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INTERACTIONS BETWEEN THE ENCELIA LEAF BEETLE AND ITS HOST PLANT, *ENCELIA FARINOSA*: THE INFLUENCE OF ACIDIC FOG ON INSECT GROWTH AND PLANT CHEMISTRY

R. A. Redak,* J. T. Trumble & T. D. Paine

Department of Entomology, University of California, Riverside, CA 92521, USA

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Abstract

The impact of acidic deposition on interactions between the plant Encelia farinosa *and the herbivorous beetle* Trirhabda geminata *(Chrysomelidae) was determined under greenhouse conditions. Acidic fogs (pH 2.75) did not significantly affect the overall foliar concentrations of water or soluble protein as compared with control fogs (pH 5.6). Nonetheless,* E. farinosa *foliage was altered by exposure to three 3-h acidic fogs such that growth and biomass gain by* T. geminata *increased by more than 30% as compared to beetles feeding on control-fogged plants. Thus, previous indications that changes in soluble proteins or water content were responsible for increased biomass gain and growth of* T. geminata *cannot be substantiated by this study. Additionally, changes in the plant defensive chemistry were not responsible for increased herbivore growth, as farinosin, encecalin, and euparin foliar concentrations did not vary significantly between fog treatments. Significant increases in CO_2 assimilation rates of* E. farinosa *exposed to acidic fogs were documented at 3, 7, and 21 days following treatment, suggesting that carbohydrate-based products of increased plant metabolism may have played a role (e.g. soluble carbohydrates). However, the key factors responsible for increasing herbivore performance on acidic-fogged* E. farinosa *remain largely unknown.* © *1997 Elsevier Science Ltd. All rights reserved*

Keywords: Acidic fog, coastal sage scrub, Insecta, *Trirhabda geminata, Encelia farinosa.*

INTRODUCTION

The impact of acid deposition on vegetation has been reviewed in several publications (Cowling, 1982; Lee, 1982; Linthurst *et al.*, 1982; Treshow, 1984; Shriner, 1986; Treshow & Anderson, 1989). Although the effects on plants can be neutral or even positive, the more typical responses include lesion development, weathering of cuticular wax, foliar leaching, premature abscission, and abnormal growth or development. However, these studies were primarily concerned with acidic rain rather than acidic fogs that often exhibit a much lower pH (Waldman *et al.*, 1982; Johnson & Siccama, 1983; Granett & Musselman, 1984). Ambient fogs in the Los Angeles Basin and Bakersfield, CA, areas average 2–12 h duration, routinely exhibit a pH of 2.0–3.0, and contain proportionally more nitric acid than the sulfate-rich acidic fogs or rains of eastern North America and Europe (Waldman *et al.*, 1982; Hoffman, 1984; Hoffman *et al.*, 1985). Such fogs potentially provide plants with a substantial nitrogen resource.

The physiological effects of acidic fog upon plant life are generally less well known than those of acid rain, but reports as early as the 1950s describe a sequence of cellular collapse progressing from the upper to lower epidermis in affected vegetation (Middleton *et al.*, 1950; Thomas *et al.*, 1952). Under both laboratory and field conditions, multiple exposure to fogs with an acidity of pH 3.0 or lower induces leaf necrosis, alters the physical characteristics of the plant surface, increases foliar cation and carbohydrate leaching, decreases net photosynthesis, reduces plant growth and crop yield, and can cause plant death (Granett & Taylor, 1981; Granett & Musselman, 1984; Musselman & Sterrett, 1988; Musselman & McCool, 1989; Paoletti *et al.*, 1989*a*,*b*; Takemoto *et al.*, 1989; McCool *et al.*, 1990; Mengel *et al.*, 1990; Trumble & Walker, 1991).

