Journal of Economic Entomology
Plant resistance
Weng et al. Induced resistance to greenbug herbivory in wheat

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Spatial and Temporal Distribution of Induced Resistance to Greenbug (Homoptera: Aphidiae) Herbivory in Preconditioned Resistant and Susceptible Near Isogenic Plants of Wheat

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Abstract

Interactions between biotype E greenbugs, *Schizaphis graminum* Rodani, and two near isogenic lines of the greenbug resistance gene *Gb3* of wheat (*Triticum aestivum* L.) were examined for 62d after infestation. By comparing aphid performance and host responses on control and greenbug-preconditioned plants, we demonstrated that systemic resistance to greenbug herbivory was inducible in the resistant genotype with varying intensities and effectiveness in different parts of the plants. Preconditioning of susceptible plants resulted in modification of within-plant aphid distribution and reduction of cumulative greenbug densities, but showed no effect on reducing greenbug feeding damage to host plant. Preconditioning of resistant plants altered greenbug population dynamics by reducing the size and buffering the fluctuation of the aphid population. Preconditioning in the first (oldest) leaf of the resistant plant had no phenotypically-detectable effect in the stem and induced susceptibility locally in the first leaf over the short term. The preconditioning-induced resistance reduced the greenbug density, delayed aphid density peaks and extended the life of younger leaves in resistant plants. Expression of induced resistance was spatially and temporally dynamic within the plant, which occurred more rapidly, was longer lasting in duration and stronger in intensity in younger leaves. It was concluded that only host resistance gene-mediated induced resistance was effective in lowering greenbug performance and reducing damages from greenbug herbivory in host plants. Results from this study supported the optimal defense theory regarding within-plant defense allocation.

Keywords: Induced Resistance, Greenbug, *Schizaphis graminum*, Wheat, Optimal Defense Theory, Near Isogenic Line

Introduction

The greenbug, *Schizaphis graminum* (Rondani), is the most important cereal aphid pest in the Great Plains of North America (Brewer and Elliott 2003). Annual losses to U.S. wheat production due to greenbug damage range from $60 million to more than $100 million (Webster et al. 2000). This pest is especially notorious due to periodic occurrence of new virulent biotypes. Greenbug biotypes are genetically distinct populations (Porter et al. 1997). Over the years, there has been a shift of prevailing biotypes from C to E and E to I in the fields of the southern Plains of the U.S. (Berzonsky et al. 2003). New greenbug strains being able to damage all known host resistance of wheat have been found (David Porter, personal communication). Although six genes (*Gb1* to *Gb6*) conferring resistance to different greenbug biotypes have been identified (Tyler et al. 1987; Porter et al. 1997), *Gb3* in the wheat cultivar TAM 110 (Lazar et al. 1997) is currently the only widely deployed gene with resistance to prevailing biotypes (E, I and K) in the field. The *Gb3*-conferrred resistance might be potentially overcome by newly emerging virulent greenbug biotypes. For effective and sustainable control of the greenbug damage, it is important to study the underlying mechanisms of host resistance against this aphid pest, which are poorly understood at present.

Of all important cereal aphids, the greenbug is probably the best explored with regard to the relationship with its wheat host (van Emden 1990; Berzonsky et al. 2003), which was believed to be on a gene-for-gene basis genetically (Puterka and Peters 1989). Previously, by examining the interactions between biotype E greenbugs and two resistant and susceptible near isogenic lines of *Gb3*, we found that antixenosis, antibiosis and tolerance were all responsible for the *Gb3*-conferred greenbug resistance in wheat (Weng et al. 2004). We reasoned that the

induced resistance upon greenbug feeding might be a common mechanism shared by the antibiosis and tolerance components of the host resistance in the resistant plants.

