Damage of pepper cultivars in Austria by the European Corn Borer (*Ostrinia nubilalis*, Hübner)

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Abstract: In the last years infestation of pepper cultivars by the European corn borer (= ECB) could be observed frequently in Austria. The damage became obvious in August – September, corresponding to oviposition of ECB begun at the beginning of August. A mass release of *Trichogramma evanescens* lead to a decreased infestation with ECB larve, although effectivity was not sufficient. Chili varieties (hot tasting cultivars of *Capsicum annuum*) showed a very low infestation by the ECB in the field, which turned out to be the result of some antibiosis factor. It was shown that Capsaicin (responsible for the hot taste) was not the source of antibiosis. It is assumed that mechanical barriers prevent the entrance of the newly hatched larvae into the chili peppers: in these cultivars the calyx fits very closely to the fruit so that there are no gaps where newly hatched larvae could enter.

Key words: Ostrinia nubilalis, Capsicum annuum, susceptibility, capsaicin

Introduction

Farmers in eastern Austria have to face increasing damage of sweet peppers (Capsicum annuum) by the European Corn Borer (Ostrinia nubilalis Hübner, = ECB). Feeding sites of caterpillars inside the fruit with crumbly faeces and webbing material make the fruits unmarketable and are therefore a serious problem for the processing and deep freezing industry. In addition to the already known very low damage during the season, heavy damage has now developed also late in the season - in August/September. First symptoms were masses of crumbly faeces coming out of the mines. When larvae are inside the fruits it is too late for control measures. Diverging times for the flight activity of ECB are reported by Fülöp and Acsadi (1994) from Hungary and Berger (2000) for Austria. It was the aim of this study to get exact information on oviposition and larval hatching period of ECB as a basis for control measures. Larvae that had developed in fruits of pepper belonged to the so called Zrace of ECB (Witzgall, pers. communication), which is characterized by a special pheromonecomposition and is commonly found on maize (Lorenz & Langenbruch 1989). Because the egg parasitoid Trichogramma evanescens has been used very successfully for the control of ECB in maize (Hassan et al., 1990), a similar biocontrol trial was also performed in peppers. The low susceptibility of hot pepper cultivars (Chili) for ECB is already known in practice and is attributed mostly to Capsaicin – responsible for the pungent taste. The analysis of the assumed "resistance" should help to understand the possible role of antibiosis factors for ECB and facilitate predicting the susceptibility of new pepper cultivars.

Materials and methods

ECB laboratory culture

A laboratory stock culture of ECB derived from diapausing larvae from Pöttelsdorf (Burgenland, Austria) was established in 2002. Larvae were reared at 25°C and 18h photoperiod individually on a semiartificial diet based on agar, yeast and germinated beans (Shorey & Hale, 1965). Adults were kept at 20°C in plastic jars and laid their eggs readily on plastic foils, which were changed two times per week and stored at 20°C for hatching.

Experimental fields

The field experiments in 2001, 2002 and 2003 were carried out on fields having a size of approximately 3,5 ha located in HALBTURN (Burgenland, Austria). 7 different sweet and hot pepper varieties (among these a yellow bellpepper "Cece", a red tomato-shaped variety, a yellow apple shaped variety and a yellow chili "Santa Fé") were grown in rows each year – the planting date was around 15^{th} May. Plants were irrigated approximately 8 hours per season and were grown organically, which means that no pesticides and no synthetic fertilizers were used. The untreated control plot in 2001 was planted with the same cultivars, had a size of 400 m² and was situated 1 km westwards in upwind direction.

Oviposition period of ECB

Row sections 50 meters long of the above mentioned pepper cultivars were harvested every week beginning with 2nd August 2001. Collected fruits were dissected in the laboratory and controlled for any traces of ECB larvae. The developmental stage of young larvae was assessed by measuring headcapsule width.