Acidic fogs can affect the susceptibility and suitability of host plants to their insect herbivores (for reviews, see Bedford, 1986; Riemer & Whittaker, 1989; Heliövaara & Väisänen, 1993). Foliage concentrations of water, nitrogen, soluble proteins, amino acids, and defensive compounds have been shown to be affected by acidic fog deposition (Trumble & Hare, 1989; Dercks *et al.*, 1990; Paine *et al.*, 1993). Previous research by the present authors investigating the impact of acidic fog on insect herbivores and their host plants has focused upon the *Encelia farinosa* Gray (Asteraceae) and *Trirhabda*

E. farinosa, a drought-deciduous shrub of the Sonoran and Mojave deserts, is also a dominant vegetation component of the coastal and interior sage scrub communities surrounding the large urban areas of southern California. Its major period of growth occurs immediately after winter rains, with flowering occurring from early April to May. With the onset of summer, plants drop their foliage and enter a stage of quiescence. The major herbivore of *E. farinosa* is the beetle *T. geminata*, which is an *Encelia* specialist and feeds on the plant both as larvae and adults. The insect is active during the winter rainy season, with larvae emerging to feed on young plant tissues in late winter. Larvae pass through three stadia and pupate in the soil. Adults are active in spring and oviposit at this time. Summer aestivation occurs in the egg stage. The reader is referred to Redak and Bethke (1995) for a more thorough review of this insect's biology.

Using this system, it has been shown that multiple exposures to acidic fog (three 3-h applications of pH-2.5 fogs) increased the foliar concentration of soluble protein and nitrogen. These changes in foliage quality were correlated with increased consumption rates of both third instar and adult *T. geminata* (Paine *et al.*, 1993). Both plant and insect effects were evident within 7 days of treatment applications. Furthermore, it has been demonstrated that the effects of acidic fog in this system are plant-mediated. When applied directly to the animal, acidic fogging does not directly affect either insect mortality or growth (Redak *et al.*, 1995). The duration of the foliage quality changes that occur with acidic fogging, the impact on plant defensive chemistry, and the plant-mediated impact on insect growth are unknown.

In this study, the objectives were to determine the magnitude and duration of the effect of acidic fog on (1) the foliage quality and photosynthetic capacity of *E. farinosa* and (2) *T. geminata* growth and total biomass gain. Foliage quality is defined as the foliar concentrations of soluble protein, water, and the plant defensive compounds encecalin, farinosin, and euparin. Farinosin, euparin, and encecalin are active plant defensive compounds used by *Encelia* species to deter and reduce insect herbivory (including that by *T. geminata*, Wisdom & Rodriguez, 1982, 1983; Wisdom, 1985, 1988). Photosynthetic potential, an indication of physiological activity, was defined by estimates of stomatal resistance, internal foliar CO_2 concentration, and the rate of CO_2 assimilation.

MATERIALS AND METHODS

Application of fog treatments

Six-hundred and sixty *E. farinosa* plants were germinated from wild seed by a commercial nursery (Tree of Life Nursery, San Juan Capistrano, CA). All plants were transferred to a greenhouse and potted in 4-liter pots with UC mix # 3 soil (Matkin & Chandler, 1957) and were watered and fertilized (N:P:K = 14:14:14 at 2.5 g per pot, Osmocote®, Sierra Chemical Co., Milpitas, CA) as necessary. One year after germination plants were randomly assigned to either an acidic (pH 2.75) or a control fog (pH 5.60) treatment. Each treatment was replicated 330 times (330 plants per treatment). Within the greenhouse, fogs were applied to each replicate plant for a 3-h period every other day for a total of three applications over 5 days. Acidic fogs were prepared by adjusting distilled water to pH 2.75 with reagent grade nitric and sulfuric acid mixed at a 2.5:1 (v:v) ratio. This acid ratio is typical of fogs in southern California (Waldman *et al.*, 1982). Control fogs consisted of distilled water adjusted with 0.1 M HCl to a pH of 5.60. The additional heavy-metal ionic components known to occur in southern California fogs were added to both control and acidic fog solutions (Munger *et al.*, 1983). During treatment applications, plants were moved from greenhouse benches to adjacent 1-m^3 fogging chambers within the same greenhouse. Fogs were created within chambers using a fogging apparatus designed by Musselman *et al.* (1985) operated at 7.03 $kg\,cm^{-2}$. Greenhouse temperatures during fogging ranged from 22 to 26°C. A shade cloth cover over the fogging chamber was used to prevent light intensities from exceeding 300 $\mu E\,s^{-1}m^{-2}$, thus more closely simulating actual conditions during an acidic fog episode. As not all treatment groups were placed in a single chamber for a particular fogging episode, six chambers were utilized, and chambers for control and acidic fogs were alternated between fogging episodes to eliminate potential chamber effects. All acidic-fogged plants exhibited visible signs of acid-fog damage (necrotic lesions on the upper surface of the leaves) within 24 h of the last treatment episode. After fogging, all plants were returned to their original position on adjacent greenhouse benches.

