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EFFECT OF Bt MAIZE MON 810 EXPRESSING CRY 1 Ab TOXIN ON *APHIDIUS COLEMANI* IN TRITROPHIC PLANT – HERBIVORE – PARASITOID SYSTEM

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Abstract: Effect of Bt maize MON 810 expressing Cry 1 Ab toxin on braconid *Aphidius colemani* in tritrophic plant – herbivore – parasitoid system was studied under greenhouse conditions by rearing the bird cherry-oat aphid (*Rhopalosiphum padi*) on genetically modified maize cultivar DKc 307 (MON 810) and its isogenic parent cultivar – Monumental of Monsanto Ltd. *R. padi* aphids reached higher population level when maintained on Bt-maize cultivar DKc 307 (MON 810) than on its isogenic parent cultivar – Monumental both in the winter and summer experiments. The braconid *A. colemani* developed higher population on *R. padi* aphid population feeding on Bt maize plants in the summer experiment and on non-Bt plants in the winter experiment. The observed effect of season on parasitation level by *A. colemani* on *R. padi* host feeding on Bt and non-Bt maize plants indicates that results obtained in a single greenhouse experiment may lead to questionable conclusions and should be confirmed by other experiments.

Key words: MON 810, unintended effects, non-target species, the bird cherry-oat aphid, *Rhopalosiphum padi*, *Aphidius colemani*

INTRODUCTION

Based on the national experience in growing genetically modified crops (GMC) with insect resistance, the Entomological Society of America (2002) has released its official statement with some key points reveal to natural enemies: (a) genetically engineered crops that express insect-pest resistance traits could facilitate a shift away from the reliance on broad-spectrum insecticides and toward biological-based pest management; (b) transgenic plants that produce insecticidal substances are and

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should continue to be subject to careful testing to ensure safety and minimise environmental risks, etc.

Studies on methods and techniques used in the evaluation of GMC effects on non-target organisms (NTOs) has started seven years ago with generous financial support from the EU programmes and national governments in the majority of the EU countries. The establishment of a new IOBC group – „*GMOs in Integrated Plant Production*" during the 2003 meeting in Praha (IOBC 2003) and its consecutive meeting in Lleida, Spain in 2005 (IOBC 2005) has stimulated discussion and sharing of expertise between European scientists on biosafety research of GMC, including unintended effects on natural enemies. There is presently consensus that the effect of GM plants on natural enemies should follow a step-wise (tiered) approach. Early tier (laboratory) tests should be conducted to determine whether an organism is susceptible to the toxin under a worse – case tests (10-times higher dose exposure) under standardized and repeatable conditions (Dutton *et al*. 2003; Hill and Sendashonga 2003). If risk have been identified or can not be ruled out, additional tier tests should follow exposing no-target organism to the toxin under more realistic conditions. Different techniques were used to measure toxic effect of GMC on natural enemies under laboratory conditions: (a) *via* prey organisms previously fed on GM plants; (b) *via* a toxin incorporated into artificial diet; (c) direct effect *via* GM plant excretes.

According to Romeis *et al.* (2006) the effects of Bt plant on hymenopterous parasitoids developing on herbivores reared on transgenic plants have been investigated in ten studies. The majority of cases were related to parasitoids of Lepidoptera species. The effects of maize and cotton cultivars expressing Cry toxins on mortality, development, weight or longevity of parasitoids were observed in cases where Bt-susceptible lepidopteran herbivores were used as hosts (Bernal *et al.* 2002; Baur and Boethel 2003; Meissle *et al.* 2004). The experiments have confirmed that host quality of a phytophagous species was most likely the cause of negative effect on a parasitoid.

It shall be noted that based on their own experiments, some other scientists have questioned a validity of laboratory tests as not reflecting realistic ecological scenarios by: providing no prey choice, single prey type and no combination of stress factors (Steinbrecher and Vidal 2005).

