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A Method to Quantify and Analyze the Foraging Activity of Honey Bees: Relevance to the Sublethal Effects Induced by Systemic Insecticides

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Abstract. The assessment of agropharmaceuticals' side effects requires more realistic simulations of field conditions than those deduced from the dose-lethality relation obtained under laboratory conditions. Because the presence of sublethal doses or concentrations may also alter the behavior of foraging insects, we attempted to devise a quantifiable and accurate protocol for evidencing various alterations in free-flying bees. Such a protocol was illustrated by testing new classes of systemic insecticides. The protocol focused on video recording to quantify the foraging activity of small colonies of honey bees confined in insect-proof tunnels. The basis of the protocol was not the colony itself but the change in each colony on a specific day and between days. First, the paradigms of attendance at a safe feeding source were established by observing 8 control colonies at different times of the season during 5 days after the necessary forager training was accomplished. Second, on three different colonies we considered the paradigms on the control day before contamination and during 4 days after the feeding source was contaminated. During the same period, one more colony was exclusively fed with safe food to serve as control. Two plant-systemic insecticides were tested at contamination levels 70 times lower than the 50% of the lethal concentration. Imidacloprid, at 6 μg/kg, clearly induced a decrease in the proportion of active bees. Fipronil, at 2 μg/kg, induced an additional decrease in attendance at the feeder. Such levels are still higher than the corresponding lowest observable effect concentration (LOEC). Our protocol, which provided intermediate conditions between field and laboratory conditions, allowed the quantification, with an enhanced level of sensitivity, of sublethal effects on foraging bees.

Environmental risk assessments are based on the measurement of pesticide exposure and its effects on living organisms (van der Werf 1996). Among them, the honey bee is considered to be a good indicator species because the colony lives as a "super-organism" of 50,000 individuals who are obliged to stock honey and pollen to overcome periods of scarcity (Moritz and Southwick 1992). Thus, the survival of the colony is fragile in a polluted environment and is related to individual performance and the quality of cooperation between classes of bees.

Sublethal doses of pesticides, especially insecticides, are known to disturb the essential activities of insects (Haynes 1988) even at concentrations not revealed by analytic chemistry (Leonardi *et al.* 1996). Sublethal doses can lead to poor individual performance and population dynamics disorders of the bee colony (MacKenzie and Winston 1989; Davis 1989). In particular, foraging activity is the starting point of intoxication for the bee because the forager is the first individual from the colony to encounter of the toxin on flower parts, in nectar, and in pollen.

Foraging is performed by a particular class of adult bees that harvest pollen and nectar from plants growing within an average radius of 5 km from the nest. The process is a complex phenomenon comprising coordinated individual performances (moving, sense perception, orientation, information acquisition, and memory) and social regulation and is based on activities including communication dances, food exchange, etc. Behaviors such as communication dances, return flights, orientation, and foraging efficacy during visits to flower heads are known to be affected by pesticides at sublethal doses (Schricker and Stephen 1970; Cox and Wilson 1984; Johansen and Mayer 1990; Vandame *et al.* 1995).

Considering the frequent risk of exposure by the forager bee to sublethal doses, especially with plant-systemic insecticides, the main aim of this study was to determine an improved method of showing how some behaviors are altered. In studying to what extent the contamination of a food source can affect the behavior of the forager bees, the following conditions were applied: (1) the colony was confined in a restricted volume so the foragers did not have the opportunity to visit food sources other than those designated and observed; (2) the colony size was created in equilibrium with the offered food resources and

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the design of the food dispenser; and (3) the insecticides were applied at sublethal doses.

To ensure that these conditions were properly met, only small colonies were kept within the confines of the bee-containing tunnels. Cox and Wilson (1984) used two-frame colonies to hold and brood 5000 adult bees, whereas we previously used only one-frame colonies with 500 to 1000 adult bees (Colin *et al.* 2001), which provided some dozens of foraging bees at the feeder. Nevertheless, the former did not allow any observation inside the hive without disturbing the colony and provide crowds at the feeding station. Concerning the latter, the precision of the statistical analysis would be improved by increasing the number of observed foragers.

However, because it is technically impossible to create strictly identical colonies, the basis of the present method was not the colony itself but the temporal change in one colony on a specific day and between days. The temporal change of the control colonies was compared with that of the experimental ones and, consequently, experimental distortion caused by possible variability of the colonies was avoided.

The second aim was to investigate the effects of two insecticides with systemic or systemic-like properties in plants, imidacloprid and fipronil, both of which exhibit acute toxicity for the honey bee. These insecticides (e.g., Gaucho and Regent) are commonly used by farmers as seed-dressing for sunflowers and various crops, respectively, or as soil treatment. In recent years they were implicated by beekeepers, who ascertained that the progressive decrease in the nectar and pollen harvest, particularly on sunflower and maize, was correlated with the increase of the crop surfaces treated with these insecticides.