First described as a plant response to pathogen infection, induced resistance to insect herbivory has been documented in many plant species (Karban and Baldwin 1997). In recent years, significant progress has been made in understanding the underlying mechanisms of induced resistance to insect damages (see Karban and Baldwin 1997, Gatehouse 2002, Kesser and Baldwin 2002 for reviews), which is particularly true in the plant interactions with chewing insects in model plants such as *Arabidopsis thaliana*, tomato and tobacco (*Nicotiana*) species. However, in the numerous, largely ecological and entomological literature on plant-insect interactions, the roles of host resistance genes in induced responses upon insect herbivory were often not explicitly indicated. Most, if not all, of these studies were based on plants that were essentially susceptible to attack by the insect pests used. Therefore, induced resistance in these plants, while of major importance in reducing the damage caused by these insect pests, is not the causative factor in most examples of plant resistance to herbivory (Gatehouse 2002). In plant breeding, only host resistant genes can provide effective protection to pest damages in the field. Therefore, it is important to investigate the roles of host resistance genes in insect herbivory-induced resistance.

There were a few reports comparing aphid performance and host responses upon greenbug feeding in cereal crops (e.g., Ryan et al. 1987, Formusoh et al. 1992, Hays et al. 1999). In a susceptible wheat cultivar, the fecundity of biotype E greenbugs was increased by preconditioning (previous infestation) with the Russian wheat aphid, *Diuraphis noxia* (Mordvilko), but not with biotype E greenbug (Formusoh et al. 1992). During a seven-hour monitoring of greenbug feeding behavior on a resistant barley (*Hordeum vulgare* L.) genotype,

Hays et al. (1999) found that the resistance gene conferred an inducible form of resistance that can be trigged by plant recognition of an avirulent greenbug biotype. The chemical basis of induced resistance to greenbug infestation in wheat has been investigated for a long time, and the cyclic hydroxamic acids, especially the 2, 4-dihydroxyl-7-methoxy-1, 4-benzoxazin-3-one (DIMBOA), were believed to be the main greenbug deterrent in wheat (Argandona et al. 1981, Niemeyer et al. 1988, Gianoli and Niemeyer 1997a). While these studies on wheat-greenbug interactions have given some insights into the complexity of aphid-cereal interactions, the roles of host resistance genes in induced resistance to greenbug herbivory were not well documented in wheat. The heterogeneous genetic background at loci other than the resistance locus might affect aphid performance on the plant materials used in these studies. In addition, most of these experiments were conducted in a relatively short period, the effects of the resistance gene beyond this time may not be observed. The near isogenic lines of *Gb3* (Lazar et al. 1996) provide a useful tool to address some of these problems. The phenotypic mechanisms of resistance conferred by *Gb3* have been extensively examined by the authors (Lazar et al. 1995, Michels et al. 1997, Fritts et al. 2000, Weng et al. 2004). Molecular marker analysis indicated that the two lines are very closely related with less than 1% difference across the whole genome (Weng and Lazar 2002). Therefore, by comparing the greenbug performance between the two genotypes under controlled conditions, the background noise caused by loci other than *Gb3* could be minimized. The objectives of this study were to (1) examine the inducibility of resistance in resistant and susceptible lines by greenbug preconditioning, (2) investigate the effects of induced resistance, if any, on aphid performance, and (3) clarify the roles of host resistance genes in the induction of resistance. We investigated the phenotypic interactions between biotype E greenbugs and the two wheat lines for 62d. The spatial and temporal expression patterns of

preconditioning-induced resistance and its effects on aphid population dynamics as well as host growth and development were studied and the results were reported below.

Materials and Methods

Insects and plant materials. Colonies of biotypes E greenbugs were reared on wheat lines in insect cages in a greenhouse at 23 ± 5 °C and a photoperiod of 14:10 (L:D)h. The greenbugs used for infestation were a mixture of apterous adults randomly chosen from a large population. Two near isogenic wheat lines for the greenbug resistance gene *Gb3*, the resistant TXGBE273 and susceptible TXGBE281 (Lazar et al 1996) were used to study the aphid-plant interactions.