Experiment A: impact of acidic fog on plant foliage quality and photosynthesis

Two-hundred and forty of the above 660 experimental plants were utilized for this experiment (120 control-fogged plants and 120 acidic-fogged plants). These plants were assigned randomly to one of the following sampling periods: 3, 7, 10, 14, 21, and 28 days after the last fogging episode. Twenty acidic-fogged and 20 control-fogged plants were assigned to each period. Individual plants were assigned to only one period of sampling. For each sampling period, all of the new foliage (leaves from terminal and terminal internode) on an individual plant was collected for tissue analysis.

Upon collection, the fresh mass of each foliage sample was immediately determined (to the nearest 0.1 mg), and the foliage was then stored in 40 ml screw-cap vials, returned to the laboratory, and frozen at −65°C. Subsequent to freezing, samples were lyophilized and their dry mass determined. Percent water for each sample was calculated from initial tissue fresh mass and final dry mass. Samples were then ground in a Wiley Mill (#20 mesh screen, A. H. Thomas, Philadelphia, PA) and returned to the freezer.

Freeze-dried samples were then used to determine the foliage quality of experimental plants. Soluble protein concentration (an estimate of the nutritional quality of the foliage) was determined using the methods of Jones *et al.* (1988). Dried foliage samples (100 mg) were extracted with 10 ml 0.1 M NaOH for 30 min at room temperature. Leaf tissue was removed by centrifugation (10 000 RCF for 10 min). The decanted supernatant was brought to 10 ml with 0.1 M NaOH. Protein concentration in this final solution was measured using Bradford reagent (Bradford, 1976) with bovine serum albumin (Sigma Chemical Co., St Louis, MO) as the standard. Triplicate readings were taken on each extraction. Values are reported as milligrams BSA equivalent protein per milligram dry mass of foliage.

The concentrations of farinosin, encecalin, and euparin were determined using the HPLC methods of Wisdom and Rodriguez (1982). Using a ten-broeck homogenizer, 50 mg of plant tissue were extracted two times with 3.0 ml of methanol each time. Extracts were combined, centrifuged to remove particulates (10 000 RCF, 10 min) and passed through a 300-mg C-18 reverse-phase extraction column (MaxiClean cartridge, Altec, Deerfield, IL) to remove non-polar contaminants. Final extracts were brought up to 10.00 ml in volume with methanol. Samples (20 μl) were injected into a Hewlett-Packard 1050 liquid chromatograph system equipped with a variable-wavelength UV-detector and a Hewlett-Packard 3396 Series II Integrator (Hewlett-Packard, Fullerton, CA). Using a 35% aqueous acetonitrile solvent flowing at a rate of 1.5 ml min^{-1}, samples were loaded onto a C-18 reverse-phase column (Sperisorb ODS2, 5 μm, 250×4mm, Hewlett-Packard, Fullerton, CA). Samples subsequently were eluted from the column using a constant solvent flow rate of 1.5 ml min^{-1} and the following solvent program: 35% acetonitrile increasing to 74% acetonitrile over 15 min, followed by an increase to 100% acetonitrile over 2.5 min, and then held at 100% acetonitrile for 2.5 min. To maximize detection of the known chromenes and sesquiterpene lactones, the following detector program was used: 254 nm for 0–6 min, 326 nm from 6 to 11.2 min, and 262 nm from 11.2 to 20 min. Concentrations of each compound were determined through the use of an internal standard (0.500 ml of 0.50 mg ml^{-1} 7-benzyloxy coumarin) added to the sample at the time of initial extraction. Concentrations of these compounds are expressed as milligrams of compound per milligram dry mass of foliage.

Two-way analysis of variance (Model I; Zar, 1984) was used to assess the effects of acidic fog and time of sampling after fog treatment on *E. farinosa* foliage quality. Acidity of the treatment fog (at two levels: pH 2.75 versus 5.60) and day of sampling after fog application (at six levels: 3, 7, 10, 14, 21, 28) were considered as treatment factors. The sample size for each treatment combination was 20 plants. Values defining the foliage quality (concentrations of soluble protein, foliar water, farinosin, encecalin, and euparin) of the *E. farinosa* plants at the two pH levels and six sampling periods were considered as dependent variables in separate two-way analyses of variance. All analyses were performed using SAS version 6.03 (SAS, 1988).

