Apidologie (1997) 28, 123–127

Original article

The effect of Apistan® on honey bee (*Apis mellifera* L). Responses to methyl parathion, carbaryl and bifenthrin exposure

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(Received 5 March 1997; accepted 13 May 1997)

Summary — Honey bees treated with Apistan Queen Tabs® exhibited greater susceptibility to bifenthrin than untreated bees in laboratory bioassays. Bifenthrin was 1.9 times more toxic to bees that were caged with Apistan® Queen Tabs than to bees held in cages without Apistan®. The toxicity of carbaryl and methyl parathion was not significantly affected by Apistan® treatment. The possibility that honey bee colonies being treated with Apistan® are more susceptible to injury by bifenthrin is supported by data obtained in this study. However, susceptibility of colonies to carbaryl or methyl parathion does not appear to be affected by prior Apistan® exposure.

honey bee / synergism / fluvalinate / insecticide / toxicity

INTRODUCTION

In 1987, the varroa mite (*Varroa jacobsoni* Oudemans) was detected in the United States (anonymous, 1987). Subsequently, varroa spread to most regions of the US, and many beekeepers now routinely treat their colonies with a miticide to protect them from varroa injury (Mussen, 1993). At present, the only miticide registered and available in the US to protect colonies from varroa is fluvalinate (Apistan®).

In addition to Apistan® treatments applied within the hive, bees can be exposed to pesticides if they forage on treated crops. If synergism exists between Apistan® and pesticides applied to crops, colonies being treated for varroa may be more vulnerable to pesticide injury than untreated colonies.

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There have been few studies of potential interactions when honey bees are exposed to more than one pesticide. Chaney (1988) observed no synergism when permethrin and fluvalinate were mixed independently with carbaryl and fed to adult bees. However, Belzunces et al (1993) found that pyrethroids in low concentrations can increase the toxicity of amitrole (Azole®) fungicides which are normally harmless to honey bees. Pilling et al (1995) also reported that ergosterol biosynthesis inhibiting fungicides can synergize the toxicity of pyrethroid insecticides to the honey bee. They suggested that their observations may result from the pyrethroid blocking the bee's detoxification mechanism for the fungicide. Immaraju et al (1990) found that sesame oil synergized fluvalinate toxicity to citrus thrips (Thysanoptera: Thripidae) up to 48 fold at the LC_{90}, suggesting the possible use of this product in the field to enhance toxicity. Most combinations of miticides used to control varroa and other pesticides have not been tested for synergistic effects (de Ruijter, 1994).

Inadequate pollen intake and varroa infestation have also been shown to influence the susceptibility of honey bees to chemical injury. Wahl and Ulm (1983) demonstrated reduced tolerance to pesticides in poorly nourished bees with lowered protein reserves. Drescher and Schneider (1988) reported a higher mortality of varroa-parasitized bees when exposed to endosulfan (Thiodan®). They also demonstrated that the negative effect of Thiodan® was enhanced by the synergism of coumafos (Perizin®).

The arrival of varroa in Nebraska, and a concomitant increase in beekeeper reports of pesticide injury prompted the following studies. This research was conducted to determine if synergism between pesticides used on crops and in beehives could be a contributing factor to honey bee losses.

MATERIALS AND METHODS

Adult workers were collected from the brood nest of a single colony of New World Carniolan honey bees in September 1995. Ten bees were placed in each of 72 Benton mailing cages for each of three bioassays. Apistan Queen Tabs® were placed in 36 cages and remained in position throughout the experiment. The remaining 36 cages did not receive Apistan Queen Tabs®. All caged bees were held without light at 20 °C for 48 h prior to conducting bioassays. Bees were provided with water twice daily throughout the experiment by brushing the cage screens with water.

Serial dilutions of bifenthrin, carbaryl and methyl parathion in acetone were applied to individual bees using a Hamilton microsyringe and repeating dispenser. The three insecticides tested represent three different insecticide classes, pyrethroid, carbamate, and an organophosphate, respectively. Bees were anesthetized with CO_2, and 0.5 µL of the test solutions was applied to their abdomens. Acetone was used as a solvent and controls treated with acetone were included in each bioassay. Six replications of ten bees were examined for each dose of both bees treated with Apistan® and untreated bees. After preliminary range-finding bioassays, six dosage levels were selected that provided mortalities ranging from zero to 100 percent. Bees were held in an incubator at 20 °C in darkness except for brief periods when water was administered. Mortality was evaluated 24 h after treatment.

