

**Zeitschrift:** Mitteilungen der Schweizerischen Entomologischen Gesellschaft = Bulletin de la Société Entomologique Suisse = Journal of the Swiss Entomological Society

**Herausgeber:** Schweizerische Entomologische Gesellschaft

**Band:** 75 (2002)

**Heft:** 3-4

**Artikel:** The safe use of thiamethoxam by drench or drip irrigation in glasshouse crops where bumble bees *Bombus terrestris* (L.) are released

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**DOI:** <https://doi.org/10.5169/seals-402833>

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## The safe use of thiamethoxam by drench or drip irrigation in glass-house crops where bumble bees *Bombus terrestris* (L.) are released

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The side effects of the thianicotinyl compound thiamethoxam on bumble bees (*Bombus terrestris* [L.]) were investigated in the laboratory, under semi-field conditions and in plastic tunnels. The feeding of contaminated sugar solution or exposure to dried residues on glassplates caused 100 % mortality of bumble bee adults at application rates between 10 and 100 g ai/ha. Dried residues on tomato or *Phacelia acetifolia* plants slightly decreased the mortality, and further reductions were obtained by allowing the deposit to age from 2 up to 14 days before exposure to bumble bees. A decisive improvement was obtained by replacing the foliar by drench or drip irrigation of thiamethoxam at rates between 100 and 172 g ai/ha. The mortality figures of all bumble bee stages dropped to the level in the untreated control. The flight activity monitored at the entrance of bumble bee hives was normal and so was the pollination activity. Thiamethoxam could be rated as being harmless to bumble bees with a single application through the irrigation system or by drench application. No spilling of this compound during the application should occur to avoid intoxication of adult bumble bees by oral uptake or contact contamination. If multiple application via the irrigation system is considered, further sequential testing of this mode of use is recommended.

Keywords: *Bombus terrestris*, thiamethoxam, tomatoes, *Phacelia acetifolia*, integrated pest management, side effects

### INTRODUCTION

The use of bumble bees (*Bombus terrestris* [LINNAEUS, 1758]) for pollination is a common practice nowadays in some 25 different covered crops world-wide (GRIFFITHS & ROBERTS 1996). Tomatoes are by far the most important crop where bumble bees are used for pollination (VAN HEEMERT et al. 1990; RAVENSTIJN & VAN DER SANDE 1991). Since bumble bees are potentially exposed to pesticides, specific data on the effect on bumble bees are required. So far data from the other important pollinator species, the honey bee (*Apis mellifera* LINNAEUS, 1758) cannot be extrapolated to cover also the risk of pesticides to bumble bees (LEWIS et al. 1998). There are two main routes of exposure under practical conditions: direct overspraying of adult bumble bees foraging on the glasshouse crop, and contamination via the uptake of treated pollen and nectar from the treated plants. An additional intoxication might occur by feeding bumble bee larvae with treated pollen or nectar.

Thiamethoxam belongs to the subclass of thianicotinyl compounds within the chemical group of neonicotinoids. It is highly active against aphids, whiteflies and some beetles (SENN et al. 1998; MAIENFISCH et al. 1998). It displays root-, leaf- and stem-systemic activity. In target insects it shows quick stomach and contact action.

Testing a compound at the maximum recommended application rate and exposing the test organism to the fresh, dried residues in the laboratory can be consi-

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dered as a worst-case scenario. This severe test condition with a maximized contact serves rather to define the possible modes of action and inherent risks of a pesticide. Nontoxicity at this level reduces the amount of further testing if not at all (HASSAN *et al.* 1992).

Where a compound has been found to be toxic at the initial level, additional testing becomes necessary in which bumble bees are exposed to pesticide residues on plants instead of inert materials. Exposure to pesticides deposits that have experienced various periods of aging provide additional information that can be useful in assessing the likely risk under field conditions.

The exposure on aged pesticide deposits allows a number of specific management options, such as the possibility of an evening application when the residual activity is very short or, when it is longer, determining a quarantine. These options are available because bumble bee hives can be closed or removed temporarily from the glasshouse area. However, removing hives for more than 2 days would not be accepted by the growers. In case of such undesirable effects of a pesticide detected on the laboratory level, semi-field testing may be conducted, in which free-flying colonies of bumble bees are confined to plots of crop in mesh-covered cages or tents. Field tests incorporate the more realistic conditions of exposure to treated crops in sizeable greenhouses.

A scheme of sequential testing procedures using laboratory, semi-field and field selectivity testing methods has been developed with the aim of evaluating all potential risks of pesticides to beneficial arthropods (HASSAN 1992). Experience with other, non-pollinating beneficial predators and parasitoids have demonstrated that laboratory toxicity data will not always be a sufficient indicator of field hazard. Factors like the application rate, the mode of application, the interval between application and the time of release of the bumble bees, the behaviour of the chemical and the vitality of the bumble bees themselves can influence their survival too. Therefore a modified approach of sequential testing has been adopted in this study of bumble bees (SECHSER & REBER 1998).

## MATERIALS AND METHODS

Eighteen separate experiments were conducted from 1994 to 1998 in the laboratory, tents and plastic tunnels. All laboratory and tent tests were carried out in the laboratories and testing facilities of the Syngenta Crop Protection AG, Basel and the tunnel tests by the Federal Agricultural Research Station, Changins, Switzerland, in collaboration with the Centre Horticole in Lullier.