Experimental design. Seeds from each line were germinated in Petri dishes for two days and planted randomly in four plastic flats (30 × 50 cm) with regular greenhouse soil. At the one-leaf stage (Zadoks' growth stage 11) (Zadoks et al. 1974), the seedlings were thinned to five plants per line in each flat in which the resistant and susceptible plants were randomly distributed. Two treatments, control and preconditioning, were applied to each line with 10 replications per treatment. At the two-leaf stage (Zadoks' 12), ten seedlings from each line were randomly selected from four flats for preconditioning which was performed by placing ten aphids on the adaxial surface of the first (oldest) leaf of each plant using a fine hair paintbrush. Immediately after infestation, each preconditioned plant was covered by a cage, which was constructed of a clear plastic tube (10 cm diameter by 40 cm high) with a nylon mesh top and ventilation holes on the sides. Control plants were also individually caged during the preconditioning phase, but remained uninfested. After infestation, all four flats were kept in a growth chamber with mixed fluorescent and incandescent lights (approximately 300 $\mu Em^{-2}s^{-1}$) on a 12h photoperiod. The

temperature was 22 ± 2 °C. Two days later, all aphids were carefully removed from each preconditioned plant with a fine hair paintbrush. Then, ten new biotype E greenbugs were transferred onto the adaxial surface of the first leaf of both preconditioned and control plants. The flats were returned to the growth chamber and all plants remained individually caged throughout the experiment. Aphid counts on the stem and each leaf of a plant were conducted daily. Great care was taken to avoid dislodging the aphids during daily counting. Observations on resistant plants were continued until all susceptible plants were killed by greenbug feeding. Twenty days after infestation, aphid counts were taken every other day until conclusion of the experiment when all resistant plants were dead.

Data analysis. This experiment was a completely randomized design with preconditioning and control as treatments in each genotype. Each aphid-plant combination was considered a replication and there were 10 replications per treatment. Aphid count data were analyzed using the SAS PROC GLM and means were separated at the 0.05 level ($P = 0.05$) using the Fisher protected least significant difference (LSD) tests (SAS Institute, 1999). The cumulative greenbug density was also used as an indicator of the effects of treatments, which was the sum of daily average number of aphids on the stem, each leaf or whole plant in a treatment throughout the experiment. In resistant plants where aphid counts were taken every other day, beginning 20d postinfestation, aphid density on the "off" day was estimated by averaging the values of the two neighboring days. Comparison of cumulative greenbug densities between the two treatments of each line was performed by χ^2 tests.

The within-plant distribution of aphids was evaluated by calculating the percentages of the greenbug population on the leaf or stem of each plant. After an arcsin transformation of the percentage data, *t*-tests (Moore and McCabe 1989) were conducted to compare the differences in

within-plant distribution of greenbugs between the two treatments of each genotype. Significance level was set to $P = 0.05$ for all tests.

Results

1. Within-plant distribution and performance aphids, and host responses in preconditioned and control susceptible plants

The average greenbug densities on the control and preconditioned susceptible plants are illustrated in Fig. 1a. No difference was found in aphid densities on the stem, the first or second leaf, and the whole plant between the two treatments in any day. However, in days 7 to 10 postinfestation, significantly fewer aphids fed on the third leaf of preconditioned plants ($P<0.05$, data not shown). In addition, there were significantly fewer cumulative greenbugs on the stem (123.4 versus 184.3), the third leaf (18.3 versus 42.2) and the whole plants (459.6 versus 566.6) of preconditioned plants versus the control ($P < 0.05$, $\chi^2_{0.05} = 3.84$, d.f. = 1).

Greenbug preconditioning affected within-plant distribution of aphids between the stem and the first leaf in the susceptible plants. Previously, it was found that significantly more aphids moved to the stem than to the first leaf within 6h after infestation when the greenbugs were placed on the first leaf (Weng et al. 2004). This was confirmed in the present study. Nearly 60% of aphids fed on the stem of susceptible control plants in the first three days after infestation, but they did not show such preference in preconditioned plants in any day during this period (data not shown).

No visually detectable difference was found in host responses in the two treatments. Both preconditioned and control susceptible plants were killed by greenbug feeding 12d postinfestation (Fig. 1a).

2. Greenbug population dynamics on preconditioned and control resistant plants

Aphid counts were taken on resistant plants until every plant was dead. Aphid population dynamics for the whole plants and the three oldest leaves of the preconditioned and control plants are compared in Figs. 1b and 2, respectively.

