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Original article

Effects of ingestion of a *Bacillus thuringiensis* toxin and a trypsin inhibitor on honey bee flight activity and longevity

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Abstract – To assess potential impacts of transgenic pest-resistant plants, newly-emerged adult honey bees from ten colonies were tagged, placed in cages at 33 °C, and fed with 625 µg/g Cry1Ba *Bacillus thuringiensis* (Bt) toxin or 2.5 mg/g aprotinin proteinase inhibitor in pollen-food (equivalent to 0.25% or 1% of total soluble protein). Control bees were given similar food without additive. All foods were consumed at similar rates. After seven days, all bees were returned to their hives. Subsequent observations showed that Cry1Ba-fed bees did not differ significantly from control bees in the timing of their first flight, the period during which flights took place or in estimated longevity. However, aprotinin-fed bees began to fly and also died about three days sooner than Cry1Ba-fed or control bees. Their flight periods were similar to those of the other bees. The effects of transgenic aprotinin-plants on honey bees will thus depend on gene expression levels in pollen.

***Apis mellifera* / *Bacillus thuringiensis* / Cry1Ba toxin / proteinase inhibitor / aprotinin / transgenic plant**

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1. INTRODUCTION

Increasingly, crop plants are being genetically modified for protection against pest attack (James, 1997, 1998). Although there are a number of genes with potential for use in pest-resistant transgenic plants, recent commercial and research attention has been focused mostly on those encoding either *Bacillus thuringiensis* (Bt) toxins or proteinase inhibitors (PIs) from a variety of plant and animal sources. The success of cultivars containing these genes will depend in part upon their safety to beneficial pollinating insects such as honey bees (*Apis mellifera* L.), which may be exposed to gene products in either pollen or nectar.

There have been few published measurements of Bt toxin or PI expression levels in pollen or nectar. Cry1A(b) Bt toxin was undetectable in pollen from maize containing a *cry1A(b)* gene under the control of cauliflower mosaic virus (CaMV) 35S promoter, but was present (as 260–418 ng/mg soluble protein) in pollen/anther preparations from maize containing the same gene on a pollen-specific promoter (Kozeil et al., 1993). Cotton plants of the commercial transgenic cultivar, Bollgard™, had lower levels of CryIA(c) Bt toxin in pollen (0.6 μg/g fresh weight) than in petals (3.4 μg/g) (Greenplate, 1997). Indirect evidence of Bt gene expression in pollen has also been provided by a report of insecticidal activity of pollen from Bt-transgenic N4640 maize (Losey et al., 1999). In contrast, the cysteine PI, oryzacystatin I (OC-I) was found in the leaves of transgenic CaMV 35S/OCI-oilseed rape plants (0.2–0.4% of total soluble protein), but was undetectable in the pollen and nectar of the same plants (Bonadé Bottino et al., 1998). This finding is in accordance with that of Jouanin et al. (1998), who also noted that Bowman-Birk soybean trypsin inhibitor (BBI) could not be detected in the nectar or pollen of transgenic oilseed rape plants with measurable expression levels in leaves (gene also on CaMV 35S promoter). Further studies are required before generalisations can be made about the tissue specificities of commonly-used promoters and the expression of pest-resistance proteins in pollen of transgenic plants.

A number of recent studies have provided information on the effects on bees of various concentrations of purified Bt and PI proteins added to bee food. Purified CryIA(c) toxin fed at a concentration of 20 μg/ml to 1–3 day-old larvae and adults of *A. mellifera* had no significant effect on the survival of these insects (Sims, 1995). Similarly, purified CryIIIB toxin (0.066% and 0.332%) fed in sugar syrup to colonies of honey bees over a two-month period had no effect on larval survival or pupal dry weight (Arpaia, 1996). Purified Cry1Ba toxin (similar to the recombinant protein that would be expressed in a plant genetically modified to contain the Cry1Ba gene) mixed into a pollen-based food at 10, 2.5 or 0.25 mg/g and fed to adult honey bees for seven days post-emergence, had no significant effect on the rate at which each food was consumed or on the longevity of the bees (Malone et al., 1999).

