Oecologia (2003) 137: 564–571
DOI 10.1007/s00442-003-1383-4

PLANT ANIMAL INTERACTIONS

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The relationship between relative growth rate and susceptibility to aphids in wild barley under different nutrient levels

Received: 3 December 2002 / Accepted: 15 August 2003 / Published online: 11 September 2003

Abstract The Resource Availability Hypothesis (RAH) states that plants with a low Relative Growth Rate (RGR) and high levels of defence against herbivores or pathogens are favoured in habitats with low resource availability, whereas plants with a high potential RGR and low levels of defence are favoured in environments with high resource availability. High levels of defence are expected to result in lower reproduction and/or growth of the herbivores or pathogens. To test this hypothesis, four accessions of each of nine natural *Hordeum spontaneum* (wild barley) populations were grown in a climate chamber under two levels of nutrient supply. Susceptibility to *Schizaphis graminum* (greenbug) was quantified by placing a single adult greenbug on each plant and measuring its realised fecundity after 8 days. Data on potential RGR were available from a previous experiment. No support for the RAH was found. The correlation between potential RGR and greenbug reproduction was not significant, neither at the high nor at the low level of nutrient supply. Furthermore, on average plants grown under high and low nutrients did not differ in susceptibility. However, accessions-within-populations differed in the way susceptibility was affected by nutrient supply, and most accessions had a higher susceptibility under nutrient-poor conditions. It could be that these accessions differed in the spectrum of secondary metabolites they produced. Whatever the cause, the genetic variation for the reaction in susceptibility to nutrient supply suggests that selection could act in favour of more or less plasticity in plants without any apparent change in potential RGR.

Keywords Aphid fecundity · Hordeum · Resource Availability Hypothesis · Relative Growth Rate · *Schizaphis*

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Introduction

Species originating from favourable environments often have an inherently high potential Relative Growth Rate (RGR), whereas those from less favourable habitats usually have an inherently low maximum RGR (Grime and Hunt 1975; Poorter and Remkes 1990). The question arises why species from unfavourable habitats cannot grow faster when they have access to ample resources (Almeida-Cortez et al. 1999). The Resource Availability Hypothesis (RAH) gives a possible explanation for this phenomenon. It states that natural selection favours plants with high maximum growth rates and low defence levels in habitats with high resource availability and plants with low potential growth rates and high levels of defence in habitats with low resource availability (Coley et al. 1985). Resource-poor habitats favour plant species with long-lived leaves (Coley 1983, 1988; Coley et al. 1985) because leaf replacement, and therefore the replacements of the lost mineral nutrients, is much more costly in these habitats (Coley et al. 1985; Lim and Turner 1996). In order to avoid leaf losses as a result of, for example, herbivory, species adapted to such habitats should be better defended against herbivore attack (Lambers and Poorter 1992). The trade-off between optimal defence and growth emerges because plants must divide resources over these two functions. The payoff of allocation to growth increases when the environment improves, whereas the payoff of defence remains the same (De Jong 1995).

Support for the RAH has been found in several studies. Light availability in the natural habitat of neotropical tree species was negatively correlated to defence investment and positively to both herbivore damage (Coley 1983) and potential RGR (Coley 1983, 1988). Furthermore, Coley (1983, 1988), Sheldon (1987) and Bryant et al. (1989) found that herbivores prefer plant species with inherently rapid growth to inherently slower growing species. The negative correlation between maximum RGR and defence investment shown by Coley (1983, 1988) and Niemann et al. (1992) is in accordance with the latter observation. Other studies, however, do not support the RAH. McCanny et al. (1990) and Rohner and Ward (1997) did not find a correlation between defence investment and resource availability in the natural habitat (soil fertility and water availability). In addition, McCanny et al. (1990) and Almeida-Cortez et al. (1999) found no correlation between defence investment and potential RGR.

To test if the RAH also applies to different genotypes within a single plant species and their defence against a pathogen, Hoffland et al. (1996) measured maximum RGR, resistance against *Fusarium* and chemical composition of non-infested plants in 15 radish (*Raphanus sativus* L.) cultivars. They found a negative correlation between maximum RGR and resistance level, as predicted by the RAH. Maximum RGR was negatively correlated with defence investment (cell wall material) in the leaves, but positively with the amount of cell wall material in the roots.

All the aforementioned studies compared species or cultivars under identical conditions. However, plants react to the ambient environmental conditions, and it is also of interest to study plastic responses in defence investment in the framework of the RAH. De Jong (1995) argued that the RAH also predicts plastic adjustment of optimal defence investment to local growing conditions. One would expect a plant to increase defence investment under nutrient-poor conditions since leaves are costly and carbon is not limiting. The main objective of this study is to investigate the possible plasticity of the relationship between potential RGR and defence investment, in reaction to nutrient limitation.