Thiamethoxam (ACTARA® WG 25) was used at rates between 10 and 100 g ai/ha as a foliar spray, and between 100 and 172 g ai/ha in a drench or drip application. Imidacloprid (CONFIDOR® LS 200) was applied at 80 and 100 g ai/ha as a foliar spray and 150 g ai/ha in a drench application as a standard pesticide in tent tests. Dimethoate (PERFEKTHION® EC 400) was used as a toxic standard at 80 g ai/ha as a foliar spray.

Statistics: The effect of the treatments on the bumble bee populations was expressed as percentage reduction/increase using Abbott's formula (ABBOTT 1925). Differences of mortality between treatments in the laboratory and tent tests were identified using a one way analysis of variance (ANOVA) assuming equal variances between two samples. Treatment means were separated by the least significant difference ( $P < 0.05$ ). Figures with the same letter are not significantly different.

## 1. Laboratory studies

### 1.1 Contact activity on glassplates

Thiamethoxam was tested at the equivalent of 10 g ai/ha (corresponding to 50 ppm; application at 200 l H<sub>2</sub>O / ha) by spraying it on glassplates of 45 x 30 cm and exposing adult bumble bee workers, which were confined within a plexiglass cylinder placed on the glassplate (SECHSER & REBER 1998). Dimethoate was included as a positive control at 80 g ai/ha. Five adults were placed within each cylinder and there were four replicates for each product. The evaluation was done 7 days after permanent exposure. This severe test is the first step in the sequential scheme according to the IOBC (HASSAN 1992).

In three trial series testing the residual activity adult bumble bee workers were exposed the same way as before four days after applications. The final mortality ratings were done 10 days after the beginning of the exposure.

### 1.2 Oral activity

Thiamethoxam was offered at the equivalent of 10 g ai/ha in 2 ml of a 70 % sugar solution (meaning 50 ppm of the 2 ml sugar solution; application at 200 l H<sub>2</sub>O / ha) which had been put into small recipients on a glassplate for feeding adult bumble bee workers. They were confined within a plexiglass cylinder placed on the glassplate. Dimethoate was included as a positive control at 80 g ai/ha. Five adults were placed within each cylinder and there were four replicates (cylinders) for each product. The food was not renewed during the testing period. The mortality was evaluated 7 days after application (DAA).

### 1.3 Contact and residual activity on tomatoes

Adult bumble bee workers were exposed on tomato plants, of which each plant had been placed inside a cage (1 x 0.8 x 0.8 m). The plants (cultivar Trust, 50 cm high) were sprayed with the equivalent of 40 g ai/ha of thiamethoxam and 800 l/ha and allowed to dry. This is four times the maximum recommended rate for foliar applications on tomatoes. In case of positive results this would mean a high safety margin for bumble bees. Each treatment had four replicates with ten adult workers for each. The bees were fed with untreated honey solution in small recipients. Temperatures were 22°C and the light:dark ratio was 16:8 h. The assessment was done 14 DAA. Water treated plants served as negative control. In the thiamethoxam treatment another series of 4 x 10 bumble bee workers were released in the cages 2 DAA, simulating a realistic residual activity (i.e. maximum accepted waiting period of glasshouse growers to reintroduce bee hives following an insecticide application). The assessment was done 12 days later.

## 2. Tent studies

### 2.1 Contact and residual activity on *Phacelia acetifolia* following foliar application

*P. acetifolia* is a very attractive plant for honey and bumble bees and was selected in the testing procedure as a severe test in order to guarantee the contact

between bumble bees and a treated plant surface. One bumble bee hive was exposed in tents of 3 x 3 x 3 m in which nine potted *P. acetifolia* plants had been placed and sprayed with thiamethoxam at 40 g ai/ha prior to the release of the bumble bees. The placement of one hive per tent was done immediately after the drying of the spray deposit and repeated with new sets of hives one week later. Imidacloprid at 80 g ai/ha was included as a positive control and untreated plants as a negative control. Each treatment was replicated three times.

The following parameters of relevant stages (adult workers, queens) were evaluated at intervals up to three weeks after application in the first series or up to two weeks after the second release, respectively: Dead workers in the tents; dead workers and queens in the hives; alive workers and queens in the hives. Immature stages were ignored for the purpose of this study.

## 2.2 Contact and residual activity on tomatoes following foliar application

In a first series thiamethoxam was applied at the rate of 100 g ai/ha as a foliar spray and compared to a water control and imidacloprid at 100 g ai/ha. Sixteen potted tomato plants (cultivar Trust, 40 cm high) were placed into a tent (3 x 3 x 3 m) and each treatment was replicated three times. One colony was placed in a tent immediately after application and another new colony after two weeks in a new tent on 14 days old thiamethoxam deposits. The whole observation period was 28 days starting from the exposure of the first series of colonies, at the end of which all colonies were analyzed for dead and alive stages.

