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Original article

Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants

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Abstract – In order to assess the potential impacts of transgenic plants on larvae of the honey bee, *Apis mellifera*, information on pollen consumption is needed. We here report on experiments that were conducted with small bee colonies kept in field cages (8 × 14 m) containing only flowering maize plants as protein source. Fully grown worker bee larvae were found to contain between 1720 and 2310 maize pollen grains in their gut before defecation, corresponding to 1.52–2.04 mg of pollen consumed per larva. On average, 74.5% of pollen grains were completely digested while 23.3% were partially digested and 2.2% remained undigested. Our data indicate that the contribution of the protein by directly feeding larvae with pollen is less than 5% in relation to the total amount of protein necessary for complete larval development. We suggest that our measurement for pollen consumption should be taken into account when establishing dose regimes to assess the risk that transgenic plants pose for honey bee larvae.

***Apis mellifera* / pollen consumption / transgenic plant / agrochemical / maize / risk assessment**

1. INTRODUCTION

Honey bees are of great ecological and economic importance as pollinators of many crop and wild plants (Free, 1993). Therefore, novel plant protection methods have to be evaluated for potential effects on this group of beneficial insects. The studies conducted to evaluate the effects of pesticides have recently been reviewed by Devillers and Pham-Delègue (2002) while Malone and Pham-Delègue (2001) have summarised studies carried out to evaluate the effects of transgenic plants. Bees in general may be exposed to transgene products or agrochemicals via pollen or nectar. Pollen is the main source of protein for bees (Crailsheim, 1990) while nectar is known to contain mainly sugars and rather low concentrations of proteins (Baker and Baker, 1986; Carter et al., 1999). As the toxins produced by transgenic plants are proteins which have not yet been detected in nectar (Malone and Pham-Delègue, 2001), potential risks for honey bees due to transgene products will most likely be due to pollen feeding. Regarding agrochemicals, potential risks may occur due to systemic insecticides and to a lesser extent, to insecticides sprayed onto flowering crops (Kubik et al., 1999; Villa et al., 2000; Tasei, 2001). Potential risks for larvae may not only occur by pollen feeding but also by nectar feeding because agrochemicals present in nectar may be delivered to larvae through the contents from the honey sac (Davis and Shuel, 1988). In the present paper, we focus on pollen consumption of honey bee larvae aiming to examine the effects of exposure to transgene products. Nevertheless, we would like to

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stress the relevance of this contribution for risk assessment of agrochemicals that are present in pollen.

Numerous studies have been carried out indicating that pollen from a wide range of plant species is collected during each bee foraging season (Alfonsus, 1933; Wille et al., 1985a, b). However, a study carried out in the low lands of Northern Switzerland showed that only five plant species including maize accounted for 51% of the total pollen collected annually (Wille and Wille, 1983; Wille et al., 1985a). Therefore, honey bees in some regions may well be exposed to the pollen of transgenic lepidopteran resistant maize containing genes from the soil bacterium *Bacillus thuringiensis* Berliner (Bt-maize) if this crop is deployed on a large scale. In order to assess the risks that transgenic plants pose on non-target organisms, both the exposure and the hazard have to be considered. Regarding honey bees, the hazard is basically determined by the toxicity of the transgene product while the exposure is dependent on the amount of pollen that adults and larvae ingest. Bioassays have been carried out to test potential effects of transgenic plants or transgene products on adult honey bees (Malone and Pham-Delègue, 2001) but few studies have considered the larval stages (Malone et al., 2002; Brodsgaard et al., 2003). In addition, studies including the larvae lacked a detailed knowledge on how much pollen is ingested during development.

During the first 3–4 days of larval development, honey bee larvae are fed with jelly produced in the hypopharyngeal glands of adult worker bees (Crailsheim, 1990). The composition of this jelly depends on whether the larva is being raised to become a queen, a worker or a drone, but is almost free of pollen. During subsequent development, larvae are fed with modified jelly that is less rich in protein but rich in sugars (honey) and contains some pollen (Planta, 1888; Kunert and Crailsheim, 1988; Malone et al., 2002). However, the amount of pollen that honey bee larvae are exposed to has never been investigated in detail. The only study on honey bees dealing with that question experimentally was carried out by Simpson (1955) who measured the amount of pollen in the guts of larvae that have fed on a diverse mixture of plants.