Several studies have shown that purified PIs fed to adult honey bees can alter digestive protease levels and, at high concentrations, cause some mortality. BBI fed to foraging (older adult) honey bees in syrup at concentrations of 1, 0.1, 0.01 or 0.001 mg/g had no effect on bee survival over four days, but trypsin activity was reduced after 3.5 days' consumption of 0.1 or 1 mg/g BBI syrup (Belzunces et al., 1994). Bonadé-Bottino et al. (1998) reported "deleterious effects" on bees fed BBI in a "long-term" bioassay with BBI supplied to bees at 100× the expression level of BBI in transgenic oilseed rape plants, but did not provide detailed methods or data. No mortality was observed three days after 15-day-old adult bees were each fed with 11 μg of BBI, OC-I or chicken egg white cystatin (Girard et al., 1998). Cowpea trypsin inhibitor (CpTI) did not have short-term (24 h)

toxicity to 15-day-old adult bees either fed with 11 μg or injected with 0.5 μg of this PI (Picard-Nizou et al., 1995). Four serine endopeptidase inhibitors, aprotinin (also known as bovine pancreatic trypsin inhibitor or BPTI), soybean Kunitz trypsin inhibitor (SBTI or SKTI), potato proteinase inhibitor I (PI-I or POT-1) and potato proteinase inhibitor II (PI-II or POT-2), have been shown to have dose-dependent effects on bee longevity when fed to newly-emerged adult bees (Malone et al., 1995, 1998; Burgess et al., 1996). Bees had significantly reduced longevity when fed BPTI or SBTI in sugar syrup ad lib. at 10, 5 or 1 mg/ml (Burgess et al., 1996). Similar reductions in longevity were noted in bees fed either POT-1 or POT-2 at 2 mg/ml in syrup ad lib. or at 10 mg/g in a pollen-based solid food for the first eight days of adult life (Malone et al., 1998). Midgut protease activity levels were also significantly altered by many of these PI treatments, in some cases even in the absence of a longevity effect (e.g. bees fed for eight days with 2 mg/g of POT-1 or POT-2 in pollen-based food had normal longevity but significantly reduced levels of trypsin, chymotrypsin and elastase) (Burgess et al., 1996). SBTI, mixed into a pollen-based food at 10, 5 or 0.5 mg/g and fed to newly-emerged adult honey bees for seven days, had no significant effect on the rate at which each food was consumed or on the subsequent longevity of the bees (Malone et al., 1999).

Honey bees kept in cages in an incubator are unable to carry out many of the activities that they would undertake in the hive, such as flying and interacting with other bees. Whether this lack of activity ameliorates or exacerbates the effects of Bt toxins or PIs is uncertain. Here we report on the effects of a Bt toxin (Cry1Ba) and PI (aprotinin) on the flight activity and longevity of adult honey bees fed these purified proteins for the first seven days of adult life and then returned to their hives.

2. MATERIALS AND METHODS

Activated Cry1Ba toxin was obtained from a large-scale fermentation of *B. thuringiensis* Bt4412, purified and cleaved according to the method described by Simpson et al. (1997). This process subjects the Bt parasporal inclusions to conditions similar to those in an insect gut, i.e. they are dissolved, releasing protoxins, which are then cleaved into toxic core fragments ("activated toxins") by proteases. Activated toxin was used as this most closely resembles the form in which Cry1Ba will be expressed in transgenic plants. Purified aprotinin was obtained from Intergen® Company, Canada/USA.

Honey bees were obtained from our apiaries at Mt Albert, Auckland, New Zealand, from ten different colonies. Each colony had been re-queened three months prior to the experiment with mated queens of similar genetic background obtained from a commercial queen breeder. Each colony was housed in a two-storey, full-depth hive, containing a plastic feeder and 17 frames, of which six on average (range, 4 to 8) contained brood and the rest contained stores of honey and pollen. Colonies were placed in irregularly spaced groups at two apiaries, which were about 500 m apart (Colonies 1, 4 and 10 were in one apiary, the others in another). Newly-emerged adult bees were collected for the experiment by taking "capped" brood combs from each colony placing them in an incubator at 33 °C. Adult bees that emerged over the next four hours were removed from the combs and tagged by gluing coloured, numbered discs to their thoraces. Four thousand eight hundred bees were tagged.