In a second series nine potted tomato plants (cultivar Trust, 40 cm high) were sprayed with thiamethoxam (40 g ai/ha) 14, 7, 2 and 1 days before the exposure date, and nine plants for the water control. A series of nine plants were placed each into one tent (3 x 3 x 3 m) at the same date, i.e. the deposits were 14, 7, 2 and 1 days old. One bumble bee colony was placed in each tent. Each treatment and rate was replicated three times. Dead bumble bees were collected daily within the tents, and all hives were checked for alive adult stages 24 days after the start of exposure.

## 2.3 Single drench application on tomatoes

Thiamethoxam was applied at 150 g ai/ha as a drench and compared to a water control and imidacloprid at 150 g ai/ha. Sixteen potted tomato plants (cultivar Trust, size 40 cm high) were placed into a tent (3 x 3 x 3 m) and each treatment was replicated three times. The irrigation system was stopped the preceding night of the application to put the plants under stress thus guaranteeing a good absorption of the products. For the application 1 liter of a previously prepared stock solution was poured into each pot. The water holding capacity had been defined in preceding trials. Two days after application, the regular irrigation system was turned on again. One bumble bee hive was placed in a tent immediately after application. The whole observation period was 28 days, at the end of which all three colonies were analyzed for dead and alive stages. The following additional evaluation parameters were used during the study: Pollination activity (shown by the marking of stamen of flowers) 0, 1, 2, 4, 7, 10, 14, 18, 21, 25 and 28 DAA and mortality assessment by collecting dead bumble bees in the tent at the same intervals.

### 3. Plastic tunnel studies

#### 3.1 First trial by a single application on tomatoes via the irrigation system

Thiamethoxam was applied via the irrigation system on tomato plants (cultivar DRW 3759, 50 cm height at the time of application) in a plastic tunnel (450 m<sup>2</sup>). 31 g WP 25 (7.75 g ai) was dissolved in a 5 l stock solution and added continuously to a mixing tank of 20 l, of which the content was renewed 5 x during the irrigation process (the 7.75 g ai applied on 450 m<sup>2</sup> correspond to 172 g ai/ha). The application was done 26 days after the installation of the colony and thereafter bumble bees were monitored for 28 days. An untreated tunnel in the neighbourhood with the same cultivar but slightly delayed planting date served as negative control.

The following parameters were evaluated during the growing season:

**a) Activity at the entrance of the hive.**

The number of bumble bees entering and leaving the hive in the treated tunnel was counted with an electronic device (BumbleSCAN from Lowland Electronics bvba, Belgium) over the whole 56 days observation period in the treated tunnel. The arrivals and departures were monitored at regular intervals for a total of 30 minutes per day. There was no second device available to monitor the untreated tunnel.

**b) Pollination activity.**

The pollination activity was monitored by visual inspections at the trial sites. When bumble bees land on tomato flowers for collecting pollen, they slightly injure the stamen. These spots turn brown by oxidation, indicating that the flower has been visited. Five times five double-branched tomato plants were marked in each tunnel and the following parameters were evaluated: Closed buds; open, unmarked flowers; open, marked (pollinated) flowers; number of cymes having one of these categories. Seven evaluations were done (-22, -15, -8, -2, +5, +13, +20 DAA).

**c) Proportion of tomato and other plant pollen collected by bumble bees.**

Pollen balls transported by five workers in each hive were removed from them at the moment of entering the hives at -8, -2, +5 and +20 DAA and analyzed, i.e. a total of 40 bumble bee specimens were examined in the two hives.

**d) Vitality of bumble bees.**

Tunnels were searched regularly for dead and paralyzed bumble bees. The hives were opened 28 days after application and analyzed for alive and dead stages. The food reserves were also evaluated.

#### 3.2 Second trial by a single application on tomatoes via the irrigation system

This trial was done in the follow up season (1998) of trial 3.1 and the outline corresponds largely to trial 3.1. Deviations in the outline are especially mentioned. Thiamethoxam was applied via the irrigation system on tomato plants (cultivar DRW 3759, 40 cm high, grown on stone wool) in a plastic tunnel (450 m<sup>2</sup>). A non-insecticidal tunnel of the same size in the neighbourhood served as negative control. 29 g WP 25 (7.25 g ai) was dissolved in a 5 l stock solution and added continuously to a mixing tank of 20 l, of which the content was renewed permanently during the irrigation process of half an hour (the 7.25 g ai applied on 450 m<sup>2</sup>



correspond to 161.1 g ai/ha). The rate is slightly different from the preceding one because exact dosaging was difficult with the machinery. The application was done 27 days after the installation of the colony and thereafter bumble bees were monitored for 35 days (36 days in the untreated control).

The following parameters were evaluated during the growing season:

**a) Activity at the entrance of the hive**

The number of bumble bees entering and leaving each hive in the treated and untreated tunnel was counted with an electronic device (BumbleSCAN) with two separate tubes for arrivals and departures. The monitoring was carried out from -17 to +29 DAA.

**b) Pollination activity**

Idem item 3.1, but in addition the fruits and the total number of cymes were counted. Evaluations were done at -18, -11, -8, -4, +4, +10, +17 and +24 DAA.

**c) Proportion of tomato and other pollen**

Idem item 3.1 but pollen balls were sampled from 10 workers in each treatment for analysis at -11, -4, +4 and +17 DAA, except at -11 DAA, when only from a total of 8 workers samples could be taken.

