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RESEARCH ARTICLE **LIFE SCIENCE**

TRITROPHIC INTERACTION BETWEEN THE INVASIVE INSECT PEST *DIABROTICA VIRGIFERA VIRGIFERA*, ITS HOST PLANT MAIZE AND ENDOPHYTIC FUNGI, *ACREMONIUM STRICTUM*

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Abstract: Root feeding experiments were performed to determine the effect of endophytic fungus, *Acremonium strictum*, on the feeding behavior of *Diabrotica virgifera virgifera* LeConte larvae (western corn rootworm = WCR) at the Institute of Plant Pathology and Plant Protection, Georg-August-University, Goettingen, Germany. Maize (*Zea mays*) plants were inoculated by endophytic fungus, *A. strictum and* the roots were fed to WCR larvae in newly adopted experimental test tubes. Three parameters, viz. weight gained by larvae after root feeding; the amount of ingested food and food conversion efficiency (ECI) were measured. The fungus reduced the root feeding of WCR larvae; however the results were not statistically significant. The weights gained by larvae were higher in control than treatment. The ECI value of control was significantly higher than *Acremonium* treatment. Results suggest that this fungus can reduce the suitability of roots of maize plants to WCR larvae.

Key words: insect, endophytic fungi, maize, alkaloids

Introduction

Diabrotica virgifera virgifera (Coleoptera, Chrysomelidae, Galerucinae), the western corn rootworm (WCR), is a major pest of maize, *Zea mays* L. (Gramineae) and the larvae feed on root-hairs and cortical tissue (Chiang, 1973), resulting in root-pruning and destruction of plant-roots (Branson, 1986). WCR was first reported to attack maize in the United States of America in 1909. At present infestation of WCR causes an approximate loss of one billion dollar per year in the USA (Pleau *et al*., 2002). In Europe, since its introduction near Belgrade in 1992, WCR is spreading rapidly causing extensive damage of maize. Today the insect cause economic damage to maize in at least 14 European countries causing an economic damage of about 500 m Euro per year in predicted in the EU states (Moeser and Vidal, 2005). Therefore the ecology, biology, feeding behaviour, interaction with other organisms and control measure of WCR need to be studied intensively.

Fungal endophytes have a very intimate and most likely co-evolutionary relationship with their host plant, and therefore have the potential to influence the plant defences (Wilson, 1993). They are able to increase the resistance of their host plants to insect herbivores via the production of toxic alkaloids (Clay, 1996). However, there are numerous species of root colonizing endophytes, whose effects on insect-plant interactions are not yet studied (Dugassa *et al*., 1998). *Acremonium*

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spp. is an endophytic fungus which colonizes in living plant tissues without causing significant visible symptoms (Dugassa *et al*., 1998). Wilson (1993) showed that this fungus can influence the outcome of plant-herbivore interactions. Endophytic fungi in grasses have been shown to confer anti-herbivore properties to their hosts (Christensen and Latch 1991; Clay, 1996). Results from laboratory feeding trials suggest that many insect herbivores preferred uninfected plants over the infected to such an extent that uninfected plants were unable to compete with infected plants (Clay, 1996). Therefore the present study was undertaken to assess the role of *Acremonium* fungi on the amount of root feeding, weight gained and food conversion efficiency of WCR larvae.

Materials and Methods

Maize seeds were sown in 20, 9 cm diameter pots. A single seed was placed in each pot containing 50% potting earth (Ökohum GmbH, Herbertingen, Germany), 50% sand. The seeded pots were placed in a green house allowing plants to grow for seven weeks. Ten plants were grown for treatment and 10 plants for the control.

A. strictum spores were added with liquid media. *A. strictum* was cultured in 2% Malt Extract Broth (Buchs, Switzerland) for one month in a shaker at 28 °C. The cultured suspension was filtered with 160 mm filter paper and with the help of water jet pump to remove the mycelium. Then the spores were counted using a Thoma chamber. The spore concentration was 7×10^6 spores ml^{-1}. The obtained spore solution was divided in two equal parts. The first half was diluted to the final concentration of $1x10^6$ spores ml^{-1}. The other half was filtered three times to remove the spores by using 70 mm filter paper and diluted to a similar degree as the spore suspension. This spore free solution was used for the control plants. Seventy mililiter solution per plant was used for the inoculation and for the control.

After 7 weeks the roots were collected by uprooting the plants and were washed properly with running water to clean them completely from soil particles that could influence the measurements. Medium sized to thicker roots (ca 2 mm) were cut (6-10 mg in weight) from the primary roots from below 5 cm of stem up to 20 cm.

WCR larvae used in this study were obtained following the protocol derived from Jackson (1986). Second instar larvae from 1.0 mg to 2.0 mg fresh weight were used. In root feeding study the protocol described by Moeser (2003) were followed: the experimental device consisted of 10 cm long and 1 cm wide glass test tube closed with plastic plug. The test tubes were filled half with plaster of Paris mixed with activated charcoal (Merck GmbH, Germany). This mixture acted as an indicator for humidity, if the moisture level was sufficient, the plaster of Paris retained its dark grey colour whereas it turned almost white when dry. Vermiculite (Klein GmbH, Zellertal, Germany) was used as a substitute for soil and also as a moisture buffer.

A single larva and a cut portion of root were weighed and put in each test tube. The roots were embedded with vermiculite. The moisture content was adjusted to a suitable level by adding 2 to 3 ml water to each test tube. The test tubes were closed with plastic plugs and kept in dark place (Chiang, 1973) at 26 °C and 60% RH for 6 days. After 6 days, the roots and larvae were collected and dried for two days at 100 °C to obtain the final dry weight. Forty replications were done for each treatment.

