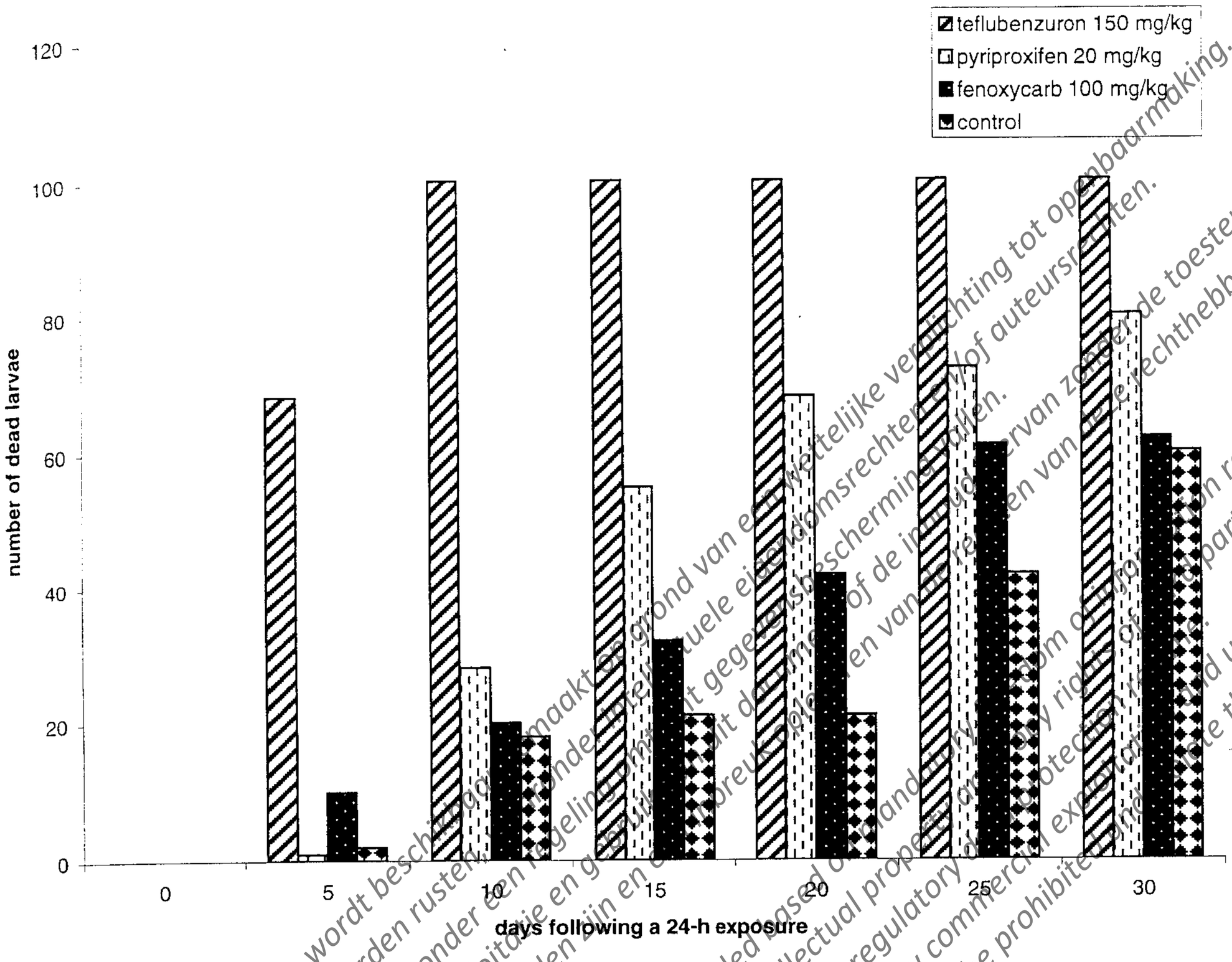

Figure 7.3 Mortality in *Bombus terrestris* larvae exposed to three IGR compounds (after de Wael *et al.*, 1995) [47].

as nonhazardous to *A. mellifera* [48]. The effects of the three IGRs, fenoxycarb, pyriproxyfen, and teflubenzuron, were compared in a laboratory study, using concentrations in bumble bee food based on the standard application rate in greenhouse, that is: 100, 20, and 150 mg/kg, respectively. After a 24-hour exposure, mortality records in larvae populations revealed no negative effects of fenoxycarb and pyriproxifen, whereas teflubenzuron killed all the larvae that were ejected by *B. terrestris* workers (Figure 7.3). This substance also arrested egg development and no developing brood appeared for 5 weeks in the treated colony [47].