In our greenhouse studies on the role of transgenic crops on tritrophic relations we have included DKc307 maize cultivar with *cry 1Ab* gene (insert MON 810) and cv. Monumental, its isogenic line as representatives of the fist level; the bird cherry-oat aphid (*R. padi*), the common phytophagous species of cereals in Poland, as the second level and *A. colemani*, as the representative of the third level. *A. colemani*, an exotic parasitoid commonly used in controlling aphids in greenhouses in Europe was chosen as the bio-indicator. From technical point of view the availability of a species used in experiments on unintended effects due to the genetic modification was mentioned as an important factor in increasing the reproducibility of the tests in different laboratories (Romeis 2004; Dąbrowski and Górecka 2006).

MATERIALS AND METHODS

Seeds of the genetically modified cultivar DKc 307 Bt (F_1) and its isogenic line Monumental were provided by Monsanto Ltd. The bird cherry-oat aphid, *R. padi* was selected as a prey for parasitic braconid, *A. colemani*. The colonies of aphids were maintained in the Experimental Greenhouse Centre of the Faculty of Horticulture and Landscape Architecture, Ursynów. The aphid colonies were maintained on a traditional maize cultivar to eliminate the negative effect of host food changes when they would be traditionally reared on barley or wheat plants.

A stock colony of parasitoid *A. colemani* was supplied by the Rol-Eko Sp. company, the largest distributor of beneficial arthropods in Poland.

Ten plants in three replications of each maize cultivar were grown in plastic pots located on two separate shelves in the isolated greenhouse cabinets. The average temperature was maintained at 25°C level and 70% relative humidity, under 16:8 h photoperiod regime. Unfortunately, during the summer period, the temperature unexpectedly increased inspite of active ventilation system.

Ten young wingless aphid females were transferred from the stock colony onto maize seedlings of 4–5 leaf growth stage of two experimental maize cultivars. Number of progeny developed on experimental plants were counted three times in two week intervals. After 8 weeks 125 parasitized aphid mumies carrying pupae of *A. colemani* were transferred into four large cages covered by insect proof fine mesh: two cages for each cultivar. In addition, a glass dish with honey water solution were kept in the cages to provide food for adult parasitoids. Number of emerged adult *A. colemani* was estrimated by counting number of mummies with emerging holes after three weeks.

Data was analysed by a one-way analysis of variance by using STATGRAPHICS Plus 4.1 statistical package.

RESULTS AND DISCUSSION

Effect of MON 810 and its isogenic line on *R. padi* population development

The distinct differences in the *R. padi* population development were observed during the winter and summer experiment. During the winter experiment, the number of developed *R. padi* aphids was always higher on the transgenic MON 810 plants, unsignificant at the first counting period after two weeks. In the two following counts, at the 4^{th} and 8^{th} week, the differences were statistically significant. After eight weeks the *R. padi* population reached av. level of 258 aphids/plant on transgenic plants in comparison to only 186 on its isogenic line (Fig. 1).

Similar differences in the *R. padi* population development on GM and non-GM maize plants was observed in the summer experiment. Unexpected rapid temperature increase in the greenhouse (in spite of effective ventilation system) caused breakdown of the aphid population between the second and fourth week of the observations. After this period, the *R. padi* population reached av. level of 600 aphids/plant on the GM plants and 459/plant on non-GM maize plants (Fig. 1).

Population development of *A. colemani* on *R. padi* aphids feeding on GM and non-GM maize plants.

