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# Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*

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**Abstract**

Laboratory bioassays were conducted to determine the contact honey bee toxicity of commercial and candidate neonicotinoid insecticides. The nitro-substituted compounds were the most toxic to the honey bee in our laboratory studies with $LD_{50}$ values of 18 ng/bee for imidacloprid, 22 ng for clothianidin, 30 ng for thiamethoxam, 75 ng for dinotefuran and 138 ng for nitenpyram. The cyano-substituted neonicotinoids exhibited a much lower toxicity with $LD_{50}$ values for acetamiprid and thiacloprid of 7.1 and 14.6 μg/bee, respectively. Piperonyl butoxide, triflumizole and propiconazole increased honey bee toxicity of acetamiprid 6.0-, 244- and 105-fold and thiacloprid 154-, 1,141- and 559-fold, respectively, but had a minimal effect on imidacloprid (1.70, 1.85 and 1.52-fold, respectively). The acetamiprid metabolites, *N*-demethyl acetamiprid, 6-chloro-3-pyridylmethanol and 6-chloro-nicotinic acid when applied topically, produced no mortality at 50 μg/bee. These results suggest that P450s are an important mechanism for acetamiprid and thiacloprid detoxification and their low toxicity to honey bees. When honey bees were placed in cages in forced contact with alfalfa treated with acetamiprid and the synergist, triflumizole, in combination at their maximum recommended application rates, no mortality was detected above that of the control.


*Keywords:* Honey bee; Neonicotinoid; Acetamiprid; Imidacloprid; Thiacloprid

## 1. Introduction

The honey bee, *Apis mellifera*, is an economically important insect worldwide, producing honey, pollen, royal jelly, propolis and wax. In addition, honey bees pollinate more than 50 of over 250 crops in the United States, some of which are important for the production of high quality, commercial seeds and fruits (Atkins, 1992). The study of pesticide effects on the honey bee is vital because of the need to control a wide variety of agricultural pests with insecticides (Atkins, 1992) without hurting bees that inadvertently come into contact with pesticides when foraging.

The neonicotinoids are a new insecticide class which include the commercial products imidacloprid, acetamiprid, nitenpyram and thiamethoxam and are important to agriculture because of their activity against sucking insects and some Heteroptera, Coleoptera and Lepidoptera (Yamamoto and Casida, 1999). The acute honey bee toxicity by contact and per os for imidacloprid is high (Suchail et al., 2000). Compared to imidacloprid, acetamiprid exhibits a broader insecticidal spectrum and has been registered against the diamondback moth and fruit moth in Japan. Acetamiprid appears to be safe to honey bees and bumble bees (Takahashi et al., 1992). Steffens and Lin (2000) and Elbert et al. (2000) found that thiacloprid exhibited a broad insecticidal spectrum like that of acetamiprid with significantly reduced activity toward honey bees.

Neonicotinoids such as imidacloprid, thiamethoxam and thiacloprid have a chloro-substituted heterocyclic group, either a chlorpyridinyl or chlorthiazolyl, joined to a second heterocyclic ring. In place of the second heterocyclic ring, neonicotinoids such as acetamiprid, nitenpyram, clothianidin and dinotefuran have an acyclic group. In general, an electron-withdrawing moiety, either a nitro or cyano group is essential for insecticidal activity (Kagabu, 1999).

Neonicotinoids act on the insect nicotinic (acetylcholine) receptor (nAChR). Binding studies using

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α-bungarotoxin showed that the receptors in both honey bee and housefly heads have the same order of magnitude affinity to acetamiprid, with $K_i$ values of 7.0 and 3.6 μM, respectively (Yamada et al., 1999; Yamamoto, 1999). These data suggest that the low susceptibility of honey bees to acetamiprid does not result from differences in the target sites between these two species.

The current study examines the susceptibility of the honey bee to the commercial neonicotinoids, related compounds in commercial development and potential metabolites of acetamiprid and examines the role of xenobiotic metabolism in reducing honey bee toxicity by this insecticide class. Synergism studies were used to investigate mechanisms of insecticide metabolism and their relative importance in pesticide susceptibility. In addition to typical insecticide synergist like piperonyl butoxide (PBO), *S*,*S*,*S*-tributylphosphorotrithioate (DEF), and diethylmaleate (DEM), we included in our studies the 14α-demethylase inhibitor- (DMI-) fungicides also referred to as ergosterol biosynthesis inhibitors (EBI), and a plant growth regulator. It was previously shown that the fungicide class included in our studies synergized pyrethroids (Colin and Belzunces, 1992; Pilling and Jepson, 1993), and in some practical applications, bees could be exposed in the field to both fungicide and neonicotinoids together. Studies were also included to examine the practical use of neonicotinoids and fungicides in combination and their effect on honey bees.