Recently, we documented that imidacloprid contaminates all the parts of sunflowers and maize in the 1–20-μg/kg range (Bonmatin *et al.* 2004). The flowers are contaminated at an average level of approximately 10 μg/kg at the time of foraging (Bonmatin 2002). Moreover, average levels of a few μg/kg of imidacloprid were measured in pollen of both crops and in sunflower nectar (Bonmatin *et al.* 2003; Stork 1999).

Both insecticides are considered highly toxic to the adult bee with regard to the 50% lethal dose (LD50). The LD50 ranges between 0.005 and 0.081 μg/bee for imidacloprid (Schmidt 1996; Suchail *et al.* 2000) and between 0.0037 and 0.006 μg/bee for fipronil (Agritox 2002). In addition, sublethal effects of 4 μg/kg imidacloprid in sucrose solution were noticed on olfactory learning performance (Decourtye *et al.* 2001), and effects of 6 μg/kg were noticed on visits to a feeding source (Colin *et al.* 2001). Because the latter concentration is 70 times lower than that used to determine the oral LD50 of bees under laboratory conditions, the risk assessment—if based on the LD50—inevitably leads to an underestimation for this eusocial insect.

Note that the concentration inducing the LD50, 420 μg/kg, was calculated from the lowest oral LD50, 0.005 μg/bee (Suchail *et al.* 2000), given in 10 μl 50% sucrose syrup (the density of the sucrose syrup was 1.19 kg/L). In the same way, we chose the concentration of fipronil to also be 70 times lower than that used for LD50 (3.7 ng/bee in 20 μl sucrose solution; see Agritox 2002), which yields 2 μg/kg sucrose solution. Thus, these levels of imidacloprid (6 μg/kg) and fipronil (2 μg/kg) clearly belong in the sublethal range of each insecticide. Indeed, we did not observe any bee mortality throughout the presented 5-day experiments. Only more or less severe clinical signs, particularly in the case of fipronil, were observed.

Materials and Methods

Bees and Nuclei

Small colonies of 2300 bees (Harbo 1986) were created at the Institut National de la Recherche Agronomique (INRA) research station in Avignon, France). This location experiences a Mediterranean-type climate with infrequent rainfall and temperatures $> 20°C$ during spring and summer. Triple-hybrid bees were chosen as one of the most representative bees reared by honey producers in France. Special nuclei were designed to hold one Dadant-type frame when covered with adult bees. The dimensions of the nucleus container were $47 \times 33 \times 4.5$ cm (7-L inner volume) with glass walls, which allowed observation without having to open the nucleus. The walls were protected from temperature variations by an external and removable insulation layer consisting of 2-cm-thick polystyrene. In addition, an insulated roof covered the nucleus. Two thousand three hundred adult bees were added to a frame completed with brood, pollen, honey, and empty cells taken from a strong colony. A young queen bee in egg-laying condition was introduced when the nucleus was created. Before she was introduced into the experimental setup, the queen's correct behavior was checked during a period lasting at least 1 week. The foraging activity of the nucleus was also observed during this same pre-experimental period.

Experimental Setup: Tunnel and Feeder

Two semicylindrical, insect-proof tunnels were used and oriented east to west. They each were 4 m high and had a ground surface area of 8×20 m. Each tunnel was divided into two identical compartments (8×10 m). This setup was created for two reasons: (1) to ensure the separation of the experimental bees from those of the nearby colonies and (2) to enable the observation of complete bee paths if necessary. Each compartment enclosed one nucleus, placed in the southeastern corner, and a feeding station placed at the opposite angle. The distance between the feeding station and the nucleus was 10 m. This distance was chosen so the bees could use local landmarks (Collett 1992; Horridge 1996; Lehrer 1997; Dyer 2002) and it was sufficient to avoid any confusion between activities at the hive entrance and activities at the approaches to the feeding station.

The feeding station consisted of two main parts. First, there was a horizontal surface of 0.2 m^2 for carrying the feeder at its center, which was protected against direct exposure to the sun by a screen. Second, there was a wooden frame bearing a video camera hanging 60 cm above the feeder. The feeder itself was a dish, 20 cm in diameter, that held 600 g of syrup and was sufficiently large to feed 150 bees without crowding. To prevent any degradation of its content by ultraviolet (UV) light, the dish was made of a material impervious to UV rays. The free surface of the syrup was protected by using a circular float that was impervious to UV light. This float allowed the bee to land on and reach the syrup through a 2-mm free space with the dish wall.

Training and Test Procedures

In all of the tests, the bees first learned to associate the food source with visual stimuli. Then they learned to synchronize their foraging activity with a defined time of the day (Beling 1929; Moore *et al.* 1989). In fact, the bees did not spontaneously visit the feeding station

when it was placed in the tunnel. To help the bees learn the location of the feeder, it was first put at the entrance hole of the nucleus. Once bees began feeding, the bees and the feeder were gently carried to the final feeding location (Moore *et al.* 1989; Nigg *et al.* 1991). This process generally required 2 days to complete. Thus, teaching the bees the daytime feeding period was achieved by placing the feeder in its final position, at the defined time, every day. This temporal learning required an additional 2 days after the period needed for the bees to learn the feeder location. Using this procedure, the bees learned a specific feeding time with associated precision of a few minutes.