## RESULTS

### *4. Laboratory studies*

#### 4.1 Contact activity on glassplates

Thiamethoxam was harmful to adult bumble bees at the tested rate of 10 g ai/ha as was the standard dimethoate at 80 g ai/ha. Both caused a mortality of 100 % (Tab. 1). Also in the residual activity test on four day old deposits, all bumble bee workers in the thiamethoxam treatments were dead in the final rating.

#### 4.2 Oral activity on glassplates

Thiamethoxam (10 g ai/ha) and dimethoate (80 g ai/ha) caused 100 % mortality at the tested rates (Tab. 1).

#### 4.3. Contact and residualt activity on tomatoes

The toxicity of thiamethoxam at 40 g ai/ha was 95 % and therefore only slightly better than in the contact and oral tests on glassplates (Tab. 1). A clear improvement was obtained by a retarded release of bumble bees two days after the foliar application of thiamethoxam. The mortality obtained 12 days after the exposure of bumble bees was 67.5 %, which is, however, still too high for commercial operations.

Tab. 1. Effect of thiamethoxam on bumble bees in laboratory and tent tests.

Ref no	Type of test	Thiamethoxam			Standard			Control		
	Tests on	Exposure DAA <sup>1</sup>	Evaluation DAE <sup>2</sup>	g ai/ha	Mortality %	Abbott %	g ai/ha of standard	Mortality %	Abbott %	Mortality %
	glassplates (G)									
	tomatoes (T)									
	Phacelia (P)									
Laboratory studies										
4.1	Contact activity (G)	0	7	10	100 b	100	80	DIMT <sup>3</sup>	100 b	100 0 a
	Residual activity (G)	4	10	10	100 b	100				3 a
4.2	Oral activity (G)	0	7	10	100 b	100	80	DIMT <sup>3</sup>	100 b	100 0 a
4.3	Contact activity (T)	0	14	40	95 b	94				20 a
	Residual activity (T)	2	12	40	68 b	63				20 a
Tent studies										
5.1	Contact activity (P)	0	21	40	92 b	78	80	IMID <sup>4</sup>	97 b	91 55 a
	Residual activity (P)	7	14	40	68 b	31	80	IMID <sup>4</sup>	67 b	24 35 a
5.2.1	Contact activity (T)	0	28	100	93 b	79	100	IMID <sup>4</sup>	86 b	64 58 a
	Residual activity (T)	14	14	100	94 b	85	100	IMID <sup>4</sup>	90 b	80 45 a
5.2.2	Residual activity (T)	14	24	40	78 b	56				50 a
	Residual activity (T)	7	24	40	88 b	75				50 a
	Residual activity (T)	2	24	40	80 b	59				50 a
	Residual activity (T)	1	24	40	79 b	58				50 a
5.3	Drench application (T)	0	28	150	50 a	- 85	150	IMID <sup>4</sup>	49 a	- 59 58 a
1	DAA = days after application									
2	DAE = days after exposure									
3	DIMT = dimethoate									
4	IMID = imidacloprid									

## 5. Tent studies

### 5.1 Contact and residual activity on *P. acetifolia* following foliar application

The impact of thiamethoxam was evaluated by the counts of alive and dead specimens of bumble bee workers and queens found in the hives at the end of the trial and of dead specimens found on the floor of the tents and in the hives. The



average mortality of adult stages was 92 % at the end of the three weeks observation period following the first exposure immediately on dried spray deposits of thiamethoxam (Tab. 1). The mortality was 68 % in the second series where bumble bees had been released one week after the spray. The mortality figure in the untreated control in the foliar contact test (55 %) was high probably due to a shortage of available flowering *P. acetifolia* plants during the third week of the whole experiment. Also parts of the bumble bee hive population start to die in the third week. The corrected mortality figures according to ABBOTT were 78 % for bumble bees exposed on fresh deposits of thiamethoxam and 31 % on one week old deposits.

The corresponding readings for imidacloprid were 97 % (ABBOTT 91 %) and 67 % (ABBOTT 24 %), respectively. Imidacloprid was used at double the rate of thiamethoxam in tomatoes.

## 5.2 Contact and residual activity on tomatoes following foliar application

**5.2.1** During a period of 28 days thiamethoxam at 100 g ai/ha reduced the adult bumble bee population in hives introduced immediately after application by 93 % (ABBOTT 79 %). The mortality remained at the same level (94 %, ABBOTT 85 %), when colonies were introduced 14 days after the application and bumble bees were exposed to aged deposits for only 14 days. The corresponding figures for imidacloprid were 86 % (ABBOTT 64 %) and 90 % (ABBOTT 80 %), respectively.

**5.2.2** The mortality of deposits of thiamethoxam at 40 g ai/ha, aged for 1, 2, 7 and 14 days, was 79 %, 80 %, 88 % and 78 %, respectively (Tab. 1). The mortality in the untreated control was 50 % which is a normal phenomenon for an observation period of 14 days. The corrected mortality (ABBOTT 1925) for the corresponding aged deposits was 58 %, 59 %, 75 % and 56 %, respectively. These readings, collected under more practice oriented conditions, are still too high to be acceptable to growers.

