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EFFECT OF INFESTATION BY APHIDS ON THE WATER STATUS OF BARLEY AND INSECT DEVELOPMENT

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Key Word Index—*Hordeum vulgare*; Graminea; barley; *Schizaphis graminum*; greenbug; water-stress; stomatal resistance; abscisic acid.

Abstract—To compare the effects of aphid infestation with some effects of wounding and drought-stress, several physiological parameters and metabolite concentrations were measured in infested, mechanically wounded or water-stressed young barley plants (*Hordeum vulgare* cv Aramir). Barley plants infested with the greenbug (*Schizaphis graminum*) had lower water potentials and CO_2 assimilation than non-infested plants. Abscisic acid content increased by 55% in leaves after 72 hr of infestation. Water potentials and stomatal resistance of barley plants changed only as a consequence of infestation by the greenbug or by drought-stress. Proline concentration increased in leaves subjected to infestation or drought by 11- and 14-fold, respectively. Leaves with artificial damage showed the same reduction in chlorophyll contents as leaves of drought-stressed plants. Greenbug infestation caused higher chlorosis than other treatments. Contents of soluble carbohydrates and proteins decreased 52 and 38% by infestation, 38 and 28% by drought, and 14 and 8% by artificial leaf damage, respectively. To study the influence of these treatments on the quality of barley plants as a food source for the aphids, developmental rates ($1/D$) and the mean relative growth rates (MRGR) of nymphs reared on these seedlings were compared. New aphids reared on previously infested seedlings had the lowest MRGR and $1/D$ (*ca* 82 and 68%, respectively) compared to aphids on control plants without previous infestation. Aphids reared on plants subjected to drought also had lower MRGR and $1/D$ (*ca* 89% and 77%). Greenbugs on wounded leaves had similar MRGR and $1/D$ rates to nymphs reared on control plants. These results show that greenbug infestation of barley produced changes similar to those observed in plants subjected to drought-stress and that aphids feeding on both groups of seedlings had lower developmental and mean relative growth rates. Water-stress caused in barley by aphid infestation or drought would probably affect greenbug development due to the effects of stress on the chemical composition of the plant.

INTRODUCTION

Environmental factors (abiotic and biotic stress) may affect plant–aphid interactions because the chemical composition of the plant under stress changes [1]. It has been suggested that barley seedlings grown under high temperatures and long photo-periods are more resistant to aphids because these factors cause an increase in gramine concentration in the youngest leaves [2, 3]. Hydroxamic acids induced by aphid feeding in wheat [4] and in maize after artificial damage [5] or larval attack [6] are some of the secondary metabolites studied whose synthesis is promoted in plants under insect attack. Other phytochemical changes, which are known to occur after infestation and leaf damage, are increases in the concentration of phenols [7], alkaloids [8], plant proteinase inhibitors [9] and amino acids [10].

Barley seedlings subjected to several days of infestation by the greenbug (*Schizaphis graminum*) showed physiological and metabolic changes. With a larger number of aphids, proline concentration increased, water potentials, chlorophyll and soluble carbohydrates contents decreased, and lower rates of CO_2 assimilation were found [11]. The greenbug also affects the aerial vegetative growth of barley by destruction of photosynthetic areas [12] and, therefore, infestation reduces chlorophyll content in leaves, affecting photosynthesis [13]. In wheat, the greenbug decreased CO_2 assimilation by decreasing photosynthetic capacity [14] and, although the advanced symptoms—prostrate growth habit and yellow and rolled leaves in infested barley—are well defined, the physiological or metabolic responses of infested plants are not; the effects of such metabolic changes on insect development are also unknown.

Extrinsic factors, such us food quality and temperature, affect developmental rates of aphids ($1/D$). The

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time required for the greenbug to develop from birth to adult (D) and the mean relative growth rate (MRGR) of an aphid, which is its growth per unit weight per unit time, are both good indirect measurements of the quality of the food supply to aphids [15]. In this paper, we show that water-stress induced by greenbug infestation causes metabolic and physiological changes in barley that are likely to be deleterious to the development of the aphid.