After the spatial and temporal teaching was accomplished, the experiments began. Every day the feeder was filled with 400 g 40% (w/w) sucrose solution. If the quantity was not entirely harvested by the bees after 120 minutes, the feeder was removed. A control sugar solution was offered to all of the colonies on the first day (day 0). During the 4 consecutive days, a contaminated sugar solution was offered to 3 nuclei (out of 4) placed in three different compartments. Under the same conditions, the control sugar solution was still offered to the fourth nucleus situated in the last compartment. This protocol is called a "run." It is therefore defined as one control nucleus and three contaminated nuclei, all observed during a 5-day period. Observations were recorded by a video camera and included the entire surface on which the feeder stood. This procedure circumvented disturbances caused by observer presence.

Attendance at the Feeder and Feeding Activity

The numbers of bees present at the feeder were counted every 3 minutes using data from the video film. Two categories of bees were distinguished: (1) "active" bees, named "A," which were sucking the sucrose solution, and (2) "inactive" bees, named "I," which were not taking food but were only present at the feeder. The first criterion was the number of active bees relative to time and was called "attendance." The second criterion was the ratio of the number of inactive bees to the number of active bees (I/A). This reflected the restlessness of the bees visiting a contaminated feeding source. Note that the restlessness was not mistaken with the normal agitation of the bees when the feeder was empty. In the latter case, the drastic decrease in active bees is counterbalanced by the increase in inactive bees.

Paradigm and Features of the Control Nuclei

The essential paradigm in the control nuclei is the maintenance or improvement of foraging activity during a day and with the passing days. Thus, attendance is constant or tends toward an increase during a day and/or with the passing days. The paradigm was verified on 8 nuclei, which were constituted during the experimental period and fed for 5 days with a simple sucrose solution.

Because the I/A ratio in any nucleus varied from day to day during the 5 days, we tried to verify whether a pattern or trend could be derived from these variations. If we could not, we compared the 8 nuclei as 8 experimental groups with each group measured during 5 days. Moreover, we compared the 5 days as 5 experimental groups with each group measured according to 8 values.

Analyses of the Nuclei Exposed to the Insecticides

The hypothesis of a constant attendance at the feeder was first tested as was done for the control nuclei. Any relation between the days was investigated. The changes in I/A ratio were considered with the passing days and particularly among days 0 (day without toxin), 1, and 4, which were the first and last experimental days, respectively, for all of the nuclei. Any relation between the days was investigated, and the data from day 4 for the three nuclei in the contaminated group were compared with those of the 8 control nuclei to verify any differences. When the effects of the insecticide were so severe that the number of active bees became too low to obtain a reliable ratio at day 4, then data from day 3 were used instead.

Insecticides

Imidacloprid (1-[(6-chloro-3-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimidine) belongs to the chloronycotinyl family. It acts as an acetylcholine agonist at several pharmacologically distinct acetylcholine receptor subtypes in insects (Liu *et al.* 1993; Buckingham *et al.* 1997). Imidacloprid (255.7 g/mol) was purchased as a standard analytic product from the Institute of Organic Industrial Chemistry (Warsaw, Poland). Because of its relatively high solubility in water (0.51 g/L at 20°C), dilution of imidacloprid was first accomplished in water. The first step consisted of dissolving 20 mg imidacloprid in 200 ml water. One milliliter of this solution was diluted in 99 ml water in a second step. Then 6 g of the latter solution was added to 994 g sucrose solution, thus resulting in a relative concentration of 6 μg/kg of imidacloprid.

Fipronil (±5-amino-1-[2,6-dichloro-α,α,α,-trifluoro-p-tolyl]-4-trifluoromethylsulfinyl-pyrazole-3-carbonitrile) is a member of the phenylpyrazole class of insecticides. It acts on GABA insect synapses for neurotransmission (Ozoe *et al.* 2000). Fipronil (437.14 g/mol) was purchased as a standard analytic product from Cluzeau Info Labo (France). Because fipronil is poorly soluble in water, a first dilution of 10 mg fipronil in 100 ml acetone was made. A second solution was prepared by diluting 1 ml of the previous solution in 99 ml acetone. Finally, 2 ml of the second solution was diluted in 1 kg sugar solution to give a relative concentration of 2 μg/kg of fipronil.

For each run, 3 kg sucrose solution with the *ad hoc* insecticide concentration was prepared on day 0. Each day the stock solution was taken out of the refrigerator and vigorously stirred for 15 minutes before the bee feeder was filled.