## 2. Materials and methods

### 2.1. Chemicals

Technical neonicotinoid insecticides (acetamiprid, imidacloprid, thiacloprid, nitenpyram, clothianidin, dinotefuran and thiamethoxam), metabolites of acetamiprid (*N*-demethyl acetamiprid, IM-2-1; 6-chloro-3-pyridiylmethanol, IM-O; and 6-chloro-nicotinic acid, IC-O), and the DMI-fungicides (triflumizole, triadimefon, epoxiconazole) were obtained from Nippon Soda Co., LTD (Japan). The purity of these compounds was >99% by high performance liquid chromatography. PBO, DEF, propiconazole (mix of isomers) and uniconazole-P were purchased from Chem Services Inc. (West Chester, PA) and DEM from Sigma Chemical Co. (St. Louis, MO). Formulated acetamiprid (NI-25, TADS 1242) was made available to us from Aventis CropScience (Research Triangle Park, NC) and was 73.9% acetamiprid. The fungicide, Procure 50 WS was purchased from DeCran Ag Supplies Inc. (Rochester, MA) and was 50% triflumizole. The primary structure of the neonicotinoids, metabolites and synergists used in our studies are given in Figs. 1 and 2.

### 2.2. Laboratory bioassays

Honey bees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae), were collected from two hives on the North Carolina State University Campus (Raleigh, NC) from June to September, 1999. The hives at the time the bees were collected were free of obvious diseases that might be observed during routine colony maintenance and bee collections. No hive treatments to control diseases were conducted prior to our studies. Hives were exposed to smoke twice for 30–60 s prior to collection. Honey bees on frames containing honey and pollen were always collected from the top super, and the bees shaken from the frames into a plastic container. The opening of the container was covered with a solid plastic lid and the bees transported to the laboratory. The bees were

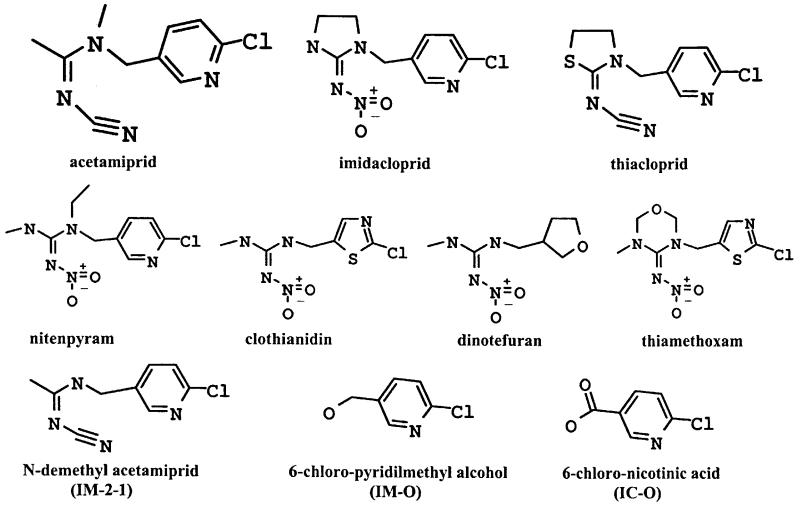


Fig. 1. Primary structure of the neonicotinoids and metabolites of acetamiprid used in this study.