As a model system we chose the grass species *Hordeum spontaneum* C. Koch. (wild barley) and the aphid *Schizaphis graminum* Rondani (greenbug), a common herbivore. *H. spontaneum* is the closely-related wild ancestor of *H. vulgare* L. (cultivated barley) (Harlan and Zohary 1966; Zohary 1969; Nevo et al. 1984) and it is widely distributed over the eastern Mediterranean basin and in western Asiatic countries. Its occurrence over a wide range of climates and soils (Harlan and Zohary 1966; Zohary 1969; Nevo 1992) makes it a suitable species for studying plasticity. Israel represents a large portion of the ecological range of wild barley in the Middle East, where it grows on a number of different soil types (Nevo et al. 1984). High genetic variation has been found for several traits, such as biomass, yield, nitrogen content, drought, salt tolerance and disease resistances (powdery mildew and leaf rust) (cf. Nevo 1992). Genetic variation was found for growth characteristics, both among and within natural populations, indicating that some accessions consistently grew faster than others (Elberse et al. 2003). Plasticity in potential RGR in reaction to nutrient limitation did not differ among populations or accessions-within-populations, i.e. all accessions reacted to nutrient limitation in a similar way. The same was found for the RGR components Net Assimilation Rate (NAR, dry mass gain per unit leaf area per day) and Specific Leaf Area (SLA, leaf area per unit of leaf dry mass). However, for the RGR components Leaf Mass Fraction (LMF, leaf dry mass per unit of plant dry mass) and Leaf Area Ratio (LAR, leaf area per unit of plant dry mass) differences in plasticity were shown, both among populations and among accessions-within-populations (Elberse et al. 2003). As far as we know, greenbug resistance of wild barley accessions from this region has not been tested.

S. graminum is a serious pest in small grain cereals and sorghum and it is spread worldwide (Auclair 1989). Aphids may severely damage barley, mainly because they remove essential nutrients, damage tissues and may transmit viruses (Corcuera 1993). Barley has several defence mechanisms that act against greenbugs and other aphids, including morphological and chemical defences. The secondary metabolite gramine (an indole proto-alkaloid) produced by *H. vulgare* has been shown to act against greenbugs (Corcuera 1990, 1993; Velozo et al. 1999), and has been shown to have a genetic basis in crosses between *H. spontaneum* and *H. vulgare* (Moharramipour et al. 1999). Gramine also occurs in *H. spontaneum* (Hanson et al. 1981) and it has a wide range of biological activities, including toxicity to mammals, insects (including several aphids), plants and bacteria (cf. Corcuera 1993). Other secondary compounds that negatively affect *S. graminum* are the phenolic compound ferulic acid (Cabrera et al. 1995), and the hydroxamic acid DIBOA (Niemeyer 1991; Barria et al. 1992; Gianoli and Niemeyer 1998). It remains to be tested whether phloem levels of these two compounds in natural barley populations are comparable to levels shown to be toxic in artificial diet studies (Corcuera 1993; Cabrera et al. 1995). Since aphids depend on the plant for their nutrition, the plant's nutrient conditions may also indirectly affect aphids through effects on plant quality (Corcuera 1993). The literature, however, is not clear on this matter. Some studies found that a high nutrient supply to the plant improved aphid performance (Gruber and Dixon 1988; Petitt et al. 1994; Kainulainen et al. 1996), while others reported a negative relationship (Salas et al. 1990; Mabry et al. 1997). Salas et al. (1990) studied *S. graminum* on barley, and we therefore expected to find a negative relationship. Furthermore, since a trade-off between potential RGR and defence level can only be present when resources are limiting (Karban and Baldwin 1997), the negative relationship between potential RGR and defence level was expected to be strongest at low nutrient levels.

In this study, the relationship between potential RGR and susceptibility of *H. spontaneum* to greenbugs was studied. With respect to herbivores, the terms resistance and susceptibility are commonly used to indicate the ability (or inability) of plants to decrease the preference or performance of the herbivore (Karban and Myers 1989; Karban and Baldwin 1997). We will use the term 'susceptibility' to indicate higher aphid fecundity on a particular plant. The RAH was originally phrased in terms of plant defence. Use of the term defence is restricted to cases where higher resistance (lower susceptibility) results in increased plant performance (Karban and Myers 1989). Since we did not estimate effects of increased aphid fecundity (susceptibility) on plant fitness, we make the assumption that higher aphid fecundity is associated with increased damage to the plant, i.e. lower defence. The following questions were asked:

1. Does the RAH apply? Are wild barley accessions with a high potential RGR more susceptible to aphids under near to optimal nutrient supply?
2. Are the plants most susceptible at the lowest nutrient level, as Salas et al. (1990) found?
3. Does nutrient limitation make the positive relationship between potential RGR and susceptibility stronger?
4. Does the change in susceptibility in reaction to nutrient limitation differ among wild barley populations or accessions-within-populations?

Materials and methods

Plant material

Eight *Hordeum spontaneum* populations from different habitats in Israel and one from Iran were studied (Elberse et al. 2003). The habitats at the sampling sites of these nine populations represent a wide range of geographical and environmental conditions. The Iranian population was included since extra genetic diversity was expected due to the geographical distance. Four randomly chosen accessions per population were used.