Statistical Analysis

No hypotheses on the structure of the data were formulated *a priori*, especially concerning their temporal distribution, thus nonparametric statistics were chosen. Concerning the paradigm of forager attendance, its consistency during the run period was tested by means of nonparametric statistics (Kruskall-Wallis test followed by Dunn test if the null hypothesis was initially rejected at $p = 0.05$). In some cases, the changes during a particular day were tested by linear regression to check if the trend was toward an increase in attendance. Thus, the paradigm of attendance in the control nuclei could simply be written as *attendance median day 0* $\leq$ *attendance median day 1* $\leq$ *attendance median day 4*.

Concerning the features of I/A ratio for a given nucleus, comparisons between the days were also made using the same nonparametric tests. The existence of a clear relation was then discussed. If there was no particular relation (as in the case of the 8 control nuclei), nuclei were compared by nonparametric statistics with each nucleus described by the medians of the days. The comparison of the days, with each day being defined according to 8 values of the control nuclei, was also tested. If any relation among the days was clear (as in the case of contaminated nuclei), day-4 days of the contaminated and control groups were compared using Mann-Whitney test.

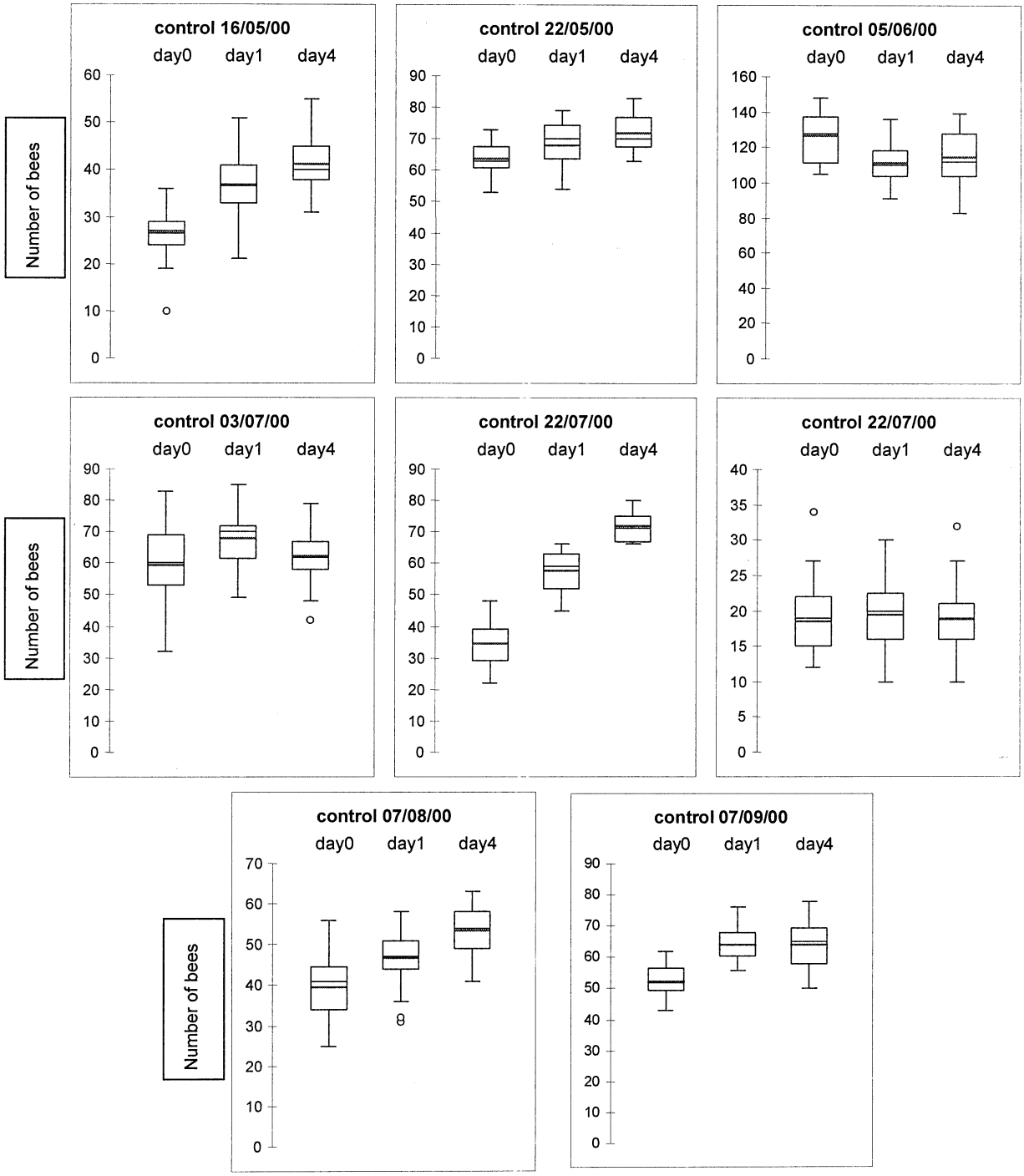

Fig. 1. Daily bee attendance of "active" bees in the control nuclei. Each day, after the recruiting period, the number of active bees was recorded every 3 minutes during the observation period. This period finished either when the feeder was empty or 120 minutes after presentation of the feeder to the bees. The mean, median, and percentiles of the daily counts are shown as one box plot. Only data for days 0, 1, and 4 are given for clarity. The bee attendance did not decrease with the passing days

Results

Paradigm and Features of the Control Nuclei

Attendance at the Feeder. We observed increased performance of several nuclei, which resulted in exhaustion of the sugar syrup during the last day (day 4). For this reason, the time interval taken at day 4 was used as the basis of comparison for days 0 and 1. The time interval began at the end of the recruiting phase. It ended after 120 minutes or just before the number of foragers decreased drastically because the feeder was empty. The recruiting phase was characterized by a rapid increase in foragers after the feeder

was put in place. The duration lasted between 9 and 18 minutes.