Comparison of *A. colemani* population development on cereal aphids feeding on MON 810 and its isogenic plants may indicate the indirect effect of GM plants on *R. padi* parasitation by *A. colemani*. Significantly higher percentage of parasitation was noted for *R. padi* population developing on non-GM maize plants than on MON 810 line during the winter experiment (Fig. 2). When eight parasitoids were released per

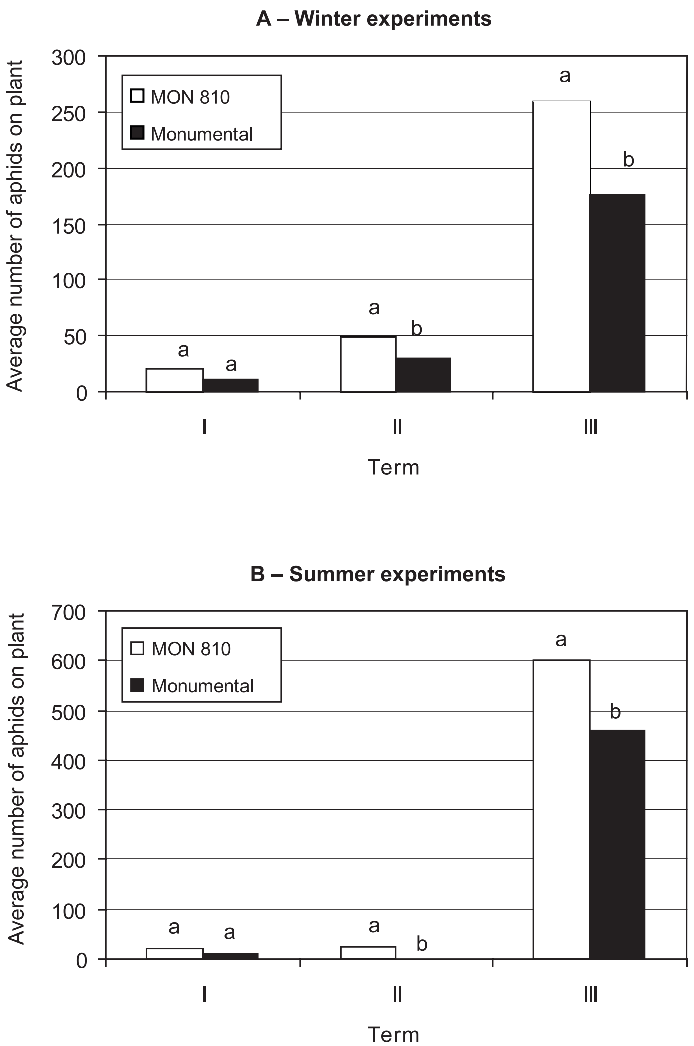

Fig. 1. Density of *Rhopalosiphum padi* population on Bt-maize cultivar DKc 307 and its isogenic parent – Monumental in: A – the winter and B – the summer greenhouse experiment.

non-GM plant at the beginning of experiment, the mummification level of aphids indicated three fold increase of *A. colemani* population and only 1.5 fold on the transgenic plants.

Opposite results were obtained during the summer experiment. Higher percentage of mummified aphids carrying pre-imaginal stages of *A. colemani* was noted on MON 810 plants. After three weeks from releasing parasitoids in cages with *R. padi* feeding on transgenic maize plants, their population increased 11 times, whereas on non-GM Monumental cultivar only 9 times (Fig. 2). However, the statistical analysis did not indicate significant differences in the level of parasitation of *R. padi* aphids reared on GM and non-GM maize plants.

Critical analysis of the percentage of mummified aphids in the total population of aphids on experimental plants showed rather low level of parasitation equalled to 20% (Fig. 2). The higher portion of mummiefied aphids in the total population of aphids was observed on the conventional control maize plants, in both winter and summer experiment. These differences between maize cultivars were unsignificant in the summer. Statistically significant lower portion of parasitized aphids by *A. colemani* on MON 810 plants than on its isogenic cultivar was noted in the winter experiment. Approximately two times higher mummification rate was observed on non-Bt maize plants than on transgenic ones (Fig. 2).