Aphid rearing

Seeds of a susceptible wild barley line were sown directly into pots (11×11×12 cm), containing a potting soil:sand mixture of 4:1. Plants were grown in a glasshouse in the Netherlands from January until March 2001 (day/night temperature 20°C/16°C, day length 16 h, average relative humidity 60%). About a month after sowing, plants were used for aphid rearing.

Greenbugs (*Schizaphis graminis* Rondani), biotype E, were obtained from Plant Research International (Wageningen, The Netherlands). Subsequently, the aphids were reared in a growth cabinet, in cages containing wild barley plants (day/night temperature 18°C/15°C, day length 16 h, relative humidity 70%). As the greenbugs reproduced asexually, they all had the same genotype. Young viviparously reproducing females were used in the experiment. They can be recognised as they are typically surrounded by a small colony of first instar nymphs. Thus the females were approximately the same age.

The experiment

A randomised complete block design was used, including eight blocks, nine populations, four accessions per population and two nutrient levels. Four blocks fitted into a climate chamber, so the experiment was conducted in two series of four blocks each.

To stimulate germination, the glumellae, lemmas and spikelet stalks were removed from the caryopses (Gutterman et al. 1996). Naked caryopses were germinated in Petri dishes, on filter paper moistened with demi water, at 4°C in dark. After 7 days, the Petri dishes were transferred to a climate chamber (day/night temperature 23°C/15°C, day length 16 h, light intensity at plant level varying between 312 and 419 μmol m^{-2} s^{-1}, relative humidity 80%). Two days later, the seedlings were planted in the same climate chamber into coarse quartz sand in pots with a diameter of 5 cm, a height of 18 cm and a volume of 250 ml.

Immediately after planting, approximately 100 ml of nutrient solution was carefully given by hand. The two nutrient levels supplied were 1/2 and 1/32 strength of a modified Hoagland's solution (see Elberse et al. 2003). The next day, each plant received 80 ml twice, and during the remainder of the experiment 80 ml was given once a day. Two days after planting, relative humidity was reduced to 70% and kept at this level until the end of the experiment.

As soon as the second leaf was fully expanded, its width was measured. For plants growing at high nutrient supply, this was 18 and 19 days after sowing (block 1–4 and block 5–8, respectively) and for plants growing at low nutrient supply this was 24 days after sowing (all blocks). Subsequently, a clip cage (diameter 2 cm) containing one young reproducing greenbug was put at the middle of the second leaf of each plant. Clip cages appeared not to have any negative effect on the plants, as the part of the second leaf above the clip cage remained green and looked healthy. Reproduction was checked 2 days after introducing the aphid onto the plant. When she had not reproduced at all, she was replaced with a new greenbug. Four days after introduction of a reproducing female, the produced nymphs were counted and removed. Subsequently, the adult female was returned to the clip cage on the same spot of the leaf. Four days later the newly produced nymphs were counted. This procedure resulted in 472 plants for which greenbug data were obtained, and 18% with missing values, mostly due to death or disappearance of mother aphids. Most of these missing values occurred after the first nymph count. Removing the nymphs after the first counts appeared to affect the mother to some extent.

Data analysis

The dependent variable in this study was the total number of nymphs produced by one mother, which we interpret as a measure of plant susceptibility. For the analysis, these data were square-root transformed. Data on potential RGR and its components, for plants growing at 1/8H and 1/32H, were available from a previous experiment (Elberse et al. 2003).

The relationship between potential RGR and susceptibility was tested using the GLM procedure (SAS 1999). An ANOVA was performed on square-root transformed number of nymphs, separately for both nutrient levels. Single degree of freedom contrasts among the 36 accessions were constructed by taking the mean RGR per accession per nutrient level minus overall mean RGR per nutrient level. Random factors in the model were block and accession, and their interaction was included in the error term. Furthermore, Pearson correlations (SAS) between RGR (components) and susceptibility were calculated and tested two-sided.

Analysis of Variance (GLM procedure, SAS) was used to assess if nutrient supply influenced susceptibility and to investigate genetic differences in plasticity of susceptibility in reaction to nutrient limitation. As the diameter of the clip cage exceeded leaf width, the latter was used as a cofactor in the ANOVA. Leaf width had no effect on the number of nymphs produced, and was left out of the analysis; similarly, neither the series effect nor any of the interactions with series was significant (data not shown), and these factors were also left out of the ANOVA model and added to the error term. In the final ANOVA, factors in the model were: block, nutrient level, population, accession-within-population and the two-way interactions. Random factors were block, accession-within-population and all interactions with these two.

The nutrient level×accession effect was studied in more detail by looking at the environmental sensitivity of the accessions. The mean susceptibility values per accession per nutrient level were regressed against the overall mean values per nutrient level. Square-root transformed data were used. The slope of a regression line represents the environmental sensitivity (or plasticity) of an accession (Falconer and Mackay 1996), with the average sensitivity over all accessions being one.