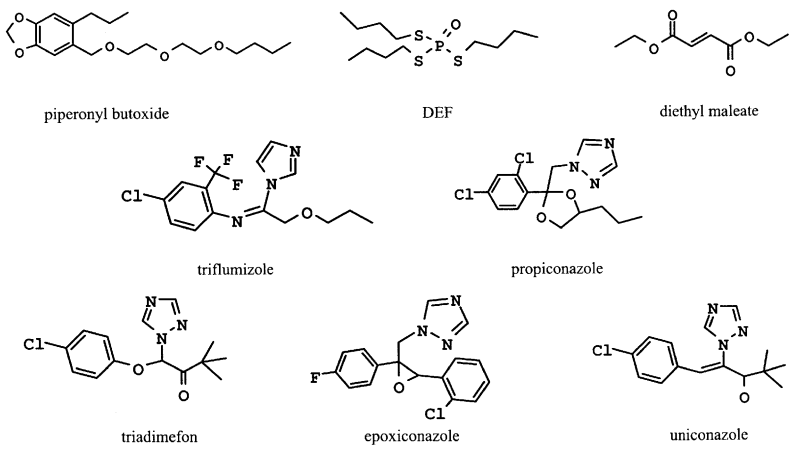


Fig. 2. Primary structure of potential neonicotinoid synergists. PBO, the DMI-fungicides (triflumizole, propiconazole, triadimefon and epoxaconazole) and the plant growth regulator uniconazole-P are putative inhibitors of P450. DEF is an esterase inhibitor, and DEM is an inhibitor of glutathione transferase.

maintained at approximately 25°C during the 10 min transportation to the lab.

Immediately upon arrival from the field, bees were anesthetized by exposure to carbon dioxide gas for no longer than 3 min, and then the newly emerged workers and drones were separated from the remaining workers. Newly emerged workers were distinguished from other workers by the appearance of abundant light yellow setae on the dorsum of the thorax. Usually, $<5\%$ of the workers were classified as newly emerged. The older workers were immediately transferred to a 177 ml plastic cup (10–15 bees/cup) covered with a nylon mesh (0.4 cm holes) held in place with a rubber band. Covering the cup with a nylon mesh was essential to obtaining low mortality in the control experiments. The bees spent most of their time on this mesh and were less likely to be injured by interactions with the container surface or with other bees. A small hole was made in the center of the cup bottom and a single Kimwipe (Fisher Scientific, Raleigh, NC) was partially extruded through the hole into the inside. The cup was then placed into a reservoir of 20% sucrose in water (wt/vol) so that the Kimwipe was soaked with the solution, and sugar was available ad libitum to the worker bees during the duration of the assay. The insects were given approximately 15 min to recover from the carbon dioxide treatment, and any bees that were not walking or flying within 15 s were replaced.

Prior to treatment with insecticide or synergist, bees (10–15/cup) were anesthetized again with carbon dioxide as described earlier. The maximum time of each of these carbon dioxide treatments was 30 s, and no differences in control mortality were found for bees anesthetized 1–3 times. After anesthetization, the mesh top was removed and the workers were treated before they became active again. The insecticides and synergists were separately dissolved in 100% (absolute) ethanol, and dilutions were made to obtain the appropriate dose per bee in 1 μl of the solvent. Solutions were vortexed vigorously before use. Controls received 1 μl of ethanol only. Topical applications to the dorsal thorax of each bee were made with a 50 μl Hamilton syringe fitted with a 1 μl repeating dispenser. These treatments were made within 30 min of the first anesthetization described earlier. In all, 10 μg of the synergist was applied to anesthetized bees 1 h prior to the insecticide application. The upper limit of the amount of synergist that could be applied was determined by the mortality produced by the most toxic compound (DEF, 29.0% corrected mortality). Corrected percent mortality for the other synergists were as follows: PBO (0%), DEM (0%), triflumizole (0%), epoxaconazole (0%), propiconazole (2.2%), triadimefon (0%) and uniconazole-P (0%). The synergist was applied before the insecticide to provide time for transport of the compound into the insect system and to maximize the likelihood of metabolic inhibition (Zhao et al., 1996 and references therein). After treatment, the container was covered with nylon mesh and bees incubated at $27\pm1$°C, 50% relative humidity and a photoperiod of 14:10 (light:dark). Mortality was assessed 24 h after the insecticide treatment in order to minimize control mortality and because in preliminary tests with acetamiprid and nitempyram, only a small or no increase in mortality was found between 24 and 48 h for doses of insecticide that produced mortality in the range of 8–100%. In our studies, bees were considered dead if they were unable to walk or fly. The control mortality averaged 3.7%. Each experiment was replicated 2–3 times per insecticide dose with a minimum of 30 insects per replicate and 5–7 doses were used to determine the $LD_{50}$. All results were corrected as appropriate for control mortality and/or mortality due to the application of the synergist by methods that are described later.

