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Greenbug systemic effect on barley phosphate influx

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Abstract

Greenbugs (*Schizaphis graminum* Rond.) cause considerable yield loss in cereals. Local feeding damage of greenbug-infested leaves includes collapsed mesophyll cells, chlorosis, alterations in photosynthesis and respiration. However, this damage cannot explain rapid changes taking place in plant metabolism (inhibition of new leaf primordia and new root differentiation, within a few hours after attack), or the early death of such plants. This study was aimed at determining whether greenbug feeding induces systemic damage to barley. The phosphate influx by roots of susceptible and tolerant barley (*Hordeum vulgare* L.) plants was evaluated as an estimate of aphid systemic damage. Phosphate (P)-influx was determined at two plant growth stages, with two levels of greenbug infestation, at two different greenbug life stages. Plants grown in hydroponics in a glasshouse were infested for 0 (control), 3, 6, 12, 24, 48, and 72 h with the Argentinean biotype C greenbug. The P-influx was not significantly affected in tolerant barley plants by greenbug infestation. In contrast P-influx was significantly reduced 6 h post-infestation in the susceptible cultivar. Plants with one expanded leaf suffered a significantly greater reduction in P-influx than plants with two expanded leaves. By 48 h after infestation, the P-influx of the two-expanded-leaf treatment was similar to that of the controls, whereas P-influx in plants with one expanded leaf remained significantly less than on the controls 72 h after infestation. A larger greenbug population resulted in greater reduction in P-influx. Adult greenbugs, but not third stage nymphs, affected P-influx. In summary, the intensity of greenbug-induced systemic damage was greater when young plant stages were infested by the aphid. Reductions of P-influx may become critical under increasing natural infestation levels. © 1997 Elsevier Science B.V.

Keywords: *Hordeum vulgare*; Barley; Greenbug; *Schizaphis graminum*; Systemic damage; P-influx

1. Introduction

Greenbug, *Schizaphis graminum* Rond., is a pest of small grains and sorghum (*Sorghum vulgare*, L.) and can cause either seedling death or yield decreases, depending on intensity of infestation on susceptible cultivars (Smith, 1989). Research of greenbug–host interactions has focused on toxicological aspects associated with salivary secretions that are injected into the plants at the feeding sites (Chatters and Schlehuber, 1951; McAllan and

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Adams, 1961; Clarkson et al., 1978; Campbell et al., 1982; Al-Mousawi et al., 1983; Campbell and Dreyer, 1985). Greenbug infestation of wheat (*Triticum aestivum* L.) diminishes total root biomass and density of root hairs, thereby reducing the root surface and volume (Ortman and Painter, 1960) in relation to the local damage caused on infested leaves (chlorosis, shortening of leaf laminae and decrease in the total leaf area; Al-Mousawi et al., 1983; Burton, 1986). Similar alterations have been found in susceptible barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.) and sorghum (*Sorghum bicolor* Moench.) (Gerloff and Ortman, 1971; Morgan et al., 1980; Castro et al., 1989). Furthermore, 5 d of greenbug feeding decreased photosynthetic capacity but not leaf conductance to water vapor; drought stress in combination with greenbug feeding also caused damage to the mesophyll capacity for photosynthesis beyond that explained by chlorosis in wheat (Ryan et al., 1987).

