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Interactive effects of host resistance and drought stress on the performance of a gall-making aphid living on Norway spruce

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Abstract Four full-sib families of Norway spruce (*Picea abies*), of which two were resistant to a galling aphid (*Adelges abietis*) and two were susceptible, were exposed to drought stress for 2 years. The primary aim was to test the hypothesis that the various life stages of the aphid differ in their response to environmentally induced changes in the host plant. The drought treatment had a significant negative effect on tree growth. This was reflected in gall size which responded in a similar way to the drought stress, thus supporting the plant vigour hypothesis. Drought affected the survival of aphid stem-mothers negatively in susceptible trees but positively in resistant trees. This result was matched by the response of an individual phenolic compound which, contrary to the total phenolic concentration, tended to increase in susceptible trees exposed to drought and decrease in similarly exposed resistant trees. Thus it is possible that this single, as yet unidentified, phenolic compound could be used as a marker of resistance. The performance of surviving stem-mothers, evaluated by measuring the diameter of the wax cover they produced (a correlate of fecundity), was not significantly affected by drought, but aphids on susceptible trees produced more wax than those on resistant trees. The change in gall density over time (analysed separately for each treatment and phenotype) correlated best with patterns of stem-mother survival. This indicates that stem-mother survival, which is closely linked to host plant quality and resistance, may play a significant role in the population dynamics of this aphid. However, drought-stress-induced changes in host plant quality affected survival, and hence gall density, less than the genetically determined level of resistance. The results also support the hypothesis that an insect may respond differently to environmentally induced changes in the host plant at different stages in its development.

Key words *Adelges abietis* · *Picea abies* · Feeding mode · Environmental change · Phenolics

Introduction

There is a concern that greenhouse warming and global pollution will increase the stress (e.g. drought) experienced by plants (Ayres 1993; Vitousek 1994; Lawton 1995). Supported by observations, populations of herbivorous insects have been suggested to reach higher densities on stressed plants (White 1974; Mattson and Haack 1987; Koricheva et al. 1998). The mechanisms responsible for increased insect densities on stressed plants are not fully understood and may differ depending on the type of insect (Mattson and Haack 1987; Larsson 1989; Waring and Cobb 1992; Larsson and Björkman 1993). One of the more frequently observed trends is the better performance of insects on stressed host plants, probably because these are of higher nutritional quality and/or have lower resistance (Rhoades 1979; Braun and Flückiger 1984; Kidd 1990). Sucking insects, such as aphids, typically respond positively to stress-induced changes in the host plant, whereas gallers typically respond negatively (Larsson 1989; Larsson and Björkman 1993; Koricheva et al. 1998). How then might insects that are both suckers and gallers, such as adelgid aphids, respond? This will most likely depend on which life stage is most sensitive to the environmental change(s) in question. Two alternative, but complementary, mechanisms for explaining increased insect densities on stressed plants are less efficient control by natural enemies (Hanski 1990) and direct positive effects of the abiotic environment (Martinat 1987).

To test hypotheses for explaining how environmental change affects the risk for increased and more variable insect densities, I chose to work with a system in which there are no known parasitoids and in which predation, at least on some life stages, seems negligible: the gall-making aphid *Adelges abietis* (Homoptera: Adelgidae) on Norway spruce (*Picea abies*) (Schwenke 1972;

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Björkman 1998). Since the impact of natural enemies should be relatively low in this system, aphid responses should mainly reflect the interaction between plant and insect. Another reason for choosing this insect species is that, like other adelgids, it is both a sucker and a galler. One can thereby determine which stage is most sensitive to stress-induced changes in the host plant at both the individual and population (density) level.

The main aim of this study was to determine whether or not the response of this adelgid to drought stress on its host plant is different in the sucking and galling phases (Björkman 1998). The predictions to be tested were (1) that drought should enhance gall initiation (i.e. stem-mother survival) because the associated stress should decrease host resistance, (2) that the performance of surviving stem-mothers should be higher on drought-stressed trees because such trees are of higher nutritional value and (3) that gall size should be negatively affected by drought stress since it largely reflects tree growth. Here, the main objective was to estimate the overall net effect of these separate responses on gall density.

Material and methods

Study organisms

The aphid *Adelges abietis* (L.) (Homoptera: Adelgidae) induces characteristic, pineapple-shaped galls (deformed buds) on Norway spruce, *Picea abies* (L.) Karst. The entire life cycle is completed on Norway spruce, and most individuals stay on the tree on which they are born (Ewert 1967; Carter 1971). For a detailed description of the insect's biology see Schwenke (1972) and Björkman (1998). The life cycle can be summarized as follows. Galls open in the autumn, whereupon gallicolae (i.e. the winged stage) crawl out of the chambers. Each gall comprises 2–30 chambers, and each chamber contains around ten gallicolae. Most gallicolae stay on the tree, where they lay eggs on the needles. The eggs hatch in autumn, and the nymphal stem-mothers crawl out to a bud into which they insert their stylet and start to feed. The stem-mothers overwinter with their stylets inserted in the bud. If the attack is successful, the bud develops into a gall. The stem-mothers lay eggs beneath the wax cover that they produce, while the bud is swelling. Nymphal gallicolae hatching from these eggs crawl into the gall chambers where their feeding enhances gall development (Rohfritsch and Anthony 1992).