### 2.3. Plant bioassays

These studies were conducted in Springborn Laboratories in Wareham, MA in July–August, 2000. Alfalfa seeds (variety Oneida) were purchased from Stekson Agway (Berkley, MA). Fourteen plots (1 $m^2$ each) were arranged in a randomized block design with each plot separated by a 0.6 m buffer zone on all sides. Six plots were treated with acetamiprid (three replicates at two sampling intervals after treatment); another six plots received a combination of acetamiprid and triflumizole (Procure$^R$)(three replicates for two sampling intervals); and there were two non-treated control plots at two sampling intervals. Control and treated plots were harvested at 3 and 24 h after the insecticide application.

Acetamiprid and acetamiprid/triflumizole in combination were sprayed on alfalfa at the rate of 168.1 g (a.i.) acetamiprid/hec and 280.2 g (a.i.) triflumizole/hec (780 l/hec) using a carbon dioxide pressurized (20 psi) hand-held sprayer with a single nozzle and a 250 ml tank. The sprayer was cleaned thoroughly with deionized water prior to use. Spraying was conducted by first applying deionized water to the control plots, followed by treatment of the acetamiprid plots, and finally by treatment of the acetamiprid/triflumizole plots. To insure accurate applications, the appropriate weight of active ingredient in water was added to the tank and dispensed evenly to an individual plot. The tank and nozzle were then rinsed with 18 ml of deionized water sprayed evenly over the same plot. This procedure was used for all plots for the control and treatments. The plots were covered after spraying and as needed before rain to prevent insecticide runoff. After application, alfalfa was collected at two time intervals, i.e. 3 and 24 h after treatment. The control foliage was collected first, followed by the acetamiprid treatment and then the acetamiprid/triflumizole treatment. The alfalfa was hand harvested by cutting just above the soil line using pruning shears. At each sampling interval, 32–72 plants were collected from each treatment and 87–115 plants from the control. The pruning shears were cleaned between plots with soap and water followed with acetone.

The honey bees used for these studies were worker bees less than 7 days old which were removed from brood frames from a single hive. The bees were purchased from Apiary Services Inc. (Wareham, MA). The test cages were $13 \times 13 \times 13\,cm^3$ constructed from sheet PVC covered with polyester mesh (3.5 mm mesh). Each cage had a glass sliding door to introduce plants into the cage and an access hole with a self-sealing rubber closure to introduce the bees. Six replicate chambers were used for each treatment and control. The bees were collected on the day prior to testing by picking workers from brood frames that had been isolated from a mature hive. The honey bees were captured at random into a glass tube and transferred (25 insects per cage) by gently blowing them by mouth. The cages containing bees were immediately transferred to the laboratory.

In the laboratory, the honey bees were fed in their cages with 50% sucrose in a PVC trough and deionized water from an inverted glass jar with a perforated lid. At each harvest interval, any weeds from the alfalfa plants that were collected were removed, and 15 g of plants selected at random from the three plots for each treatment and 15 g from a single control plot were placed separately in cages containing 25 bees per cage. This amount of plant material filled more than 75% of the cage volume, and the majority of the time, the bees were in contact with the plant surface. The studies were replicated six times at both the 3 and 24 h post-insecticide treatment intervals. The honey bees were anesthetized with carbon dioxide just long enough to allow for the addition of the alfalfa. The insects were maintained at $25 \pm 2^{\circ}C$ with the relative humidity between 77% and 84% under constant darkness except at the observation intervals. Criteria for mortality were defined earlier.

### 2.4. Statistics

Abbot's correction (Abbott, 1925) was applied to all data from dose–response experiments. $LD_{50}$ values were estimated by plotting log dose versus probit plus five mortality (Sokal and Rohlf, 1995; Finney, 1971; Microsoft Excel, 1997). Confidence intervals for toxicity ratios were determined by the method of Robertson and Preisler (1992). Means tests were conducted using Student's *t*-test ($P < 0.05$).

## 3. Results and discussion

### 3.1. Honey bee susceptibility to neonicotinoids

The toxicity of the neonicotinoid insecticides (Fig. 1) applied topically to worker honey bees in the laboratory (Table 1) may be classified into two groups based upon the presence of a nitro versus a cyano substitution (Fig. 1) and their corresponding toxicity to the honey bee. The most toxic neonicotinoids contained a nitro substitution and within this group, imidacloprid was the most toxic with an $LD_{50}$ of 17.9 ng/bee. Clothianidin and thiamethoxam demonstrated a similar level of toxicity to imidacloprid with $LD_{50}$ values of 21.8 and 29.9 ng/bee, respectively. Dinotefuran and nitenpyram were slightly less toxic with $LD_{50}$ values of 75.0 and 138 ng/bee, respectively.