Data were analyzed by Probit analysis using POLO (LeOra Software, 1991). LD_{50}'s with 95% confidence intervals that did not overlap were considered significantly different (LeOra Software, 1991). All synergistic ratios calculated in this study were significant at the 95% probability level (g statistics generated were less than 0.5). However, synergistic ratios were only meaningful if the LD_{50}'s being compared were significantly different.

RESULTS

Honey bees treated with Apistan® exhibited significantly greater susceptibility to bifenthrin than untreated bees in laboratory bioassays (table I). Significant differences in methyl parathion and carbaryl toxicity

Table I. Responses of bees treated with Apistan® and untreated bees to three commonly used insecticides.

Compound	n	*Slope*	LD_{50} *(CL)*[a]	*Synergistic Ratio*
Bifenthrin	360	3907 ± 0.332	0.034 (0.023-0.058)	
Bifenthrin + Apistan®	360	5434 ± 0.564	0.018 (0.016-0.020)	1.9*
Carbaryl	360	2828 ± 0.293	0.232 (0.190-0.279)	
Carbaryl + Apistan®	360	4384 ± 0.453	0.175 (0.137-0.209)	1.4
Methyl Parathion	360	10415 ± 1.187	0.041 (0.037-0.044)	
Methyl Parathion + Apistan®	360	11449 ± 1.695	0.039 (0.033-0.044)	1.1

[a] µg/µL. * All synergistic ratios calculated in this study were significant at the 95% probability level (g statistics generated were less than 0.5). However, synergistic ratios were only meaningful if the LD_{50}'s being compared were significantly different.

were not observed in similar tests. Bifenthrin was 1.9 times more toxic to bees that were caged with Apistan Queen Tabs® than to bees held in cages without Apistan®. The calculated LD_{50}'s for carbaryl and methyl parathion were 1.4 and 1.1 times higher for untreated bees, respectively, however, the differences in relative toxicity were not significant.

DISCUSSION

Data from this study support the hypothesis that honey bees being treated with Apistan® are more susceptible to injury by bifenthrin. Data reported in this study do not support the hypothesis that Apistan® treatment affects the susceptibility of colonies to carbaryl or methyl parathion injury. However, the most important result is that Apistan® did not have a large effect on toxicity for any of the three compounds tested. Even though the effect of bifenthrin was significant, the difference was small, and it would be difficult to extrapolate to field conditions. Nevertheless, as a precaution, beekeepers should avoid treating colonies with Apistan® strips at times of the year when bees are likely to forage on bifenthrin-treated crops. Bifenthrin and fluvalinate are both pyrethroids and have similar modes of action.

Furthermore, since Drescher and Schneider (1988) have demonstrated that varroa infestation can result in a lower threshold for pesticide injury, beekeepers should maintain varroa populations at low levels as well as avoiding Apistan® treatment during times when bees are likely to forage on pesticide-treated crops. During the spring in Midwestern agriculture, Apistan® treatments can be made with little risk of concomitant bee exposure to crop pest control chemicals. Spring treatments are also compatible with beekeeping management practices and with beekeeper's need to have low mite populations (Ellis and Baxendale, 1996). Beekeepers relying on late-summer applications of Apistan® can have both high mite levels and the presence of a pesticide in the hive (Apistan®) during the main crop pests control period.

ACKNOWLEDGMENTS

The authors thank Elf Atochem for their support of this study.