## 5.3 Single drench application in tomatoes

Low mortality was observed in all treatments during the whole observation period (Tab. 1). The highest number of dead bumble bees was found 18 days after the drench application. This is a normal phenomenon with bumble bees kept in hives. Bumble bee workers with strong pollination activity start to die after two to three weeks. After 28 days, the mortality figures were 50 % for thiamethoxam and 49 % for imidacloprid, and 58 % in the water control. The highest number of alive bumble bees was found in the hives of the thiamethoxam treatment. The corrected effect (ABBOTT 1925) on the whole bumble bee population treated with thiamethoxam was -85 % and for imidacloprid -59 %, i.e. there was a relative increase of bumble bee numbers compared to the untreated control, i.e. thiamethoxam was harmless to bumble bees at the rate of 150 g ai/ha as a drench application.

## *6. Plastic tunnel studies*

### 6.1. First trial by a single application on tomatoes via the irrigation system.

#### **a)** Activity at the entrance of the hive.

At the beginning of the trial the daily activity measured at the hive entrance was 178 departures, increasing to a peak of 737 per day at 9 days before applica-

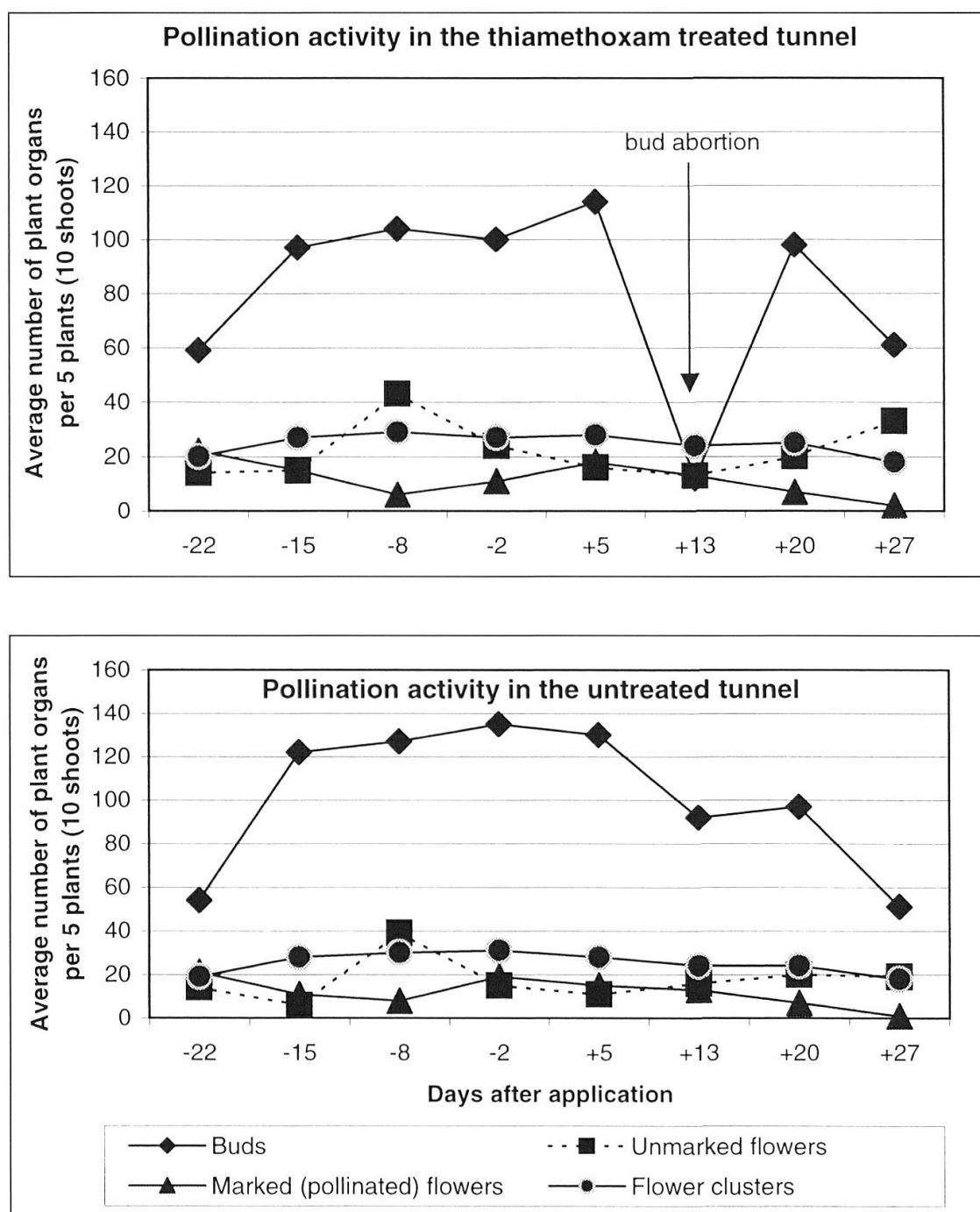


Fig. 1. Pollination activity of bumble bees in tunnels with tomato crops following a single application of thiamethoxam via the irrigation system. Centre Horticole, Lullier, 1997.

tion and of 654 per day at 1 day before. The activity decreased progressively ending with 181 departures daily at the end of the trial (+24 DAA). Between -13 and +16 DAA there were 24 days with more than 400 daily departures. The general trend of the activity of this bumble bee population seems not to be affected by the application of thiamethoxam.