These tagged bees were then assigned to three different treatments (control, aprotinin or Cry1Ba). They were placed in wooden cages ($9 \times 8 \times 7$ cm) with mesh on two sides, 80 bees per cage and 20 cages per treatment. While an attempt was made to keep the bees from different colonies in separate cages, so that they could be returned to

the colonies from which they had been taken, shortages of suitably-aged bees in some colonies meant that some cages contained bees from more than one colony. Each cage was supplied with about 5 g of pollen-food (0.33 parts pollen, 0.08 parts sodium caseinate, 0.16 parts brewer's yeast, and 0.43 parts sucrose mixed with water to a paste) to which the gene products had been added. The pollen used in this food was bee-collected from unknown floral sources and stored at –20 °C. In addition, each cage was fitted with two gravity feeders, one containing water and the other sugar syrup (50% w:v sucrose solution), which were replenished as necessary during the experiment. One thousand six hundred bees (160 bees for each colony) each received pollen-food without additive (controls), with 2.5 mg/g aprotinin added or with 625 μg/g Cry1Ba added. These dosage levels approximated 1% aprotinin and 0.25% Cry1Ba of total protein in the pollen-food (assuming that this food was 25% protein). They were chosen to represent Bt and PI expression levels reported from leaves of transgenic plants effectively protected from pest attack (Perlak et al., 1990; Greenplate, 1997; McManus et al., 1994; Benedict et al., 1996; Duan et al., 1996; Lee et al., 1999; McManus and Burgess, 1999; Voisey et al., 1999). The caged bees were incubated in darkness at 33 °C for seven days.

The number of dead bees and the weight of pollen-food consumed by the bees in each cage were recorded after seven days. Pollen-food consumption was estimated by dividing the weight lost from each food container by the number of live bees. Mean food consumption rates (mg per bee) and mean numbers of surviving bees per cage were compared using ANOVA.

Six cages of bees (two each of the controls, aprotinin- and Cry1Ba-fed bees) were then released directly onto the brood combs of each of the ten colonies. As explained earlier, an attempt was made to return bees to the colony from which they had been taken, in the hope that this would reduce rejection. However, this was not possible for the majority of tagged bees, since there had been insufficient bees of a suitable age ready in each colony on the day allotted for tagging. Colonies 1 and 4 received only bees from the same colony, but the others received mixtures of bees from several colonies. Because each bee in the colony had a unique tag, its treatment was known and its subsequent behaviour and survival could be determined.

The onset of flight activity and the total length of time over which flights took place were estimated for each surviving tagged bee. Flight activity of the tagged bees was observed from the day after the bees had been introduced into the hives. Hive entrances were blocked with a piece of foam plastic for 10 min and the numbers and colours of any tagged bees that had landed on the front of each hive were noted. Observations were made three times daily for the first five days and then six times daily until no more tagged bees were seen. The presence of pollen in the corbiculae of tagged bees was also noted. Since the hives were located in adjacent apiaries in a suburban area, all the bees had similar access to flowering plants and were able to forage from many different garden plant species during the experiment.

A "census" of tagged bees in each hive was conducted 3, 7, 14 and 21 days after their return to the hives by opening each hive at dusk and recording all tagged bees that could be seen. Each frame in the hive was removed, both faces and all edges were examined thoroughly for tagged bees twice, as were the inner walls and bottom board. The whole process took about 15 to 20 min per hive. By combining these observations with those made on bees outside the hive during the day, an estimate of minimum longevity (last day seen alive) was obtained for each tagged bee. Bees not recorded in a preceding census were assumed to have been alive if they were observed in a later census or flight observation.

To determine whether any of the treatments had influenced the rejection of bees released into the colonies, the numbers of bees from each treatment group recorded in the day 3 census were compared using ANOVA.

To compare the longevity of bees from different colonies receiving different treatments, survival curves were constructed in which the proportions of each type of tagged bee alive in each colony were plotted against time in days from their return to the hive. Mantel-Haenzel (log-rank) tests (Kalbfleisch and Prentice, 1980) were carried out to compare Kaplan Meier estimates of survival distribution, S(t), for bees receiving each treatment and from each colony. The mean longevity of bees from each colony and of those subjected to each treatment was also calculated.

The mean day of first observed flight and the mean number of days over which flights were observed (from the first to the last day upon which flight was observed) were calculated for bees from each treatment group and colony and compared using ANOVA. There were too few bees observed carrying pollen for analysis to be carried out on these observations.

3. RESULTS

During the first seven days of adult life, tagged bees kept in cages had similar mortality and consumed similar amounts of pollen-food, regardless of whether they had received Cry1Ba, aprotinin or no food additive (Tab. I).