*Susceptibility of non-*Apis *bees to field applications of pesticides*

Few experiments in field conditions have been reported. The earliest one showed that a population of *M. rotundata* females reared in an alfalfa field was not reduced significantly after a treatment with trichlorfon in late evening. However, the number of cells completed per day was reduced during the post-treatment period (Figure 7.4) and the number of dead immature individuals was a maximum the day following application. It was concluded that trichlorfon was a short residual substance [58]. In a similar

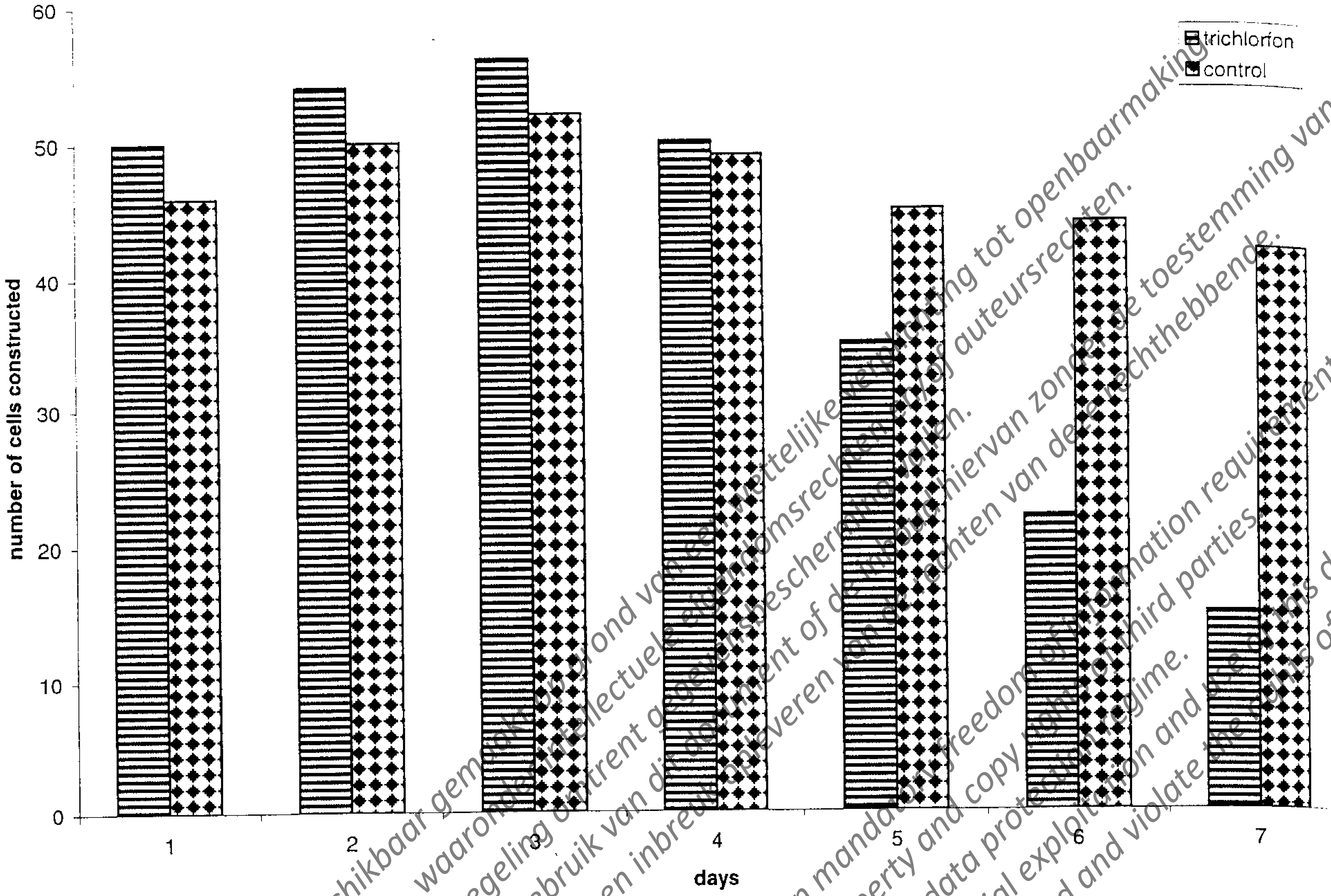

Figure 7.4 Egg-laying activity of *Megachile rotundata* in 50 nests in each of two experimental alfalfa plots, one of which was treated on Day 3 with trichlorfon (after Torchio, 1983) [58].