RESULTS

Physiological parameters and metabolite concentrations

After five days, the population of aphids on plants infested initially with 20 adults had increased to 158 ± 32 individuals per plant (mean of 10 replicates ± s.e.). The water potentials of barley plants changed significantly as a consequence of greenbug infestation or of drought-stress treatments (*ca* 100%). Mechanical wounding did not cause significant changes in the water potential of the plants (Table 1). Carbon dioxide assimilation decreased 26% compared to non-infested plants. After five days of greenbug infestation, the chlorophyll content was 55% of the control plants of the same age. Leaves with artificial damage showed the same reduction in chlorophyll as drought-stressed plants after five days of treatment (20% reduction). Contents of soluble carbohydrates and soluble proteins decreased 52 and 38% by preinfestation, 38 and 28% by drought, and 14 and 8% by artificial leaf damage. Proline concentration increased in barley leaves subjected to infestation and drought-stress by 11- and 14-fold, respectively. Sucrose content decreased in barley plants in relation to the levels of infestation (Fig. 1). In relation to non-infested plants, the sucrose content was 49 and 23% in plants infested with 20 and 40 initial aphids, respectively.

Diurnal patterns of stomatal conductance of infested and drought-stressed plants were different compared with non-infested controls. Wounding by artificial damage did not affect the diurnal stomatal conductance pattern, compared to non-infested controls (Fig. 2). When barley leaves of infested and control plants were excised and placed in solutions with ABA at several concentrations, the response of stomatal conductance to exogenous ABA was similar for both treatments (Table 2); ABA was quantified only on infested and control plants. A net increase of ABA was found in the first group after 48 hr of infestation. However, after 96 hr, the amount of ABA in infested leaves decreased and was lower in relation to levels found in control plants (Fig. 3).

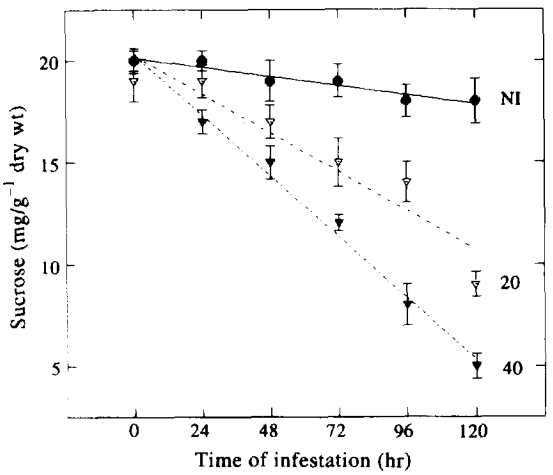

Fig. 1. Time-course of sucrose content in leaves of non-infested and infested barley plants (cv. Aramir). Groups of 10-day-old barley seedlings were infested with 20 or 40 adults of *S. graminum*. Levels of sucrose were measured by HPLC in non-infested control and infested plants every 24 hr for 5 days. Non-infested (●, NI); 20 initial aphids per plant (▽, 20); 40 initial aphids per plant (▼, 40). After 5 days of infestation with 20 and 40 initial aphids per plant, the numbers of aphids per plant were 164 ± 28 and 295 ± 56, respectively (means of $n = 10$ plants ± s.e.).

Table 1. Effects of infestation, wounding and drought on physiological parameters and metabolite concentrations in barley plants

Pretreatment	Water potential (MPa)	Soluble sugar (mmol kg^{-1} dry wt)	Soluble protein (mg g^{-1} dry wt)	Free proline (mmol kg^{-1} dry wt)	Chlorophyll (mg g^{-1} dry wt)	CO_2 assimilation (mg CO_2 mg^{-1} Chl)
Control (A)	− 0.28 ± 0.06 *a*	122 ± 4 *a*	143 ± 6 *a*	4.2 ± 0.4 *a*	8.1 ± 0.4 *a*	2.37 ± 0.08 *a*
Control (B)	− 0.26 ± 0.08 *a*	117 ± 4 *a*	140 ± 5 *a*	3.8 ± 0.3 *a*	7.9 ± 0.3 *a*	2.54 ± 0.04 *a*
Infested	− 0.84 ± 0.09 *c*	56 ± 5 *c*	87 ± 5 *b*	42.3 ± 4.4 *c*	4.4 ± 0.4 *c*	1.89 ± 0.06 *b*
Wounding	− 0.40 ± 0.10 *a*	101 ± 4 *a*	129 ± 4 *a*	10.3 ± 2.0 *a*	6.5 ± 0.3 *b*	N.D.
Drought	− 0.78 ± 0.05 *c*	73 ± 4 *b*	102 ± 6 *b*	53.3 ± 4.8 *c*	6.5 ± 0.4 *b*	N.D.