The three different foods received by the tagged bees did not affect their subsequent acceptance by other bees when released into the colonies (Tab. II). There was a significant colony effect however, with more tagged bees surviving their first three days in colonies 1 and 2 than in colonies 3, 5, 6, 8, 9 or 10 ($P < 0.001$). Colony 4 had an intermediate number of survivors at day 3 (post-release).

When results from all colonies were combined, log-rank tests showed that there was a significant reduction in survival of aprotinin-fed bees from day 3 (post-release) onwards ($P < 0.001$; Fig. 1). (Colony 3 was omitted from the analysis as only two bees had survived introduction into the hive, both from the Cry1Ba treatment.) There were also significant colony effects on bee survival within each treatment group (controls, $P = 0.0002$; Cry1Ba-fed, $P < 0.001$; aprotinin-fed, $P = 0.006$). However, there were no consistent patterns among these survival curves, i.e. no single colony consistently had longer- or shorter-lived bees regardless of treatment. Mean estimated bee longevity figures were in accordance with the findings of the log-rank tests. Data pooled over all colonies gave the following mean bee longevities: controls, 19.3 days, aprotinin-fed bees, 15.5 days and Cry1Ba-fed bees, 18.0 days. Data pooled over all treatments gave the following longevities: colony 1, 17.7 days, colony 2, 21.9 days, colony 4, 15.6 days, colony 5, 12.7 days, colony 6,

Table I. Numbers of surviving adult bees and their consumption of pollen-food with and without additives after seven days in cages.

Treatment	Number of survivors (out of 1600)	Food consumed (mg per surviving bee)
Control	1 457	46.6
Cry1Ba, 625 µg/g	1 438	46.5
Aprotinin, 2.5 mg/g	1 496	50.4

Table II. Survival of tagged bees three days after their release from cages into ten different colonies (number alive after three days/number released into colony).

Colony number	Treatment received while in cage			
	Control	Cry1Ba, 625 μg/g	Aprotinin, 2.5 mg/g	All treatments
1	53/125	48/113	72/146	173/384
2	49/120	28/157	24/158	101/435
3	0/156	2/155	0/156	2/467
4	4/142	5/98	13/125	22/365
5	5/156	6/150	1/155	12/461
6	1/146	3/147	6/134	10/427
7	19/149	19/158	27/159	65/466
8	9/151	5/149	23/157	37/457
9	5/155	13/156	4/150	22/461
10	1/157	1/155	5/156	7/468
All colonies	146/1457	130/1438	175/1496	451/4391

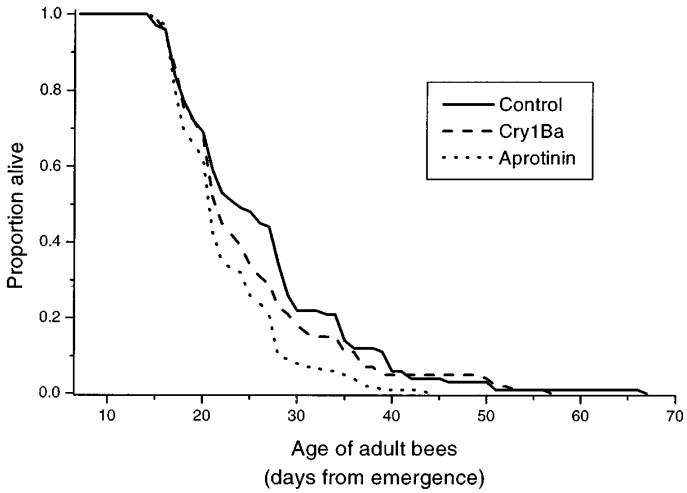

Figure 1. Survival of adult bees after their release into colonies in the field (pooled data from ten colonies). Before release, the bees had been fed for seven days post-emergence with pollen-food containing 625 μg/g Cry1Ba Bt toxin, 2.5 mg/g aprotinin proteinase inhibitor or no additive (control).

14.8 days, colony 7, 13.7 days, colony 8, 14.8 days, colony 9, 17.8 days and colony 10, 13.3 days.

When flight data from all colonies were pooled, ANOVA showed that bees fed with aprotinin began flying significantly sooner (by 2.8 days on average) than the other bees ($P = 0.003$) (Tab. III). (Colonies 3, 5, 6 and 10 were omitted from the analysis as there were insufficient flying bees in some categories from each.) The day of first flight also varied significantly from colony to colony when all treatment groups were pooled ($P < 0.001$). For example, colony 4 bees began flying sooner than colony 2 bees (Tab. III).