Results

The RAH predicts a positive correlation between potential RGR and susceptibility for *S. graminum* under near optimal growth conditions for the plant. No such correlation was found (Fig. 1A). None of the RGR components showed a significant correlation with susceptibility either (Table 1). For NAR, the correlation coefficient of +0.332 would be significant at the $P<0.05$ level, but after Bonferroni correction for eight tests, the P significance threshold of 0.006 per individual test was not reached.

Susceptibility of *H. spontaneum* to greenbugs was expected to decrease with nutrient supply. However, nutrient level had no significant effect on this susceptibility (Table 2). On average, plants grown at the low nutrient level had 24.3 nymphs (237 plants, SE=0.46) while for the high nutrient plants the mean was 22.8 (235 plants, SE=0.50).

Under the RAH, nutrient limitation would strengthen the positive relationship between potential RGR and susceptibility. However, similar to the high nutrient environment, at the low nutrient level no correlation was found between potential RGR and number of nymphs per plant ($r=0.002$, ns). Therefore, nutrient limitation did not influence the relationship between potential RGR and susceptibility. The same situation was observed for the different RGR components and susceptibility (Table 1).

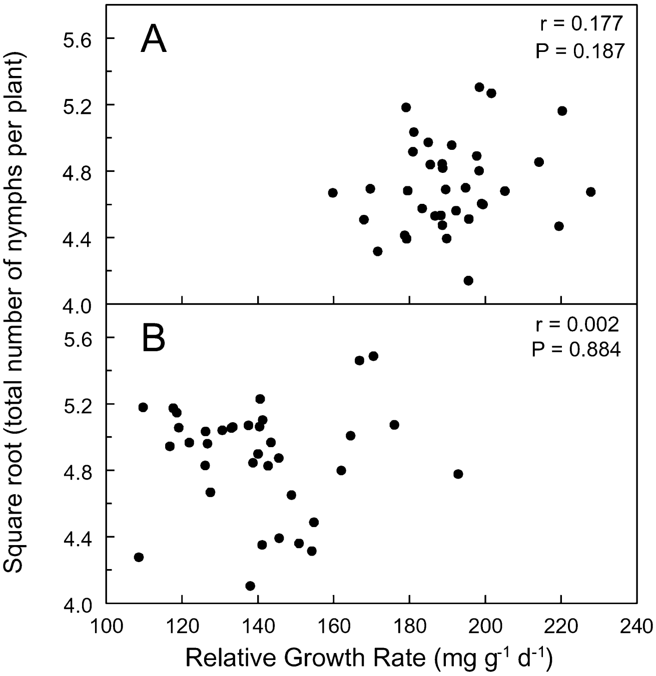

Fig. 1 Correlation between accession means for potential Relative Growth Rate and aphid fecundity (square-root transformed) at high (**A**) and low (**B**) nutrient level. The correlation coefficients, *r*, and the corresponding *P* values are given in the upper right corners

Differences among populations or accessions-within-populations in greenbug reproduction in response to nutrient supply would show up in ANOVA as interactions with nutrient level. Indeed, a significant nutrient×accession-within-population effect was found (Table 2). This plasticity was further studied via environmental sensitivity graphs in which the average numbers of nymphs per nutrient level represent the environmental (x-) values. Since, in our study, this average number was highest for the low nutrient level, a positive slope for an accession (representing the environmental sensitivity) would mean that for that particular accession the susceptibility was highest at the low nutrient level and vice versa. A summary of the environmental sensitivities for all accessions is given in Fig. 2. Supporting the results given in Table 2, no clear differences between populations can be seen, but accessions-within-populations did differ in plasticity. In most populations, accessions with both positive and negative environmental sensitivities were observed. The Mt. Hermon population was the only one for which the plasticity of all four accessions had the same sign (positive).

Discussion

The Resource Availability Hypothesis

The RAH assumes that a trade-off exists between potential RGR and resistance to herbivores and pathogens. This would imply a positive relationship between growth rate and susceptibility. In our study, no such correlation was found. The RAH is usually tested by comparing different species. The lack of support for RAH in the current study could imply that variation within species in the way they allocate resources is too small to be detected, or too small to result in differences in protection against herbivores.

The driving mechanism envisaged by the RAH is that leaves are more worth defending when mineral nutrients are in short supply. As aphids remove photosynthate, rather than leaf material, one could argue that the RAH is not applicable to sapsuckers like aphids, in agreement with our results. But, on the other hand, it is known that aphids are often deterred by secondary metabolites (see Introduction), so that if plants vary in the amount of defence at the cost of a lower growth rate, a response in aphid growth rate is to be expected, albeit not leading to the preservation of leaf material.