The 'pale green biotype', the most common greenbug in Argentina (Arriaga et al., 1984), inhibited seedling aerial and root growth in susceptible barley, oat and sorghum cultivars (Castro and Rumi, 1987; Castro et al., 1988, 1989, 1990, 1991; Castro, 1994). The extent of this damage was related to the inhibition of differentiation of new leaf primordia and nodal roots in susceptible cultivars (Castro et al., 1988, 1991; Castro, 1994). These inhibitory effects were observed 48 h post-infestation and were independent of the chlorosis produced later by greenbugs at the feeding sites (Castro and Rumi, 1987; Castro et al., 1988, 1989, 1990, 1991; Castro, 1994). The response of infested susceptible barley, oat and sorghum cultivars was an accumulation of higher dry matter in roots with a lower water relationship between shoot and root (Castro et al., 1988, 1991; Castro, 1994). It has been suggested that these alterations to the aerial and root growth could be related to nutritional and or hormonal imbalances that would be a direct effect of greenbug extraction or injection of substances. These imbalances would be related to disturbances in the plant absorption and or transport capacity (Castro et al., 1988, 1991; Castro, 1994). It has been reported that after 3 h of infestation, greenbug feeding decreased the phosphate transport from root to aerial parts in susceptible barley seedlings, without modifying the P-influx (Giménez et al., 1990). It has not been reported whether the absorption capacity is affected by greenbug infestation in susceptible barley plants and there are no reports on tolerant ones. Phosphorus is a part of essential molecules (e.g. ATP, membrane phospholipids, nucleic acids) and is absorbed by an active mechanism (Nissen, 1974) (the binding and release of a molecule at a specific site on a protein that occur in carrier-mediated transport are similar to the binding and release of molecules from an enzyme in an enzyme-catalyzed reaction), and as it is rapidly translocated within the plant, it is well suited for short-term nutrient uptake studies (Marschener, 1983). The objective of the project was to determine whether greenbugs can systemically alter plant absorption capacity by studying the P-influx in two barley cultivars, a greenbug-susceptible and a tolerant one. We also sought to determine if plant absorption capacity is quantitatively affected by increasing the intensity of infestation and by using two different aphid developmental stages (immature and adult).

2. Materials and methods

2.1. Plant material

Two commercial barley varieties were used, a greenbug-susceptible (Bordenave Ranquelina MAG) and a tolerant one (La Plata Bordeba FA) (Arriaga, 1977). Two-day-old seedlings of both cultivars were placed into small baskets (20 mm diameter × 25 mm height) (1 seedling/basket). These were put onto a supporting plank (0.70 × 0.50 m) (Asher and Edwards, 1983) floating on aerated Hoagland's nutrient solution (Hoagland and Arnon, 1950) in a 20 l plastic tray (8 plants/l solution). The solution was replaced every 5 days. The pH was checked daily and adjusted to 5.6 with H_2SO_4 1N, if needed. Every 2 days, distilled water was added to maintain a constant concentration of solution. The trays were kept in a greenhouse until the plants reached either the first or second fully expanded leaf. Plants were grown under sunlight with maximum photosynthetically active radiation (PAR) of 1400 to 1600 mmol m^{-2} s^{-1} at midday

and a 12 h photoperiod. Air temperature ranged from 18 to 24 ° C during daytime, and from 14 to 17 °C at night.

2.2. Experiment 1. Influence of plant developmental stage

Susceptible plants with one expanded leaf (S-1) or two expanded leaves (S-2) were subjected to different infestation periods. Tolerant barley plants with only one expanded leaf (T-1) were also used.

2.3. Greenbug source and infestation

The pale green ecotype of greenbug, biotype C (Castro, 1994), was obtained from the Insectary Collection of Cereal Science, Facultad de Ciencias Agrarias y Forestales, U.N.L.P. La Plata, Bs As, Argentina (34° 54′ S lat.) and was reared on a susceptible barley cv. (Bordenave Ranquelina MAG). Twenty-five adult greenbugs were placed onto each plant ('infested treatment'). Progeny of greenbugs was eliminated daily with a fine paintbrush. For each experiment, uninfested plants were used as controls. Plants selected at random were subjected to infestation periods of 0 (control), 3, 6, 12, 24, 48 or 72 h. The maximum infestation period was 72 h, since by then local damage in feeding zones has become significant (Al-Mousawi et al., 1983). The treatments were begun in a staggered fashion, thereby allowing simultaneous harvest of all treatments with the control at the end of the experiments.

2.4. Experiment 2. Effects of greenbug intensity of infestation and developmental stage

Plants from susceptible and tolerant cultivars, with one expanded leaf, were divided into three sets. One set was infested with 25 adults/plant, the normal intensity (S-1N, T-1N). Another set was further infested with 10 more adults 24 and 48 h later (total 45 greenbugs/plant), the high intensity of infestation (S-1H and T-1H) treatments. The third set remained uninfested as controls in each cultivar (S-1C, T-1C). Infestations lasted for 72 h.