Groups of 20 barley plants cv Aramir (10 days old) were used for experiments. Controls, without treatment: (A) are 10-day-old plants and (B) 15-day-old plants. The third group was infested with 20 adult aphids of the greenbug per plant for 5 days. The aphid population reached a mean of 158 aphids per plant. Leaf damage was caused by wounding leaves twice per day with glass microcapillary tips for 5 days. The drought-stress treatment was done by suspending watering 7 days after sowing. Each value is the mean of 4 samples ± s.e. N.D.: not determined. Letters within columns differ significantly at (*a*: $P > 0.05$; *b*: $P < 0.05$; *c*: $P < 0.001$ ANOVA).

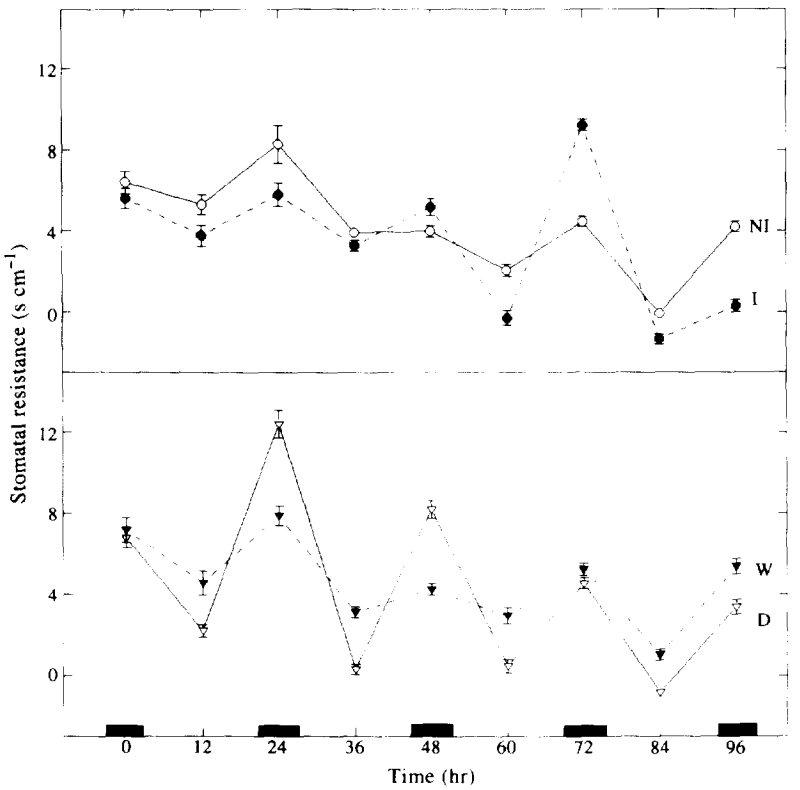

Fig. 2. Time-course of stomatal resistance under a light/dark regime of non-infested (○, NI) and infested (●, I) or wounded (▼, W) and drought-stressed (▽, D) barley seedlings. Treatments were done on 10-day-old plants for 5 days as described in Table 1 and Experimental. Each value is the mean of 8 samples ± s.e.

Table 2. Time-course of stomatal resistance in excised leaves of non-infested (NI) and infested (I) barley seedlings in solutions with different ABA concentrations

	ABA solutions ($M \times 10^5$)	Stomatal resistance (s cm^{-1})		
		0 hr	3 hr	6 hr
NI leaves	0	4.7 ± 0.8	0.3 ± 0.2	− 1.0 ± 0.3
	3.8	4.4 ± 0.2	7.2 ± 0.4	6.0 ± 0.9
	7.6	3.9 ± 0.3	12.0 ± 1.1	11.1 ± 0.8
I leaves	0	1.1 ± 0.3	0.9 ± 0.2	− 0.2 ± 0.3
	3.8	0.5 ± 0.4	1.5 ± 0.7	5.2 ± 0.6
	7.6	0.7 ± 0.5	6.5 ± 0.5	6.6 ± 0.4

Excised leaves were incubated with the cut end for 6 hr in control solution of distilled water without ABA or with ABA. Measurements were made at 0, 3 and 6 hr after incubation with ABA solutions was started. Each value of stomatal resistance is the mean of 8 samples ± s.e.