Neonicotinoids with a cyano substitution were in some cases almost three orders of magnitude less toxic than those containing a nitro group (Table 1). The two

Table 1
Mortality 24 h after the topical application of neonicotinoid insecticides and acetamiprid metabolites to the dorsum of the honey bee thorax

| Insecticide or acetamiprid metabolites | $n$[a] | $LD_{50}$ (μg/bee)[b] | 95% CI[c] | Chi-square | Slope±SE |
|---|---|---|---|---|---|
| Acetamiprid | 465 | 7.07 | 4.57–11.2 | 0.826 | 1.77±0.105 |
| Imidacloprid | 137 | 0.0179 | 0.0092–0.0315 | 0.303 | 1.70±0.176 |
| Thiacloprid | 195 | 14.6 | 9.53–25.4 | 0.480 | 2.73±0.371 |
| Nitenpyram | 132 | 0.138 | 0.0717–0.259 | 1.17 | 1.77±0.105 |
| Clothianidin | 174 | 0.0218 | 0.0102–0.0465 | 2.22 | 2.60±0.259 |
| Dinotefuran | 133 | 0.0750 | 0.0628–0.0896 | 0.0704 | 2.28±0.076 |
| Thiamethoxam | 144 | 0.0299 | 0.0208–0.0429 | 0.1619 | 3.06±0.201 |
| IM-2-1 | 57 | >50 (0%) | — | — | — |
| IC-O | 50 | >50 (0%) | — | — | — |
| IM-O | 50 | >50 (0%) | — | — | — |

[a] Number of insects tested.
[b] Results were corrected for control mortality. Dose is given in micrograms of active ingredient. (0%) = percent mortality at 50 μg/bee.
[c] CI, confidence interval.

cyano-substituted compounds tested, acetamiprid and thiacloprid, had $LD_{50}$ values 395 and 816 times larger than that for imidacloprid. The $LD_{50}$ for acetamiprid was 7.1 μg/bee and for thiacloprid, 14.6 μg/bee (Table 1). Stark et al. (1995) and Suchail et al. (2000) previously reported $LD_{50}$ values for imidacloprid in the range of 6.7–23.8 ng/bee, Senn et al. (1998) estimated the $LD_{50}$ for thiamethoxam as 24 ng/bee, and Elbert et al. (2000) reported that the $LD_{50}$ for thiacloprid was 24.2 μg/bee. It is apparent that the nitro group in neonicotinoids produces high honey bee topical toxicity in the laboratory and that neonicotinoids like acetamiprid and thiacloprid with the cyano substitution have significantly lower bee activity.

### 3.2. Toxicity of acetamiprid metabolites

Tomizawa et al. (1995) previously reported that the binding affinity of acetamiprid for the nicotinic acetylcholine receptor from the honey bee head was in the same order of magnitude as that from the house fly. These results suggest that the reduced honey bee toxicity of the cyano-substituted neonicotinoids like acetamiprid cannot be explained by differences in the affinity of the insecticide for its target.

Another potential mechanism for the differential susceptibility of honey bees to neonicotinoids is differences in metabolism and/or the toxicity of metabolites. The major plant metabolites of acetamiprid are IM-2-1, IM-O and IC-O (Tokieda et al., 1997) (Fig. 2). Although we do not yet know if these metabolites are produced by honey bees, it is clear from the present study that they are not highly toxic when applied topically. At a dose of 50 μg/bee, no mortality was found above that of the control for bees treated with IM-2-1, IM-O and IC-O (Table 2). Since the metabolites of acetamiprid that were investigated were not toxic, any acetamiprid metabolism that would produce these products would be a detoxification mechanism for the honey bee.