Study site

The study was performed in a spruce plantation established at Pustnäs (59°50' N, 17°40' E) southeast of Uppsala in 1987. The plantation consisted of five full-sib families of Norway spruce arranged in a randomized complete block design with 14 blocks (Fig. 1). Each full-sib family was represented by five plants in single-tree plots. The full-sib families originated from a phytotron study in which responses to various temperature treatments applied during the first growth period were studied in the second and third growth periods (Kang et al. 1994). Families of different origin had been selected to ensure wide variation in phenology and growth traits (Kang et al. 1994). Parents originated from northern Sweden for the F1 crossing, central Sweden for the F2 crossing, northern Sweden and Belgium for the F3 crossing, and Belgium and Germany for the F4 crossing. The 14 blocks were arranged in two rows. The two rows were oriented from south to north. Plots in the left row, with odd numbers (1, 3, 5, 7, 9, 11 and 13), were situated lower than plots in the right row, with even numbers (2, 4, 6, 8, 10, 12 and 14), because of a weak natural slope in the landscape. The soil was sandy and fertile. The 14 plots were adjacent to each other, and the whole plantation was surrounded by a 3-m-wide zone without any (or only sparse) vegetation. Trees were planted 1 m apart.

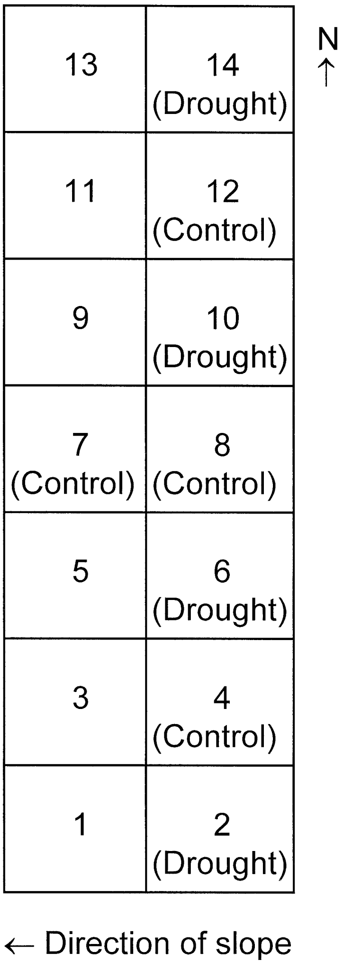

Fig. 1 Arrangement of plots with Norway spruce (*Picea abies*) used in the drought stress experiment. Each plot contained five trees of each of five different full-sib families, planted randomly 1 m apart

A clear difference in gall density (see method for estimating density below) among the five full-sib families was observed in 1993 and in previous years. Two of the families had low gall densities, two had high densities, and the fifth had intermediate densities. To simplify the study, I decided to include only the two families with the lowest gall densities (most resistant) and the two with the highest densities (most susceptible). Gall densities on these four families in 1993 are presented in Fig. 2.

Preference of gallicolae

To determine whether differences in gall density among spruce families could be due to differences in gallicolae preferences, I performed a preference experiment in August 1993. One shoot without galls from each of the four families was cut and placed around the edge of a water-filled bucket (diameter 12 cm) with equal distances between shoots. In the middle, I placed a shoot with a gall from which gallicolae had started to emerge. The buckets (*n*=20) were placed on 1.5-m-high poles, 5 m from the edge of the plantation. Gallicolae were counted on the experimental shoots

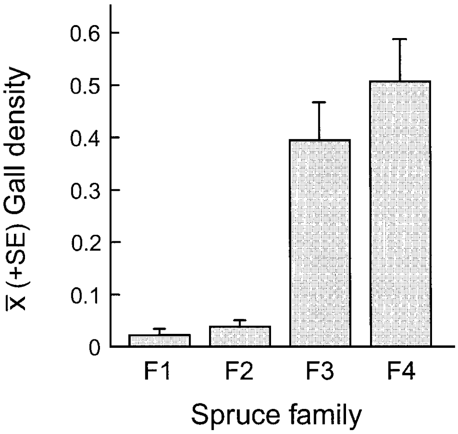

Fig. 2 Density of galls induced by the aphid *Adelges abietis* on four full-sib families of Norway spruce (*P. abies*). Gall densities are expressed as the proportion of available shoots with galls on the five uppermost whorls of each tree sampled (n=16 trees per family). The F1 and F2 did not differ significantly (P>0.05, Tukey's test), nor did F3 and F4. F1 and F2 were therefore designated "resistant" and F3 and F4 as "susceptible" based on the obvious difference in gall density. The data are from 1993, the year before half of the trees were exposed to drought stress for 2 years

daily for a week. For each replicate (bucket), the results from the day on which the total number of gallicolae on the four experimental shoots was highest were used in the statistical analysis. All replicates were pooled and analysed with a χ^2-test.

