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ScienceDirect
European Journal of Soil Biology 42 (2006) S132–S135
EUROPEAN JOURNAL OF SOIL BIOLOGY
http://france.elsevier.com/direct/ejsobi

Original article

# Preference tests with collembolas on isogenic and Bt-maize

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Available online 14 July 2006

**Abstract**

Collembolas are important members of belowground food webs. There is little information available on the effects of the plant residues of transgenic maize expressing *Bacillus thuringiensis* (Bt) toxin on soil animals, including collembola. This is why two questions were addressed in laboratory feeding experiments with three collembolan species: (i) Are collembola equally distributed on residues of isogenic and Bt-maize? and (ii) Do collembola show feeding preference to either of the maize types? Bt-maize (producing Cry1Ab toxin) proved to be a less preferred food source for *Folsomia candida* than the isogenic one. No similar phenomenon was found in the case of *Heteromurus nitidus* and *Sinella coeca*. *F. candida* reacted to as low as 3.45 (± 0.8 mg $g^{-1}$) Bt-toxin content of the maize. Our results show that the effect of the Bt-toxin producing maize on the collembolan is species specific.


*Keywords:* Collembola; Bt-maize; Preference

## 1. Introduction

Environmental impacts on soil biota of the *Bacillus thuringiensis* (Bt) Cry toxin produced by maize plants are poorly known. This is a matter of regret, because about 40–50% of the Bt-maize production enter the soil on 9.1 million ha worldwide [4] as dead residue. There is little information available on the differences in effects of decomposing isogenic and Bt-maize for collembola. Bt-toxin producing cotton (Cry1Ab and Cry1Ac, 10–25 µg $g^{-1}$ fresh weight) as food did not have a significant effect on oviposition, number of eggs and final body lengths of *Folsomia candida* [11]. Four Bt-toxins (Cry1Ab, Cry1Ac, Cry2A, Cry3A) in a concentration of 200 µg $g^{-1}$ did not influence the mortality of adults and the number of juveniles per adults in the case of *F. candida* and *Xenylla grisea* [8]. A recent study shows that population growth and reproduction of *Protaphorura armata* was similar when reared on Bt-maize varieties compared with non-Bt-maize [2]. However, *F. candida* reproduction was lower feeding on a mixture of Nutrient Agar, *B. thuringiensis* spores, parasporal crystals and vegetative cells than feeding on yeast [1].

Some more data are to be found in registration documents where the experimental conditions are not detailed. NOEC of Cry1Ab toxin was found to be 125 µg $g^{-1}$ in a study of adult survival and reproduction

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1164-5563/$ - see front matter 
doi:10.1016/j.ejsobi.2006.06.005

of *F. candida* [6]. No effect on mortality and reproduction of *F. candida* was found when lyophilized maize leaf (Cry1Ab toxin content estimated at 50.6 μg $g^{-1}$) was mixed by 50% to the diet [8]. NOEC of Cry1F toxin in maize leaves was higher than 12.5 μg $g^{-1}$ for *F. candida* mortality [8].

The results of our field experiments (not published data) showed that the feeding activity of soil biota (measured by bait lamina test) was lower in the Bt-maize than in the control plot soil. This is why laboratory feeding experiments were set up with collembola. Two questions were addressed:

- Are collembola equally distributed on residues of isogenic and Bt-maize?
- Do collembola show feeding preference to either of the maize types?

## 2. Material and methods

Isogenic and Bt-maize DK-440 BTY "YieldGard" (event MON810) were grown in an experimental field in 2002. The genetically modified maize plant we used produced Cry1Ab toxin. At harvest uniform senescent leaves of similar size and position on the plant were collected, dried, grinded and 1–2 mm long pieces were sieved. Plant material was stored at 4 °C until used. Some main parameters of the plants are presented in Table 1. The C and N content of plants were determined by Carlo-Erba NA 1500 elemental analyzer. Bt-toxin content was determined by ELISA test.

Three collembola species, *F. candida*, *Heteromurus nitidus* and *Sinella coeca* were used in experiments. Only adult individuals were tested. Animals were fed continuously before the experiment. All individuals were used only once in the experiments. Paired-choice assays were conducted in Petri-dishes. White filter paper was put on the bottom and kept wet continuously. Hundred milligrams of isogenic or Bt-maize was placed oppositely in the arena at the edge of dish. Two experiments were made. In the first experiment 20 collembolas were put in a Petri-dish of 7 cm diameter and two parameters:

- the number of animals;
- fecal pellets were counted after 12 hours.

The number of collembolas on the plant and the number of fecal pellets in a distance of 1 cm around the maize was counted (replicate number = 5). In the second experiment one collembola was put in a Petri-dish of 3 cm diameter. Fecal pellets of individually tested collembola were counted after 8 days. In the case of *F. candida* fecal pellets were counted the 4th and the 8th day after the beginning of an experiment which aimed at comparing maize preference of starved and not starved individuals. The number of fecal pellets near by the maize plant was regarded as representative of the food consumption (replicate number = 45–48). Preference of the starved *F. candida* was also tested. Collembolas were kept individually on a drop of water in small tubes without food for 10 days before the experiment (replicate number = 23). Experiments were conducted in total darkness at 20 ± 0.2 °C. The number of animals on maize and fecal pellets was compared for each species by paired *t*-test [3].