study deltamethrin and phosalone applications on four alfalfa plots resulted in low but significant losses of leafcutting bee females, compared to two control plots (Figure 7.5). From pollen analysis it was estimated that 70 and 60 percent of females, respectively, were exposed to the spray. When nests and larval development were completed the authors found a significant increase in the number of dead old larvae when bees were exposed to test compounds (Table 7.5) [59]. Leafcutting bee females were more affected by alphamethrin sprays applied to alfalfa fields at 10 g/ha.

Table 7.5 Mortality rates of different stages of *Megachile rotundata* progeny (after Tasei and Carre, 1985) [59]

	Deltamethrin	*Phosalone*	*Control*
Sample size	640	618	775
% Eggs and young larvae	2.8	5.2*	1.6
Prepupae	17.3*	12.8*	7.5
Pupae	1.9	0.5	0.9
Adults	0.6	0.5	0.5
Total	22.6*	18.9*	10.5

Note
*Mortality significantly higher than in control.

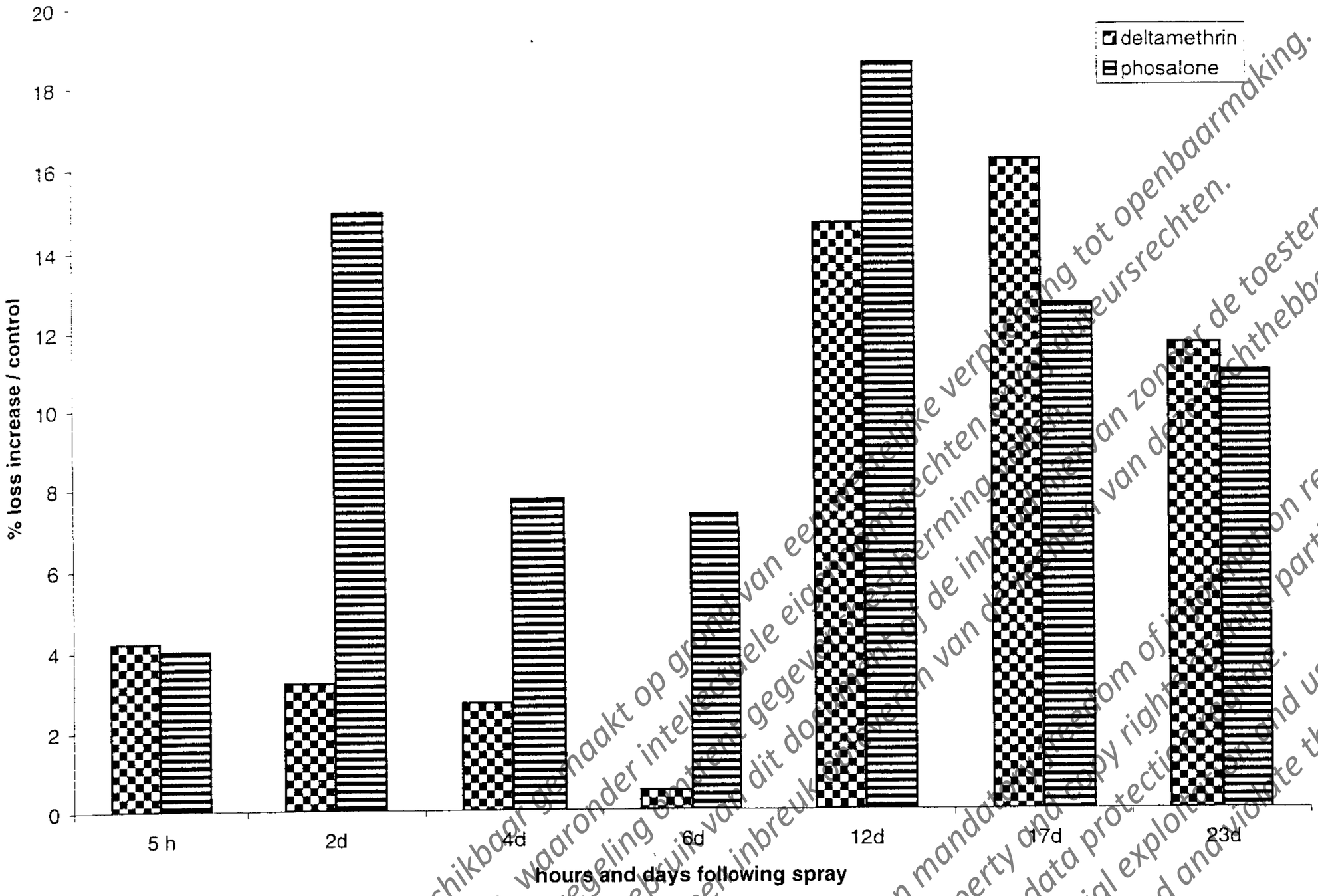

Figure 7.5 Increase of female losses in *Megachile rotundata* compared to a control population, when shelters were placed in alfalfa fields treated with deltamethrin and phosalone (after Tasei and Carre, 1985) [59].

The day following application 20 percent of nesting females had disappeared compared to 5.5 percent in the field treated with phosalone and 0.8 percent in the control. At the time of application, exposure rates of foragers were estimated at 82 and 74 percent. Larvae were slightly affected in treated fields during the 3 days post-treatment, mortality reaching 4.8 and 5.4 percent, respectively, while it was 1.2 percent in the control [36]. Observations of *B. terrestris* colonies moved to two sunflower fields, one treated with imidacloprid as a seed dressing, the other being nontreated, showed that 60 percent of foragers in the treated field and 50 percent in the control visited sunflowers during the 9-day test period. Losses of workers accounted for 33 and 23 percent of the population marked the day of introduction into fields, but the difference was not significant. It was concluded that *B. terrestris* was not affected by the systemic properties of imidacloprid seed dressing [49].

Short- and long-term impact of agrochemicals on native populations

Because of difficulties in determining the loss of bees in the native non-*Apis* bee population, the impact of agrochemicals on native pollinators

