INTRODUCTION

Herbivorous insects need to find suitable host plants for oviposition and larval feeding. In addition to finding the right plant species, they also need to assess the quality of the individual plants within a given species. Host plant choice in insects is governed by many factors (Bernays 2001). Factors directly relating to the nutritional quality of the food plant include the levels of primary as well as secondary metabolites. Although the former category includes basic nutrients, secondary metabolites can interfere with digestion or act as toxins. In addition, host plant choice may be driven by ecological factors, such as competition and mortality risk.

Inter- and intraspecific competitive interactions have recently been identified as important drivers of the performance and distribution of phytophagous insects (Kaplan and Denno 2007). These interactions can be direct, for example, through interference and exploitative competition. However, many interactions between herbivores were found to be indirect, often mediated by plant responses to herbivory (Ohgushi 2005; Kaplan and Denno 2007). These indirect interactions depend on the spatial distribution and the temporal persistence of the plant response (Wäckers and Bezemer 2003; Kaplan and Denno 2007).

Many plants respond to herbivory by activating induced resistance mechanisms. Induced resistance may benefit plants in 3 ways. First, it can reduce the plant’s attractiveness to ovipositing herbivores. Second, it can reduce herbivory by the phytophagous stages (larvae and/or adults). Third, it may allow plants to unload larval herbivores onto neighboring plants with which the induced plant competes for light and nutrients (Edwards and Wratten 1983; Tuomi et al. 1994; van Dam et al. 2001). Induced resistance mechanisms include the production of both volatile and nonvolatile secondary metabolites (Karban and Baldwin 1997; Turlings and Wäckers 2004). These herbivore-induced compounds can be a key factor in mediating herbivore-herbivore interactions and in structuring insect communities (Kessler and Baldwin 2004). Only few studies to date have linked plant-mediated competition between herbivores to the well-documented induction of plant secondary metabolites in response to herbivory (Kaplan and Denno 2007). For example, it has been shown that wild radish plants damaged by Pieris rapae show increased glucosinolate levels, corresponding with poorer performances by Lepidoptera, aphids, and a leafminer (Agrawal 1999). Herbivore damage induced volatiles as well as extrafloral nectar have been shown to be widely exploited by natural enemies of herbivorous insects to locate plants with host prey (e.g., Dicke et al. 2003; Turlings and Wäckers 2004). There are also examples showing that these volatiles can be attractive or deterrent to herbivorous insects (e.g., Bolter et al. 1997; De Moraes et al. 2001; Kessler and Baldwin 2001). However, relatively, little is known about how herbivores respond to
damage-induced changes in plant chemistry during host plant choice (Dicke et al. 2003).

Most studies on host plant choice have focused on above

ground interactions (van Dam et al. 2003). There is increasing appreciation that induced plant responses can also drive interactions between organisms below- and aboveground (e.g., van der Putten et al. 2001; Wardle et al. 2004; Bezemer and van Dam 2005; Erb et al. 2008). There are now many examples showing that the growth and development, and one example where oviposition decisions (Soler et al. 2010), of aboveground herbivores are affected by belowground her

bivory. Furthermore, it has been shown that belowground herbivory can induce changes in the plant that influence the behavior and performance of natural enemies of above

ground herbivores (Wäckers and Bezemer 2003; Rasmann and Turlings 2007; Soler et al. 2007).

In cotton, a number of studies have linked secondary metab

olite induction to adverse fitness consequences for the induc

ing herbivore (Alborn et al. 1996; McAuslane and Alborn 2000; Bezemer et al. 2005). Induction of terpenoid aldehydes in cotton has also been reported to underlie plant-mediated indirect interactions, both between spider mites and a fungal pathogen (Karban et al. 1987) and between root- and shoot

feeding herbivores (Bezemer et al. 2004). Moreover, detailed studies on the distribution patterns of induced secondary metabolites in cotton showed that the site of herbivory has a distinct impact on the pattern in which cotton secondary metabolites are allocated (Bezemer et al. 2003). Belowground feeding by wireworm larvae Agrotis lineata L. (Coleoptera: Elateridae) moderately increases the levels of terpenoids in all true leaves, whereas aboveground feeding by the Egyptian cotton leaf worm, Spodoptera littoralis Bods. (Lepidoptera: Noctuidae), elicits induction in the developing leaves only (McAuslane et al. 1997; Bezemer et al. 2003). These induced changes in plant metabolites and their specific allocation pat

terns subsequently affect performance of herbivore larvae (Bezemer et al. 2004; Anderson and Agrell 2005).