Table III. Flight activity of tagged bees after three different treatments and subsequent release from cages into colonies. Data were pooled across colonies for treatment comparisons and pooled across treatments for colony comparisons. Numbers without a letter in common within each column differ at $P \leq 0.05$ (ANOVA).

Treatment received or colony number	Mean number of days until first flight observed (after release into colony)	Mean number of days over which flights were observed
Control	17.0a	4.5a
Cry1Ba	17.0a	3.7a
Aprotinin	14.2b	3.8a
Colony 1	16.9cd	3.3cd
Colony 2	20.3c	6.4b
Colony 4	10.6e	5.1bc
Colony 7	13.6de	1.6d
Colony 8	13.8de	3.6cd
Colony 9	12.6e	5.9bc

There were no significant differences attributable to treatment in the length of time over which bee flights took place, although there was a significant colony effect on this parameter ($P < 0.001$) (Tab. III). For example, colony 7 bees had extremely short flight periods compared with colony 2 bees (Tab. III).

Very few bees were observed carrying pollen, although there were representatives from each treatment group among them.

4. DISCUSSION

The high rate of rejection of bees returned to the hive after being kept in the laboratory for seven days was a major drawback with the method employed in this study. Nestmate recognition among worker honey bees appears to be influenced not only by relatedness but also by odours acquired in the hive (Saleh, 1991). For example, Breed et al. (1988) noted that newly-emerged bees were generally accepted by both laboratory-kept groups and field colonies of bees, regardless of whether they were related or not. However, laboratory-kept bees older than 12 h were not accepted by field colonies, whether they were related or not. This fits with the observations made during the present study. Although an attempt was made to match some of the released bees to their colonies of origin, in order to avoid problems with unrelated bees encountering each other, it may have been more beneficial to employ some technique to introduce the bees into the hive slowly so that they could acquire an appropriate "colony odour" before being exposed to other bees. In preliminary tests to the present study, high rejection rates were also observed when week-old bees from the laboratory were introduced into hives inside queen cages sealed with candy (unpublished observations). Furthermore, attempts to place larger cages containing tagged, newly-emerged bees supplied with the proteins and syrup directly inside hives, rather than keeping them in an incubator for seven days, resulted in very high and immediate bee mortality (unpublished observations). Lower rejection rates may have resulted if the week-old, treated bees had been introduced into the hive behind a screen of paper. This is a technique commonly used by beekeepers when combining hives (e.g. "the newspaper method" described by Matheson, 1997)

and the time taken for the bees to chew through the paper seems sufficient for them to acquire similar odours and to recognise each other as nestmates.

The high rejection rate in the present study meant that most colonies had too few bees for a meaningful colony-by-colony statistical analysis of observations. However, the treatment effects revealed by analysis of data pooled across all colonies were also demonstrated in separate analyses of colonies 1 and 2, the only individual colonies with reasonable numbers of accepted bees. This tends to support the conclusions of the study, i.e. that bees fed aprotinin began flying and died sooner than the other bees.

Bees fed with purified Cry1Ba toxin at a rate approximating expression in pollen of 0.25% (of total soluble protein) had similar longevity and flight activity to the control bees in this study. This confirms previous studies showing that bees were unaffected when fed on purified Bt gene products (Sims, 1995; Arpaia, 1996; Malone et al., 1999). The safety to honey bees of commercial Bt biopesticide formulations, provided they do not contain exotoxins, is well-established (Cantwell et al., 1972; Celli, 1974; Buckner et al., 1975; Cantwell and Shieh, 1981; Vandenberg, 1990). It seems likely that transgenic Bt-plants will be similarly harmless to bees, even though these plants may expose bees to specific Bt toxins in soluble and cleaved form ("activated toxins"), rather than a mixture of toxins, spores and vegetative stages, as is the case with Bt biopesticides.