We here aimed to quantify the amount of pollen that honey bee larvae ingest during development by exposing them exclusively to maize pollen under near-field conditions. This bioassay makes use of the fact that honey bee larvae do not defecate until development of the larva is finished. Such a measurement for pollen consumption will allow for a more realistic estimation of the exposure of honey bee larvae to transgene products expressed in pollen and insecticide residues. It should furthermore be used to determine meaningful amounts of toxins in future feeding experiments to assess potential risks of genetically modified plants or agrochemicals to honey bee larvae.

2. MATERIALS AND METHODS

2.1. Field cages

Large field cages (8 × 14 m, height in the centre 3.5 m, mesh size 1 mm) were set up at the Swiss Federal Research Station for Agroecology and Agriculture in Zurich, Switzerland. Eight rows of maize (variety Monumental) with 80 cm space between rows were sown resulting in a total of about 800 plants per cage. Maize was sown at three different times in biweekly intervals beginning on May 8 in order to set up three replicates over time. Fertilisation and weed control were done in agreement with standard agricultural practices in Switzerland. At the beginning of flowering, the tent was installed and two colonies of *Apis mellifera* L. were introduced per cage as soon as 5–10% of the maize anthers were open. From the total of six bee colonies, only five yielded brood and were subjected to further analysis.

2.2. Bee colonies

Bee hives were set up in the field cages close to the soil on small posts surrounded by paraffin to protect them against ants. They were also protected against rain and partly against direct sunlight by a brick (25 × 40 cm) put on top of the hive. Each hive contained one queen and about 1000 worker bees of varying age that had ad libitum access to a protein-free sugar patty and water.

The hives were composed of three combs of 10 × 10 cm embedded into a Styrofoam box (Apidea™). At the start of the experiment, one comb was empty while the second one contained predominantly open and capped brood. The third comb contained eggs < 48 h old laid by the queen used in the experimental hive. Larvae from this comb hatched shortly after introducing the hives into the field cage. Larvae were sampled for experimental purposes from this comb

only. At the beginning of the experiment, all combs were checked for pollen stores and the few cells that contained pollen were filled with liquid bee wax to prevent the bees from gaining access to this pollen.

2.3. Sampling of bee larvae

When larvae were fully grown, i.e. five to six days after the hives had been introduced into the cages, the third comb of each colony was checked for capped cells. If such cells were found, the comb was temporarily transferred to the laboratory and all larvae that were partly or completely capped, but had not yet started cocoon formation and defecation, were removed, weighed on a microbalance (Mettler Toledo M × 5, d = 1 μg; ± 2 μg) and stored at 4 °C in 1.5 mL Eppendorf tubes. Afterward, the comb was placed back into the hive. This procedure was repeated three times a day until about 30 larvae were obtained per colony. In addition, we found drone larvae in one of the hives (colony 5) which were also sampled the same way. The experiment lasted for a maximum of 8 days during which time bees had abundant access to pollen. This was also indicated by numerous cells filled with maize pollen in the course of the experiment. Fresh weights of all worker bee larvae were compared using single factor ANOVA among the five colonies. Means were subsequently separated using the Tukey HSD test.

2.4. Counting of pollen

Larvae were dissected and each complete gut was frozen at –18 °C in 200 μL 0.5 M sugar water. For pollen counts, each thawed gut was homogenised with a pestle and a10 μl aliquot of the homogenate transferred onto a counting device and covered with a coverslip (18 × 18 mm). The number of pollen grains in the 10 μl sample was determined by microscopic examination (magnification 50 ×). Complete pollen grains and fragments larger than half of a pollen grain were counted (cf. Crailsheim et al., 1992). Three times 10 μL aliquots were taken from each sample and counted. The mean was multiplied by 20 to estimate the number of pollen grains in a single larval gut. Between 26 and 35 larvae were analysed per colony.

In order to estimate the weight of a single pollen grain, six samples of fresh pollen pooled from ten maize plants each (variety Monumental) were collected by hand from a field that bordered the field cages. These were weighed immediately and then all pollen grains (300–720 pollen grains per sample) were counted under a microscope at 50 × magnification. Dividing the weight of the samples by the number of grains we found the weight (± SE) of a single maize pollen grain to be 882 ± 2.2 ng.

Mean weight of pollen grains in the guts of larvae among the three different field cages set up on consecutive dates were compared using single factor ANOVA. Since no effects of the cages were found ($F_{2,147} = 1.24$, $P = 0.293$), a single factor ANOVA was conducted on the mean weight of pollen grains in the guts of larvae among the five colonies. Regression analysis was carried out to reveal a potential relationship between larval weight and the weight of pollen grains in the gut.