Unlike in the susceptible plants, preconditioning did not affect within-plant distribution of aphids between the first leaf and the stem of resistant plants. The aphid population dynamics on control plants was a curve with two prominent peaks, which occurred at days 16 and 27 postinfestation, respectively (Fig. 1b). After the second peak, the greenbug population declined, and all control plants were killed 54d after infestation. In contrast, the curve of greenbug population development on preconditioned plants was smoother and nearly single peaked (Fig. 1b), indicating a less dramatic fluctuation of aphid populations over time. From 8 to 49 days postinfestation, aphid density on preconditioned plants was always lower than that on the controls in each day, and the difference was significant in 10 of the 42 days (days 13 to 18, and 27 to 30) ($P < 0.05$).

Preconditioning on the first leaf had no phenotypically detectable effects on aphid performance on the stems between the two treatments. There were significantly more aphids feeding on the first leaf of preconditioned plants in the first two days after infestation (Fig. 2a) indicating induced susceptibility by preconditioning. No difference in aphid density was found between the two treatments in the rest of the experiment. In fact, the control plants outnumbered the preconditioned ones in aphid numbers on the first leaf from days 9 to 17, although not significantly (Fig. 2a) suggesting preconditioning-induced resistance might playing some role in this process.

Preconditioning negatively affected aphid performance on the second, third and fourth leaves. Overall, preconditioning in the resistant plants lowered the peak density of the aphid population, reduced cumulative aphid numbers, and delayed the aphid density peaks. Based on data illustrated in Figs, 1b and 2, four indicators for aphid performance on each leaf, the stem and the whole plant were compared between the control and preconditioned resistant plants and the results are presented in Table 1. It is clear that preconditioning in the first leaf did not affect the aphid performance in the stem, but lowered performance on younger leaves. It seems that the younger the leaf was, the poorer the greenbug performance on that leaf.

3. Growth and development of control and preconditioned resistant plants

Preconditioning seemed to extend the life of the resistant plants with varying effects on different leaves. All control plants were dead 54d after infestation, but it was 8d later (day 62) when preconditioned plants were killed (Fig. 1b). No phenotypically-visible difference was observed on the stems in the two treatments. The first leaves of both control and preconditioned plants were dead on the same day (day 39, Fig. 2a). However, compared to the control, preconditioning extended the life of the second, third and fourth leaves, and the younger the leaf was, the more it was protected by preconditioning (Table 1). In addition, while no control resistant plant reached the five-leaf stage, 20% (2/10) of the preconditioned seedling plants had the fifth leaf emerged before they were killed.

Discussion

In this study, two near isogenic wheat lines for the greenbug resistance gene *Gb3* were employed, and the aphid-plant interactions were examined for 62 days. The aphid population dynamics on

control plants and host responses in this study were consistent with our previous observations (Weng et al. 2004), suggesting effective control of environmental factors in these experiments. While the extended time of investigation enabled us to have a better view of the actions of host resistance gene against greenbug feeding, the use of closely related resistant and susceptible near isogenic lines helped to elucidate the roles of host resistance genes in preconditioning-induced systemic resistance to greenbug herbivory.

Both control and preconditioned susceptible plants were killed by aphid feeding 12d postinfestation (Fig. 1a). In contrast, the control and preconditioned resistant plants outlived the susceptible plants by 42 and 50d, respectively. This observation has two important implications: first, the greenbug resistance gene *Gb3* played an essential role in combating greenbug herbivory; second, the *Gb3*-mediated induced resistance was effective in lowering greenbug performance and extending the life of host plants.

In some plant-insect interaction studies, the lower performance of insects on plants with previous infestation was attributed to reduced nutrition quality of the host plant by the first infestation (e.g., Broady and Karban 1989, Wool and Hales 1996). This seems unlikely to be true in the present study. If preconditioning degraded the food quality for the greenbugs, aphid performance on the preconditioned first leaf should be poorer (lower aphid density) than on the control plant, which was not found in this study, however. In fact, the greenbugs are able to modify the feeding sites (Burd 2002) and change the composition of the phloem sap (e.g., increase free amino acids) to their benefit (Dorschner et al. 1987, Sandstorm et al. 2000). The ability of greenbugs to improve nutrition quality locally may be the reason why there was an "induced susceptibility" in the first two days on the first leaf of preconditioned resistant plants (also see discussion below).