Drought treatment

The drought treatment, performed in 1994 and 1995, was applied by putting transparent corrugated plastic sheets (6×1 m) between rows of trees just above ground level. The sheets were slightly wider than the distance between trees in a row**.** To be able to remove and replace the plastic sheets without damaging the tree trunks, they were shaped to form shallow Us by tying strings (90 cm) and connecting the edges after first drilling holes through which the strings could be inserted. These shallow Us (gondolas) could easily be moved back and forth between the upper and lower row of blocks by pulling long ropes attached to the ends of the sheets.

The sheets were made movable in order to minimise the risk that effects caused by factors other than drought, such as increased soil temperature or decreased soil evaporation, would confound the results. Such effects might have been responsible for unexpected results obtained in earlier drought experiments (cf. Larsson and Björkman 1993). The plastic sheets were placed over the drought-stressed plots when it rained and were removed as soon as possible after the rain had stopped. The plastic sheets intercepted about 80% of the throughfall. This resulted in severe drought stress for the trees in the centre of the plots. Because of the treatment severity, I decided not to include measurements of water potential in the analysis of treatment effects.

Four of the plots in the plantation (2, 6, 10 and 14) were allocated to the drought treatment. These four blocks were chosen because they were not adjacent to each other and were situated in the upper row of plots. Thus, water removed from these plots could be automatically drained to the four corresponding plots in the lower row (i.e. plots 1, 5, 9 and 13). Four plots were chosen as controls (4, 7, 8 and 12). See Fig. 1.

Tree growth

Trees were cut down in January 1996. Their growth was estimated by measuring annual ring widths for the years 1993, 1994 and 1995 in thin radial-stem sections. Two measurements per section per tree per year were taken at right angles to each other, and their means were used in the data analyses.

Gall diameter

The diameter of galls was measured with the aid of a digital slide calliper. Fifteen galls per tree were measured at various positions in the tree canopy. If there were fewer than 15 available on a tree, as many as possible were measured. If galls were irregular in shape, the mean of two measurements (the widest and narrowest) was used. The galls were measured once the gallicolae had emerged from them, i.e. after the gall had stopped growing.

To determine whether gall diameter is correlated to fitness, 32 galls, representing a wide range of gall diameters, were selected for study before they had started to open. Each gall was enclosed in a small sleeve cage made of mist-net. After the gallicolae had emerged, the cages were brought to the laboratory where gall diameters were measured and gallicolae counted.

Stem-mother performance: wax cover diameter

The diameter of the wax cover produced by stem-mothers was measured with the aid of a digital slide calliper. Fifteen stem-mother wax covers were measured per tree, unless fewer were available in which case as many as possible were measured. If waxes were irregular in shape, the mean of two measurements (the widest and narrowest) was used.

To avoid killing the few living stem-mothers on resistant trees, a separate study was performed to determine the extent to which wax cover diameter was correlated to stem-mother size and hence to fecundity. After measuring the diameter of the wax cover of 20 randomly selected stem-mothers from trees in the control plots in the experimental plantation, the buds with the stem-mothers were cut off and transferred to vials with 70% alcohol. The collection was made when the diameter of the wax cover appeared to have reached its maximum size. In the laboratory, the length of the hind tibia was measured under a stereo-microscope (magnification ×80), having first removed the stem-mothers from the buds with a camel's hair brush.

Stem-mother survival

Stem-mother survival was estimated by counting numbers of living and dead stem-mothers around the time when the first galls were starting to form in early summer. The living proportion of stem-mothers was used as the measure of survival. This procedure may have overestimated survival somewhat because some of the dead stem-mothers may have had disappeared by that time. However, predation was low (Björkman 1998), and dead stem-mothers are difficult to remove from the buds (shoots) once they have inserted their stylets into the plant, suggesting that this estimate should be fairly accurate. Furthermore, the relative difference between treatments and phenotypes in this case was considered to give as good a picture of the effects as the absolute values.

Gall density

The density of *A. abietis* galls was estimated by counting the number of galls on two trees of each full-sib family that were closest to the centre of each plot. These trees were used in all of the studies. Thus, the maximum number of trees studied was 64. Only these two trees per plot and hybrid were used so that the trees most representative of the treatment could be included (drought and control); trees close to the edge of a plot were probably not as representative. Because *A. abietis* seemed to occur on the upper whorls of the spruce trees, the counts were limited to the five uppermost whorls each year. This made it possible to minimize the effects of shading. The number of galls was divided by the number of preferred galling sites, i.e. the number of shoots with a diameter >1 mm. This measurement of gall density, i.e. proportion of galled shoots, was arcsine transformed before performing statistical analyses.

Chemical analyses

Buds and parts of shoots were analysed in January 1996 after the trees had been cut down and the samples flash frozen in liquid nitrogen. These samples did not contain any *A. abietis* or other insects.