In several control nuclei, the hypothesis of day equality could not be rejected at $p = 0.05$ (dates [mm/dd] 05/22, 06/05, 07/03, and 07/22). In the other nuclei, the differences clearly tended toward an increase in the number of active bees with the passing days (Figure 1). Note that for day 0 of the 09/07 control nucleus and for days 0 and 1 of the 08/07 control nucleus, a linear regression could be proposed with a positive slope (not shown), thus indicating behaviors of the nuclei that were even better. Finally, the paradigm—*attendance median day 0* $\leq$ *attendance median day 1* $\leq$ *attendance median day 4*—was verified for all of the control nuclei under these conditions.

Inactive Versus Active Bees. For the reasons mentioned previously, the time interval taken at day 4 served as the basis of comparison for days 0 and 1. First, when comparing the days for each nucleus, the hypothesis of day equality was rejected at $p = 0.05$ except for both 05/22 and 06/05 dates nuclei. No relation linking the days, valuable for all of the nuclei, could be proposed. Second, the equality of the nuclei, defined by the medians of their 5 days, could not be rejected ($H = 13.20$, $p = 0.07$, $df = 7$) as shown in Figure 2. Third, the equality of the days, defined by the medians of the eight nuclei, could not be rejected ($H = 8.870$, $p = 0.064$, $df = 4$). One day, day 4 of the 05/16 was excluded from the calculation because it clearly lied outside the distribution of the other days (Figure 3). Because the contaminated and control nuclei came from the same apiary, the changes in the contaminated nuclei during the experimental run periods could be compared with those of the 8 control nuclei.

Analyses of the Nuclei Exposed to the Insecticides

Bee Attendance at Imidacloprid Solution. When comparing days 0, 1, and 4 of the contaminated nuclei, the equality of the days was rejected at $p = 0.05$, and there was no common relation linking the days (Figure 4). Therefore, it was concluded that imidacloprid does not have an effect on attendance at 6 μg/kg.

Bee Attendance at Fipronil Solution. When comparing the same run days of the contaminated nuclei, the equality of the days was rejected at $p = 0.05$ (Figure 5). However, conversely to imidacloprid, there was a common relation for each contaminated nucleus. The relation—*median day 0 = median day 1 > median day 4*—was verified and was opposite to the relation observed in all of the control nuclei. In fact, attendance was zero or close to zero in two contaminated nuclei on day 4. Thus, we concluded that fipronil has a strong effect on bee attendance at a concentration of 2 μg/kg.

I/A Ratio at Imidacloprid Solution. Equality of the days was rejected for the three contaminated nuclei. Moreover, the relation—*median day 0 = median day 1 < median day 4*—was clearly established compared with the lack of relation observed for the control nuclei. When comparing the experimental day-4 days with the control days, the equality of the groups was rejected ($T = 122$, $p = 0.008$). Thus, the effect of the imidacloprid resulted in a clear increase in the I/A ratio on day 4.

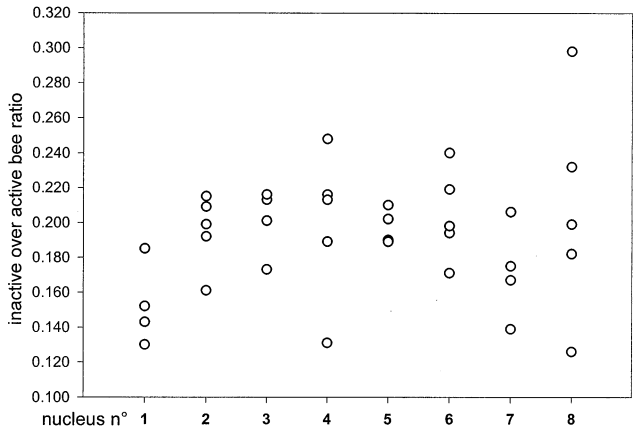

Fig. 2. Comparison of the I/A ratio of the 8 control nuclei during the 5 days. One control nucleus is described by five points. One point is the daily median of the I/A ratio for a given nucleus. No significant difference was noticed between the control nuclei