The initial weight of the larvae and roots were fresh weight while the final weights were taken after drying. Therefore, aliquots were done. Thirty samples of roots and larvae of each treatment were used for aliquots. To obtain aliquots for the roots or larvae, the fresh weight was measured. After a drying period of two days at 100 °C the dry weight was taken. The correlation between the

variables was calculated and a regression was obtained, which was used to calculate the initial dry weight of the samples in the experiment (Fig. 1).

The utilization of food was measured by calculating gravimetrically determined nutritional indices, E.C.I (Efficiency of conversion of ingested food) (Waldbauer, 1968) using the following equation:

$$E.C.I = \frac{Wt\ gained\ larvae(dry\ weight)}{Wt\ \ food\,ingested\ (dry\ weight)} \times 100$$

Wt gained: The difference between initial and final weight of the larvae.

Wt food ingested: The difference between initial and final root weight.

Results

The weight gained by larvae feeding on the roots of *Acremonium* control was higher than that of *Acremonium* treated. But the difference was not statistically significant ($F = 0.28$, $df = 1$, $p = 0.599$; Fig. 2). A similar result was also found with regard to the amount of ingested food by larvae feeding on *Acremonium* treated roots compared to the controls ($F = 1.183$, $df = 1$, $p = 0.283$; Fig. 3). Here, WCR larvae were eaten more root tissue of *Acremonium* control than that of *Acremonium* treated. However, a little difference was observed between ECI values. The ECI value of *Acremonium* control was higher ($F = 1.792$, $df = 1$, $p = 0.188$) than *Acremonium* treated, though that was non significant (Fig. 4).

Discussion

A negative effect of endophyte fungi was found, on the feeding behavior and food conversion rate of WCR larvae, though it was statistically non significant. But still this result supports the results from several other studies, which showed that endophyte fungi confer anti-herbivore properties to their hosts (Christensen and Latch 1991, Clay 1991).

The endophyte fungi can establish a very intimate relationship with the root of plant (Wilson, 1993). So there might be some mechanisms, which are very closely related to the anti-herbivore properties. Clay (1996) stated that the endophytic fungi produce a variety of alkaloids that make the host plants toxic or distasteful to herbivores. We observed that the amount of eaten root tissue by WCR larvae was higher in control plants compared to the infected plants. A similar result was also seen in weight gain. An even bigger difference was found in the food conversion rate where the ECI value of infested treatment was lower than control. It shows that the efficiency of converting the root tissue into body substances was reduced by *A. strictum* infestation. Perhaps, the alkaloid substances produced by endophytes change the contents of root tissue, and decrease the conversion efficiency to body biomass. Another mechanism is that endophytic fungi can change the biochemical composition of plant. Dugassa *et al*. (1998) observed that endophytic fungi can change the phytosterol composition of brussel sprouts, which resulted in reduced growth rate of diamondback moth. Our mean value (± SE) results show the trend (Fig. 2, 3 and 4), which support several evidences. But ANOVA did not show significant differences, probably due to sample size. The findings of the present research work can act as guidelines for conducting future works in the use of *Acremonium* fungus as control agent of WCR.

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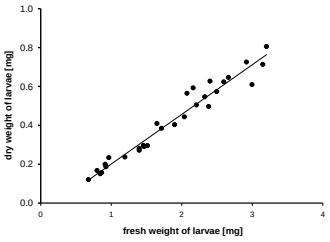

Fig. 1. Correlation between the fresh and dry weight of WCR larvae (f = -0.0547+0.2555*x, r = 0.96, p <0.001).

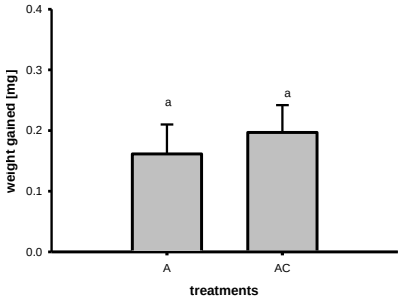

Fig. 2. The mean value of dry weight gained (± SE) by *D. v. virgifera* larvae feeding on the roots treated by *Acremonium* (A) and control (AC). Same letters above bars indicate non significant (ANOVA).

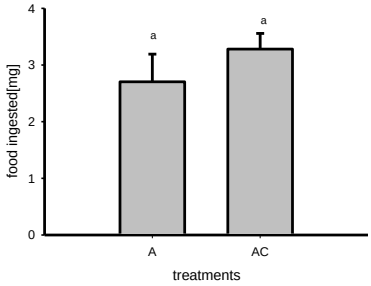

Fig. 3. The mean value of the amount of ingested food in dry weight (± SE) by *D. v virgifera* larvae feeding on the roots treated by *Acremonium* (A) and control (AC). Same letters above bars indicate non significant (ANOVA).

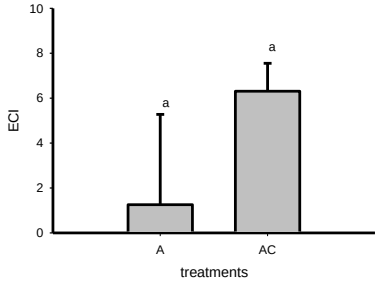

Fig. 4. The mean ECI values (± SE) of root feeding experiment from *D. v. virgifera* feeding on the roots treated by *Acremonium* (A) and control (AC). Same letters above bars indicate non significant (ANOVA).