Biocontrol of ECB in sweet pepper by mass release of Trichogramma evanescens

Trichogramma evenscens was released on the experimental field in 2001. As the parasitoids are able to disperse actively or drifted passively by the wind, the trial was not carried out in a field-plot design, but without replications and with an untreated control plot located in some distance. The commercial product "Trichokarte" produced by "AMW-Nützlinge GmbH" (Außerhalb 54, D-64319 Pfungstadt, Germany) was used as source of *Trichogramma* wasps. These had been reared on *Sitotroga cerealella* in the laboratory; parasitized eggs had been glued on cardboard, so that from each "Trichokarte" 2000 *Trichogramma evanescens* could escape over a period of 2 weeks continuously. The "Trichokarten" were distributed in the field on 2nd, 16th and 30th August 2001, so that at each date 200 000 *T. evanescens* hatched per ha. For the evaluation of infestation by ECB 50 meter long row sections of pepper cultivars were harvested every week, beginning with 2nd August 2001. Fruits were dissected in the laboratory and controlled for larvae, entrance holes, faeces and webbing of ECB larvae.

Susceptibility of sweet and hot pepper cultivars

Pepper fruits of the experimental field were infested manually on 1st and on 6th August 2002. Hereby 60 newly hatched larvae from the laboratory culture were transferred with a fine brush to ripening fruits both of a sweet (Cece) and a pungent (Santa Fé) pepper variety. Afterwards each fruit was covered by a small gauze bag. For evaluation the fruits were dissected 3 weeks later and controlled for infestation by ECB larvae.

To analyse possible antibiotic effects ripening fruits (having reached their final size) were harvested from the experimental field, cut to pieces of approximately 5 x 15 mm. Each piece was put into a glassvial. After transferring one newly hatched larvae into each of these vials it was closed with cottonwool and stored at 20° C / 18 h photoperiod. This test was done with 50 newly hatched larvae on a sweet (Cece) and a pungent (Santa Fé) pepper cultivar each. As a control 50 larvae living in vials filled with a semiartificial diet (Shorey & Hale 1965) were used. Development of larvae was controlled on the 6th day. Larvae with a head capsule width of approximately 0,3 mm were classified as 1st larval stage, those with 0,5 mm as 2nd stage.

Results and discussion

Oviposition period of ECB in a pepper field 2001

The first ECB larvae were found on 16th August 2001 during the weekly sampling programme (Fig. 1). The width of their head capsules was about 0,3 mm, which means that they were in the first larval stage. ECB-larvae need approximately 6 days to finish the first larval stage at a temperature of 20°C (see Tab. 2). As the mean daily temperature in Halbturn was 23°C (Anonymus, 2001) in that period, it can be concluded that oviposition of ECB in Halbturn started after the beginning of August, 2001. As hatching of adults from overwintering larvae starts in June (Berger, 2000) it is clear, that these egg-laying females belong to a second generation.



Fig. 1: Course of infestation of pepper fruits (*Capsicum annuum*) by ECB (*Ostrinia nubilalis*) in a field in Halbturn (Burgenland, Austria), 2001. The total number of observed ECB larvae was 143.

In Austria *O. nubilalis* usually develops one generation per year (Berger, 2000). When the temperatures are very high, a second generation can also be observed (Cate, 2003). It seems that damage to sweet pepper is correlated with the appearance of this second generation: when females hatch from overwintering larvae in June only very few pepper plants with ripening fruits are available whereas corn plants are already well developed. For females hatching from 1st generation larvae most pepper plants are in the appropriate condition for development of ECB larvae, whereas corn is already becoming dry. Fülöp & Acsádi (1994) also report that ECB has several generations in Hungary with flight activities in pepper during May/June, July and late August.

Biocontrol of ECB in sweet pepper by mass release of Trichogramma evanescens

Preceding observations had shown different susceptibility of various pepper cultivars to ECB, so that the infestation level had to be recorded separately for each cultivar. In our experiment the release of *Trichogramma evanescens* reduced the infestation of pepper fruits by ECB larvae by 72,3 - 50% (Tab. 1). For practical purposes this is not sufficient, because remaining infestation of 8-13,6 % is far too high. This weak effect is in contrast with good effectiveness

of *Trichogramma evenscens* in corn reported by many authors (e.g. Hassan et al., 1990). This minor effectiveness possibly is the consequence of the exposure to wind, which is characteristic for the plains of eastern Austria. In contrast cornfields form a more structured ecosystem similar to a forest, where wind is retarded and *Trichogramma* wasps are likely to be more protected.