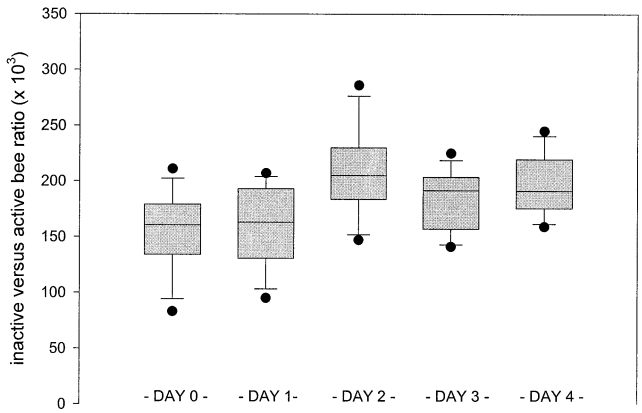

Fig. 3. Comparison of the I/A ratio of the 5 days for the 8 control nuclei. One day is represented by one box. Each box plot is created from the percentiles and medians of the 8 nuclei for a given day. The lowest and highest values are shown as extreme points. No significant differences were noticed between the days

I/A Ratio at Fipronil Solution. On day 4 only a very weak number of active foragers, often none, remained, which indicated a strong effect of fipronil (Figure 5). Thus, the I/A ratio tended toward infinity. The relation, median day 0 $\leq$ median day 1 $<$ median day 3, was clearly established. This result contrasted with that seen in the control nuclei. When comparing experimental day-3 days with control days, the equality of the groups was rejected ($T = 30$, $p = 0.012$). Thus, fipronil induced a drastic decrease in the number of foragers coupled with an increase in inactive bees at the feeder. In addition, we noticed the presence of bees showing evident clinical signs of intoxication.

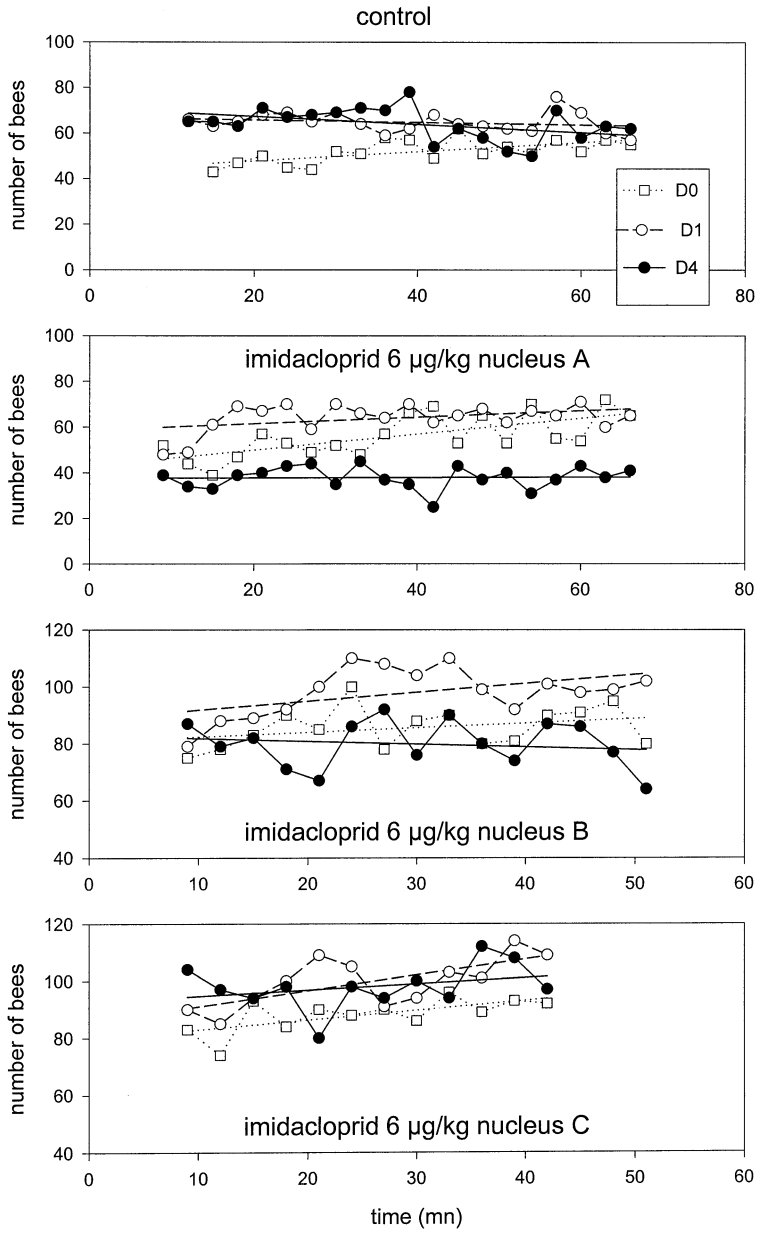

Fig. 4. Daily attendance of "active" bees when the feeder was contaminated with imidacloprid at a concentration of 6 µg/kg. Data from three nuclei (A, B, and C) can be compared with that of the pooled control nuclei. Only data and slopes for days 0 (control day), 1, and 4 are depicted for clarity. A slight improvement is seen for all the nuclei at day 1. Then a significant decrease in the number of active bees (submitted to the contaminated source) occurred for nuclei A and B, but not for nucleus C, as shown on day 4