Developmental (1/D) and mean relative growth (MRGR) rates of aphids

Nymphs of *S. graminum* reared on preinfested barley had the lowest developmental (*t*-test: $t = 7.205$, D.F. = 8, $P < 0.001$; *ca* 68%) and mean relative growth rates (*t*-test: $t = 5.303$, D.F. = 8, $P < 0.001$; *ca* 82%) compared to nymphs reared on control plants without pretreatment. Drought-stressed plants, as well as the preinfested group, are a poor quality food supply for development of the greenbug, as suggested by the $1/D$ values ($t = 3.856$, D.F. = 8, $P < 0.001$; *ca* 77%); MRGR values were similar ($t = 1.886$, D.F. = 8, $P = 0.096$; *ca* 89%). Nymphs of the greenbug reared on artificially damaged plants had a developmental rate ($t = 0.930$, D.F. = 8, $P = 0.280$; *ca* 92%) and a mean relative growth rate ($t = 1.886$, D.F. = 8, $P = 0.707$; *ca* 97%) similar to aphids reared on control, undamaged barley plants (Fig. 4).

DISCUSSION

Barley seedlings infested with *S. graminum* have drought-stress symptoms, such as lower water potentials and lower relative water contents, even in the presence of ample root moisture [11]. Our study suggests that some of the metabolic changes in barley could be due to water-stress caused by aphids. Stomatal conductance is an important factor in regulating water losses by the plant. It has been suggested that *S. graminum* induces a decrease in CO_2 assimilation in wheat by decreasing the photosynthetic capacity in the mesophyll rather than by decreasing leaf conductance to water vapour [14]. In our experiments, leaf stomatal resistance of barley seedlings infested with the greenbug showed similar diurnal patterns to those of control plants without infestation between 0 and 48 hr after infestation. After this time, stomatal resistance in infested plants was higher during the dark (72 hr) and lower during the light periods (60, 84 and 96 hr) than in control plants. These results are similar to those found with *Diuraphis noxia* in barley [16]. Excised barley leaves of infested and control plants placed in solutions with different ABA concentrations gave similar responses in stomatal resistances to water vapour. Restoration of stomatal physiology in infested leaves incubated with exogenous ABA may be indicative that greenbug infestation, in addition to the disruption of the mesophyll chloroplasts that affects light-absorbing systems and CO_2 assimilation, would also decrease the capacity for the synthesis and transport of ABA, because chloroplasts appear to be the major site of synthesis and storage of this hormone [17]. The low level of ABA found in leaves of infested plants at 96 hr supports this hypothesis (Fig. 3). These results suggest that greenbug infestation could affect the regulation of water balance in leaves by affecting stomatal physiology. Stomatal closure is initiated by the redistribution of stored ABA from mesophyll chloroplast into the apoplast [18]. Altered compartmentation of ABA provides possible explanations for patterns of stomatal closure in water-stressed barley during the first 60 hr of drought-stress. These changes in stomatal conductance in the light period in early days may be a result of leaf dehydration with redistribution and increase of ABA synthesis in water-stressed plants.

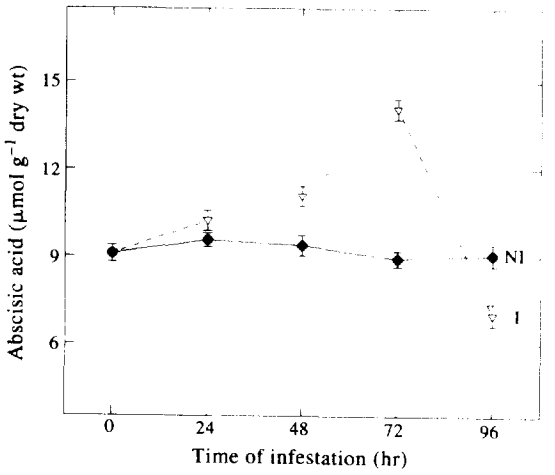

Fig. 3. Time-course of ABA levels in control non-infested (●, NI) and infested (▽, I) leaves of barley plants. Ten day-old seedlings were infested with 20 aphids per plant and contents of total ABA in plants were measured by HPLC every 24 hr for 5 days. Each value is the mean of 3 samples ± s.e.