Résumé — Action de l'Apistan® sur les réactions des abeilles (*Apis mellifera* L) exposées au parathion-méthyl, au carbaryl et à la bifenthrine. Cette étude visait à déterminer si le traitement à l'Apistan® augmentait la vulnérabilité des abeilles à trois insecticides, utilisés couramment en protection des cultures. Les tests biologiques ont été faits sur 720 abeilles adultes prélevées dans une même colonie pour chacun des trois insecticides étudiés. Les abeilles ont été placées dans des cages d'expédition de Benton à raison de dix abeilles par cage. Les cages ont été divisées en deux groupes: une moitié a reçu les inserts Apistan Queen Tabs®, l'autre n'a pas été traitée. Des dilutions en série de bifenthrine (pyréthrinoïde), de carbaryl (carbamate) et de parathion-méthyl (organophosphoré) ont été administrées aux abeilles avec une micro-seringue Hamilton, à raison de de 0,5 µL par abeille. L'acétone a été utilisée comme solvant et des témoins traités à l'acétone ont été inclus dans chaque test. Six répétitions de dix abeilles ont été faites pour chacune des doses d'insecticide pour le lot traité à l'Apistan® et autant pour le lot non traité. La mortalité a été évaluée 24 h après le traitement, les données ont été analysées par la méthode Probit à l'aide du logiciel POLO. Les DL_{50} avec des intervalles de confiance qui ne se chevauchent pas ont été considérées comme significativement différentes. Tous les taux de synergie ont été significatifs au seuil de probabilité de 95 %; néanmoins, ils n'avaient de valeur que si les DL_{50} comparées étaient significativement différentes. Les abeilles traitées à l'Apistan® ont montré une sensibilité plus grande à la bifenthrine que les non traitées (tableau I). Mais la sensibilité des colonies au carbaryl et au parathion-méthyl ne semble pas être affectée par une exposition préalable à l'Apistan®. La bifenthrine a été 1,9 fois plus toxique pour les abeilles traitées que pour les non traitées. Les DL_{50} calculées pour le carbaryl et le parathion-méthyl sont respectivement 1,4 et 1,1 fois plus élevées pour les abeilles non traitées, mais les différences ne sont pas significatives. Le résultat le plus intéressant est que l'Apistan® n'a eu d'action importante sur la toxicité d'aucun des trois insecticides testés. Même si l'effet concernant la bifenthrine est significatif, la différence est faible et il est difficile d'extrapoler aux conditions de plein champ. Néanmoins, par précaution, les apiculteurs devraient éviter de traiter leurs colonies à l'Apistan® pendant les périodes où les abeilles sont susceptibles de butiner des cultures traitées à la bifenthrine.

***Apis mellifera* / fluvalinate / synergie / insecticide / toxicité**

Zusammenfassung — Auswirkung von Apistan® auf toxische Wirkungen der Pflanzenschutzmittel Methylparathion, Carbaryl und Bifenthrin auf Honigbienen. Honigbienen, die mit Apistan® Queen Tabs behandelt wurden, zeigten in Laborversuchen gegenüber Bifenthrin eine größere Empfindlichkeit als unbehandelte Bienen (Tabelle I). Die Toxizität von Carbaryl und Methylparathion wurde durch die Apistanbehandlung nicht signifikant beeinflußt. Nach den Ergebnissen dieser Untersuchung ist es wahrscheinlich, daß mit Apistan® behandelte Bienenvölker leichter durch Bifenthrin geschädigt werden können. Dagegen scheint die Empfindlichket der Völker auf Carbaryl oder Methylparathion nicht durch eine vorherige Apistanbehandlung beinflußt zu sein. Bifenthrin war für mit Apistan® Queen Tabs gekäfigte Bienen 1,9 mal mehr toxisch als für Bienen, die in Käfigen ohne Apistan® gehalten wurden. Die berechneten LD_{50} Werte für Carbaryl und Methylparathion waren bei unbehandelten

Bienen 1,4 bzw 1,1 mal höher, diese Unterschiede waren jedoch nicht signifikant. Das wichtigste Ergebnis ist jedoch, daß die Wirkung von Apistan® auf die Toxizität bei den 3 getesteten Substanzen nur gering war. Auch wenn der Effekt von Bifenthren signifikant war, war die Differenz klein und es würde schwierig werden, sie auf Feldbedingungen zu übertragen. Trotzdem sollten Imker aus Vorsorge vermeiden, Völker zu den Zeiten mit Apistanstreifen zu behandeln, zu denen sie möglicherweise auf mit Bifenthrin behandelte Pflanzungen fliegen. Bei der Landwirtschaft des Mittelwestens der USA kann die Apistanbehandlung der Bienen im Frühling mit nur geringem Risiko der gleichzeitigen Exposition durch der Pflanzenschutzmittel durchgeführt werden. Behandlungen im Frühling sind mit der Imkerpraxis und mit dem Anspruch der Imker auf eine geringe Milbenpopulation gut zu vereinbaren (Ellis und Baxendale 1996). Imker, die sich auf die Spätsommerbehandlung mit Apistan® verlassen, riskieren zusätzlich zu einem hohen Milbenbefall die Belastung durch ein Pestizid (Apistan®) im Stock während der Hauptzeit der Pflanzenschutzmaßnahmen.

Honigbiene / Apistan® / Synergie (Zusammenwirken) / Fluvalinat

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