Interactions between honey bees and plants expressing PIs are likely to be more complex. Earlier studies have shown that PIs can reduce bee digestive protease activity and that they have dose-dependent effects on bee survival (Burgess et al., 1996; Malone et al., 1998). In an earlier study with aprotinin, bees were kept in cages and supplied with the pollen-food without additive (same recipe as in the present study), but were fed continuously with the PI added to sugar syrup (10, 5, 1, 0.1 or 0.01 mg/mL) (Burgess et al., 1996). Those receiving the two lowest concentrations had similar longevity to the controls (37 days on average), but those receiving 10, 5 or 1 mg/mL aprotinin had their lifespans reduced by an average of 17, 18 or 9 days respectively. In the present study, bees fed 2.5 mg/g aprotinin in pollen-food for seven days died between 2.7 and 4.8 days sooner than control bees.

If we assume that aprotinin has its major impact on the bee during the first week of adult life in a caged bee, when proteolytic activity peaks (Crailsheim and Stolberg, 1989), then we may compare these two studies by estimating a "daily dose" of PI received by each bee during this period. In the caged bee study, this can be calculated by multiplying the concentration of PI in syrup by the amount of syrup consumed per bee per day (0.032 mL, unpublished observation). In the present study, a comparable figure may be obtained by multiplying the concentration of PI in the pollen-food (2.5 mg/g) by the average amount of food consumed per day (7.1 mg, Tab. I). Thus bees that were kept in cages and consumed 32 µg of aprotinin per day via syrup had significantly reduced longevity (by nine days on average), but those that received 3 µg aprotinin per day in syrup did not (Burgess et al., 1996). From these data we may speculate that the "threshold value" above which aprotinin will cause adult bee mortality lies somewhere between 3 and 32 µg per bee per day. In the present study, colony 1 and 2 bees that received 18 µg of aprotinin per day in pollen-food and were returned to their hives died significantly sooner (by 2.8 days) than control bees in the same colonies. This places the "threshold value" between 3 and 18 µg per day and suggests that the effects of aprotinin on bee survival in the hive are not dramatically different from its effects on bees kept entirely in cages.

The aprotinin dose given to newly-emerged bees in this experiment was chosen

to simulate exposure to transgenic pollen expressing 1% aprotinin (of total protein) continuously for seven days. While this is an approximation of a potential field situation, there are several factors complicating it. Firstly, bees in field colonies may consume more pollen over a longer time than bees kept in cages, since the presence of brood has been shown to stimulate protein ingestion by adult bees (Crailsheim, 1990). Secondly, the composition of pollen stores in a hive and the proportions of transgenic pollen they contain will vary greatly depending on the types of flowering plants grown in the area where the bees are kept, the quantity and age of stored pollen and the provision of protein supplements, if any, to the bees. Finally, without comprehensive data on expression levels of PIs and other proteins in transgenic pollen, it remains difficult to estimate the concentrations of transgene products to which bees may be exposed in the field.

PI expression levels in leaves of transgenic plants protected from pest attack range from 0.05 to 2.5%. For example, rice expressing 0.5 to 2% of a potato PI was resistant to pink stem borer (Duan et al., 1996), *Spodoptera litura* were killed by feeding on leaves of tobacco expressing 0.4 to 1% soybean trypsin inhibitor McManus and Burgess, 1999), rice expressing 0.05 to 2.5% soybean trypsin inhibitor had improved resistance to brown planthopper (Lee et al., 1999), and *Wiseana* spp. growth was reduced on white clover expressing 0.07% aprotinin Voisey et al., 1999). Therefore, a leaf expression level of 1% might realistically be expected in a pest-resistant transgenic PI-plant, although lower levels of expression may suffice for pest control. It remains to be seen whether a pollen expression level of 1% is also realistic, since PI expression levels in the pollen of pest-resistant PI-plants have not yet been conclusively established (Bonadé-Bottino et al., 1998; Jouanin et al., 1998).

While experiments using purified gene products mixed into bee food have the potential to provide useful information on the impacts of transgenic plants prior to release, the current lack of data on expression levels in pollen limits the conclusions we can draw and is a constraint on rational experimental design.

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Résumé – Effets de l'ingestion d'une toxine de *Bacillus thuringiensis* et d'un inhibiteur de trypsine sur l'activité de vol et la longévité de l'abeille domestique. Afin d'évaluer l'impact potentiel sur l'abeille domestique (*Apis mellifera* L.) des plantes transgéniques résistantes aux ravageurs, des abeilles adultes fraîchement écloses provenant de 10 colonies ont été marquées, encagées à 33 °C et nourries pendant sept jours avec du pollen auquel avait été mélangé soit 625 µg/g de toxine Cry1Ba de *Bacillus thuringiensis* (Bt), soit 2,5 mg/g d'aprotinine, inhibiteur de protéase.