and the consequences on crops and long-term effects on vegetation have not been well documented [66]. The main case story is that presented in 1979 on the effects of fenitrothion used for protecting Canadian forests. During a 3-year period beginning in 1971 this compound was sprayed on a large scale, then its use was discontinued. Monitoring studies over 8 years were conducted on the impact of forest sprays on bees and on the pollination of blueberries, the main crop in the spray areas of New Brunswick. After the depopulation following the 3 years of treatment, a slow recovery appeared where sprays were stopped. Migration, mostly for bumble bees, and resident reproduction, mostly for solitary bees, were the two causes of this recovery [67]. During the first period, a survey of pollinators in blueberry fields cultivated at various proximities to fenitrothion sprays showed that the diversity and abundance index in nearby crops was 5–6 times less than in distant ones. Collected bees were bumble bees, solitary ground nesting species belonging to *Andrenidae* and *Halictidae* families. The author presumed that blueberry production failures might be attributed in part to fenitrothion sprays which reduced blueberry pollinators [22]. A later study in the same Canadian province showed that bumble bees exposed in cages to fenitrothion sprays were strongly affected. The negative effects were observed at least 150 m from the flight path of the spray aircraft, thus indicating serious spray drift. A survey of the bumble bee population demonstrated that reduction in their densities was associated with fenitrothion sprays. This reduction persisted for at least 2 years after these treatments were discontinued, and the author presumed that 3 to 4 years were necessary for a total recovery. Apparent recovery may be due to movement of queens from unsprayed areas or local individuals emerging later [57]. Other compounds such as carbaryl, trichlorfon, acephate, and diflubenzuron were used in North America to control the spruce budworm and Douglas-fir tussock moth in Maine, Montana, and Pacific Northwest forests. In Maine, carbaryl sprayed at 0.84 kg/ha sometimes resulted in more than 50 percent mortality of native bees of *Andrena*, *Dialictus*, and *Nomada* genus. In addition, in sprayed areas the bee population depletion was associated with a lower fruit set of *Viburnum cassanoides*. In Montana, wild bee densities were not affected by carbaryl and trichlorfon sprays at 1.12 kg/ha but there was a significant reduction in the proportion of native bees of small size, belonging to the families *Megachilidae*, *Andrenidae*, and *Halictidae* [66]. In the Pacific Northwest, carbaryl and acephate depressed foraging populations of wild bees observed on flowering "sight units" and fruit production of bluebells (*Mertensia paniculata*) was significantly reduced in an area treated with acephate. Conversely, diflubenzuron at 0.275 kg/ha did not affect the native bee population and was not hazardous to the honey bee brood. This IGR was thus recommended for moth control [61].