Discussion

An Improved Method

Our method dealt with the activity of bees using two relevant criteria: attendance and the I/A ratio of foragers. These criteria can be objectively quantified by using video records. First, we used comparisons between (1) a pool of control nuclei observed at several times during spring and summer and (2) several nuclei exposed to a contaminated food during the same time. Second, statistical analysis could be performed on the raw data at a definite period during a day and throughout the 4-day run. Thus, the method not only evaluated levels of activity, it also evaluated qualitative and quantitative evolution in activity levels, during a day and between days, within a nucleus and between several nuclei. By this method, clear effects on the foraging activity were documented in the cases of imidacloprid and fipronil at contamination levels of 6 and 2 µg/kg, respectively. Imidacloprid only induced a significant change in the I/A ratio, whereas fipronil affected both attendance and I/A ratio. Because such relative concentrations were far lower than the LC50 for the two insecticides (by approximately 70 times), the method appeared to be very sensitive and well suited for the study of sublethal effects on foraging activity.

The use of 8 control nuclei allowed verification of the reproducibility of the evolution of each nucleus. To accomplish this, two conditions were required. One concerned nucleus strength, and the second concerned tunnel length. Concerning nucleus strength, the number of bees and the the balance between bees of various ages needed to be checked to ensure that there was an adequate number of foragers in relation to the hosting capacity of the feeder and avoid crowding. Concerning

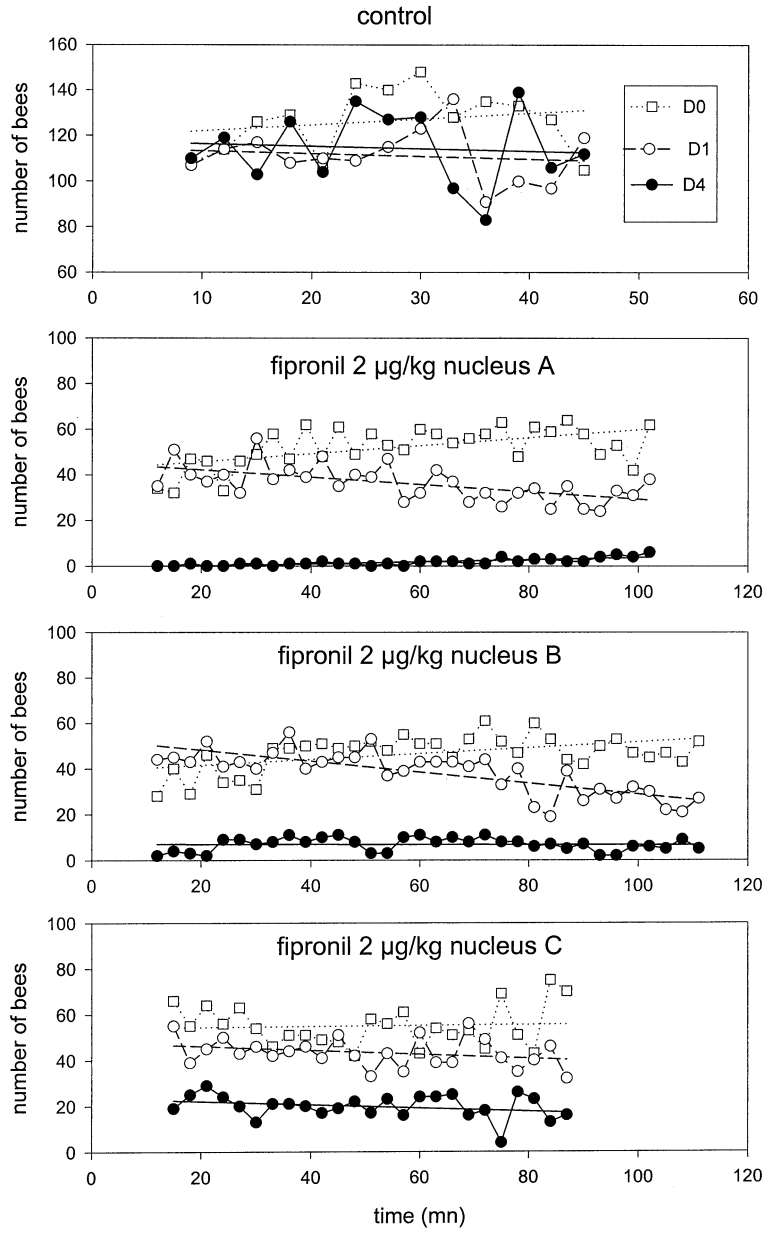

Fig. 5. Daily attendance of the "active" bees when the feeder was contaminated with fipronil at a concentration of 2 µg/kg. Data from three nuclei (A, B, and C) can be compared with that of the pooled control nuclei. Only data and slopes for days 0 (control day), 1, and 4 are depicted for clarity. A first significant change in slopes is depicted from day 1. Then the number of active bees (submitted to the contaminated source) sharply decreased as shown on day 4