Water-stress has important effects on carbohydrate metabolism in plants [19]. Infested barley leaves had the lowest contents of sucrose, soluble sugars, total chlorophyll and the lowest photosynthetic rates when com-

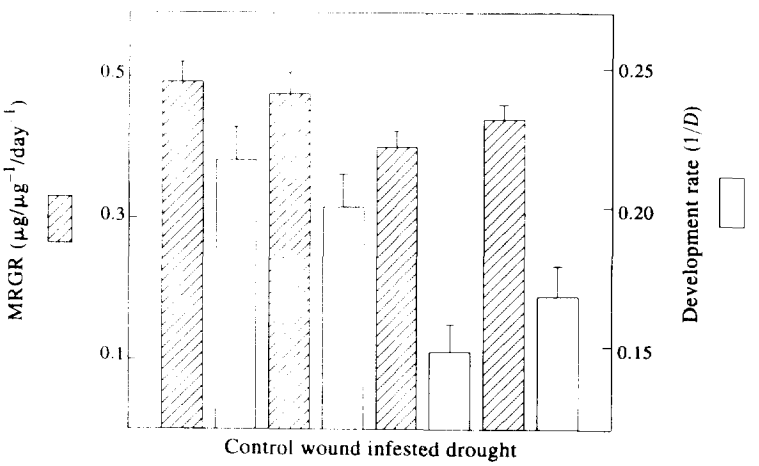

Fig. 4. Mean relative growth rates (MRGR) and development rates ($1/D$) for aphids reared on control, wounded, preinfested and drought-stressed barley seedlings. Nymphs of aphids weighed at 1st or 2nd instar were placed on barley leaves (20 plants each with a single aphid per treatment). MRGR was calculated from aphid weights over a 72 hr period and development rates were calculated with the expression $1/D$, where D is the time taken by an aphid to reach the adult stage and onset of reproduction. Each value is the mean ($n = 10$) ± s.e.

pared with damaged leaf, drought or control treatments (Fig. 1 and Table 1). Since leaves of plants previously infested with aphids had the lowest sucrose levels, it is possible that greenbug infestation may affect the capacity of barley plants to adjust osmotically to drought-stress. In addition, the poor carbohydrate status in barley plants after several days of infestation may limit the bulk of carbon for proline synthesis and may be a possible reason why barley plants infested with the greenbug accumulated less proline (an osmotic solute) than plants with a similar reduced water potential, but without infestation [11].

Water-stress caused by greenbug infestation induces several metabolic changes in barley. These changes would affect aphids at the nutritional–physiological level or may alter behavioural responses, such as the length of time for feeding and preference for specific plants or location on plants [20, 21]. *Schizaphis graminum* biotype C feeds preferentially from vascular tissues in sorghum [22] and phloem in barley [20]. It is therefore likely that nymphs of greenbugs reared on preinfested and drought-stressed plants had lower sap phloem ingestion. The lower levels of soluble proteins and sucrose found in infested plants may be important nutritional factors affecting development of the nymphs. Additional studies of greenbug nutrition on well defined diets where levels of constituents can be varied independently would be especially valuable in establishing a more complete understanding of the role of plant metabolites in the relationship between plant water-stress provoked by the greenbug and insect performance.

EXPERIMENTAL

Plant growth conditions, infestation treatments and metabolite contents. Groups of 20 10-day-old barley seedlings (*Hordeum vulgare* cv Aramir) grown in pots with vermiculite, were irrigated twice per week with a Hoagland soln and infested with 20 adult individuals of the aphid, *S. graminum* Rondani biotype C per plant. After 5 days, the final population was counted and the aphids removed. Individual aphids were collected from colonies maintained on barley, kept at 22 ± 3° with a 16 hr photo-period. The same conditions were used for all expts. The physiological and metabolic state of controls and infested plants for 5 days on 10- and 15-day-old barley seedlings (with 2 and 3 leaves, respectively) was measured by parameters, such as H_2O potential and CO_2 assimilation, and concns of free proline, soluble carbohydrates, soluble proteins and total chlorophyll. Contents of abscisic acid (ABA) and sucrose were measured in controls and infested plants at 0, 24, 48, 72 and 96 hr after initial infestation with 20 and 40 aphids per plant.