Phenolics were analysed by high-performance liquid chromatography (HPLC) after first weighing the buds. The chemical constituents were extracted in 80% ethanol for 1 min and then filtered through cotton. The rest was extracted in 95% ethanol which was also filtered through cotton. The two extracts were combined and the ethanol was evaporated at 30°C. The precipitate was dissolved in methanol and then analysed by HPLC as described in Lindberg et al. (1992). Concentrations were estimated by peak integration; i.e. the area under all peaks or a certain peak was divided by the fresh weight of the sample.

Resistance in Norway spruce to *A. abietis* has been suggested to be coupled to a single phenolic compound (Tjia and Houston 1975). To obtain evidence supporting this notion, all major (i.e. with a concentration of >10% in resistant trees) phenolics were studied further to identify those that responded in opposite directions (with respect to concentration) to drought stress in resistant and susceptible trees.

Total nitrogen concentrations were determined, on a dry weight basis, with an NA 1500 Elemental Analyser (Rodano, Italy).

Statistics

In all analyses, a single measurement per tree or the mean of several measurements per tree were used as the experimental units to avoid pseudoreplication. This is particularly important in this system because all (or many) aphids on a tree (or part of a tree) may be the descendants of the same ancestor.

For traits measured for several years on the same trees, a repeated-measures analysis of variance (in the SAS GLM procedure) was adopted. For traits measured only once, i.e. chemical traits which were only measured using 1995 data, an analysis of variance (in the SAS GLM procedure) was used. The same was true for the analysis of gall density prior to treatment (Fig. 2) in which a pairwise comparison was tested with Tukey's test. Data in the form of proportions were arcsine transformed before statistical analysis.

I used phenotype (susceptible vs resistant) instead of the four families in the analyses because for some of the traits too many values were missing to reach sample sizes large enough for proper statistical analysis. The use of phenotypes is justified (1) because of the similarity in gall density (a strong indicator of resistance) prior to treatment between families within each phenotype and (2) because in the cases where families could be used, there were never any significant (*P*>0.05) difference found between families within phenotypes. Thus, phenotypes are used in all analyses for the sake of clarity, even though families could have been used in some.

Missing values were also the reason for not dividing the whole experimental plot into blocks. This probably made it more difficult to achieve significant differences between treatments, phenotypes and years. Thus, differences were, if anything, underestimated.

Results

Preference of gallicolae

There was no difference between numbers of gallicolae recorded on shoots of the four spruce families arranged around an *A. abietis* gall (χ^2=2.7, *P*>0.4, *df*=3); the grand total number of gallicolae was 21 on F1, 35 on F2, 36 on F3 and 26 on F4.

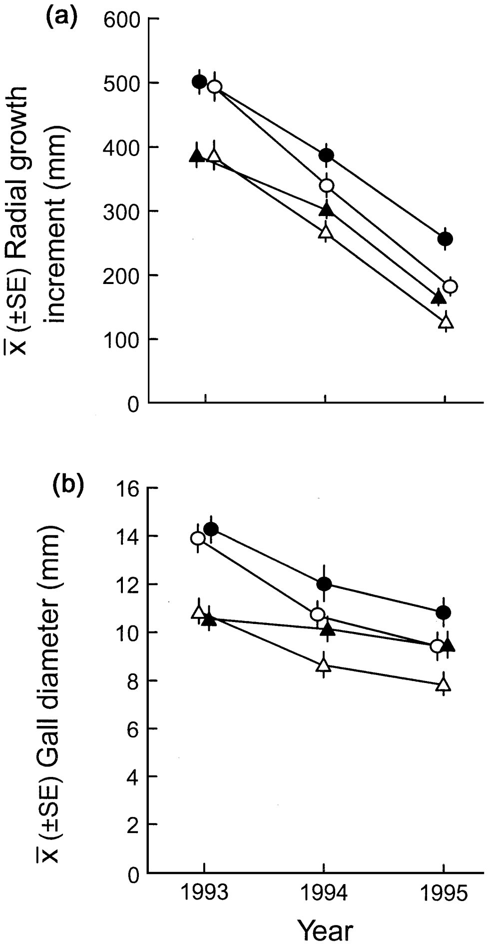

Fig. 3 Year-ring growth of two phenotypes of Norway spruce trees, i.e. susceptible (*circles*) or resistant (*triangles*) to the gall-forming aphid *Adelges abietis*, exposed to drought stress (*open symbols*) and untreated controls (*filled symbols*) (**a**) and the diameter of galls induced by *A. abietis* on these trees (**b**). The drought treatment started in April 1994 (*n*=16 trees per point)

Resistance of spruce families

The four spruce full-sib families used in this study differed significantly in gall density (Fig. 2, $F_{3,59}$=28.45, *P*<0.001). Families F1 and F2 did not differ significantly (*P*>0.05, Tukey's test) and were designated "resistant" because of their low gall densities. Likewise, families F3 and F4 did not differ significantly (*P*>0.05), but had much higher gall densities than F1 and F2 and were therefore designated "susceptible". Because there was no preference for any particular spruce family (see above) and the dispersal rate of aphids among trees is normally low (Ewert 1967; Carter 1971), this categorizing of families seems justified (cf. Eidmann and Eriksson 1978).