Table 1
Chemical characteristics of the Bt and non-Bt-maize used in the experiment

| | N (%) | C (%) | C/N | Cry1Ab-toxin (μg $g^{-1}$) |
|---|---|---|---|---|
| Isogenic | 0.27 | 41.92 | 155.3 | 0 |
| Bt | 0.29 | 40.52 | 139.7 | 3.45 (± 0.8) |

## 3. Results

In the first experiment significantly less *H. nitidus* individuals were found after 12 hours on Bt-maize than on isogenic one. The distribution of the two other species was not different (Table 2). Counting fecal pellets gave different results. *F. candida* produced significantly less fecal pellets ($P < 0.05$) at the Bt-toxin producing maize than at the isogenic maize. No such difference was observed in the case of the two other collembolan species (Table 2).

Similar results were found in the second experiment. The number of fecal pellets of *F. candida* was significantly ($P < 0.001$) higher at isogenic maize than at Bt-

Table 2
Number of collembolas on maize and number of fecal pellets around the maize (average ± S.D.) in the first experiment (see text)

| | | Individuals on maize (number ± S.D.) | Fecal pellet (number ± S.D.) | *N* |
|---|---|---|---|---|
| *F. candida* | Isogenic | 6.4 (± 6.2) | 30.0 (± 19.3)* | 5 |
| | Bt | 6.0 (± 4.0) | 21.8 (± 21.9) | |
| *H. nitidus* | Isogenic | 4.4 (± 2.2)** | 69.6 (± 26.8) | 5 |
| | Bt | 2.0 (± 0.7) | 61.6 (± 19.6) | |
| *S. coeca* | Isogenic | 6.6 (± 2.6) | 110.5 (± 14.4) | 5 |
| | Bt | 5.4 (± 3.7) | 120.5 (± 34.1) | |

*N* = number of individuals tested. Results of a paired *t*-test for each species. * $P < 0.05$; ** $P < 0.01$.

Table 3
Number of fecal pellets of three collembolan species, in the experiment with one collembolan per Petri-dish, 8 days after the beginning of the experiment

| | | Fecal pellet (number ± S.D.) | *N* |
|---|---|---|---|
| *F. candida* | Isogenic | 68.0 (± 28.9)*** | 48 |
| | Bt | 48.8 (± 26.2) | |
| *H. nitidus* | Isogenic | 37.7 (± 19.9) | 45 |
| | Bt | 34.1 (± 19.7) | |
| *S. coeca* | Isogenic | 30.7 (± 17.2) | 47 |
| | Bt | 31.3 (± 17.2) | |

Dishes without fecal pellets were excluded. *N* = number of individuals tested. Results of a paired *t*-test for each species. *** $P < 0.001$.

Table 4
Number of fecal pellets of individually tested *F. candida* 4 and 8 days after the beginning of the experiment. Starved and not starved individuals are compared. Dishes without fecal pellets have been excluded

| | | | Fecal pellet (number ± S.D.) | *N* |
|---|---|---|---|---|
| Not starved | After 4 days | Isogenic | 9.8 (± 10.7)* | 40 |
| | | Bt | 6.9 (± 7.5) | |
| | After 8 days | Isogenic | 68.0 (± 28.7)*** | 48 |
| | | Bt | 48.8 (± 26.2) | |
| Starved | After 4 days | Isogenic | 24.9 (± 18.6) | 23 |
| | | Bt | 22.1 (± 17.9) | |
| | After 8 days | Isogenic | 65.6 (± 28.6) | 23 |
| | | Bt | 59.3 (± 25.0) | |

*N* = number of individuals tested. Results of a paired *t*-test for each species. * $P < 0.05$, *** $P < 0.001$. Results of statistical comparison between non-starved and starved individuals are in the text.

maize, but *H. nitidus* and *S. coeca* specimens produced equal number of pellets at both maize types (Table 3).

Normally fed *F. candida* individuals produced significantly more fecal pellets at isogenic maize than at Bt-maize after 4 and 8 days ($P < 0.05$ and $P < 0.001$, respectively) (Table 4). The numbers of fecal pellets produced by starved individuals of the two maize types were not different. Significantly more pellets were left by starved than not starved collembolas after 4 days both at the isogenic ($P < 0.01$) and Bt ($P < 0.001$) maize. This difference disappeared after 8 days (Table 4).

## 4. Discussion

Differences in substrate selection of *H. nitidus* and preference of *F. candida* for isogenic maize proved that some collembolan species are able to discriminate between the two types of maize. The reason of this is not clear. Besides the toxin and lignin content of isogenic and Bt variant of maize varieties may also be different [7]. Bt-maize seems to be a less preferred and therefore probably a less usable food source for *F. candida* than the isogenic one. This may explain why Ke and Krogh [5] found lower reproduction of *F. candida* feeding on similar Bt-maize (MON810).

Bt-toxin concentration of the maize leaves used in this experiment was very low. Cry1Ab protein level in YieldGard maize plant is more than one order of magnitude higher (10.34 μg $g^{-1}$) according to the official data [9]. Therefore, food choice seems to be a sensitive measure of Bt-maize effect on collembolas. The number of fecal pellet is a more reliable measure of substrate/food choice than the number of animals. Our results show that the effect of the Bt-toxin producing maize on the collembolan is species specific. The results of the food preference tests were not influenced by the number of animals per Petri-dish. In spite of this fact, testing one collembola per arena seems to be a more simple and reliable method because intraspecific effects (e.g. pheromones [10]) are excluded using this type of tests.

## Acknowledgements

We are grateful to Prof. B. Darvas for involving us into the project tests and for two anonymous reviewers for valuable comments and improving the manuscript. This work was supported by the OMFB-00605/2004 grant.

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