Some other studies have also found no support for the RAH. McCanny et al. (1990) and Rohner and Ward (1997) did not find any correlation between defence (fertility) investment and resource (water) availability in the natural habitat. In addition, McCanny et al. (1990) and Almeida-Cortez et al. (1999) observed no correlation between

Table 1 Pearson correlations for the square-root of the total number of nymphs per plant with RGR components at different nutrient levels. Low nutrient level is 1/32 Hoagland and high nutrient level implies 1/2 Hoagland for the aphid experiment and 1/8 Hoagland for the RGR experiment.*P* values of the RGR component contrast are given in brackets. As eight tests were performed (two-sided), the Bonferroni correction results in an individual *P* significance threshold of 0.006 and an overall level of 0.05

Nutrient level aphid experiment	Nutrient level RGR experiment	NAR	LAR	SLA	LMF
High	High	0.332 (0.037)	−0.132 (0.527)	−0.021 (0.859)	−0.108 (0.459)
Low	Low	0.037 (0.725)	−0.050 (0.832)	0.107 (0.393)	−0.151 (0.304)

Table 2 ANOVA results for square-root transformed total number of nymphs per plant. Mean squares and *F* values and their significance are presented. *B* block, *N* nutrient level, *P*population, *A(P)*accession-within-population, *r* random factor, *f*fixed factor

Source	r/f	*df*	MS	*F*
B	r	7	1.50	0.66
N	f	1	1.30	0.54
P	f	8	0.18	0.70
A(P)	r	27	0.49	0.50
B×N	r	7	2.17	4.48***
B×P	r	56	0.38	0.62
B×A(P)	r	182	0.63	1.29
N×P	f	8	0.83	1.06
N×A(P)	r	27	0.81	1.67*
Error		146	0.49	

$^*P<0.05$
$^{***}P<0.001$

maximum RGR and defence investment. Therefore, the RAH may not be generally valid.

A positive correlation between susceptibility and potential RGR is expected because a low susceptibility to generalist herbivores will be caused by a high defence investment in secondary compounds, which will lead to a low potential RGR. In this experiment, susceptibility was measured instead of defence investment assuming a negative relationship between those two traits. This assumed negative correlation requires that the defence compound has a negative effect on the herbivore. Furthermore, the defence should be costly to the plants. The first requirement is met, as the secondary metabolite gramine is known to be harmful to greenbugs (Corcuera 1990, 1993; Velozo et al. 1999). Other secondary compounds that may make *H. spontaneum* resistant to greenbugs are ferulic acid (Cabrera et al. 1995), and DIBOA (Niemeyer 1991; Barria et al. 1992; Gianoli and Niemeyer 1998).

Many researchers have investigated whether defence is costly, mostly via searching for a link between resistance and a growth component (like biomass or number of leaves) or a measure of fitness (e.g. total seed mass produced). Some studies have reported costs of resistance, and some studies have found no such costs (Simms 1992; Karban and Baldwin 1987; Strauss et al. 2002). Windle and Franz (1979) grew near-isogenic greenbug-resistant and susceptible *H. vulgare* cultivars, without the presence of aphids, both in monocultures and cultivar mixtures. In the monocultures, resistant and susceptible cultivars were not found to differ in biomass, indicating no costs of resistance. In the cultivar mixtures, however, the susceptible cultivars were better competitors than the resistant ones. Van Dam and Baldwin (1998) state that costs to defence should be investigated in plants growing in competition. When they grew defended and undefended

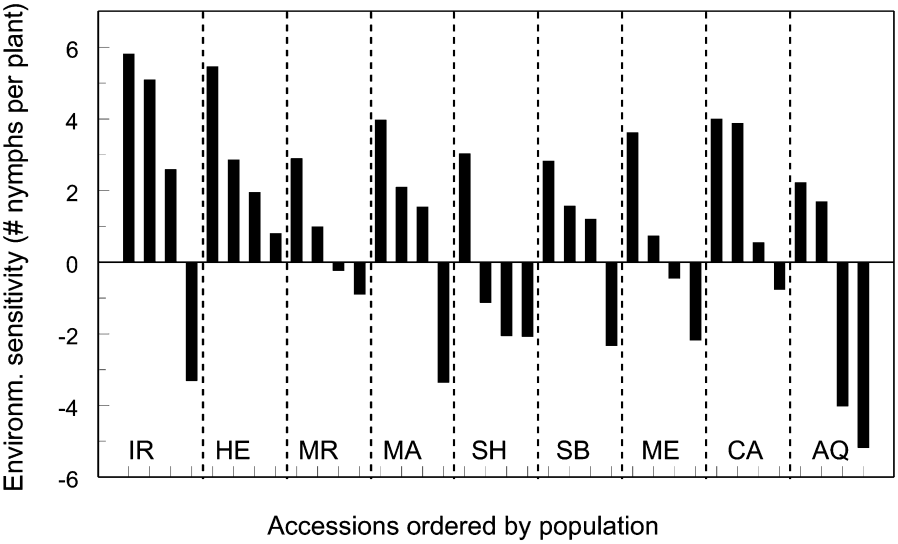

Fig. 2 Environmental Sensitivity (ES) in the number of greenbugs produced in reaction to nutrient level for each of the 36 accessions. ES values are shown as the slope of regression of greenbug production per accession per nutrient level against the mean values per nutrient level (for further explanation see text). The accessions are ranked from high to low ES value within each site of origin, and sites of origin are ranked arbitrarily (so the *x*-axis has no biological meaning). *IR* Iran, *HE* Mt. Hermon, *MR* Mt. Meron, *MA* Maalot, *SH* Shechem, *SB* Sede Boqer, *ME* Mehola, *CA* Caesarea, *AQ* Asquelon. Further details of locations are given in Elberse et al. (2003)