2.5. Digestion of pollen

To estimate the proportion of pollen grains that were digested by the larvae, 10 μl of each gut sample were checked under a transmission microscope at 100 × magnification. For each sample, 50 randomly selected pollen grains were classified into one of three groups: fully digested (at most an estimated content of 10%), shrunken, i.e. all grains that contained some material (see Crailsheim et al., 1992) and undigested (no detectable difference to fresh pollen). To illustrate our classification, a few typical images are shown in Figure 1.

3. RESULTS

The mean weight of one fully grown honey bee larvae varied from 132 to 155 mg and significant differences in the weight of worker bee larvae among the five colonies were detected ($F_{4,145} = 15.22$, $P < 0.001$, Fig. 2A). Between 1720 and 2310 maize pollen grains were found in the guts of worker honey bee larvae. Based on the weight of a single pollen grain, we calculated the amount of pollen consumed by one honey bee worker larvae to be between 1.52 ± 0.108 and 2.04 ± 0.104 mg (Fig. 2B) with significant differences among the five bee colonies ($F_{4,145} = 3.44$, $P = 0.010$). Significantly more pollen grains were found in larval guts of colony 1 compared to colony 5 (Tukeys HSD test, $P = 0.021$).

There was no relationship between the number of pollen grains consumed and larval weight if whole colonies were studied (regression analysis, $F_{1,3} = 0.34$, $P = 0.60$). We found significant relationships between the number of pollen grains consumed and the weight of individual larvae in colonies 2, 3, 4 but not in colonies 1 and 5. As shown in Table I, the coefficients of determination (R^2) were generally low with a maximum of 0.22.