The influence of greenbug developmental stage on P-influx in susceptible plants after 12 h of infestation was investigated. One set of plants was infested with 25 adult greenbugs per plant (S-1G), while another set was infested with 25 nymphs (third stage) per plant (S-1Nym). A third set remained untreated, as control (S-1C).

2.5. Procedures

Chlorophyll concentration, dry weight, P-concentration and P-influx determination

Twenty minutes before each treatment was completed, the plants were moved to 2 l plastic pots containing Hoagland's nutrient solution at 25 °C, with 1.11 10^6 Bq l^{-1} of ^{32}P as H_3PO_4 (^{32}P was provided by the Comisión Nacional de Energia Atómica, CONEA). Roots were exposed to the solution for 20 min with gentle bubbling of air. Immediately afterwards, they were removed to other pots with Hoagland's solution without ^{32}P, and kept at 2 to 4 °C for another 20 min. Plants were divided into shoots and roots which were kept separate; leaf area was determined using a portable leaf area meter (Model LI-3000, Li-Cor) and chlorophyll concentration was determined following Moran and Porath (1980). Plant material was oven-dried for 48 h at 80 °C, weighed and wet ashed with a nitric–perchloric acid mixture (3:2 v/v). Phosphorus (P) concentration was determined colorimetrically by the phospho-vanado-molybdate complex procedure (Jackson, 1964) for shoots and roots. Radioactivity in the samples was determined by Cerenkov counting in a Beckman LS 100-C liquid scintillation counter. Phosphorus influx was calculated as described by Cogliatti and Santa Maria (1990). The methodology used (20 min of ^{32}P uptake followed by 20 min of free space rinsing) results in underestimation of P-influx, due to ^{32}P-efflux during the loading period and ^{32}P-exchange from the inner space during free space rinsing. Because the P-influx underestimation is negligible at 1 mol m^{-3} phosphate Hoagland nutrient solution at 14 to 24 °C (Cogliatti and Santa Maria, 1990) but not at temperatures lower than 15 °C (Clarkson, 1985), readings were taken at the previously mentioned warmer temperatures.

2.6. Data analysis

Data were subjected to analysis of variance (ANOVA) in a completely randomized design. In Experiment 1 there were three independent trials, one for every plant developmental stage and cultivar. Dry weight, P-concentration and P-influx for every infestation treatment within plant developmental stage and cultivar were replicated ten times; every replicate consisted of four plants. Chlorophyll concentration was determined in another ten individual plants with one expanded leaf for every treatment in both cultivars. Means of plant measures in each treatment for different developmental stages within cultivars (S-1, S-2 and T-1) were compared by the least significant difference test (LSD) at the 0.05 and 0.01 probability levels when ANOVA showed a significant treatment effect.

In Experiment 2, the effect of the intensity of infestation on the plant dry weight, P-concentration and P-influx was studied in two independent trials, one for every cultivar. The effect of aphid developmental stage on the same plant features was studied in the susceptible cultivar in a third independent trial. Every treatment was replicated four times, with three plants per replicate. Means of each treatment within cultivars (S-1C, S-1N, S-1H; T-1C, T-1N, T-1H; S-1C, S-1G, S-1Nym) were compared by the LSD test at the 0.05 and 0.01 probability levels when ANOVA showed a significant treatment effect.

3. Results

In Experiment 1, infested susceptible plants with a single expanded leaf (S-1) showed a significant reductions in P-influx with a 6, 12, 24, 48 ($P<0.01$) and 72 h infestation period ($P<0.05$), (Figure 1). Phosphate influx in plants with two expanded leaves (S-2) was also significantly reduced ($P<0.05$) after 6 h infestation and became highly significant ($P<0.01$) in plants after 12 and 24 h of greenbug attack. Later, P-influx increased and reached values similar to that of the controls. In contrast, infes-