Nicotiana attenuata plants in competition, they found a benefit for non-defended plants when no herbivores were present. They argue that the costs of defence should be considered in terms of competitive strength and resource acquisition, rather than a direct physiological trade-off between defence and biomass production. Their result supports the RAH, since RAH expects fast growers from productive habitats to have a low defence. These plants cannot afford a high defence, because they would lose the competition with other plants. In the present study, the populations studied came from natural habitats which varied from very unfavourable to rather favourable for *H. spontaneum* growth. Thus, these habitats might have very different levels of competition. Therefore, the lack of evidence for the RAH may be because the plants were not grown in competition.

Nutrient effect on susceptibility

No general nutrient effect on susceptibility was found. In the literature, both positive (Gruber and Dixon 1988; Petitt et al. 1994; Kainulainen et al. 1996) and negative (Salas et al. 1990; Mabry et al. 1997) effects of nutrient supply on aphid performance have been described for a number of plant-aphid combinations. This is most likely because of the types of chemical defence involved. Usually, under nutrient-limited conditions non-structural carbohydrates accumulate, leading to an excess of carbon in the plant, so accumulation of carbon-based defences is expected. Similarly, in the case of high nutrient availability, accumulation of nitrogen-based secondary compounds is predicted. This carbon/nutrient balance theory (Bryant et al. 1983) has been confirmed in several studies. Plants grown at a low nutrient availability showed an increase in the concentration of the carbon-based tannins, phenolics and/or phenolic glycosides (Waring et al. 1985; Bryant et al. 1987; Nicolai 1988; but see also Denslow et al. 1987). Results concerning nitrogen-based chemicals were not that clear. Frischknecht et al. (2001) report a negative correlation between growth and pyrrolizidine alkaloid formation, whereas Johnson et al. (1987) observed the expected positive correlation between nitrogen supply and the concentration of alkaloids. The latter also seems to apply to *Hordeum*, given that Salas et al. (1990) found that a high KNO_3 supply to *H. distichum* plants negatively affected *S. graminum* feeding on those plants. Aphid population growth rate decreased when the nitrate concentration in the nutrient solution was higher than 15 mM. This effect seemed to be caused both by an increase in gramine (nitrogen-based) and by a direct negative effect of KNO_3 itself (toxic concentration).

The nutrient×accession-within-population effect was significant. Most accessions were more susceptible to greenbugs at the lowest nutrient level (i.e. positive plasticity), but several others were more susceptible at the highest level of nutrient supply. It is possible that the plants switched from carbon-based protection under low nutrient conditions to nitrogen-based protection at a high nutrient level, with virtually no net effect on greenbug reproduction when averaged over all accessions. Some accessions probably invested more in one of the two types of defence.

Effect of nutrient supply on the relationship between potential RGR and susceptibility

Nutrient supply did not influence the relationship between RGR (components) and susceptibility: in fact, there was no relationship for either nutrient level. Thus, no shift in allocation towards higher potential RGR or more resistance was shown. Frischknecht et al. (2001) studied the plasticity of growth and pyrrolizidine alkaloid formation of *Senecio vulgaris* in response to nutrient level. They observed a negative correlation between growth and defence in both environments, but no clear influence of nutrient level on the correlation between growth and defence was detected in their study.

Differences in plasticity of susceptibility

The change in susceptibility with nutrient limitation varied among accessions-within-populations. Between populations, no significant differences were found, and apparently the population×nutrient effect did not exceed differences among accessions-within-populations in their reaction to nutrient limitation. The highest amount of genetic variation for plasticity was found within populations, confirming the results on other traits that exhibit a significant amount of genetic variation within *H. spontaneum* populations (Elberse et al. 2003; Nevo et al. 1986; Dawson et al. 1993; Zhang et al. 1993; Baum et al. 1997). Accessions-within-populations were sampled randomly, so an ecological explanation of why certain accessions reacted with more plasticity than others remains unclear. Thus, there is scope for natural selection for plasticity of susceptibility (or defence investment) within the sampling sites.

In summary, this study found no support for the RAH and no influence of nutrient availability on the relationship between potential RGR and susceptibility. Conducting a similar experiment with plants growing in competition, though, might reveal support for the RAH. Nevertheless, variation for plasticity of susceptibility was detected between accessions-within-populations. Thus, at the local level, there is scope for evolution of plasticity of susceptibility to greenbugs.

Acknowledgements The authors thank Ab Wijlhuizen for developing and constructing the system for water and nutrient supply. Eviatar Nevo is thanked for kindly supplying seed material and Arjen Biere for his valuable comments on previous versions of this manuscript. This study was supported financially by the Earth and Life Science Foundation (ALW) of the Netherlands Organisation for Scientific Research (NWO).