Although there is increasing evidence that herbivores in
teract indirectly by altering plant nutritional composition, very little is known about how plant-induced changes mediate behavior of different herbivores using the same host plant. We need to understand the full complement of these behavioral impacts in addition to the nutritional implications if we want to fully appreciate the functioning of induced resistance. Herbivore-induced changes in cotton elicted by conspecific larvae have been shown to influence oviposition and feeding behavior of S. littoralis (Anderson and Alborn 1999; Anderson et al. 2001; Anderson and Agrell 2005). In this study, we examine whether the behavioral re

sponse of an aboveground herbivore reflects the distinctly different patterns of induced plant responses in wireworm and Spodoptera induced plants. To study these indirect interspecific interactions, we used the same methods and herbi

vores as used in our previous studies, that is, S. littoralis as aboveground herbivore and A. lineata as belowground her

bivore. Specifically we investigate if systemic responses in cotton due to root feeding by wireworms influenced host choice by the mobile stages of S. littoralis, addressing both the ovipositing behavior of gravid females and plant leaving behavior by feeding larvae.

**MATERIALS AND METHODS**

**Insects**

The S. littoralis were obtained from a laboratory culture and reared on a semisynthetic diet (Hinks and Byers 1976) in Alnarp at 25 °C, 70% relative humidity (RH) and a 16:8 h light:dark cycle. Larvae were reared individually from the third instar until pupation. The pupae were sexed and the sexes kept in different rearing chambers until used in the experiments. Wild-collected moths are annually intro

duced into the culture. Wireworms, A. lineatus, were collected in pastures close to the Wageningen University in The Nether

lands. In the laboratory, they were kept in planting soil at the same environmental conditions as the S. littoralis culture and were provided potato as food. Before each experiment, the wireworms were extracted from the soil and transferred to the experimental plants.

**Plants**

Cotton plants, Gossypium hirsutum (var. Delta Pineland 90), were grown individually from seeds in pots (Ø = 14 cm) and kept in a climatized greenhouse at 25 ± 2 °C and 70 ± 10 RH. During October to March, artificial light (Osram Powerstar, HQI-T, 400 W/D, Daylight) was provided in addition to natural light. The plants had 7–8 true leaves when used in the experiments and had not started to develop flower buds. True leaves were numbered from below.

**Oviposition experiment**

Eight late stage wireworms were transferred on to the soil surface of the pots containing cotton plants with 4–5 true leaves. The wireworms went down into the soil within minutes from the transfer. The infested plants together with the plants designated to be control plants were moved to an experimen
tal greenhouse where the experiments were performed. The wireworms were allowed to feed on the roots for 3 weeks. At the start of the oviposition experiments, 2 treated plants and 2 control plants were placed at opposite sides of a cage (120 × 80 × 60 cm). The treated plants did not show any visible effects of the wireworm feeding and did not visually differ from the control plants. Plastic cups, containing 6 female and 8 male pupae of S. littoralis that were about to emerge, were placed between the 2 treatments in each cage. After the first egg batch was found in a cage, the number of egg batches on each plant was recorded for another 4 days. Each morning, the egg batches were gently collected and weighed individually. The number of eggs in each batch was estimated based on the weight of the egg batch. Student’s t-test (paired samples) was used for analyzing the results from the oviposition experiments.

**Larval feeding experiment**

In a 2-choice feeding bioassay, larvae were allowed to feed on and move between a damaged plant and an undamaged cotton plant. The plants were either damaged by wireworms or by S. littoralis larvae. The infestation by wireworms followed the same procedure as in the oviposition experiments except that the wireworms were allowed to feed 8 days on the plants before the start of the experiment. To prepare S. littoralis–damaged plants, two third to fourth instar larvae were put in a 3l plastic bag, which was placed over the second true leaf. The bag was sealed around the leaf petiole close to the stem of the plant using a twister. The plastic bag and the larvae were removed after 24 h when the larvae had eaten approximately half of leaf tissue. For both treatments, the feeding experiments were started 8 days after the plants had been exposed to the initial herbivores. This time period has been found to be sufficient for cotton plants to exhibit induced changes that affect S. littoralis larval feeding behavior (Anderson et al. 2001). The plants had 7 true leaves when damaged and about 8 true leaves when the experiment
started. As a control, we also performed an experiment where we recorded the larval movement between 2 initially undamaged plants.