Sublethal effects

Very few authors have investigated the sublethal effects of pesticides on non-*Apis* bees. Reported studies deal with repellency, knock-down, fecundity, longevity, lifespan, food uptake in adults, and growth rate of larvae.

Repellency

This symptom was first reported in 1948 on *Andrena flavipes* which was repelled by a DDT application [16]. Further assessments were those on *M. rotundata* [23] and *B. terrestris* [25], which reacted negatively to residues of the three pyrethroids: fenvalerate, deltamethrin, and lambda-cyhalothrin. Visits of plants treated with pyrethroids by *M. rotundata* females were reduced by 50 percent compared to control. Generally, bees approached the flowers but did not touch them, or if they did, the contact was very brief. This repellency lasted more than 1 hour with fenvalerate and more than 3 hours with deltamethrin [23].

Knock-down

One hour following a fenvalerate spray at 50 g/ha, more than 80 percent of *M. rotundata* males were "knocked down" and counted dead, but 62 percent of the treated population recovered within 14 hours. This was not the case for males sprayed with deltamethrin at 7.5 g/ha which were all killed. When males were maintained on dry residues of both compounds, the knock-down effect was also observed and recovery affected 44 and 59 percent of males treated with deltamethrin and fenvalerate, respectively [23].

Fecundity

The fecundity of *M. rotundata* females foraging in a field sprayed with trichlorfon was significantly affected, since 4 days after application the number of cells completed per female dropped by 66 percent compared to control [58].

Longevity

When applied to male leafcutting bees a dose of deltamethrin equal to 0.04 × LD 1 reduced their survival rate by 50 percent after 6 days. According to the authors, females reacted similarly [27]. In a chronic feeding test where sucrose solution and pollen dough contained 10 and 6 μg/kg imidacloprid, the longevity of *B. terrestris* workers was affected only during the first month of the 3-month trial and the survival rate was reduced by 10 percent [46]. Conversely, the lifespan of bumble bees was prolonged considerably by chronic ingestion of deltamethrin at 0.2 mg/kg [45].

Food consumption

Topical application of 0.01–0.02 μg deltamethrin per worker resulted in a significant increase in sucrose solution intake, whereas a reduced consumption was observed when workers were fed solutions containing 0.1–0.2 mg/kg deltamethrin [45].

Growth rate of larvae

In *M. rotundata*, the duration of the larval development was 2 days longer if pollen balls were contaminated with 0.1 mg/kg deltamethrin [27].