References

Almeida-Cortez JS, Shipley B, Arnason JT (1999) Do plant species with high relative growth rates have poorer chemical defences? Funct Ecol 13:819–827

Auclair JL (1989) Host plant resistance. In: Minks AK, Harrewijn P (eds) Aphids, their biology, natural enemies and control. World Crop Pests, 2C Elsevier, Amsterdam, The Netherlands, pp225–265

Barria BN, Copaja SV, Niemeyer HM (1992) Occurrence of DIBOA in wild *Hordeum* species and its relation to aphid resistance. Phytochemistry 31:89–91

Baum BR, Nevo E, Johnson DA, Beiles A (1997) Genetic diversity in wild barley (*Hordeum spontaneum* C. Koch) in the near east: a molecular analysis using random amplified polymorphic DNA (RAPD) markers. Genet Resour Crop Evol 44:147–157

Bryant JP, Chapin III FS, Klein DR (1983) Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357–368

Bryant JP, Clausen TP, Reichardt PB, McCarthy MC, Werner RA (1987) Effect of nitrogen fertilization upon the secondary chemistry and nutritional value of quaking aspen (*Populus tremuloides* Michx.) leaves for the large aspen tortrix (*Choristoneura conflictana* (Walker)). Oecologia 73:513–517

Bryant JP, Kuropat PJ, Cooper SM, Frisby K, Owen-Smith N (1989) Resource availability hypothesis of plant antiherbivore defence tested in a South African savanna ecosystem. Nature 340:227–229

Cabrera HM, Muñoz O, Zúñiga GE, Corcuera LJ, Argandoña VH (1995) Changes in ferulic acid and lipid content in aphid-infested barley. Phytochemistry 39:1023–1026

Coley PD (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecol Monogr 53:209–233

Coley PD (1988) Effects of the plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. Oecologia 74:531–536

Coley PD, Bryant JP, Chapin III FS (1985) Resource availability and plant antiherbivore defense. Science 230:895–899

Corcuera LJ (1990) Plant chemicals and resistance of cereals to aphids. Ambio 19:365–367

Corcuera LJ (1993) Biochemical basis for the resistance of barley to aphids. Phytochemistry 33:741–747

Dawson IK, Chalmers KJ, Waugh R, Powell W (1993) Detection and analysis of genetic variation in *Hordeum spontaneum* populations from Israel using RAPD markers. Mol Ecol 2:151–159

De Jong TJ (1995) Why fast-growing plants do not bother about defence. Oikos 74:545–548

Denslow JS, Vitousek PM, Schulz JC (1987) Bioassays of nutrient limitation in a tropical rain forest soil. Oecologia 74:370–376

Elberse IAM, van Damme JMM, van Tienderen PH (2003) Plasticity of growth characteristics in wild barley (*Hordeum spontaneum*) in response to nutrient limitation. J Ecol 91:371–382

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics. Longman, Harlow, UK

Frischknecht PM, Schumacher K, Müller-Schärer H, Baumann TW (2001) Phenotypic plasticity of *Senecio vulgaris* from contrasting habitat types: growth and pyrrolizidine alkaloid formation. J Chem Ecol 27:343–358

Gianoli E, Niemeyer HM (1998) DIBOA in wild poaceae: sources of resistance to the Russian wheat aphid (*Diuraphis noxia*) and the greenbug (*Schizaphis graminum*). Euphytica 102:317–321

Grime JP, Hunt R (1975) Relative growth-rate: its range and adaptive significance in a local flora. J Ecol 63:393–422

Gruber K, Dixon AFG (1988) The effect of nutrient stress on development and reproduction in an aphid. Entomol Exp Appl 47:23–30

Gutterman Y, Corbineau F, Côme D (1996) Dormancy of *Hordeum spontaneum* caryopses from a population on the Negev desert highlands. J Arid Environ 33:337–345

Hanson AD, Traynor PL, Ditz KM, Reicosky DA (1981) Gramine in barley forage—effects of genotype and environment. Crop Sci 21:726–730

Harlan JR, Zohary D (1966) Distribution of wild wheats and barley. Science 153:1074–1080

Hoffland E, Niemann GJ, Van Pelt JA, Pureveen JBM, Eijkel GB, Boon JJ, Lambers H (1996) Relative growth rate correlates negatively with pathogen resistance in radish: the role of plant chemistry. Plant Cell Environ 19:1281–1290

Johnson ND, Liu B, Bentley BL (1987) The effects of nitrogen fixation soil nitrate and defoliation on the growth alkaloids and nitrogen levels of *Lupinus succulentus* (Fabaceae). Oecologia 74:425–431

Kainulainen P, Holopainen J, Palomäki V, Holopainen T (1996) Effects of nitrogen fertilization on secondary chemistry and ectomycorrhizal state of Scots pine seedlings and on growth of grey pine aphid. J Chem Ecol 22:617–636