Our experimental results showing that the transgenic maize provided better conditions for the *R. padi* population development than its isogenic line may suggest that this phenomenon is specific to the original parent maize cultivar. Differences in biochemical composition of Bt and non-Bt maize lines were found for lignin content, indicating some unexpected phenotypic changes in biochemical composition of Bt maize tissues (Saxena and Stotzky 2001). Some authors carrying out field experiments did not observed a higher aphid population on Bt maize lines, others did. Lumbierres *et al.* (2004) noticed a higher density of *R. padi*, particularly alates and young nymphs on Bt plots at very young maize development stages, corresponding to the settlement period, in the 3 years field studies. The development and pre-reproductive times of the offspring of the first generation of alatae were shorter and the intrinsic rate of natural increase (rm) higher when aphids fed on Bt maize. However, no differences on the aphid population parameters were found by the authors among the offspring of apterous aphids maintained on Bt or non-Bt maize for several generations (Lumbierres *et al.* 2004). Their results agreed with those reported by Lozzia and co-workers (1998) and Lozzia (1999) in the sense that any differential effect that appears between Bt and non-Bt maize appears when the whole season was considered. Finally, the results obtained by these authors suggest that the economic effects on maize crops should not be expected.

Lathan and Wilson (2006) based on a large scale survey of the scientific literature were of opinion that the unexpected phenotypic consequences are common in transgenic plants. The authors however emphasized that most unexpected phenotypes reported had no known biosafety implications.

Bourguet and co-workers (2002) in their field studies carried out in two locations did not found significant differences (t – test, p = 0.05) between numbers of *Metapolophium dirhodum* (Walker), *R. padi* and *Sitobion avenae* (F.) on cv. Elgina (Monsanto hybrid MON 810) and cv. Cecilia (non-Bt maize). Under Southern Bohemia (Czech Republic) conditions, *R. padi* or *M. dirhodum* seemingly preferred either the Bt or the non-Bt maize, but statistical evaluation of the data collected over the season revealed that these differences were insignificant (Sehnal *et al.* 2004). However, under Spain field conditions, aphids of three species (*R. padi; S. avenae and M. dirhodum*) were in general more abundant on Bt maize (event 176) than on non-Bt control cultivar (Eizaguirre *at al.* 2006).