There were always slightly more departures than arrivals. This small difference can be attributed to natural losses (death outside the hive, disorientation).

Tab. 2. Final analysis of bumble bee colonies 28 days after the single application of thiamethoxam via the irrigation system, 1997. Centre Horticole, Lullier, Switzerland. One colony per treatment.

Category / stages of development	Treated tunnel		Untreated tunnel	
	Total no. of cells with	%	Total no. of cells with	%
Eggs	27	2.9	106	15.6
Dry eggs	0	0	57	8.4
Larvae L1	19	2	25	3.7
Larvae L2	12	1.3	15	2.2
Larvae L3	15	1.6	13	1.9
Larvae L4	18	1.9	12	1.8
White nymphs	42	4.5	26	3.8
Black nymphs	23	2.5	16	2.3
Unhatched adults	8	0.9	1	0.1
Empty cells	453	48.4	190	28
Nectar cells	284	30.4	46	6.8
Preyed cells	34	3.6	10	1.5
Cells preyed by the wax moth	0	0	162	23.9
Total cells with bumble bees	935	100	679	100
Adults	134		78	
Queens	1		1	
Net weight of remaining bumble bee food ' BeeHappy ' in ml	105		980	
Net weight of remaining pollen in g	0		0	
Small wax moth larvae	38		0	
Big wax moth larvae and cocoons	0		99	

#### b) Pollination activity

Fig. 1 shows the pollination activity in the treated and untreated tunnel. Bud formation is rather normal everywhere throughout the trial period. The general trend of marked and unmarked flowers is similar in both tunnels. Apparently the thiamethoxam treatment did not increase the number of unmarked (unpollinated) flowers.

#### c) Proportion of tomato and other plant pollen collected by bumble bees

More pollen from other plants than from tomatoes was collected by the bumble bees in the two tunnels before and after the application. The pollination of the tomatoes by the bumble bees was good in spite of the fact that only 15 % of the bumble bees (6 of 40 observed) had sampled tomato pollen. The following percentage of pollen sampled by these six specimens originated from tomato plants: <1%, 78%, 99%, >99%, 100% and 100 %.

#### d) Vitality of bumble bees

During the various and repeated evaluations in the tunnels neither paralyzed nor dead bumble bees could be found on the ground close to the plants or around the hives. Tab. 2 gives the details of the final hive analysis. In the treated tunnel the single colony had a higher total number of cells with bumble bees (935) than the untreated one (679). This can be attributed largely to the wax moth, *Galleria mellonella* (LINNAEUS, 1758). This is a lepidopterous predator of hives which was present