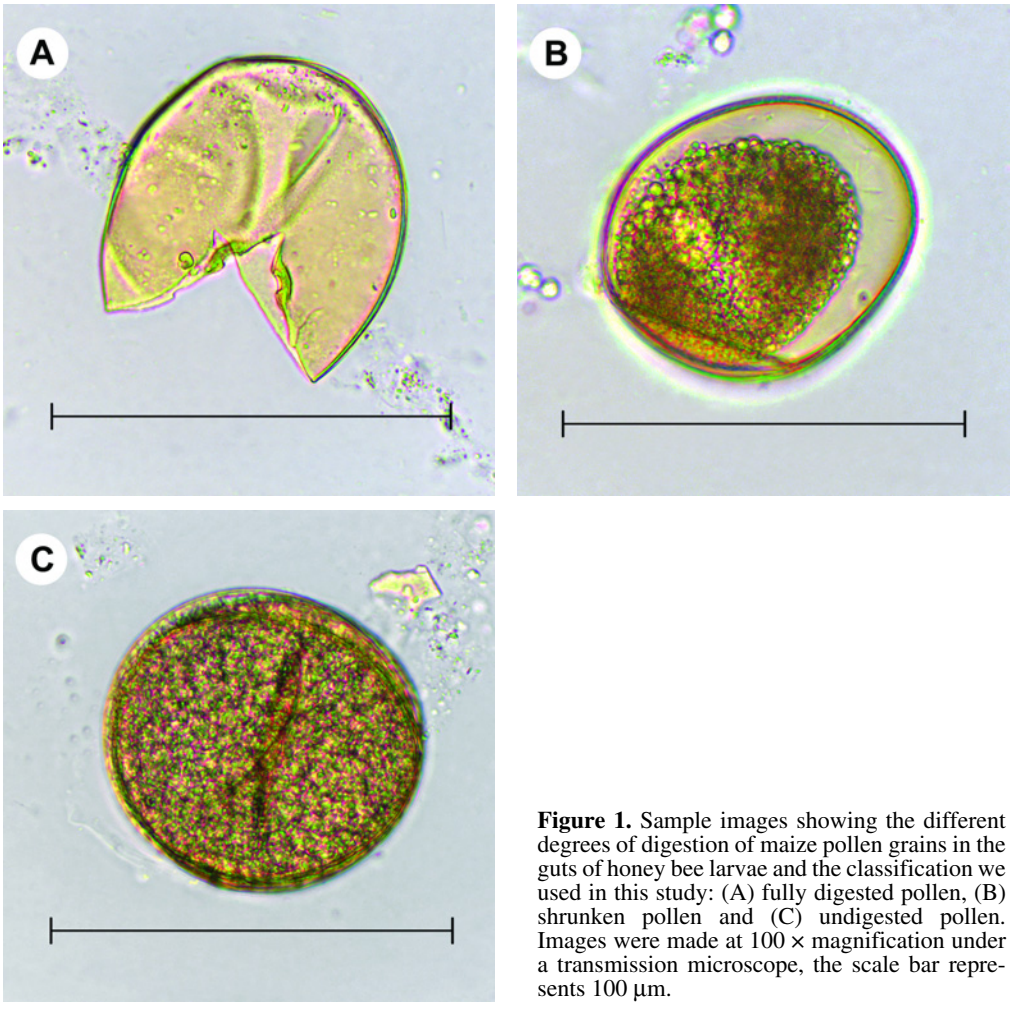

Figure 1. Sample images showing the different degrees of digestion of maize pollen grains in the guts of honey bee larvae and the classification we used in this study: (A) fully digested pollen, (B) shrunken pollen and (C) undigested pollen. Images were made at 100 × magnification under a transmission microscope, the scale bar represents 100 µm.

The degree of digestion of maize pollen by honey bee larvae was in the same range for the five colonies investigated (Fig. 3). On average, 74.5% of pollen grains were completely digested while 23.3% were partially digested and 2.2% remained undigested.

4. DISCUSSION

The aim of this study was to assess the total amount of pollen consumption by honey bee larvae in colonies restricted to foraging on maize plants and to estimate the contribution of pollen relative to the total amount of protein larvae receive during development. We have shown that worker honey bee larvae under our experimental conditions were fed with a total amount of 1.5 to 2 mg maize pollen during their complete development. In the present study, drone larvae were 39% heavier than worker bee larvae and their gut contained 36% more pollen, suggesting that drone larvae are fed with similar quantities of pollen compared to worker larvae. However, because the drone brood is normally not produced during August and those we measured may have been underweight, these results should be regarded as preliminary.

As the colonies in our experiments were allowed to collect pollen only from maize plants, it can not be ruled out that different

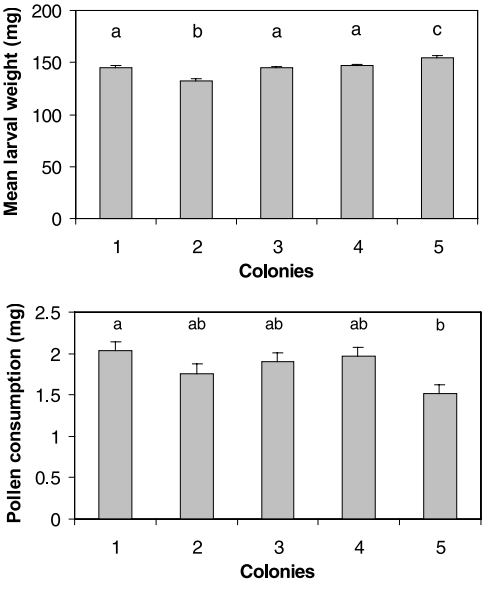

Figure 2. (A) Mean weight of fully grown larvae (+ SE) and (B) mean consumption of maize pollen (+ SE) per honey bee larva for the five bee colonies investigated (N = 26–35 larvae). Different letters indicate significant differences among colonies (Tukeys HSD test, $P < 0.05$).

Table I. Results of a regression analysis to test for a relationship between the weight of the larvae and the number of pollen consumed for each of the colonies.

	R^2	F	df	*P*
Colony 1	0.046	1.59	1, 33	0.217
Colony 2	0.201	6.04	1, 24	0.022
Colony 3	0.216	8.82	1, 32	0.0056
Colony 4	0.176	5.55	1, 26	0.026
Colony 5	0.00032	0.008	1, 25	0.929

quantities of pollen may be fed to honey bee larvae under field conditions where bees may forage on a variety of plant species. Such differences may relate to the quality (N-content, amino-acid composition) as well as to the size of different pollen grains. Most pollen grains are smaller than pollen from maize (Stanley and Linskens, 1974, p. 28), thus small larvae may not be able to uptake the relatively large maize pollen grains but may easily ingest smaller ones. If such an effect would occur, pollen consumption of bees fed only larger

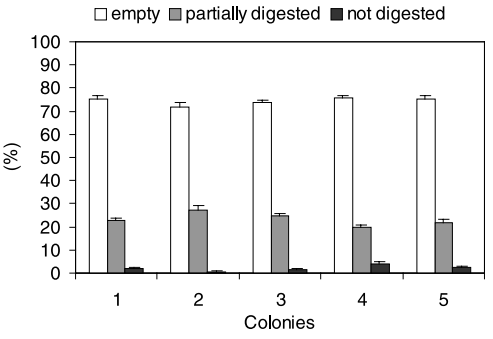

Figure 3. Percentage of digested maize pollen grains in the guts of honey bee larvae for the five bee colonies (N = 26–35 larvae).

pollen grains, such as maize, may be underestimated.