In each bioassay, the pots of the 2 plants were placed approximately 10 cm apart, so that there was contact between leaves of both plants on 2–3 places. The larvae could move between the plants through these leaf connections but also on a cardboard “platform” (50 × 20 cm) that was placed on top of the pots. Thus, there was contact between the leaves of the 2 treatments but no root contact.

At the start of the experiment, a late third instar *S. littoralis* larvae from the rearing culture was placed on the third true leaf of the damaged plant or in the control experiment on one of the undamaged plants. The position of the larvae was then noted every second hour for 10 h the first day. This recording schedule was repeated on the second and third day. The experiment was ended after 72 h. At each time, the estimated amount of leaf tissue eaten and the feeding site were also noted. At the end of the experiment, the total amount consumed and the position of the eaten plant material was measured. This was done by collecting the leaves, photocopying them, and subsequently estimating the amount consumed. This was done by collecting the leaves, photocopying them, and subsequently estimating the amount consumed on a 5 × 5 mm grid. Chi-square tests were used for the analysis of the larval distribution data and Student’s *t*-test (paired samples) was used for analyzing the results from the consumption of leaf material.

**RESULTS**

The females laid significantly more eggs on the undamaged plants compared with plants damaged by wireworms (*P* < 0.001, *n* = 10) (Figure 1A). On average, 81% of the eggs were deposited on the undamaged plants. The difference in the distribution of eggs was manifested both in the number of eggs batches deposited (Figure 1B) and in the mean number of eggs per batch deposited (Figure 1C). On average, the 2 undamaged plants in a cage received 6.0 ± 1.7 egg batches, whereas 2.7 ± 1.4 batches were deposited on the wireworm-damaged plant (*P* < 0.001, *n* = 10). On undamaged plants, the females deposited an average of 424 ± 178 eggs/batch (*n* = 60), whereas on the wireworm-damaged plants, the average number of eggs/batch (*n* = 27) was 213 ± 104 eggs (*P* < 0.001).

A higher percentage (88%) of the larvae left the plants damaged by wireworms when compared with plants damaged by *S. littoralis* larvae (31%) or movement between undamaged plants (25%) (degrees of freedom [df] = 1, *P* < 0.01, *n* = 16) (Figure 2). Larvae did not show an increased tendency to leave *Spodoptera*-damaged plants when compared with movement between the 2 undamaged plants (df = 1, *P* > 0.05, *n* = 16). Most of larvae left the wireworm-damaged plants early in the experiment. Already 34 h into the experiment, 75% of the larvae had left the wireworm-damaged, whereas only 19% had left the *S. littoralis*-damaged plants or the undamaged plant in the control experiment (df = 1, *P* < 0.01, *n* = 16). The proportion of feeding on the release plant relative to the overall feeding was also different between the treatments (Figure 3). In the *S. littoralis*-damaged plants, 87% of the leaf material was consumed on the damaged plant, in contrast to 44% in case of the wireworm damaged (*P* < 0.05). This pattern was already evident after 24 h, with an estimated 93% proportion of the feeding on the *S. littoralis*-damaged release plant and 54% on wireworm-damaged plants (*P* < 0.05). In the control experiment, the percentage consumption allocated to the release plant was 85% after 24 h and 91% after 72 h, which was significantly different from the wireworm damaged (*P* < 0.05) but not from the *Spodoptera* damaged (*P* > 0.05).

**DISCUSSION**

This study shows that feeding by a belowground herbivore influences behaviors connected to host plant choice of an aboveground herbivore. Root feeding by wireworms affects both oviposition decisions by adult *S. littoralis* and increases plant-leaveing behavior by feeding larvae. These indirect interactions are mediated through induced responses in the shared host plant. In nature, plants are normally exposed to multiple attackers that are likely to induce plant resistance mechanisms. Being able to recognize the presence of both intra- and interspecific competitors or their impact on host plant quality allows ovipositing herbivores to optimize conditions for their progeny. Behavioral interactions between herbivores inhabiting the same plant have been found previously, but these are examples of herbivores that feed on the same part of the plant (Shiojiri et al. 2002; Rodriguez-Saona et al. 2002).