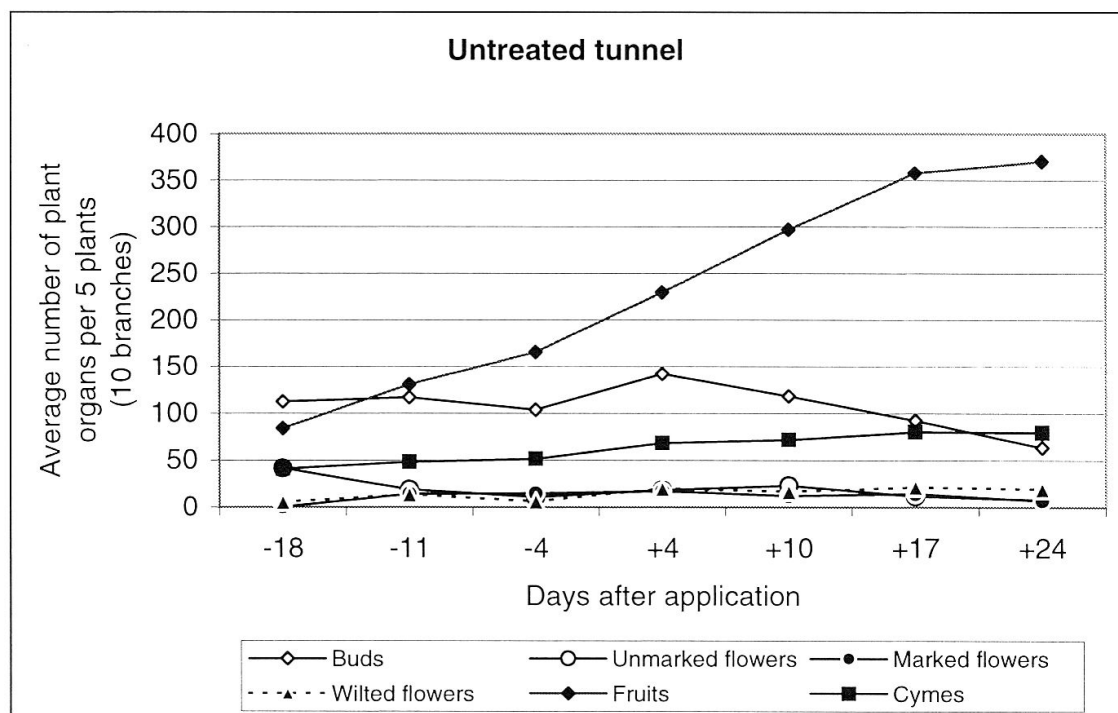
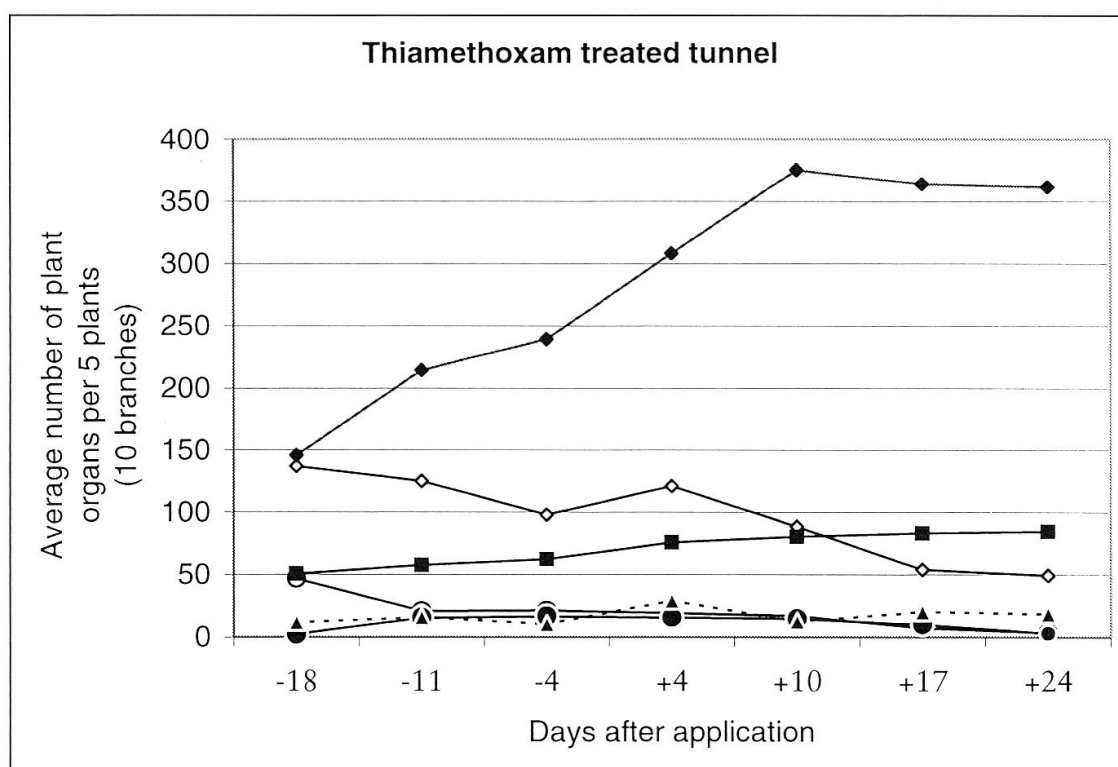


Fig. 2. Number of plant organs and pollination activity of bumble bees following the single application of thiamethoxam on tomatoes via the irrigation system. Centre Horticole, Lullier, 1998.

in higher numbers (99) in the untreated hive than in the thiamethoxam treated one (38). This situation does not allow to draw any conclusion on an eventual effect of the product on bumble bee populations but a negative effect of thiamethoxam on bumble bees looks unlikely. Since no cells had yet been preyed upon by the wax moth in the thiamethoxam treatment, infestation by this predator had obviously started later than in the untreated tunnel. There were more alive bumble bee adults (134) in the thiamethoxam hive than in the untreated one (78). Much more bumble bee food was consumed in the thiamethoxam hive (only 105 ml remaining) than in the untreated one (980 ml remaining).

## 6.2. Second trial by a single application on tomatoes via the irrigation system

### **a) Activity at the entrance of the hive**

The monitoring was done from -17 to +30 DAA (-16 to +29 DAA in the untreated control). Only departures of workers could be measured properly because guard bumble bees disturbed the counting process of arriving workers. The disturbance of guard bees by constantly crossing the entrance hole forwards and backwards, as observed also in the preceding trial, is overcome by installing an extension tube at the entrance. But the bees undergo a learning process and the length of the tube has to be extended again and again. If the adaptation is delayed, bees start to disturb the counting process again, what had happened several times during this evaluation.

Most times bumble bees were more active in the treated than in the untreated tunnel as demonstrated by the number of departures. Between 200 to 600 departures after the installation of the hive increased to about 1800 in the treated and about 1000 in the untreated tunnel at the time of application. Immediately before and several days after application the number of departures dropped sharply in both the treated and the untreated tunnel which can be explained by the decline of temperatures, and thereafter by a normal increase of natural mortality. The general trend of the activity of this bumble bee population was not affected after the application of thiamethoxam. Thus the decrease in the daily activity at the entrance of the hive was not linked to an impact of the chemical.

### **b) Pollination activity**

The curves of the buds, and unmarked, marked (pollinated) and wilted flowers in the treated and untreated tunnel are similar in both treatments (Fig. 2). The delayed fruit formation in the untreated tunnel reflects only the later planting date of this crop. The thiamethoxam treatment did not alter the percentage of marked flowers.