Our study has further shown that by far the most pollen grains are digested which is in general agreement with other studies on honey bee larvae (Simpson, 1955) and adults (Schmidt and Buchmann 1985; Crailsheim et al., 1992). This suggests that larvae were able to utilise most of the protein that maize pollen contained. In addition to pollen, however, honey bee larvae are fed with worker jelly which is provided by nurse bees. Although the relative protein contributions of pollen and worker jelly are not exactly known as yet, several studies have shown that the latter is a substantial source of protein and needed for the protein balance of the larvae (see Haydak, 1970 for a review). If one is aiming to assess potential risks of transgene products for honey bee larvae, information is needed on whether the worker jelly contains any toxins expressed by the transgenic plant. This appears to be unlikely because such proteins would have to pass the insects guts and become incorporated into the hypopharyngeal glands. Even for insecticides that are much smaller molecules, only traces were found in the hypopharyngeal glands of worker bees when fed the compound in a sugar solution (Davis and Shuel, 1988). Consequently, direct feeding on pollen may be the only significant source of transgene products for honey bee larvae.

Earlier estimations revealed that about 160 mg of pollen is necessary to rear a honey bee (Alfonsus, 1933; Wille et al., 1985b). However, this estimate includes all the protein that

adults need for their own turn over and significant amounts of protein is needed for adults during both summer and winter (Schmidt and Buchmann, 1985; Crailsheim, 1990). We believe that the amount of protein, and thus pollen, needed for development of honey bee larvae can be best estimated from the total amount of nitrogen a larva contains after finishing development. Data by Haydak (1959) and Imdorf et al. (1998) show that a single pupa of *A. mellifera* contains 1.87 mg and 1.85 mg nitrogen, respectively. Since defecation has occurred already at this stage, a loss of nitrogen of 20% has to be taken into account (Schmidt and Buchmann, 1985). Based on the conversion factor of nitrogen to protein of 6.25 (Maynard and Loosli, 1969) and the protein content of maize pollen of 16.7% (variety Monumental, unpublished data), we calculate that approximately 86 mg maize pollen is necessary to rear a single honey bee larva. A slightly lower amount of pollen is needed if the calculation is based on the average protein content of pollen of 20% (Imdorf et al., 1998). Our data indicate that the contribution of the protein by directly feeding larvae with pollen is less than 5% in relation to the total amount of protein necessary for complete larval development. Thus pollen constitutes only a minor part of the protein supply of honey bee larvae. This is very much in agreement with findings of Simpson (1955) who estimated from a large sample of dissected honey bee larvae that on average 0.078 mg N (corresponding to 2.2 mg pollen) was present in the guts of larvae which were fed with pollen from different plants. Focussing on a small sub-sample where bees collected virtually only red clover pollen, Simpson (1955) calculated that 5.4 mg pollen were present in larval guts which still is in qualitative agreement with our findings. Our data and the results of Simpson (1955) may indicate that a low pollen consumption rate is a general feature in honey bee larvae. The data furthermore indicate that the larval stages of the honey bee are less exposed to transgene products than the adults as the latter uptake large quantities of pollen not only for their own requirements but also for the rearing of the brood.

From the measurements obtained in the present study, it is possible to calculate the amount of e.g. Bt-toxin that a larva would ingest during development if fed solely with pollen from a transgenic plant. For example, pollen from Bt-maize 'Event 176' contains a relatively high amount of the Bt-toxin (5–11 µg Cry1Ab/g fresh weight pollen) as the *cry1Ab* gene is expressed under a green leaf tissue- and a pollen-specific promoter (Fearing et al., 1997). Based on this it can be calculated that a honey bee larva would ingest a maximum of 10–22 ng of Cry1Ab when fed exclusively on Bt-maize pollen (Event 176). This amount is not toxic for honey bees according to published information (Malone and Pham-Delègue, 2001). Other commercialised Bt-maize varieties (Event Bt11 and Event MON 810) contain even less of the transgene product in the pollen as it is expressed under a constitutive promoter (CaMV 35S) that has little activity in the pollen. Thus, ingestion of Bt-toxin is reduced by nearly two orders of magnitude if larvae are fed with pollen of the latter two varieties (Sears et al., 2001).