**Figure 1**

Two-choice oviposition experiments where *Spodoptera littoralis* females could lay eggs on either undamaged cotton plants (filled bars) or plants infested with wireworms, *Agriotes lineatus* (open bars): (A) the percentage of eggs deposited; (B) the mean number of egg batches; and (C) the mean number of eggs in each egg batch. Paired *t*-test, *n* = 10. Bars indicate standard deviation.

**Figure 2**

Larvae were allowed to move between and choose to feed on a damaged or on an undamaged cotton plant. The plants were either damaged belowground by wireworms (*Agriotes lineatus*) or aboveground by *Spodoptera littoralis* larvae. In each tested pair of plants, a single third instar *S. littoralis* larva was placed on the third true leaf of the damaged plant. In addition, larval movement between 2 undamaged plants was observed. The graph shows the cumulative number of experiment plant pairs where the larva had left the first plant and moved to the other plant at the different observation times. Differences between the 2 damage treatments were tested after 34 (df = 1, *P* < 0.01, *n* = 16) and 72 h (df = 1 *P* < 0.01, *n* = 16) using chi-square test.
et al. 2005). In the latter study, Rodríguez-Saona et al. (2005) found that females of *S. exigua* laid fewer eggs on plants damaged by conspecific larvae than on undamaged plants, whereas they laid more eggs on plants damaged by aphids compared with undamaged plants. Furthermore, they found that feeding by aphids disrupted resistance induction by conspecific larvae.

In this study, we find interspecific behavioral interactions between 2 herbivores that do not compete directly as they feed on different parts of the plant. Another recent study has also found that root herbivory can affect the distribution of eggs of aboveground herbivores (Soler et al. 2010). These results corroborate the findings by Kaplan and Denno (2007) that interspecific competition between different herbivore species may be an important factor during host plant choice by insects. Furthermore, as postulated by Kaplan and Denno (2007), it does not seem that competition is damped by resource partitioning. Niche division does not function as an escape from competition in situations in which the plant response to herbivory is systemic (Wäckers and Bezemer 2003; Kaplan and Denno 2007). Our study shows that effects of systemically induced resistance mechanisms influence the distribution and population dynamics of herbivore species occupying a distinctly separate niche.

We found that feeding by wireworms not only reduces the number of egg batches laid by *S. littoralis* females on the damaged plants but also the number of eggs per batch. This implies that the female recognizes host plant quality and that this is expressed both in terms of the decision whether or not to oviposit as well as in terms of the number of eggs laid. Oviposition behavior can be divided into different phases that include behaviors performed both pre- and postalighting (Renwick 1989; Janz 2002). From our experiments, we cannot conclude whether the decision to lay eggs and the size of the egg batch is taken prealighting or after direct contact with the host plant. However, our results suggest that 2 different decisions are involved: first, whether to lay eggs and second, what proportion of the overall egg load to allocate to the plant. Induced volatiles from herbivore-damaged plants have been shown to affect upwind flight and plant approach in insects (e.g., Bolter et al. 1997; De Moraes et al. 2001; Kessler and Baldwin 2001). However, the selected plant for landing in a cohort of plants may not be the plant that eventually is chosen for oviposition (Landolt 1993). Thus, further assessment of plant suitability takes place after alighting.

It is not well known that clutch size decisions during oviposition are influenced by host plant quality (Asmack and Leather 2002; Bergström et al. 2006). There are examples where females adjust clutch size to the size of the plant, thus accounting for the resources available for the progeny (Janz 2002). It has also been found that females lay larger egg batches on preferred host plants compared with low-ranked plants (Bergström et al. 2006). Here, we found that females of *S. littoralis* adjust clutch size depending on herbivore-induced changes of individual plants within the same plant species. This is also in line with the finding that cotton leaves that are preferentially chosen for oviposition by *S. littoralis* receive larger egg masses than less-preferred leaves (Sadek 2011). Reduced clutch size allocation to damaged plants can be an adjustment to lowered plant quality as food (Karban and Baldwin 1997) and/or a mechanism to minimize resource competition (Visser 1996).