Metabolism and toxicity

Synergism and detoxification processes in non-*Apis* bees have been investigated by several scientists. One of the earliest articles discussed the selective toxicity of trichlorfon to honey bees and *M. rotundata*. This compound was 18–34 times more toxic to *A. mellifera* than to leafcutting bee females and it was hypothesized that this differential toxicity could be associated with the pH of the body fluid which is 6.0 and 6.8 in *A. mellifera* and *M. rotundata*, respectively, and induces a greater stability of the molecule in the honey bee body [21]. The speed of penetration in *M. rotundata* was investigated with radioactive carbaryl which showed a 14 percent penetration after 5 minutes, 24 percent after 1 hour, and 41 percent after 8 hours. In addition, eight metabolites were recovered in the organosoluble fraction of the leafcutting bees [68]. Carbaryl served as a model for some studies that aimed at understanding variations in the toxicity to *M. rotundata*. When leafcutting males aged, their susceptibility increased rapidly, the LD_{50} for 1-, 2-, 3-, 4-day-old males being 240, 166, 109, and 51 μg/g bee, respectively. In both sexes the lipid content and microsomal enzyme activity decreased with aging. Three drugs were tested on leafcutting bees prior to carbaryl application. The first one, piperonyl butoxide, resulted in a strong synergy since LD_{50} dropped from 245 to 11 μg/g bee for 1-day-old females. The synergist ratio (LD_{50} of carbaryl alone/LD_{50} of carbaryl + piperonyl) decreased when females aged. With the second drug, chlorcyclizine, the LD_{50} for males was doubled whereas the third, aminopyrine, reduced the LD_{50} for females. The LC_{50} of carbaryl was 81.8 mg/kg for the compound alone and 47.5 mg/kg when piperonyl was added [20, 69, 70]. Experiments with radioactive carbaryl revealed a maximum persistence of the molecule when aminopyrine was used while chlorcyclizine reduced persistence and phenobarbital had no effect on it [71]. Chlorcyclizine modified the midgut structure and increased the susceptibility of *N. melanderi* to parathion [72]. More recent biochemical studies have shown that *M. rotundata* possesses seven enzymes susceptible to organophosphate inhibition [73]. Since serine esterases are the major target of organophosphate insecticides, they were used to establish the

kinetics of cytosolic esterases of *M. rotundata* females and to estimate the effects of four organophosphorus compounds: naled, trichlorfon, oxydemeton methyl, and paraoxon. The method was based on the measurement of hydrolysis of *p*-nitrophenylacetate by cytosolic preparations of leafcutting bees. It was demonstrated that a mixed mechanism of inhibition was involved and that the order of toxicity, based on inhibition constants, was: naled > paraoxon > trichlorfon > oxydemeton methyl [74]. The similarity of LD_{50} for trichlorfon and oxydemeton methyl [26] suggests that these compounds have different penetration speeds and, in addition, other enzyme systems such as polysubstrate mono-oxygenases or glutathione *S*-transferases, which may metabolize the insecticides before they reach their target, have been removed [74].

Conclusion

The use of domestication techniques has made it possible to assess the toxicity of some compounds used for pest control to a restricted number of non-*Apis* bee species. Standardized laboratory tests have enabled comparative studies to be performed which demonstrate that susceptibility to a compound can vary according to species; for example, *B. terrestris* is 60 to 90 times more tolerant to deltamethrin than *A. mellifera*. In addition, large differences in toxicity to a bee species appear within the same insecticide category. An example among IGRs is the toxicity of diflubenzuron to bumble bees which is 85 times higher than that of fenoxycarb. Owing to deficiencies in method harmonization, authors who used the same bee species have not always agree with one another when attributing toxicity ranks to identical series of pesticides.

A great variety of materials and procedures have been used for estimating hazards of pesticides to bees by either the semi-field or the field method. Cages and greenhouses were generally preferred because exposure rates cannot be controlled in the field.

Studies on detoxification in *M. rotundata* did not agree with previous toxicological data and thus expected hazards in the field. It was assumed that before a pesticide reaches its biochemical target several factors of major importance intervene in the contamination process. In particular, one should pay attention to the following:

- Insecticide pick-up depends on the insect size and is related to the ratio "surface/volume." Therefore, small bees are more sensitive than large ones.
- Penetration speed through the cuticle may be variable.
- Aged bees are more susceptible than callow individuals.
- Males are less tolerant than females.
- Degradation of pesticide in bees may depend on the pH of insect fluid, which may vary between species.

Low doses of several compounds, deltamethrin, trichlorfon, and imidacloprid, tested on *M. rotundata* and *B. terrestris*, resulted in various sublethal effects: repellency, knock-down, reduced fecundity, longevity or food consumption, and prolonged larval development.

Although the assessment of ecological consequences of temporary or permanent pest control by insecticides met technical difficulties, it has been shown that a population of small bees was more likely to be depleted than that of large species (bumble bees). Recovery was also more rapid with bumble bees, due to migration of queens from untreated areas, while solitary species recovered mostly through local reproduction. Reduction of fruit sets in some crops pollinated by native bees was associated with depression of pollinator population.

Additionally, exposure profiles of honey bees, bumble bees, and solitary bees differ significantly, due to respective flight activity hours, flight seasons, foraging habits, and nesting behavior, which result in different ecological impacts of pest management by agrochemicals.

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