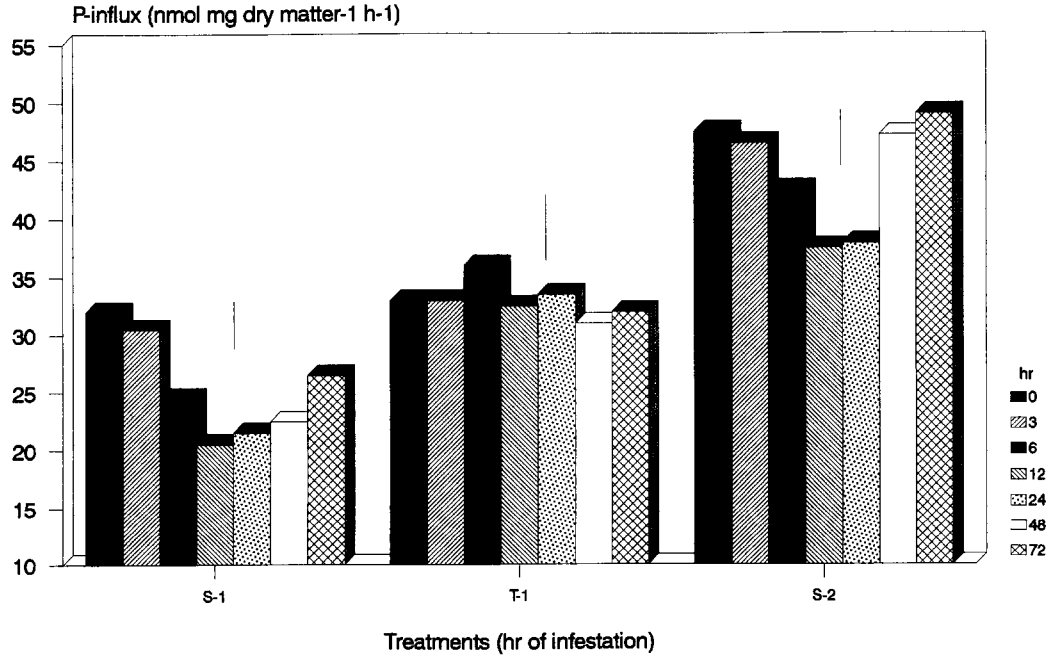

Fig. 1. Phosphate influx for susceptible barley plants with one (S-1) or two (S-2) expanded leaves and tolerant plants with one expanded leaf (T-1) subjected to different periods of greenbug infestation. Zero hour corresponds to the control treatment. Vertical bars represent LSD values at 0.01 level.

tation did not significantly alter P-influx in tolerant plants with a single leaf (T-1) relative to controls at any time (Fig. 1). Control plants with one expanded leaf of either cultivar displayed similar P-influx values.

Dry weight and P-concentration per plant did not differ from controls for either cultivar and plant growth stage at any time of infestation (Fig. 2). Control plants in the three trials displayed very different dry weights and P-concentrations (Fig. 2). There were no statistically significant differences in the concentrations of total chlorophyll among the control and treatment regimes within cultivars (Table 1). Nevertheless, there were differences between treatments. The 12 and 48 h treatments in the susceptible plants and the 6 and 24 h treatments in the tolerant ones showed significantly different ($P<0.05$) chlorophyll concentrations. Greenbug number per unit leaf area was used to estimate the density of infestation. At 72 h there were 1.6 adults cm^{-2} in S-1, 1.1 adults cm^{-2} in S-2 and 2.7 adults cm^{-2} in T-1.

In Experiment 2, the effect of greenbug intensity of infestation and aphid developmental stage were evaluated. There were no significant differences in plant dry weight between treatments within cultivars (Table 2). Phosphorus partition between shoot and root did not differ from controls for any treatment in both cultivars. P-influx was significantly reduced ($P<0.01$) in plants with a high density greenbug infestation (S-1H), and in plants with normal infestation (S-1N) ($P<0.05$). P-influx was 33% lower in S-1H compared with their controls (S-1C) after 72 h (Table 1). In contrast, the tolerant cultivar showed no significant differences in P-influx among the different degrees of infestation. Although in treatment S-1H greenbug density was 69% that of treatment T-1H (2.9/4.2) (Table 1), P-influx in susceptible plants was significantly reduced.