tunnel conditions, a maximum of 10% of the adult bees of the nucleus was considered as foragers. In other experimental designs using crops as the feeding source in the tunnel, one should verify that there are no more than 10 foragers/m^2. Otherwise, foragers probably would be less exposed to the toxin than expected. Concerning tunnel length, the distance between the nucleus and the feeder needed to be at least 8 m. This distance allowed unambiguous separation of the three different phases of the pathway of the foraging bee: (1) taking off from the nucleus or from the feeder, (2) flying straight in the direction of the goal, and (3) circling (or zigzagging) before landing at the feeder or at the nucleus entrance.

Further improvements of the method could be made to study the individual behavior of bees. Hence, attendance and I/A ratio dealt with general activity at the feeder because foragers were not individualized. By marking the bees, an ethogram—according to Markov's analytic procedure—could be made when examining the video films (digitized video data). Thus, a better description of the effects of the 2 insecticides on foraging activity could be obtained. Another improvement related to digitizing the measurements would be to substitute the video camera with web cameras placed at different locations and heights. This would allow the study to be conducted in three dimensions as well as allow the observation of entire or partial flight paths. This possibility could be useful especially for fipronil-like insecticides because numerous foragers do not correctly locate the entrance of the nucleus.

Biological Effects Induced by the Systemic Insecticides

Two modes of intoxication were by these two insecticides. They can be described by way of the two criteria relating to

food source frequentation and proportion of inactive bees relative to active bees (I/A ratio). When only I/A ratio was affected (i.e., attendance unchanged), bees were able to maintain the physical aptitudes necessary to accomplish their tasks. This was the case for imidacloprid. Here, the behavioral problems were attributed to effects on the central nervous system, which do not contradict the report of Sone *et al.* (1994). These investigators demonstrated "a loss of force in the feet of the American cockroach due to blockage of the 6th abdominal ganglion, but at elevated doses." Such doses are far greater than 6 μg/kg (Mayer and Lunden 1997). In the present case, the manner of absorption of the contaminated syrup is modified by imidacloprid. This is consistent with reports of Nauen *et al.* (1998a,b), who described an antifeed effect in certain types of aphids at several μg/kg levels of imidacloprid. However, the investigators did not indicate whether (1) the insects immediately detected the presence of the insecticide (i.e., did not absorb the contaminated food) or (2) the insects stopped feeding as a result of absorbing the toxin in the food and/or just after contact with the contaminated surface. Note that decreased sucking activity of aphids has already been mentioned by Abraham and Epperlein (1999). Furthermore, Smith and Krischik (1999), as well as Vincent *et al.* (2000), demonstrated decreased mobility of the ladybird larvae (*Harmonia axyridis*) after they walked on contaminated surface.

When both attendance and I/A ratio were disturbed by the toxin at a given concentration, the intoxication was at a subacute stage, i.e., without mortality but with evident clinical signs of impairment. This was the case for fipronil at a concentration of 2 μg/kg. Clinical signs of disruptive motor activity, such as convulsions or paralysis, meant that bees were physically unable to accomplish foraging tasks. Furthermore, the huge decrease of foragers on day 4 was correlated with the progressive appearance of convulsion episodes. In fact, fipronil neither exhibits an antifeeding effect nor produces foodstuff aversion in bees even at relatively high doses (Franc and Cadiergues 1998; Gahlhoff *et al.* 1999; Mayer and Lunden 1999; Patourel 2000). That is why bees can absorb doses sufficiently high to be even more intoxicating.

Conclusion

The present method allowed the quantification of some features of foraging activity in several small bee colonies. It also provided two reliable and reproducible quantitative parameters independent of the observer. These parameters were clearly related to biologic effects on bees.

The experiment was designed to simulate realistic conditions occurring in fields, particularly concerning the presence of toxic levels of a few μg/kg in pollens and nectars. It provided data complementary to those of chronic exposure under laboratory conditions, which revealed a delayed mortality at concentrations < 1 μg/kg for imidacloprid (Suchail *et al.* 2001). Clinical signs, highly characteristic of intoxication and already observed in sunflower fields, were reproduced in the experimental tunnel conditions. Therefore, this method provided an indispensable interface between controlled conditions in the laboratory and the field. The potential uses of this method are numerous because the study of global activity among foragers at a feeder may be further completed by observations at both the nucleus entrance and its interior, especially if the bees are individually marked.

The quantitative effects of imidacloprid and fipronil were linked to a global disturbance in the main task of the colony, i.e., feeding activity. Investigation of toxic effects is not limited to counting dead adult bees but instead deals with sublethal effects on feeding and, consequently, on the food supply of the colony, both of which affect its long-term survival. Such sublethal effects induced by systemic insecticides should be considered in risk assessment schemes when considering beneficial insects such as honey bees.

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