### 3.3. Synergism of neonicotinoid toxicity

To determine the importance of metabolism in honey bee susceptibility to neonicotinoids, we measured acetamiprid toxicity for insects pretreated with different metabolic inhibitors and imidacloprid/thiacloprid toxicity pretreated with PBO. The synergists used in these studies are shown in Fig. 2. Based on a comparison of the estimated $LD_{50}$ values and their 95% confidence intervals, no significant increase in acetamiprid toxicity was noted for bees pretreated with 10 μg of DEF or DEM (Table 2). However, a statistically significant synergistic ratio of 2.96 (95% confidence interval of 1.83–4.76) was found for DEF. DEF and DEM are inhibitors of esterase and glutathione transferase activity, respectively. PBO, uniconazole-P (plant growth regulator) and especially the DMI-fungicides (triflumizole, propiconazole, triadimefon and epoxiconazole) had a much greater effect on acetamiprid toxicity than either DEF or DEM. The synergistic ratios ranged from 6.04 (95% confidence interval of 4.29–8.51) for PBO to 244 (95% confidence interval of 171–347) for triflumizole (Table 2). PBO, triflumizole and propiconazole had an even more dramatic effect on thiacloprid toxicity in the honey bee with an increase in toxicity of 154-, 1141- and 559-fold, respectively (Table 2). In contrast, the synergistic ratios for these same compounds for imidacloprid were 1.70, 1.85 and 1.52, respectively. No significant differences were found in the $LD_{50}$ values between imidacloprid alone and bees pretreated with PBO, triflumizole and propiconazole (based on comparison of the 95% confidence intervals) (Table 2).

The DMI-fungicides obtain their fungicidal activity by disrupting ergosterol biosynthesis via cytochrome P450 inhibition, and these compounds can also inhibit insect P450s (Brattsten et al., 1994). Colin and Belzunces (1992) and Pilling and Jepson (1993) found that DMI-fungicides synergized pyrethroid toxicity in the honey bee. Considering that both PBO, a well know inhibitor of insect P450s, and the DMI-fungicides, especially

Table 2
Pretreatment effect of general insecticide synergists, DMI-fungicides, and a plant growth regulator on honey bee toxicity of neonicotinoid insecticides

| Insecticide synergist[a] | $n$[b] | $LD_{50}$ (μg/bee)[c] | 95% CI[d] | Chi-square | Slope ± SE | SR[e] | 95% CI[c] |
|---|---|---|---|---|---|---|---|
| Acetamiprid | | | | | | | |
| Alone | 465 | 7.07 | 4.57–11.2 | 0.826 | 1.77 ± 0.105 | 1 | |
| PBO | 202 | 1.17 | 0.342–3.79 | 1.18 | 1.55 ± 0.181 | 6.04 | 4.29–8.51 |
| DEF | 124 | 2.39 | 0.278–12.4 | 5.85 | 2.96 ± 0.736 | 2.96 | 1.83–4.76 |
| DEM | 123 | 6.94 | 4.10–13.2 | 0.278 | 1.46 ± 0.140 | 1.02 | 0.783–1.33 |
| Triflumizole | 215 | 0.0290 | 0.0080–0.102 | 3.46 | 1.91 ± 0.240 | 244 | 171–347 |
| Propiconazole | 201 | 0.0675 | 0.0231–0.197 | 2.63 | 2.30 ± 0.242 | 105 | 76.7–143 |
| Triadimefon | 131 | 0.0844 | 0.0431–0.176 | 0.693 | 2.05 ± 0.198 | 83.8 | 64.2–110 |
| Epoxiconazole | 156 | 0.500 | 0.156–1.66 | 4.42 | 2.74 ± 0.404 | 14.1 | 10.0–20.0 |
| Uniconazole-P | 156 | 1.12 | 0.270–4.96 | 3.66 | 2.05 ± 0.349 | 6.31 | 4.22–9.45 |
| Imidacloprid | | | | | | | |
| Alone | 137 | 0.0179 | 0.0092–0.0315 | 0.303 | 1.70 ± 0.176 | 1 | |
| PBO | 152 | 0.0105 | 0.0061–0.0172 | 0.0889 | 1.66 ± 0.112 | 1.70 | 1.29–2.26 |
| Triflumizole | 125 | 0.0097 | 0.0052–0.0168 | 0.694 | 2.76 ± 0.284 | 1.85 | 1.67–3.09 |
| Propiconazole | 145 | 0.0118 | 0.0038–0.0303 | 1.01 | 2.12 ± 0.272 | 1.52 | 1.04–2.24 |
| Thiacloprid | | | | | | | |
| Alone | 158 | 14.6 | 9.53–25.4 | 0.480 | 2.73 ± 0.371 | 1 | |
| PBO | 193 | 0.0948 | 0.0406–0.211 | 0.424 | 1.64 ± 0.134 | 154 | 115–207 |
| Triflumizole | 160 | 0.0128 | 0.0031–0.0415 | 1.66 | 2.32 ± 0.363 | 1141 | 752–1740 |
| Propiconazole | 159 | 0.0261 | 0.0083–0.0690 | 1.05 | 2.27 ± 0.298 | 559 | 388–811 |