Matter dry weight, P-partition between shoot and root, and P-influx were not significantly affected in susceptible plants infested with nymphs (S-1Nym). After 12 h of infestation, P-influx was 33.77 nmol P mg^{-1} dry wt. h^{-1} in S-1C, 30.97 in the S-1Nym plants, and 26.6 in the S-1G plants ($LSD_{0.01}=3.92$; $LSD_{0.05}=2.88$). Adults, but not nymphs, caused a significant decrease in P-influx.

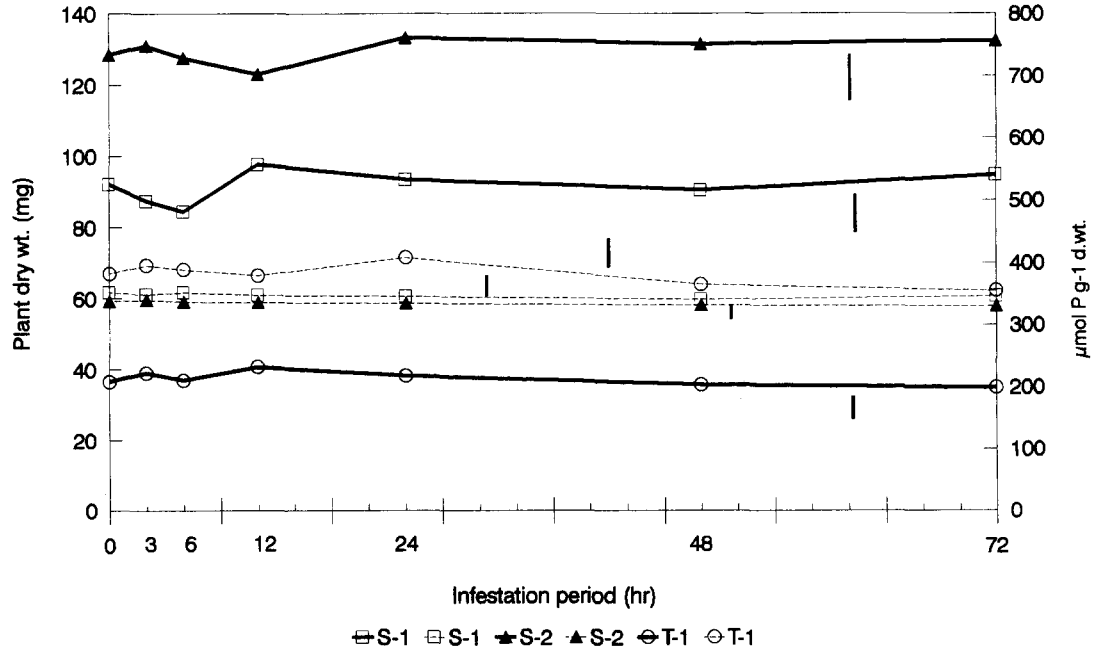

Fig. 2. Dry weight (———) and P (- - -) contents of susceptible barley plants with one (S-1; □) or two (S-2; ▲) expanded leaves and tolerant plants with one expanded leaf (T-1; O) subjected to greenbug infestation. Zero hour corresponds to the control treatment. Vertical bars represent LSD values at the 0.01 level.

Table 1
Chlorophyll concentration ($\mu g\ mg^{-1}$ of fresh weight) determined in susceptible and tolerant barley plants with one expanded leaf subjected to different periods of greenbug infestation (in hours)

	Infestation duration (h)					
	0[a]	6	12	24	48	72
Susceptible	0.93[b,c]	1.18[b,c]	0.917[c]	1.12[b,c]	1.42[b]	1.23[b,c]
SD[d]	0.1896	0.1288	0.1847	0.2187	0.3067	0.2193
Tolerant	1.18[b,c]	0.87[c]	0.95[b,c]	1.29[b]	1.29[b,c]	1.18[b,c]
SD	0.1812	0.1174	0.1214	0.2188	0.2197	0.1232

[a]Corresponds to control treatment.
[b,c]Means sharing a common letter within a row do not differ significantly (LSD: $P>0.05$).
[d]Standard deviation.