Table 1: Infestation of fruits (in %) of seven	al pepper cultivars by	ECB larvae a	after release of
Trichogramma evanescens			

Cultivar	"Cece"	Apple-shaped	Tomato-shaped	"Santa Fé"
Untreated	18,8	15,5	22,8	0,5
Trichogramma	13,6	7,9	11,9	0

Susceptibility of sweet and hot pepper cultivars

In the biocontrol trial with *Trichogramma* different infestation levels of sweet and hot pepper cultivars were observed (Tab. 1). This agrees very well with farmers' experiences. This could be the result of avoidance during oviposition as well as of antibiosis factors preventing the development of ECB larvae. To exclude the first-mentioned factor, ripening sweet and hot pepper fruits in the experimental field were infested manually with newly hatched larvae of ECB. Three weeks later the sweet pepper variety was infested to a very high degree whereas the hot pepper cultivar (Santa Fé) only at a insignificant level (Fig. 2). The result was nearly the same when the experiment was replicated one week later.



Fig. 2: Infestation of a sweet and hot pepper cultivar by ECB larvae 3 weeks after manual infestation with newly hatched larvae from a laboratory culture. The trial was conducted on 2 different dates

This shows that the low infestation of hot pepper cultivars for ECB in the field is caused mainly by antibiosis factors. Nevertheless avoidance of the fruits during oviposition may appear additionally. At first sight the capsaicin which is responsible for the hot taste was thought to be this antibiosis factor. Weissenberg et al., 1986, had already shown that capsaicin has some growth-retarding activity for caterpillars of the noctuid moth *Earias insulana*. Nevertheless, other factors could also be responsible for low susceptibility of pungent pepper varieties. In order to analyse possible antibiosis effects in a first step, newly hatched ECB larvae were transferred to pieces of the fleshy peel of sweet and hot pepper fruits. For evaluation of this experiment the development of the first larval stage of ECB larvae was recorded.

Larval food	Number of L ₁	Number of L ₂	Number of dead larvae
Semiartificial diet	23 (47,9%)	25 (52,1%)	2
Sweet pepper (Cece)	25 (53,2%)	22 (46,8%)	3
Hot pepper (Santa Fé)	27 (56,2%)	21 (43,7%)	2

Table 2: Numbers of each larval stage of ECB after developing at 20°C in fruits of sweet and hot pepper varieties, 6 days after hatching ($L_1 = 1^{st}$ larval stage, $L_2 = 2^{nd}$ larval stage).



Fig. 3: Shape of the calyx region of a susceptible pepper cultivar (variety "Cece" on left side) and a resistant one (variety "Santa Fé" on the right side). Take notice of the gaps between the fruit calyx and the peel of the fruit (arrow) which probably facilitates the penetration by newly hatched ECB larvae. (Photo AGES/Kahrer)

The development after 6 days was very similar for a mild (Cece) and for a hot cultivar (Santa Fé) (Tab. 2). This shows that possible chemical antibiosis factors in the fleshy peel of the fruit play only an inferior role for the development of the larvae. It seems very probable that the main antibiosis factor is rather a mechanical barrier which prevents newly hatched ECB larvae from entering the fruit. Under field conditions 95 % (sample size 143) of the entrance holes for ECB larvae were found in the region of the fruit calyx.

This region is shaped differently in susceptible and resistant varieties: in hot pepper varieties (as Santa Fé) the cuticle of the fruit fits very closely to the fruit calyx whereas sweet pepper varieties (as Cece) show many gaps (Fig. 3, arrow), which allow newly hatched ECB larvae to enter. The capsaicin content of pepper fruits was shown to be responsible for deterrence of small mammals like mice (Tewksbury, 2001) which are thought to affect propagation of the seeds, whereas it is shown here to have practically no or at least only minor influence on newly hatched ECB larvae.

Acknowledgement

I am greatly indebted to Mrs. Ottendorfer, Tabandeh, Michalsky and Hinnert for their assistance in practical works, Mr. Stekovics for making the experimental field available and Mr. Cate for reading and commenting the manuscript.

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