### **c) Proportion of tomato and other plant pollen collected by bumble bees**

Tab. 3 demonstrates the percentage of tomato pollen collected by the bumble bees in the two tunnels before and after the application. If possible the sampling was carried out as long until 10 bees with pollen had been counted. Then the percentage of bees carrying tomato pollen was evaluated of all the observed bee adults.

Tomatoes are not the preferred sampling source of pollen for bumble bees. The bee population was higher in the hive in the untreated tunnel at the beginning of the observation period. Therefore there was a stronger incentive and pressure for the bumble bees in the untreated tunnel to collect more tomato pollen than from oth-

Tab. 3. Percentage of bumble bees with tomato pollen sampled in two tunnels before and after one single application of thiamethoxam via the irrigation system. Centre Horticole, Lullier, 1998.

Days after application	Percentage of bees with tomato pollen	
	Treated tunnel	Untreated tunnel
- 11	0 (2)	58 (6)
- 4	8 (10)	52 (10)
+ 4	19 (10)	51 (10)
+ 17	28 (10)	21 (10)
( ) number of bumble bees from which pollen samples were collected		

er host crops. As the bee population in the treated tunnel was catching up after the application, the percentage of sampled tomato pollen approached to about the same level as in the untreated tunnel at the last sampling date 17 days after application (28 % versus 21 %).

## DISCUSSION

Thiamethoxam belongs to the neonicotinoids and has quick stomach and contact action against target sucking insects and beetles (SENN et al. 1998). It acted also strongly against bumble bees when it was tested for its contact and oral activity on glassplates. The toxicity on treated tomato plants was only slightly less pronounced. Mortality figures of thiamethoxam following exposure on two days old residues were still high. Unacceptable long removal period of bumble bee hives from the glasshouses would become necessary and therefore this mode of application proved to be unsuitable for thiamethoxam. For this reason further options had to be examined.

If no effects are seen on the semi-field level (e.g. in tents), a low-risk category can be assigned without further testing (HASSAN et al. 1992). Although thiamethoxam became less harmful, still significant effects were seen in the semi-field (tent) foliar application test when it was sprayed on the bee-attractive plant *P. acetifolia*. Exposure between 1 and 14 days following the foliar application on both tomatoes and *P. acetifolia* exerted still unacceptable mortality of thiamethoxam to bumble bees. The aging of thiamethoxam deposits following a foliar application contributed little to improve the selectivity of the product. Therefore the application via irrigation was chosen as a possible viable alternative of product application. A single drench application in a semi-field test demonstrated already complete harmlessness of thiamethoxam to bumble bees.

These findings had to be verified under more realistic, practice oriented conditions. Therefore field testing became necessary to define risk classification. The impact of thiamethoxam was evaluated by various parameters in a single drip application through the irrigation system in two series. The measurement of the bumble bee activity at the entrance of the hives did not demonstrate any reduction of flight activity in the thiamethoxam treated tunnels. The analysis of collected pollen revealed no difference in the proportion between tomatoes and other host plants nor any



discrimination of tomato pollen. The ratio of unmarked and marked (i.e. pollinated) flowers did not differ between thiamethoxam and untreated tomato plants. The detailed analysis of the content of the hives showed that there was a comparable proportion of the various developmental stages (larvae, nymphs, adults) of bumble bees present in both treated and untreated ones. The mortality was also about the same in both and was largely due to natural population decline. No dead or paralyzed bumble bees could be found on the ground of thiamethoxam treated tunnels. The single application of thiamethoxam as a drench or drip via the irrigation system proved to be acceptable safe to bumble bees. These findings suggest that thiamethoxam would be acceptable to the growers for use in tunnels and glasshouses with bumble bee populations.

The appropriate recommendations for use of thiamethoxam with regard to bumble bees is based on the implementation of a proposed sequential testing system (SECHSER & REBER 1998). The findings as described here mean that thiamethoxam is harmless to bumble bees in a single application as drench or via drip irrigation. Based on the findings in our studies, thiamethoxam is harmless according to the IOBC guidelines (HASSAN 1992) to the bumble bee *B. terrestris* (IOBC field category 1 from 0% to <25% reduction) in a single drench or drip application via the irrigation system at a rate of up to 172 g ai/ha.

If multiple application during a growing season is practised, further equivalent testing may be required for proper management of supposed risks. Since thiamethoxam has proved harmfulness in contact and feeding tests by foliar application, strict recommendations have to be communicated to tunnel and glasshouse growers in order to exclude risks caused by careless spillage of thiamethoxam in the course of a drench or drip application.

## CONCLUSIONS

Thiamethoxam applied at up to 172 g ai/ha is safe for bumble bees pollinating tomato plants when it is used in a single drench or drip application via the irrigation water. Attention has to be paid not to spill any contaminated water because of the potential oral and contact toxicity of the compound.

## ACKNOWLEDGEMENTS

The authors are grateful to M. POHL from Novartis Crop Protection, and N. MESSMER and S. FISCHER from the Federal Research Station in Nyon for their technical assistance.

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(received May 1, 2002; accepted October 18, 2002)