[a] In all, 10 μg of synergist was applied to the dorsal thorax of each worker honey bee 1 h prior to insecticide application.
[b] Number of insects tested.
[c] Results were corrected for control mortality. Dose is given in micrograms of active ingredient.
[d] CI, confidence interval.
[e] SR, synergism ratio (the $LD_{50}$ of insecticide alone/$LD_{50}$ of synergist and the insecticide).

triflumizole, produced the greatest synergistic effect among the metabolic inhibitors tested for acetamiprid and thiacloprid (Table 2), it appears that oxidation is an important neonicotinoid detoxification pathway in the honey bee for the cyano-substituted neonicotinoids. This is also in agreement with the results reported earlier (Table 1) where the metabolites of acetamiprid (IM-2-1, IM-O and IC-O, Fig. 1), which are potential products of P450s, were non-toxic to the honey bee when applied topically (Table 1). Esterases and glutathione transferases appear to be less important in detoxification.

Suchail et al. (2001) found that the metabolites of imidacloprid in the honey bee were a hydroxy derivative at the 5′ position and an olefin derivative in the imidazolin ring. The olefin has higher not lower insecticidal activity than the parent (Nauen et al., 1998). In the house fly, PBO increased imidacloprid toxicity 10.7-fold (Liu et al., 1995) while *O*-propyl-*O*-(2-propynyl) phenylphosphate (PPP) increased both imidacloprid and acetamiprid toxicity (Yamamoto et al., 1998). These findings suggest that metabolism and detoxification pathways may vary between insect species which can affect insect susceptibility to neonicotinoids. P450 inhibitors produced only a minimal increase in imidacloprid activity in the honey bee in our studies (Table 2), indicating that this was not an important detoxification pathway.

### 3.4. Triflumizole acetamiprid toxicity to honey bees when applied to alfalfa

The DMI-fungicides are an important group of fungicides widely used in crop protection. Therefore, the fact that compounds like triflumizole can increase toxicity of the cyano-substituted neonicotinoids like acetamiprid against the honey bee, as much as 244-fold (Table 2), is of some concern because of potential non-target effects when these compounds are used in combination. Colin and Belzunces (1992) and Pilling and Jepson (1993) found that the DMI-fungicides synergized pyrethroids at practical field rates. Triflumizole in our laboratory studies synergized thiacloprid activity in the honey bee 1141-fold (Table 2).

To evaluate the field implication of our laboratory studies, acetamiprid alone and acetamiprid and triflumizole in combination were applied at the maximum recommended field rates of 168.1 g/hec for acetamiprid and 280.2 g/hec for triflumizole on alfalfa. Acetamiprid and triflumizole in combination was sprayed as a tank mix. At 3 and 24 h after application, the plants were harvested and honey bees exposed to the treated plants in cages in the laboratory. At 3 h after application, average mortality for the treated plants was 4%, and this was not significantly different by a *t*-test from that obtained for acetamiprid alone or the non-treated control. At 24 h after treatment, the average mortality

with the tank mix was 2%, and again this was not significantly different from the acetamiprid treatment or control plots. These results suggest that despite the high synergism ratios observed in the laboratory between acetamiprid and triflumizole, no practical adverse effect occurs against the honey bee when exposed to treated plants at the maximum recommended treatment levels. More studies will be needed in the future in order to further validate this conclusion. Schmuck et al. (2003) also found at recommended use rates that thiacloprid poses a negligible lethal risk to honey bees when applied either alone or in tank mixes with fungicides from different chemical classes.

## 4. Summary

The current study found that the cyano-substituted neonicotinoids were less toxic to honey bees by more than two orders of magnitude as compared to the nitro substitution in laboratory studies where the insecticides were topically applied. This reduced toxicity appears to be the result of increased metabolism by P450s and the fact that the metabolites have low bee toxicity. The DMI-fungicides in laboratory studies greatly increased the bee toxicity of acetamiprid and thiacloprid by as much as 1141-fold. However, in cage studies where acetamiprid and triflumizole were applied in combination to alfalfa at the maximum recommended rate, no bee mortality was detected, suggesting that certainly acetamiprid alone and even acetamiprid in combination with a potent P450 inhibitor, is safe to honey bees. More research is needed to further validate this conclusion.

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