However, we here like to stress that the same calculation is not restricted to Bt-maize but could be applied for any potential toxin present in pollen. The few studies carried out so far on potential risks of transgenic plants on honey bee larvae have assumed that considerably higher amounts of pollen are ingested by larvae. As our data indicate a very low pollen consumption for larvae, exposure to toxins produced by transgenic plants has most probably been overestimated so far (e.g. Malone et al., 2002; Brødsgaard et al., 2003). Similarly, exposure to agrochemicals via pollen has been overestimated in the few studies dealing with potential risks for bee larval stages (Villa et al., 2000). We suggest that our measurement for pollen consumption should be taken into account to establish more realistic dose regimes in further risk assessment studies of honey bee larvae.

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Résumé – La consommation de pollen par les larves d'abeilles : une étape vers l'évaluation des risques présentés par les plantes transgéniques. En tant que pollinisateurs de nombreuses plantes cultivées et sauvages, les abeilles domestiques (*Apis mellifera* L.) ont un rôle très important du point de vue écologique et économique. Les nouvelles méthodes de protection des plantes, que ce soit l'utilisation de pesticides ou de plantes transgéniques, doivent en conséquence être testées quant à d'éventuels effets négatifs sur ces insectes auxiliaires. Puisque les abeilles ne consomment les produits de transgènes que via le pollen, des données sur la quantité de pollen consommé par les larves et par les adultes sont d'une extrême importance pour l'évaluation des risques. De telles données manquant en particulier pour les larves, nous avons mesuré la quantité de pollen qui est consommée par des larves d'abeilles durant tout leur cycle de développement. Pour cela les larves ont été exposées en conditions semi-naturelles à du pollen de maïs uniquement.

On a semé du maïs (variété Monumental) sous trois grandes cages et obtenu environ 800 pieds par cage. Dès le début de la floraison du maïs, deux petites ruchettes d'environ 1000 abeilles ont été apportées sous chaque cage. Les abeilles ont été nourries ad libitum avec une pâte sans protéines et de l'eau. Les rayons ne contenaient aucune réserve de pollen afin que les abeilles ne se nourrissent que de pollen de maïs. Il était important pour cette expérience que les larves ne puissent déféquer qu'après leur développement complet et donc que l'on puisse prélever des larves pleinement développées qui renfermaient encore dans leur intestin la quantité de pollen consommé. Dans chacune des cinq colonies qui ont élevé du couvain, on a prélevé entre 26 et 35 larves dans les cellules fraîchement operculées, puis on les a pesées et disséquées. Les grains de pollen ont tous été comptés et répartis en trois classes : entièrement digérés (au maximum une teneur de 10 %), partiellement digérés ou non digérés (aucun différence reconnaissable avec le pollen frais, voir Fig. 1). Pour chaque colonie on a trouvé en moyenne entre 1720 et 2310 grains de pollen de maïs par intestin de larve d'abeille. Cela correspond à une quantité de pollen comprise entre 1,52 ± 0,108 et 2,04 ± 0,104 mg (Fig. 2B). En moyenne 74,5 % des grains de pollen étaient totalement digérés, 23,3 % l'étaient partiellement et 2,2 % pas du tout. Aucune relation n'a pu être établie entre le poids des larves et le nombre de grains de pollen dans l'intestin (Tab. I).

Nos résultats montrent que la contribution du pollen à l'alimentation des larves en protéines durant tout leur cycle de développement ne représente qu'une petite partie (moins de 5 %). Ils montrent en outre que jusqu'à présent l'exposition des larves d'abeilles aux toxines produites par les plantes transgéniques ou aux pesticides a été surévaluée. Nos données sur la consommation de pollen devraient être utilisées comme base pour de futures études de risques pour les larves d'abeilles, afin de garantir que des quantités réalistes de toxines sont utilisées dans les expériences de nourrissement.