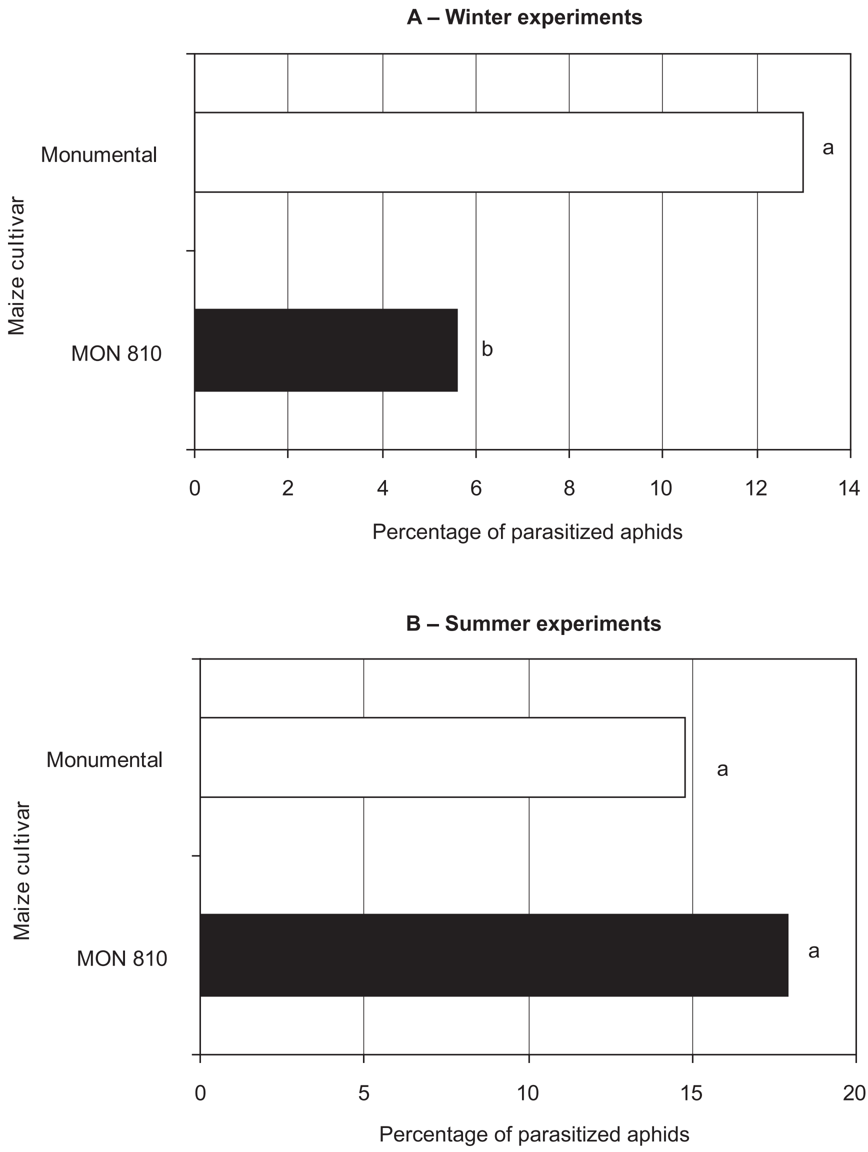

Fig. 2. Total parasitation of *Rhopalosiphum padi* aphids feeding on Bt and non-Bt maize plants by braconid *Aphidius colemani* as indicated by percentage of mummiefied aphids in: A – the winter and B – the summer experiment.

CONCLUSIONS

1. *R. padi* aphids reached higher population level when maintained on Bt-maize cultivar DKc 307 (MON 810) than on its isogenic parent cultivar – Monumental both in the winter and summer experiments.

2. The braconid *A. colemani* developed higher population on *R. padi* aphid population feeding on Bt maize plants in the summer experiment and on non-Bt plants in the winter experiment.
3. The observed effect of season on parasitation level by *A. colemani* on *R. padi* host feeding on Bt and non-Bt maize plants indicated that results obtained in a single greenhouse experiment may lead to questionable conclusions and should be confirmed by other experiments.

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POLISH SUMMARY

WPŁYW KUKURYDZY Bt MON 810 Z EKSPRESJĄ TOKSYCZNEGO BIAŁKA CRY 1 Ab NA PASOŻYTNICZĄ BŁONKÓWKĘ *APHIDIUS COLEMANI* W SYSTEMACH POWIĄZAŃ TRÓJTROFICZNYCH: ROŚLINA − SZKODNIK − PARAZYTOID

Wpływ kukurydzy MON 810 na błonkówkę *Aphidius colemani* w systemach trójtroficznych (roślina – szkodnik – parazytoid) był badany w warunkach szklarniowych poprzez hodowlę mszycy zbożowo – czeremchowej (*Rhopalosiphum padi*) na modyfikowanej genetycznie odmianie kukurydzy DKc 307 (MON 810) i jej linię izogeniczną Monumental z Monsanto Ltd. Mszyce osiągnęły wyższą liczebność populacji, gdy żerowały na kukurydzy Bt odmiany DKc 307 (MON 810) w porównaniu z linią izogeniczną, zarówno w doświadczeniach prowadzonych w terminie letnim, jak i zimowym. Pasożytnicza błonkówka *A. colemani* rozwinęła liczebniejszą populację na mszycach *R. padi* rozwijających się na kukurydzy Bt w terminie letnim, natomiast w terminie zimowym większą liczbę mumii uzyskano na roślinach niemodyfikowanych. Zaobserwowany wpływ sezonu prowadzenia doświadczenia na poziom spasożytowania *R. padi* karmionej kukurydzą z genem Bt i izogeniczną linią bez genu Bt przez *A. colemani* wskazuje, iż wyniki otrzymane w trakcie jednego doświadczenia w warunkach szklarniowych mogą prowadzić do niejednoznacznych wniosków i wymagają potwierdzenia dodatkowymi eksperymentami.