Table 1 Effects of phenotype (resistant vs susceptible), treatment (drought vs control) and time (year), and their interactions on annual radial growth increments of Norway spruce trees as revealed by a repeated-measures analysis of variance between and within subjects. Note the significant interaction between time and treatment, indicating that the two treatments differed in their response over time (see Fig. 3a)

Source	*df*	MS^{a} or Wilks' lambda^{b}	*F*	*P*
Between subjects				
Phenotype	1.54	317.040^{a}	52.64	<0.001
Treatment	1.54	48.986^{a}	8.13	<0.001
Phenotype×treatment	1.54	4.762^{a}	0.79	0.38
Within subjects				
Time	2.53	0.039^{b}	656.9	<0.001
Time×phenotype	2.53	0.892^{b}	3.20	<0.05
Time×treatment	2.53	0.819^{b}	5.85	<0.01
Time×phenotype× treatment	2.53	0.959^{b}	1.13	0.33

Table 2 Effects of phenotype (resistant vs susceptible), treatment (drought vs control) and time (year), and their interactions on the diameter of galls induced by the aphid *Adelges abietis* on Norway spruce trees as revealed by a repeated-measures analysis of variance between and within subjects. Note the similarity of these results to that of year ring growth (Table 1)

Source	*df*	MS^{a} or Wilks' lambda^{b}	*F*	*P*
Between subjects				
Phenotype	1.54	218.1^{a}	54.32	<0.001
Treatment	1.54	38.84^{a}	9.68	<0.01
Phenotype×treatment	1.54	0.058^{a}	0.01	0.91
Within subjects				
Time	2.53	0.317^{b}	60.25	<0.001
Time×phenotype	2.53	0.850^{b}	4.95	<0.05
Time×treatment	2.53	0.859^{b}	4.59	<0.05
Time×phenotype× treatment	2.53	0.996^{b}	0.41	0.67

Tree growth

Drought had a significant negative effect on annual radial growth (Fig. 3a, Table 1). The growth of susceptible trees was significantly higher than that of resistant trees. There was a steady decrease in tree growth with time. Trees of the two phenotypes responded differently over time, as did trees exposed to the two treatments (revealed by the significant time×phenotype and time×treatment interactions).

Gall diameter

The diameter of galls followed a pattern similar to that for tree growth (compare Fig. 3b and Table 2 with Fig. 3a and Table 1, respectively). This means that drought had a significant negative effect on gall diameter (Fig. 3b, Table 2). The galls on susceptible trees were significantly larger than those on resistant trees. There was a steady decrease in gall diameter (and tree growth) with time. Trees of the two phenotypes responded differently over time, as did trees exposed to the two treatments (revealed by the significant interactions with time). Gall diameter was positively correlated to the number of emerging gallicolae (Fig. 4; r=0.819, P<0.001, n=32).

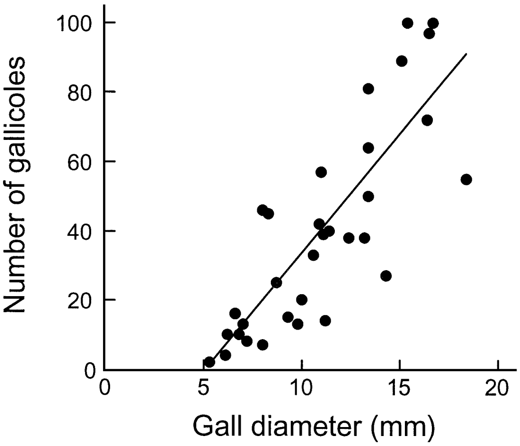

Fig. 4 Relationship between gall diameter and number of gallicolae emerging from individual galls induced by the aphid *A. abietis* on Norway spruce (*P. abies*). r=0.819, P<0.001, n=32

Table 3 Effects of phenotype (resistant vs susceptible), treatment (drought vs control), time (year) and their interactions on the diameter of the wax cover produced by stem-mothers of the gall-making aphid *A. abietis* on Norway spruce trees as revealed by a repeated-measures analysis of variance between and within subjects

Source	*df*	MS^{a} or Wilks' lambda^{b}	*F*	*P*
Between subjects				
Phenotype	1.54	46.22^{a}	673.7	<0.001
Treatment	1.54	0.204^{a}	2.98	0.09
Phenotype×treatment	1.54	0.008^{a}	0.12	0.73
Within subjects				
Time	2.53	0.859^{b}	4.56	<0.05
Time×phenotype	2.53	0.985^{b}	0.41	0.66
Time×treatment	2.53	0.946^{b}	1.58	0.21
Time×phenotype× treatment	2.53	0.994^{b}	0.18	0.84

Stem-mother performance: wax cover diameter

The diameter of the wax cover produced by stem-mothers was 50% larger on susceptible compared with resistant trees (mean±SE=2.95±0.27 mm vs 1.96±0.24 mm), indicating that performance was better on susceptible trees (Table 3). Although drought had no significant effect on wax cover diameter (Table 3), stem-mothers on drought-stressed trees tended to produce wider (12%,