Results from this study clearly indicated that systemic resistance was inducible by preconditioning the resistant plants, which reduced the greenbug population size and extended the life of host plants. In the susceptible plants, aphid performance was also negatively affected by preconditioning. There were significantly fewer greenbugs feeding on the third leaf of preconditioned plants between days 7 to 10 after infestation. Preconditioning significantly reduced cumulative greenbug densities on the third leaf, the stem and the whole plant. It is possible that systemic resistance might also be inducible in the susceptible plants, although it was weaker and less effective as compared with that in the resistant ones. Induced resistance in the susceptible host may represent a basal defense mechanism common to all plants against insect herbivory (Gatehouse 2002).

From the aphid performance and host responses in preconditioned and control resistant plants (Table 1), it was evident that expression of induced resistance in the resistant plants was temporally and spatially dynamic within the plant. No phenotypically-detectable effect on the stem was found when preconditioning was applied on the first leaf. This was also observed by Gianoli (1999) in examining the interactions between wheat and the birdcherry oat aphid, *Rhopalosiphum padi* (L.). Preconditioning on the first leaf led to induced susceptibility in the first two days but induced resistance afterwards. The localized response on the first leaf to greenbug preconditioning were different from that found in a study of the interactions between the spider mites (*Tetranychus urticae*) and two cucumber (*Cucumis sativus*) lines differing in constitutive cucurbitacin accumulation (Agrawal et al. 1999). In Agrawal et al. (1999), previous infestation of spider mites on the first leaf of the resistant plant induced resistance first, which then decayed and eventually led to induced susceptibility in this leaf. The differing responses observed in the two studies may reflect different resistance mechanisms mediated by host

resistance genes in the wheat and cucumber lines used. Nevertheless, from aphid performance and host responses observed in this study, the *Gb3*-mediated induced resistance in the first leaf was weak in intensity and late in timing.

The expression of preconditioning-induced systemic resistance in the second, third and fourth leaves of the resistant plants seems to follow a fix pattern (Figs. 2b, c, Table 1). That is, the effects on lowering the greenbug performance were stronger in intensity, faster in timing and lasted longer in younger leaves, and accordingly, the newer leaves were better protected from greenbug feeding and lived longer. This within-plant distribution pattern of induced responses upon greenbug infestation could well be explained using the source-sink relationships in plant physiology proposed by Coleman and Jones (1991). They reasoned that expanding younger leaves act as sinks for metabolites from mature leaves, which are used to complete leaf development and expansion. Damage to younger leaves would not affect undamaged older leaves, because old leaves have completed development and receive very little, if any, metabolites from younger ones. Furthermore, mature leaves would be unlikely to exhibit biochemical changes after damage, because these leaves do not usually show much plasticity to environmental conditions. On the other hand, if older leaves are damaged, it is expected that stronger, more rapidly induced responses of plants to the damage would occur on younger leaves.

In plant-insect interaction studies, the optimal defense (OD) theory (McKey 1979, Rhoades 1979) was proposed to explain the variation of defense within plants. The OD theory predicates that tissues of high fitness value will be better defended than less valuable tissues to insect herbivory by constitutive or induced resistance. The theory also predicts that defense allocation should change dynamically as fitness value and risk of attack of plant tissues change during plant development. Since younger leaves have greater relative fitness values than older ones, the

distribution of defense should mirror the distribution of relative fitness values (McKey 1979, Krischik and Denno 1983). The spatial distribution of induced resistance based on greenbug population dynamics and host responses on resistant plants in this study supported the OD theory well. However, the chemical basis of the dynamics of induced systemic resistance observed in this study is not known.