***Apis mellifera* / consommation de pollen / plante transgénique / pesticide / maïs / estimation des risques**

Zusammenfassung – Pollenaufnahme von Larven der Honigbiene: ein wichtiger Aspekt für die Risikoanalyse von transgenen Pflanzen. Als Bestäuber von vielen Kultur- und Wildpflanzen sind Honigbienen (*Apis mellifera* L.) sowohl ökologisch als auch ökonomisch von grosser Bedeutung. Neue Pflanzenschutzmassnahmen müssen daher auf mögliche schädliche Nebenwirkungen für Bienen getestet werden. Dies betrifft sowohl Pestizide als auch transgene Pflanzen. Da Bienen die transgenen Produkte nur über den Pollen aufnehmen, sind Daten zur aufgenommenen Menge an Pollen sowohl für die Larven als auch für die adulten Bienen äusserst wichtig für eine Risikoanalyse. Da solche Daten insbesondere für die Larven fehlen, haben wir in dieser Studie die Pollenmenge quantifiziert, die von einzelnen Honigbienenlarven während der gesamten Entwicklungsdauer aufgenommen und verwertet wird. Hierzu wurden die Larven unter Halbfreilandbedingungen ausschliesslich gegenüber Maispollen exponiert.

In grossen Feldkäfigen (n = 3) wurde Mais gesät (Sorte Monumental), wobei insgesamt etwa 800 Pflanzen pro Käfig wuchsen. Sobald die Maispflanzen zu blühen begannen, wurden je zwei kleine Bienenvölker mit ca. 1000 Bienen in den Käfigen etabliert. Die Bienen wurden ad libitum mit einem Protein-freien Futterteig sowie Wasser versorgt, hatten jedoch keine Pollenvorräte in den Waben. Dies stellte sicher, dass die Larven nur mit Maispollen gefüttert wurden. Wichtig für diesen Versuch war die Tatsache, dass die Larven der Honigbiene ihren Kot erst nach vollendeter Entwicklung abgeben und daher ausgewachsene Larven gesammelt werden konnten, die noch die insgesamt aufgenommene Pollenmenge im Darm enthielten. Für jedes der fünf Völker, die Bruterfolg hatten, wurden zwischen 26 und 35 Larven aus den frisch verdeckelten Zellen entfernt, gewogen und seziert. Alle Pollenkörner wurden gezählt und in drei Klassen eingeteilt: vollkommen verdaut (maximal 10 % Inhalt), teilweise verdaut oder unverdaut (kein erkennbarer Unterschied zu frischem Pollen, siehe Abb. 1). Gemittelt über die Daten aus je einem Volk wurden zwischen 1720 und 2310 Maispollenkörner im Darm der einzelnen Honigbienenlarven gefunden. Dies entspricht zwischen 1,52 ± 0,108 und 2,04 ± 0,104 mg Pollen (Abb. 2B). Durchschnittlich waren 74,5 % der Pollenkörner vollkommen verdaut, während 23,3 % teilweise und 2,2 % unverdaut waren. Zwischen dem Larvengewicht und der Anzahl Pollenkörner im Darm wurde keine Beziehung gefunden (Tab. I).

Unsere Daten zeigen, dass der Beitrag des Pollens an der Proteinversorgung der Larven während der gesamten Entwicklungsdauer nur einen kleinen Teil

ausmacht (weniger als 5 %). Sie zeigen weiter, dass bisher die Exposition der Honigbienenlarven gegenüber den von transgenen Pflanzen produzierten Toxinen oder auch Pestiziden überschätzt wurde. Unsere Daten zum Pollenkonsum sollten als Grundlage für weitere Risikostudien zu den Larven der Honigbienen herangezogen werden um zu gewährleisten, dass realistischere Toxinmengen in Fütterungsversuchen verwendet werden.

***Apis mellifera* / Pollenaufnahme / transgene Pflanzen / Pestizide / Mais / Risikoanalyse**

REFERENCES

Alfonsus E.C. (1933) Zum Pollenverbrauch des Bienenvolkes, Arch. Bienenkd. 14, 220–223.

Baker H.G., Baker I. (1986) The occurrence and significance of amino-acids in floral nectar, Plant Syst. Evol. 151, 175–186.

Brødsgaard H.F., Brødsgaard C.J., Hansen H., Lövei G.L. (2003) Environmental risk assessment of transgene products using honey bee (*Apis mellifera*) larvae, Apidologie 34, 139–145.

Carter C., Graham R.A., Thornburg R.W. (1999) Nectarin I is a novel, soluble germin-like protein expressed in the nectar of *Nicotiana* sp., Plant Mol. Biol. 41, 207–216.

Crailsheim K. (1990) The protein balance of the honey bee worker, Apidologie 21, 417–429.

Crailsheim K., Schneider L.H.W., Hrassnigg N., Bühlmann G., Brosch U., Gmeinbauer R., Schöffmann B. (1992) Pollen consumption and utilisation in worker honeybees: dependence on individual age and function, J. Insect Physiol. 38, 409–419.