Ces concentrations équivalent environ dans la nourriture à 0,25 % et 1 % respectivement de la protéine totale soluble. Les abeilles témoins ont reçu de la nourriture dépourvue d'additifs. Le taux de consommation a été le même pour tous les types de nourriture (Tab. I). Au bout de sept jours, les abeilles des trois groupes de traitement avaient le même taux de survie (Tab. I). On a ensuite réintroduit entre 98 et 159 abeilles de chaque groupe de traitement dans chacune des 10 colonies. Beaucoup d'abeilles

marquées ont été rejetées par les colonies au cours des trois premiers jours, mais il n'y avait aucune relation significative avec le type de nourriture qu'elles avaient reçues dans les cages (Tab. II).

Les observations suivantes ont montré que les abeilles du groupe Cry1Ba ne se différenciaient pas significativement des abeilles du groupe témoin en ce qui concerne l'âge de leur premier vol, la durée pendant laquelle avaient lieu les vols et la longévité estimée (Tab. III, Fig. 1). Pourtant les abeilles du groupe aprotinine ont commencé à voler environ 2,8 jours plus tôt que les témoins et sont mortes aussi 3,6 jours plus tôt (Fig. 1, Tab. III). Par contre elles ont effectué des vols sur une période semblable à celle des autres groupes (Tab. III). Ces résultats, ainsi que ceux d'autres études antérieures sur l'aprotinine, suggèrent que les effets des plantes transgéniques exprimant l'aprotinine doivent très vraisemblablement dépendre des niveaux d'expression du gène dans le pollen.

***Apis mellifera* / *Bacillus thuringiensis* / toxine Cry1Ba / inhibiteur de protéase / aprotinine / plante transgénique**

Zusammenfassung – Wirkungen eines Giftes von *Bacillus thuringiensis* und eines Trypsin Hemmers im Futter auf die Flugaktivität und Lebensdauer von Honigbienen. Um mögliche Schäden durch transgene befallsresistente Pflanzen auf Honigbienen (*Apis mellifera* L.) einzuschätzen, wurden frisch geschlüpfte Bienen von 10 Völkern markiert, in Käfigen bei 33 °C gehalten und 7 Tage lang mit einer Mischung aus Futterteig und Pollen mit dem Zusatz von 625 µg/g Gift (Cryl1Ba) des *Bacillus thuringiensis* (Bt) oder von 2,4 mg/g des Eiweißhemmers Aprotinin gefüttert. Diese Konzentrationen entsprechen etwa 0,25 % bzw. 1 % der Gesamteiweißmenge des Futters. Die Kontrollbienen erhielten ähnliches Futter ohne Zusatz. Das Futter wurde von allen Bienen in ähnlicher Menge aufgenommen (Tab. I). Nach 7 Tagen hatten alle Bienen der drei verschiedenen Behandlungen ähnliche Überlebensraten (Tab. I). Zwischen 98 und 159 Bienen aus jeder der 3 Behandlungsgruppen wurden dann in eines der 10 Bienenvölker umgesetzt. Viele der markierten Bienen wurden in den ersten 3 Tagen abgetrieben, aber das war nicht signifikant abhängig von dem Futter, das sie in den Käfigen erhalten hatten (Tab. II).

Die nachfolgenden Beobachtungen zeigten, dass die mit Cryl1Ba gefütterten Bienen sich weder in Bezug auf das Alter bei den ersten Ausflüge signifikant von den Kontrollbienen noch in der Zeit der Ausflüge oder der Lebensdauer (Abb. 1, Tab. III) unterschieden. Bienen dagegen, die mit Aprotinin gefüttert worden waren, flogen 2,8 Tage früher als die Kontrollbienen und starben 3,6 Tage früher (Abb.1, Tab. III). Ihre Flugperioden waren ähnlich wie die der anderen Bienen (Tab. III). Diese Ergebnisse sowie die von früheren Untersuchungen sprechen dafür, dass die Wirkung von transgenen Aprotininpflanzen sehr stark davon abhängen werden, wieviel Aprotinin von dem entsprechenden Gen im Pollen erzeugt wird.

***Apis mellifera* / *Bacillus thuringiensis* / Cryl1BA Gift / Proteinhemmer / Aprotinin**

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