Analysis of compounds and physiological parameters. Proline was extracted by homogenizing 1 g leaves in 10 ml 3% aq. sulphosalicylic acid and quantified as described in ref. [23]. Extracts (2 ml) were reacted with the same vol. of 3% (w/v) acid ninhydrin and 60% (v/v) of HOAc at 100° for 1 hr. Proline was extracted with 4 ml of toluene; A was measured at 520 nm. Soluble carbohydrates were quantified by the anthrone method described in ref. [24]. Leaves (0.5 g) in 3 ml of 85% (v/v) EtOH were placed at 25° for 24 hr and then filtered; 0.1 ml of the alcoholic extract was reacted with 3 ml freshly prepd anthrone reagent (1.5% w/v in 72% v/v H_2SO_4) at 100° over 10 min. The reaction was stopped by cooling with ice and A measured at 625 nm. Sucrose content was quantified by HPLC. Fresh leaves (1 g) were placed in 6 ml 85% (v/v) EtOH at 25° for 24 hr, passed through a Millipore filter (0.45 μm) and determined as described in ref. [25]. Chlorophyll contents were measured by extracting plant leaves with 96% (v/v) EtOH. The extract was filtered through Whatman No. 2 paper and the A of the extract measured at 649 and 665 nm as described in ref. [26]. The equation used for determinations of total chlorophyll is: Total Chl (μg of Chl ml^{-1} extract) = $18.08A_{649} + 6.63A_{665}$.

Soluble protein concn in leaves of barley was quantified by the method of ref. [27]. Leaves (0.5 g) were frozen and homogenized with 2.5 ml 0.1 M Tris–HCl (pH 6.8) buffer. The extract (1 ml) was centrifuged at 7000 *g* for 10 min at 4°. The supernatant (100 μl) was dild with 500 μl cold Me_2CO, stored at −20° for 1 hr and then centrifuged at 7000 *g* for 5 min at 4°. The pellet was evapd to dryness *in vacuo* for 2 hr and then resuspended in 1 ml 1 N NaOH; 100 μl of this soln was reacted for 10 min at 25° with 2.5 ml Bradford reagent. A was measured at 595 nm. Protein concn is expressed in equivalents of BSA from the calibration curve. For ABA determinations, leaves (1 g) were cut into 1 cm sections and placed in 5 ml 85% (v/v) EtOH for 24 hr at 25°. The extract was filtered through a Millipore prefilter (0.45 μM) and acidified with 0.5 N H_3PO_4 to pH 4. Samples (20 μl) were injected on to a Lichrosphere 100 RP18 column (Merck). Isocratic elution was with MeCN–H_2O (7:3) at 1.5 ml min^{-1}. Quantitation was performed by measuring A_{236}. A standard curve was made with (±) *cis, trans*-ABA standard (Sigma) in 85% (v/v) EtOH, with a R_t of 8.5 min [28]. Water potentials were measured with a pressure-pump as described in ref. [29]. Plants were excised at the lower zone of the shoot and placed in a sealed pressure chamber with the cut surface outside. CO_2 exchange was measured with an IR gas analyser at 25° with a light intensity of 700 μmol m^{-2} sec^{-1}. The area and total chlorophyll content of the leaves was measured and photosynthesis calculated as described in ref. [30]. Abaxial leaf stomatal conductance was measured with a steady state porometer as described in ref. [31]. Statistical analyses were performed using the SYSTAT-package. The significance of the effect of treatments on plants was assessed by one-way analysis of variance (ANOVA) and the effect on aphids performance by a *t*-test between treatments and control. Results are given as means ± s.e.

Development and mean relative growth rates of the greenbug. Immediately after removal of the initial populations, individual aphids (1st and 2nd instar nymphs) were weighed and placed on to the abaxial surface of

preinfested and non-preinfested leaves of barley seedlings. These aphids were taken directly from the colonies. Aphid developmental rate was calculated as $1/D$, where D = time in days from birth to adulthood. The mean relative growth rate (MRGR) was calcd with the aphid wts for a 72 hr period as follows [32]:

$$\text{MRGR } (\mu g\,\mu g^{-1}\,\text{day}^{-1}) = \frac{\log_e(\text{final wt. } \mu g) - \log_e(\text{initial wt. } \mu g)}{3}$$

The weights of aphids were obtained with a microbalance.

Development of aphids on artificially-damaged and water-stressed plants. Groups of 20 10-day-old barley seedlings, grown under the same conditions described above, were punctured with opened glass microcapillary tips. Since aphid probing is continuous, artificial puncturing was performed with 20 punctures per leaf, twice per day for 5 days. Another group of plants of the same age was not watered until it reached the same water potential of preinfested plants (*ca* − 0.8 MPa). The control was a group of plants of the same age without leaf damage or water-stress. Plants for each treatment were numbered, and one weighed aphid per plant was placed on the leaf. After 72 hr, aphids were weighed to determine MRGR rates and placed again on the respective plants to obtain $1/D$.

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