Our results show that behavioral responses to wireworms are not restricted to the ovipositing females. Also larvae of *S. littoralis* show an increased tendency to abandon an already infested plant when this is wireworm infested compared with plants previously infested by conspecific larvae. The increased tendency to leave wireworm-infested plants is apparent within a few hours. This response became even more pronounced over the following 34 h at which point most larvae had left the wireworm-damaged plants, whereas only a few larvae had left the *S. littoralis*-damaged plants.

Females of *S. littoralis* may benefit from avoiding wireworm-attacked plants in several ways. By choosing undamaged plants, the progeny avoids higher doses of deleterious secondary compounds (Bezemer et al. 2004). The females may also lower the risk for the progeny to be attacked by natural enemies (Shiojiri et al. 2002; Halitschke et al. 2004; Soler et al. 2007; Sadek et al. 2010; but see Rasmann and Turlings 2007). Furthermore, the progeny may suffer from reduced nutrient levels on damaged plants (Asmack and Leaether 2002). Studies in other systems show lowered levels of primary metabolites in foliage after root herbivory (Masters et al. 1993, but see also Johnson et al. 2008).

The larger behavioral avoidance of wireworm-infested plants reflects the different patterns in which cotton expresses its induced resistance when exposed to root and shoot herbivores. Our studies have repeatedly shown that herbivory by root-feeding wireworms results in increased concentrations of gossypol, hemigossypolone, and heliocides 1–4 in mature cotton leaves, whereas no significant systemic increase was found in these leaves after foliar feeding (Bezemer et al. 2003, 2004). On the other hand, foliar feeding by *S. exigua* larvae enhanced terpenoid concentrations (in particular concentrations of hemigossypolone and the heliocides 1 and 4) in developing leaves, whereas these immature leaves showed only a small increase in terpenoid levels after root herbivory. The increased concentration of terpenoid aldehydes throughout the wireworm-damaged plant could explain the enhanced leaving tendency of *S. littoralis* larvae on these plants. Caterpillars feeding on *S. littoralis*-damaged plants, on the other hand, would still feed on mature leaves showing little sign of induction (Bezemer et al. 2004). This was confirmed by the observed movement of *S. littoralis* larvae from developing leaves to mature leaves in our study (see also Bezemer et al. 2003; Anderson and Agrell 2005). In maize, feeding by larvae of the belowground herbivore, *Diabrotica virgifera*, reduced growth of *S. littoralis* larvae (Erb et al. 2009, 2011). The resistance was found to be connected to changed water content induced by root feeding and not to the induction of secondary compounds. Thus, abiotic factors may also be important for growth and development of *Spodoptera* larvae.
The induced response to wireworm feeding provides the cotton plant with effective resistance against secondary attack by aboveground herbivores. The possible ultimate functions of this aboveground induction following belowground herbivory have been discussed by Wäckers and Bezemer (2005). In the present study, we show that there are a number of behavioral mechanisms by *S. littoralis* underlying this resistance. First, by being less attractive to ovipositing females, induced plants receive significantly lower levels of *S. littoralis* eggs. Second, larvae emerging from these eggs show a lower feeding rate, and third, they exhibit an increased tendency to leave the plant. These 3 factors need to be considered in combination in order to appreciate the impact that root-induced resistance has on aboveground herbivory.

The positive impact on the induced plant will be further compounded by the knock-on effect that induced resistance has on those plants with which it competes for nutrients and light. Neighboring plants may receive higher numbers of eggs from ovipositing herbivores rejecting the wireworm-induced plant. In addition, they are also the likely destination of larvae abandoning induced plants. The potential competitive benefit of a plant obtained from “unloading of its herbivores” on neighboring plants will depend on the herbivore’s mobility and its host range (Wäckers et al. 2007). Competitive benefits are most likely when the herbivore is relatively polyphagous (as in the case of *S. littoralis*) or if the plant grows in a stand of unrelated conspecifics.

Only if we consider the various ways in which induced resistance mechanisms impact relevant organisms of various trophic levels (competing plants, herbivores, predators, and parasitoids) will we be able to fully understand the evolution and functioning of induced plant responses. Over the last decade, a range of studies have generated an extensive overview of the direct and indirect interactions mediated by cotton-induced resistance making this plant an excellent model to address the ecology of antiherbivore defense.

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**REFERENCES**