Table 2
Greenbug density, dry weight, P-concentration and P-influx values for barley plants with one expanded leaf of a susceptible (S-1) and a tolerant (T-1) cultivar, with no (S-1C, T-1C), normal (S-1N, T-1N) and high (S-1H, T-1H) greenbug infestation, after 72 h of infestation; each value is a mean (± 2 SD) of 4 replicates with 3 plants per replicate

Treatment	Greenbug density (greenbugs cm^{-2})	Dry wt. mg $plant^{-1}$	P-concentration μmol P g^{-1} dry wt.		P-influx nmol P mg^{-1} root dry wt. h^{-1}
			Root	Shot	
S-1C	0	70.98±4.83	424.7±27.4	320.5±25.3	33.77±1.14
S-1N	1.6±0.3	65.34±4.92	420.3±29.5	314.7±24.6	30.35±1.21
S-1H	2.9±0.5	67.63±5.12	412.5±30.6	303.5±27.8	22.83±1.29
$LSD_{0.05}$		NS[a]	NS	NS	2.84
$LSD_{0.01}$					4.01
T-1C	0	36.50±3.06	495.4±35.3	371.3±30.1	33.04±2.35
T-1N	2.7±0.6	34.65±3.12	488.7±38.6	365.6±31.5	31.25±2.43
T-1H	4.2±0.7	37.16±3.16	481.3±41.5	358.3±29.3	27.91±268
$LSD_{0.05}$		NS	NS	NS	NS

[a]Not significant.

4. Discussion

Our data support the postulate that the greenbug damages its susceptible host systemically. After 6 h of infestation P-influx is reduced in susceptible barley plants; a previous report indicated that 3 h of infestation reduced the P-transport without any alteration in the P-influx (Giménez et al., 1990). Since chlorophyll concentration, dry weight and P per plant in the infested plants were not significantly affected, the reduction in the P-influx may be attributed to an alteration in the phosphate uptake system. Phosphate absorption and transport are active processes (Epstein, 1976; Clarkson, 1985); consequently this reduction might be related to chemical mediation produced in response to infestation. The variations determined in chlorophyll concentration would be related to temporal alterations in the plant water relationship that seem to be affected by greenbug infestation (Castro et al., 1988, 1991; Castro, 1994). Susceptible plants with one expanded leaf suffered a significant reduction in P-influx over a longer period than those with two expanded leaves. Phosphate influx in the latter was similar to that of the controls at 48 h, whereas in plants with one expanded leaf, it remained significantly lower for the 72 h infestation period. The recovery observed in plants with two expanded leaves might be explained by homeostatic and non-allosteric mechanisms that activate the P-influx

(Drew and Saker, 1984). These mechanisms might be undeveloped or overcome by the stress caused by infestation in the seedlings with one expanded leaf; moreover when they were infested with an extra greenbug load, P-influx at 72 h of infestation was reduced remarkably.

The results show that in T-1 plants greenbugs did not reduce P-influx despite their smaller aerial biomass and, consequently, higher aphid density, compared with S-1 plants. In the tolerant plants with a higher density greenbug infestation, P-influx was not altered. Tolerant plants might be less sensitive to the constraints imposed by aphid infestation. It has been reported that the growth pattern of tolerant plants is not inhibited by greenbugs; since differentiations of leaf primordia in the main apex (Castro and Rumi, 1987; Castro et al., 1989, 1990) and of new nodal roots far from feeding sites (Castro et al., 1988, 1991; Castro, 1994) were not stunted, the P requirements and consequently the P-influx are kept constant.

The stages of aphid development have different effects on plant P-influx. Compared with the nymphs, the reduction caused by adults may be attributed to the greater stress they provoked. It has been reported that greenbug infestation produced a significantly increase in plant ethylene production (Miller et al., 1994; Giménez et al., 1996) in susceptible and tolerant cultivars, but while the susceptible plants were immediately affected by the toxic level of that hormone, the tolerant plants had higher thresholds (Giménez et al., 1996).

Phosphate influx may be affected permanently under greenbug infestation in field conditions due to the reduced root growth and the continuous increase in greenbug number per plant.

Greenbugs affected the systemic absorption capacity of its susceptible hosts. This is an important feature because a detailed understanding of plant–greenbug physiological interactions might provide one of the most effective tools for breeding tolerant plants.

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