Significantly more greenbugs moved to the stem than to the first leaf of control susceptible plants after infestation in this experiment. The preference of stem and lower leaves as the initial feeding sites was also found in other cereal aphids such as *R. padi,* (Leather and Lehti 1982). This pattern of within-plant distribution was thought to have important adaptive consequences for the aphids. Wiktelius et al. (1990) suggested that the lower part of cereal seedlings provides a more suitable food source for *R. padi* enabling a higher growth rate after landing in the field. By moving down to the base of the plant, the aphids could also avoid the extreme temperatures (Wiktelius 1987) or predators (Hopkins and Dixon 1997) in the field, or induced resistance within the plant, which will occur if aphids feed on the upper leaves (Gianoli 1999, present study). Then, why the greenbugs did not show their preference to the stem in the resistant plants in this study? It is not known if this is correlated with the synthesis of defense compounds such as DIMBOA in the host plants. In wheat, the majority of DIMBOA is synthesized and distributed in the roots (Wu et al. 2000) which is translocated from the stem to the leaves (Gianoli and Niemeyer 1997b). In resistant plants, there may be higher constitutive DIMBOA synthesis in the stem deterring the greenbugs from feeding there. The induced susceptibility in the first leaf of the resistant plant after preconditioning seemed to be inevitable due probably to the time delay between aphid feeding initiation and the induction of systemic resistance including increased DIMBOA synthesis (Gianoli and Niemeyer 1997a). In susceptible

plants, while preconditioning improved the diet quality of the first leaf, it might also induce synthesis of DIMBOA, increasing its concentration in the stem and causing aphids to go upwards, feeding on the first leaf. Further experiments are necessary to confirm the roles of DIMBOA during this process.

In the present study, aphid performance on resistant and susceptible near isogenic lines was observed in a growth chamber under controlled conditions. Insect population dynamics are complex and influenced by many intrinsic and extrinsic factors (Price 1997). Results from this study clearly indicated that the host resistance gene *Gb3* played the primary role in regulating the aphid population dynamics on the resistant plants. In addition, although systemic resistance seemed to be inducible in both resistant and susceptible plants, only the *Gb3*-mediated induced resistance in the resistant plants was effective in reducing the performance of the greenbugs and protecting the hosts. Yet the resistant plants cannot eliminate the greenbugs after infestation and were eventually killed by aphid feeding. In practice, the *Gb3*-bearing wheat cultivar TAM 110 (Lazar et al. 1997) is providing effective protection against greenbug damage. Obviously, the deployment of resistant cultivar, the natural enemies (predators and parasitoids) and environmental factors may all contribute to the control of the greenbug population in the field (Brewer and Elliott 2003).

Acknowledgements

We are indebted to Peihua Yan and Gary Peterson for technical help. This research was supported by a USDA-NRICGP grant (2002-35301-12044) to Y. W.

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Table 1 Effects of greenbug preconditioning on aphid performance and host responses in resistant plants of TXGBE273 as compared with the control[a]

Indicators	Stem	First leaf	Second leaf	Third leaf	Fourth leaf	Whole Plant
% Peak density of aphid population[b]	95.0	102.8	86.7	67.8	55.3	75.8
% Cumulative aphid density[c]	98.4	97.1	90.6	72.3	56.8	88.0
Days aphid peak appearance delayed	0	-2[d]	5	14	7	4
Days with significant fewer aphids	0	-2[e]	7	20	15	10
Days leaf life extended	0	0	4	7	8	8

[a] Numbers for the five indicators in the table were based on comparison of data in preconditioned plants with the corresponding ones in the control.

[b] Percentages = (peak density in preconditioned plants/corresponding peak density in control plants) * 100. Number for the whole plant was calculated for the first peak.

[c] Percentages = (Cumulative aphid density in preconditioned plants/corresponding cumulative aphid density in the control) * 100. Cumulative greenbug densities for the second (1820.6), third (1305.2) and fourth (262.0) leaves, and the whole plant (4269.9) of preconditioned plants were significantly lower than corresponding ones in the control ($P < 0.05$, $\chi^2_{0.05} = 3.84$, d.f. = 1).