Karban R, Baldwin IT (1997) Induced responses to herbivory. University of Chicago Press, Chicago, USA

Karban R, Myers JH (1989) Induced plant responses to herbivory. Annu Rev Ecol Syst 20:331–348

Lambers H, Poorter H (1992) Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. Adv Ecol Res 23:187–261

Lim WHL, Turner IM (1996) Resource availability and growth responses to defoliation in seedlings of three early-successional tropical woody species. Ecol Res 11:321–324

Mabry CM, Jasienski M, Coleman JS, Bazzaz FA (1997) Genotypic variation in *Polygonum pensylvanicum*: nutrient effects on plant growth and aphid infestation. Can J Bot 75:546–551

McCanny SJ, Keddy PA, Arnason TJ, Gaudet CL, Moore DRJ, Shipley B (1990) Fertility and the food quality of wetland plants—a test of the resource availability hypothesis. Oikos 59:373–381

Moharramipour S, Takeda K, Sato K, Yoshida H, Tsumuki H (1999) Inheritance of gramine content in barley. Euphytica 106:181–185

Nevo E (1992) Origin evolution population genetics and resources for breeding of wild barley *Hordeum spontaneum* in the Fertile Crescent. In: Shewry PR (ed) Barley: genetics, biochemistry, molecular biology and biotechnology. CAB International, Wallingford, UK, pp19–43

Nevo E, Beiles A, Gutterman Y, Storch N, Kaplan D (1984) Genetic resources of wild cereals in Israel and vicinity. II. Phenotypic variation within and between populations of wild barley *Hordeum spontaneum.* Euphytica 33:737–756

Nevo E, Beiles A, Zohary D (1986) Genetic resources of wild barley in the Near East: structure evolution and application in breeding. Biol J Linn Soc Lond 27:355–380

Nicolai V (1988) Phenolic and mineral content of leaves influences decomposition in European forest ecosystems. Oecologia 75:575–579

Niemann GJ, Pureveen JBM, Eijkel GB, Poorter H, Boon JJ (1992) Differences in relative growth rate in 11 grasses correlate with differences in chemical composition as determined by pyrolysis mass spectrometry. Oecologia 89:567–573

Niemeyer HM (1991) Secondary plant chemicals in aphid-host interactions In: Peters DC, Webster JA, Chlouber CS (eds) Proceedings aphid-plant interactions: populations to molecules. USD Agr Res Service, Oklahoma State University, Stillwater, USA, pp101–111

Petitt FL, Loader CA, Schon MK (1994) Reduction of nitrogen concentration in the hydroponic solution on population growth rate of the aphids (Homoptera: Aphididae) *Aphis gossypii* on cucumber and *Myzus persicae* on pepper. Environ Entomol 23:930–936

Poorter H, Remkes C (1990) Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia 83:553–559

Rohner C, Ward D (1997) Chemical and mechanical defense against herbivory in two sympatric species of desert *Acacia.* J Veg Sci 8:717–726

Salas ML, Corcuera LJ, Argandoña VH (1990) Effect of potassium nitrate on gramine content and resistance of barley against the aphid *Schizaphis graminum.* Phytochemistry 29:3789–3791

SAS (1999) SAS Release 8.02. SAS Institute, Cary, USA

Sheldon SP (1987) The effects of herbivorous snails on submerged macrophyte communities in Minnesota lakes. Ecology 68:1920–1931

Simms EL (1992) Costs of plant resistance to herbivory. In: Fritz RS, Simms EL (eds) Plant resistance to herbivores and pathogens: ecology evolution and genetics. University of Chicago Press, Chicago, USA, pp392–425

Strauss SY, Rudgers JA, Lau JA, Irwin RE (2002) Direct and ecological costs of resistance to herbivory. Trends Ecol Evol 17:278–285

Van Dam NM, Baldwin IT (1998) Costs of jasmonate-induced responses in plants competing for limited resources. Ecol Lett 1:30–33

Velozo JA, Alvarez RI, Wachter GA, Timmermann BN, Corcuera LJ (1999) Increase in gramine content in barley infested by the aphid *Schizaphis graminum*. Phytochemistry 52:1059–1061

Waring RH, McDonald AJS, Larsson S, Ericsson T, Wiren A, Arwidsson E, Ericsson A, Lohammar T (1985) Differences in chemical composition of plants grown at constant relative growth rates with stable mineral nutrition. Oecologia 66:157–160

Windle PN, Franz EH (1979) The effects of insect parasitism on plant competition: greenbug and barley. Ecology 60:521–529

Zhang Q, Saghai Maroof MA, Kleinhofs A (1993) Comparative diversity analysis of RFLPs and isozymes within and among populations of *Hordeum vulgare* ssp *spontaneum.* Genetics 134:909–916

Zohary D (1969) The progenitors of wheat and barley in relation to domestication and agricultural dispersal in the Old World In: Ucko PJ, Dimbleby GW (eds) The domestication and exploitation of plants and animals. Duckworth, London, UK, pp47–66