Table 4 Effects of phenotype (resistant vs susceptible), treatment (drought vs control), time (year) and their interactions on the survival of stem-mothers of the gall-making aphid *A. abietis* on Norway spruce trees as revealed by a repeated-measures analysis of variance between and within subjects. Note the significant interaction between phenotype and treatment between subjects, and between time, phenotype and treatment within subjects, indicating that the drought treatment affected the two phenotypes in opposite directions (see Fig. 5a)

Source	*df*	MS[a] or Wilks' lambda[b]	*F*	*P*
Between subjects				
Phenotype	1.48	6.581[a]	314.8	<0.001
Treatment	1.48	0.005[a]	0.24	0.63
Phenotype×treatment	1.48	0.135[a]	6.45	<0.05
Within subjects				
Time	2.47	0.812[b]	5.44	<0.01
Time×phenotype	2.47	0.760[b]	7.41	<0.01
Time×treatment	2.47	0.937[b]	1.59	0.22
Time×phenotype× treatment	2.47	0.830[b]	4.80	<0.05

P=0.09) covers than stem-mothers on control trees. Wax cover diameter was positively correlated to the size (i.e. length of the hind tibia) of the stem-mother (r=0.566, P<0.01, n=20).

Stem-mother survival

The survival of stem-mothers was higher on susceptible than on resistant trees (Fig. 5a, Table 4). There was no simple (unidirectional) effect of drought on survival (Table 4). Instead, the effect of drought on the survival of stem-mothers on susceptible trees was the opposite to that on resistant trees, i.e. drought resulted in decreased survival on susceptible trees compared with controls in 1995, after 2 years of drought treatment. However, drought had a slightly positive effect on the survival of stem-mothers on resistant trees in both 1994 and 1995 (Fig. 5a, Table 4; the significant phenotype×treatment interaction).

Gall density

The change in gall density over time and its response to the treatment were similar to the response of stem-mother survival (compare Fig. 5b and Table 5 with Fig. 5a and Table 4, respectively). In other words, drought did not have a unidirectional effect on gall density (Table 5). Instead, the effect of drought on gall density on susceptible trees was opposite to its effects on resistant trees; i.e. drought resulted in decreased gall densities on susceptible trees compared with controls, whereas it had a slightly positive effect on the gall density of resistant trees [Fig. 5b, Table 5; a trend (P=0.09) for the time×phenotype×treatment interaction, and a significant (P<0.05) phenotype×treatment interaction].

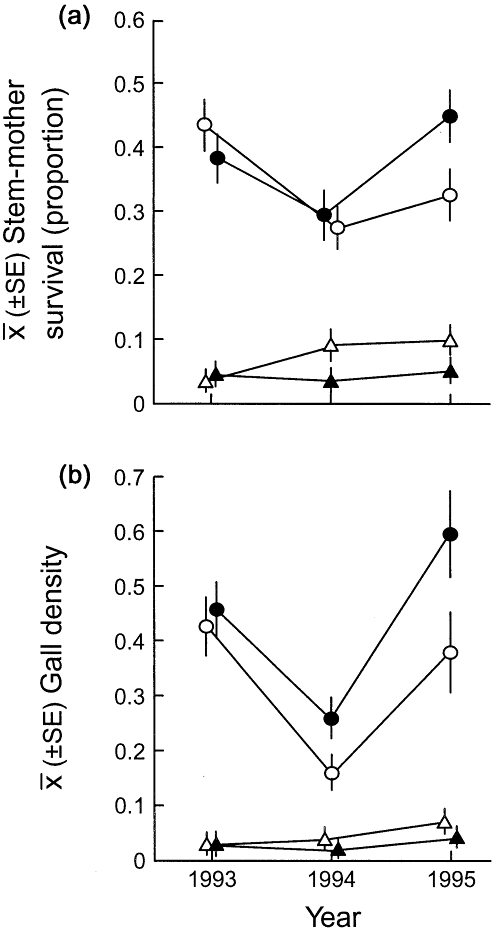

Fig. 5 Survival (i.e. a measurement of resistance) of stem-mothers of the gall-forming aphid *A. abietis* on drought-stressed susceptible (*open circles*) and resistant (*open triangles*) Norway spruce families compared with untreated controls (*filled circles* susceptible, *filled triangles* resistant) (**a**) and density of galls induced by *A. abietis* on these trees (**b**). The drought treatment started in April 1994 (n=16 trees per point)

Table 5 Effects of phenotype (resistant vs susceptible), treatment (drought vs control), time (year) and their interactions on the density of galls induced by the aphid *A. abietis* on Norway spruce trees as revealed by a repeated-measures analysis of variance between and within subjects. Note the similarity of these results to those for stem-mother survival (Table 3)