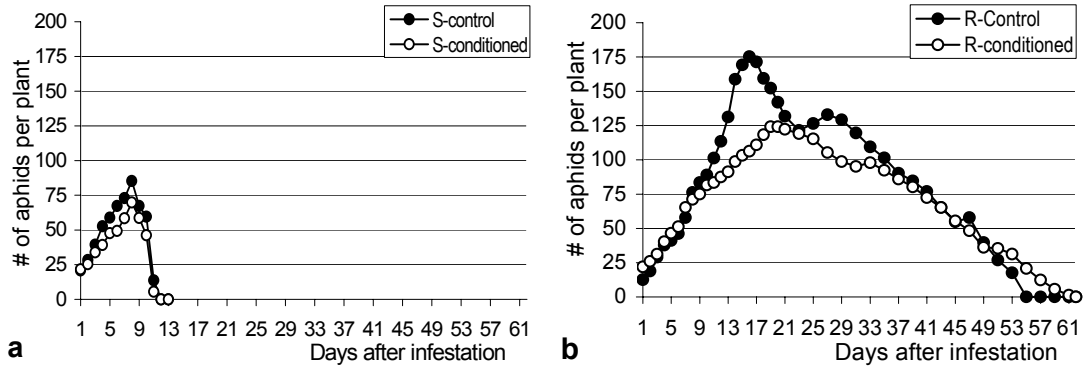
[d] The aphid density peak on the first leaf of preconditioned plants appeared two days earlier than that of the control.

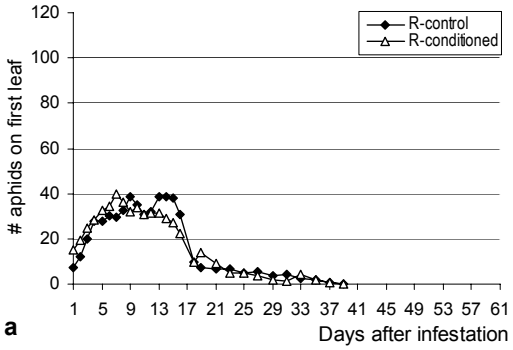
[e] Aphid density on the first leaf of preconditioned plants was significantly higher than that of control in the first two days postinfestation.

Figure legends

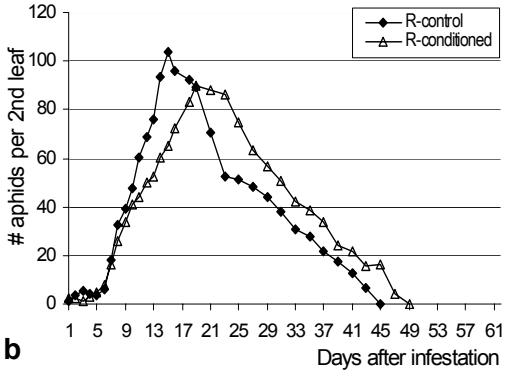
Fig.1 Biotype E greenbug population dynamics over time for the preconditioned (conditioned) and control susceptible (S) (a) and resistant (R) (b) near isogenic plants of wheat. Ten biotype E greenbug was infested on each plant. Each data point was the average of ten plants.

Fig.2 Population dynamics of biotype E greenbugs over time on the first (oldest) (a), second (b) and third (c) leaves of the control and preconditioned (conditioned) resistant plants during a 62d observation. Ten aphids were infested initially. Each data point was an average of 10 plants.

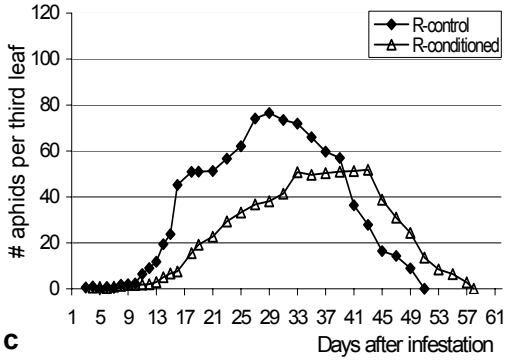

S-control
S-conditioned
# of aphids per plant
200
175
150
125
100
75
50
25
0
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61
Days after infestation
a
R-Control
R-conditioned
# of aphids per plant
200
175
150
125
100
75
50
25
0
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61
Days after infestation
b


R-control
R-conditioned
aphids on first leaf
120
100
80
60
40
20
0
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61
Days after infestation

a


R-control
R-conditioned
aphids per 2nd leaf
120
100
80
60
40
20
0
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61
Days after infestation

b


R-control
R-conditioned
aphids per third leaf
120
100
80
60
40
20
0
1 5 9 13 17 21 25 29 33 37 41 45 49 53 57 61
Days after infestation

c