Davis A.R., Shuel R.W. (1988) Distribution of C-14-labeled Carbofuran and Dimethoate in royal jelly, queen larvae and nurse honeybees, Apidologie 19, 37–50.

Devillers J., Pham Delègue M. (2002) Honey bees: Estimating the environmental impact of chemicals, Taylor and Francis Inc., New York, 332 p.

Fearing P.L., Brown D., Vlachos D., Meghji M., Privalle L. (1997) Quantitative analysis of CryIA(b) expression in Bt maize plants, tissues, and silage and stability of expression over successive generations, Mol. Breed. 3, 169–176.

Free J.B. (1993) Insect pollination of crops, Academic Press, London, UK.

Haydak M.H. (1959) Changes with age in weight and nitrogen content of honeybees, Bee World 40, 225–229.

Haydak M.H. (1970) Honey bee nutrition, Annu. Rev. Entomol. 15, 143–156.

Imdorf A., Rickli M., Kilchenmann V., Bogdanov S., Wille H. (1998) Nitrogen and mineral constituents of honey bee worker brood during pollen shortage, Apidologie 29, 315–325.

Kubik M., Nowacki J., Pidek A., Warakomska Z., Michalczuk L., Goszczynski W. (1999) Pesticide residues in bee products collected from cherry trees protected during blooming period with contact and systemic fungicides, Apidologie 30, 521–532.

Kunert K., Crailsheim K. (1988) Seasonal changes in carbohydrate, lipid and protein-content in emerging worker honeybees and their mortality, J. Apic. Res. 27, 13–21.

Malone L.A., Pham-Delègue M.H. (2001) Effects of transgene products on honey bees (*Apis mellifera*) and bumblebees (*Bombus* sp.), Apidologie 32, 287–304.

Malone L.A., Tregidga E.L., Todd J.H., Burgess E.P.J., Philip B.A., Markwick N.P., Poulton J., Christeller J.T., Lester M.T., Gatehouse H.S. (2002) Effects of ingestion of a biotin-binding protein on adult and larval honey bees, Apidologie 33, 447–458.

Maynard A.B., Loosli J.K. (1969) Animal Nutrition, McGraw-Hill, New York, 613 p.

Planta A. v. (1888) Über den Futtersaft von Bienen, Z. Physiol. Chem. 12, 327–354.

Schmidt J.O., Buchmann S.L. (1985) Pollen digestion and nitrogen-utilization by *Apis-mellifera* L. (Hymenoptera, Apidae), Comp. Biochem. Physiol. 82A, 499–503.

Sears M.K., Hellmich R.L., Stanley-Horn D.E., Oberhauser K.S., Pleasants J.M., Mattila H.R., Siegfried B.D., Dively G.P. (2001) Impact of Bt Corn Pollen on Monarch Butterfly Populations: a Risk Assessment, Proc. Natl. Acad. Sci. 98, 11937–11942.

Simpson J. (1955) The significance of the presence of pollen in the food of worker larvae of the honeybee, Q. J. Microsc. Sci. 96, 117–120.

Stanley R.G., Linskens H.F. (1974) Pollen: Biology, Biochemistry, Management, Springer-Verlag, Berlin.

Tasei J.N. (2001) Effects of insect growth regulators on honey bees and non-*Apis* bees. A review, Apidologie 32, 527–545.

Villa S., Vighi M., Finizio A., Serini G.B. (2000) Risk assessment for honeybees from pesticide-exposed pollen, Ecotoxicology 9, 287–297.

Wille M., Wille H. (1983) Vergleichende pollenanalytische Untersuchungen des Rückbehaltens in Pollenfallen, Ermittlungsjahre 1981 und 1982, Schweiz. Bienenztg 5, 253–268.

Wille H., Wille M., Kilchenmann V., Imdorf A., Bühlmann G. (1985a) Pollenernte und Massenwechsel von drei *Apis mellifera* Völkern auf demselben Bienenstand in zwei aufeinanderfolgenden Jahren, Rev. Suisse Zool. 92, 897–914.

Wille H., Imdorf A., Bühlmann G., Kilchenmann V., Wille M. (1985b) Beziehung zwischen Polleneintrag, Brutaufzucht und mittlerer Lebenserwartung der Arbeiterinnen in Bienenvölkern (*Apis mellifera* L.), Mitt. Schweiz. Entomol. Ges. 58, 205–214.