Source	*df*	MS[a] or Wilks' lambda[b]	*F*	*P*
Between subjects				
Phenotype	1.57	11.47[a]	250.9	<0.001
Treatment	1.57	0.049[a]	1.08	0.30
Phenotype×treatment	1.57	0.195[a]	4.27	<0.05
Within subjects				
Time	2.56	0.445[b]	34.92	<0.001
Time×phenotype×	2.56	0.542[b]	23.64	<0.001
Time×treatment	2.56	0.969[b]	0.897	0.41
Time×phenotype× treatment	2.56	0.921[b]	2.40	0.09

Chemical changes

The chemical composition was only analysed for tissue formed in 1995. Thus, the results reveal the summed effect of 2 years of drought stress. Total phenolic concentrations were not significantly affected by the treatment, nor did spruce phenotypes differ (Fig. 6a, Table 6). Only a single phenolic compound met the criteria of (1) being significantly higher in concentration in resistant than in susceptible trees (Table 6; $P<0.01$ for phenotype), (2) constituting a substantial part (>10%) of the total phenolics in resistant trees (Fig. 6; it accounted for an average of 20–25% of the total concentration) and (3) responding in opposite directions to drought stress in resistant and susceptible trees [Table 6; weak trend ($P=0.15$) for a phenotype×treatment interaction].

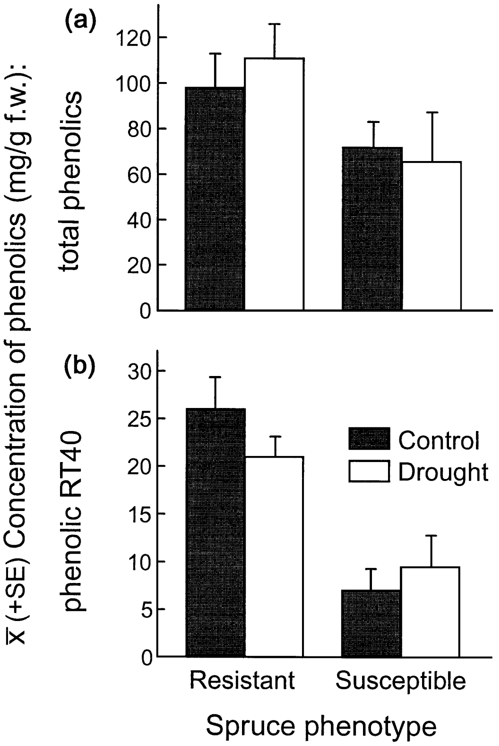

Fig. 6 Effect of 2 years of drought stress on the concentration (mg/g fresh weight) of total phenolics (**a**) and the concentration of an as yet unidentified phenolic compound with a retention time of ≈40 min (**b**) in the buds of Norway spruce families susceptible or resistant to the gall-forming aphid *A. abietis.* Data from 1995 ($n=16$ trees per mean value)

The concentration of total nitrogen did not differ significantly between phenotypes and was unaffected by treatment (Table 6). The average concentration for all trees was 0.85% dry weight (SE=0.04).

Discussion

The hypothesis that a given insect species may respond differently to environmentally induced changes in the host plant depending on the phase of the insect's life cycle (Björkman 1998) was supported by the results presented here. The negative effect of drought on gall size and the tendency for drought to enhance stem-mother performance, i.e. wax cover diameter, exemplifies this dual and opposite effect. A further complication in this study was that the effect of drought stress on stem-mother survival, which may be directly correlated to population dynamics, on resistant trees (slightly positive) was opposite to its effect on susceptible spruce trees (negative after 2 years of drought).

The good correspondence between stem-mother survival and gall density may seem surprising because the population dynamics of many, if not most, herbivorous insects is more affected by natural enemies than by the quality (or availability) of the host plant (Cappuccino and Price 1995). However, some features of *A. abietis*, in particular, and adelgids, in general, make this connection seem realistic. First, *A. abietis* does not alternate between host plant species as do many other adelgids (Schwenke 1972). It is therefore possible for this species to complete its entire life cycle on a single tree, and this appears to be a common occurrence (Ewert 1967; Carter 1971) although there are also observations of gallicolae leaving the tree on which they emerged (Carter and Barson 1973). Second, adelgids completely lack parasitoids (Schwenke 1972). This means that predators are their only enemies. Predation seems, however, to be negligible on stem-mothers (Schwenke 1972; Björkman 1998). Although the level of predation on galls and gallicolae may be higher, it is unlikely that this could have affected the results presented here. Had this been the case, predators must have become more common (or efficient) on drought-stressed, susceptible trees and less common (or efficient) on drought-stressed, resistant trees – the

Table 6 Analyses of variance for effect of Norway spruce phenotype (susceptible vs resistant to the gall-making aphid *A. abietis*) and treatment (drought stress vs control) on the concentration of total, low-molecular-weight phenolics, an unidentified phenolic substance with a retention time of approximately 40 min (*RT40*) and total nitrogen. All data from 1995

Source of variation	Total phenolics				Phenolic RT40				Total nitrogen			
	df	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*
Phenotype	1	10.3×10^{10}	1.68	0.21	1	$1{,}349\times10^{4}$	32.07	<0.01	1	0.04167	3.40	0.08
Treatment	1	5.04×10^{10}	0.82	0.38	1	6.92×10^{4}	0.16	0.69	1	0.01927	1.57	0.22
Phenotype× treatment	1	4.63×10^{10}	0.75	0.40	1	92.0×10^{4}	2.19	0.15	1	0.01707	1.39	0.25
Error	20	6.13×10^{10}	–	–	20	42.1×10^{4}	–	–	20	0.01227	–	–

second scenario is possible to envision because galls were smaller on drought-stressed trees, but I have no observations from the plantation that smaller galls were attacked more by predators than large ones. Another, more parsimonious scenario is that resistant trees became less resistant when drought stressed and susceptible trees became more resistant. This hypothesis is supported by the tendency for the response of the phenolic RT40 to differ between drought-stressed susceptible trees and drought-stressed resistant ones.

According to the growth differentiation balance hypothesis (Loomis 1932; Herms and Mattson 1992), moderate drought stress would give rise to increased concentrations of secondary metabolites, as e.g. phenolics. Severe drought stress would, according to the same hypothesis, result in reduced concentrations of phenolics. Because all trees in this study were exposed to the same level of drought stress, my results (Fig. 6) suggest that resistant and susceptible trees differ in their responsiveness to drought.

The decrease in concentration of the phenolic RT40 in drought-stressed resistant trees corresponded to the increased survival of stem-mothers, and the increase of this phenol in drought-stressed susceptible trees corresponded to the decrease in stem-mother survival. If this connection between resistance and the concentration of a single phenolic compound, yet to be identified, is genuine, it would support the data from a previous study (Tjia and Houston 1975). These authors provide the only known example suggesting that a single phenolic compound accounts for resistance against a herbivorous insect. However, even though the concentration of the phenolic RT40 was three- to fivefold higher in resistant than in susceptible trees, it seems unlikely that a single phenolic could account for the resistance; rather than being toxic, most phenolics act more as digestibility reducers (Rosenthal and Berenbaum 1992). Nevertheless, if the patterns found here are robust and reproducible, the compound could be used as a marker for resistance.

Resistance against gall-forming insects often seems to involve some kind of hypersensitivity reaction (Fernandes 1990). There are indications that the hypersensitivity reaction observed in the spruce/adelgid system studied here (Thalenhorst 1972; Rohfritsch and Anthony 1992) becomes weaker in stressed trees (Thalenhorst 1974).

For gall-forming and other insects that attack active meristematic tissue, bud break phenology is a factor that could play a role in resistance (Tuomi et al. 1989; Akimoto 1998). Of the four families studied here, one of the resistant ones had very early bud break, whereas one of the susceptible ones had very late bud break. The other two families had a similar, intermediate bud break. Thus, it seems unlikely that phenological differences could be an important factor explaining observed differences in resistance among families or why drought stress affected susceptible and resistant trees differently.

One complementary hypothesis for the observed change in gall density could be changes in nutritional value, which often affect the performance (growth and fecundity) of herbivorous insects. For example, there could have been a change in sugar content, which, however, was not measured here. Furthermore, the response of nitrogen to drought was small, and only in the case of susceptible trees was it in the same direction as the change in density. Additionally, the performance response of stem-mothers to drought was weak. However, stem-mothers on susceptible trees seemed to perform better than those on resistant trees. This could have contributed to the observed difference in gall density between resistant and susceptible trees because larger stem-mothers probably produce more offspring.

Gall diameter was correlated to tree growth, in accordance with the modified plant stress hypothesis (Larsson 1989) and the plant vigour hypothesis (Kimberling et al. 1990; Price 1991). Although the effect of drought on gall diameter was significant, after 2 years of treatment there was no difference between drought-stressed, susceptible trees and control, resistant trees. However, galls were, on the whole, much larger on susceptible than on resistant trees. This could have contributed somewhat to the difference in gall density between spruce phenotypes, because larger galls may be less vulnerable to attack by predators (Price and Clancy 1986; Abrahamson and Weiss 1997). However, the reverse relationship, i.e. that larger galls are more frequently attacked than smaller ones by enemies, is not uncommon (Clancy and Price 1987; Abrahamson and Weiss 1997).

The drought stress in this experiment was rather severe. The effect of any change in the environment, purely natural or enhanced by human activities, is likely to be less dramatic. However, if the change is in the same direction for many years, the end result could be similar to that observed here.

To conclude, our ability to predict the consequences of changes in the environment, e.g. global warming, will depend on how well we understand ecological interactions (Ayres 1993; Lawton 1995). There is a risk that we could make incorrect predictions simply by not studying the interactions of an organism during all of its life stages. As shown here, the response of an early life stage to environmental change may be opposite to that of a later life stage, and neglecting to study one or several life stages could lead to predictions that are wrong and (or) too drastic. Another factor that could render the outcome of an environmental change less dramatic is the complex interaction between genotype (plant and insect) and environment, as indicated by the observation that aphids on resistant spruce trees seemed to gain from drought stress, whereas those on susceptible trees were affected negatively by the same environmental change.

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