The impact of acidic fog on photosynthetic potential was assessed independently on an additional 120 fog-treated plants (60 acidic-treated and 60 control-treated plants). At 3, 7, and 21 days after fog application, two fully expanded equal-aged leaves were randomly selected from each of 40 plants (20 control-fogged and 20 acidic-fogged). Stomatal resistance, leaf internal CO_2 concentration, and leaf CO_2 assimilation rate were determined using a Li-Cor 6200 Photosynthesis Measurement System (Li-Cor, Lincoln, NB). Measurements were made under a metal halide lamp providing a minimum of 1000 $\mu E\, s^{-1} m^{-2}$. Prior to photosynthetic determinations all plants were allowed to acclimatize to laboratory conditions (21°C, ambient humidity, and CO_2 concentration under the aforementioined metal halide lamp for *ca* 1 h). For the photosynthetic variables estimated, initial analyses indicated no significant impact of time since fogging. Consequently, to determine the impact of acidic fog on photosynthesis, a *t*-test was employed to compare treatment means for each dependent variable at each sampling period.

Experiment B: impact of acidic fog on insect growth

An additional 300 of the above 660 experimental plants were utilized for this experiment (150 control-fogged and 150 acidic-fogged plants). These plants were assigned randomly to one of the following insect bioassay periods: 3, 7, 10, 14, 21, and 28 days after the last fogging episode. Thirty acidic-fogged and 30 control-fogged plants were assigned to each of the 3-, 10- and 28-day post-fog sampling periods; 20 acidic- and 20 control-fogged plants were assigned to each of the 7-, 14- and 21-day post-fog sampling perids. Differences in sample sizes among sampling periods were necessary to conduct subsequent experiments investigating the simultaneous impact of herbivory and acidic fog upon *E. farinosa* physiology (data not shown here; R.A. Redak *et al.*, unpubl. data, 1997). Each individual plant was utilized only once in a single bioassay trial. At the appropriate post-fog application time, plants were utilized in a feeding bioassay consisting of placing five second instar *T. geminata* upon each plant. *T. geminata* larvae (second instar) were collected from areas of interior coastal sage-scrub habitat on the Box Springs Mountains located adjacent to and southeast of the University of California, Riverside, CA. Larvae were brought into the laboratory and maintained on *E. farinosa* cuttings taken from the sampling areas. Cuttings were replaced as needed and kept alive for *ca* 1 week in plastic vials (50 ml) containing water.

Prior to placement on experimental plants, larvae were starved for 24 h and weighed to the nearest 0.01 mg. To prevent larvae from moving from their assigned host plant, insects were held on experimental plants using plastic acetate confinement cones coated with teflon (Redak *et al.*, 1995). Insects were allowed to feed *ad libitum* on experimental plants for 7 days. Larvae

were then removed from their host plants, starved for 24 h, killed, and their biomasses determined to the nearest 0.01 mg. At the time of removal (7 days after initial placement), larvae were in the third instar. Using the average initial ($n = 5$ insects/plant) and final ($n = 0$–5 surviving insects/plant, depending on mortality) insect biomass estimates, the average larval biomass gain and larval growth rate were calculated for each experimental replicate plant (Waldbauer, 1968; Kogan & Parra, 1981).

Two-way analysis of variance (Model I; Zar, 1984) was used to assess the effects of acidic fog and time since fogging on *T. geminata* biomass gain and growth. Acidity of the treatment fog (at two levels: pH 2.75 versus 5.60) and time since fog application that the bioassay was performed (at six levels: 3, 7, 10, 14, 21, 28) were considered as treatment factors. The sample size for each treatment combination was 20 plants. Values defining total larval biomass gain (mg) and insect growth rate (mg biomass gain per mg body mass per day) at the two pH levels and six bioassay periods were considered as dependent variables in separate two-way analyses of variance. All analyses were performed using SAS version 6.03 (SAS, 1988).

The approach of conducting two separate experiments to evaluate the effect of acidic fog on host-plant quality and insect success allowed the authors to avoid the potential confounding influences of insect herbivory interacting with acidic fog to alter host-plant quality. The foliage quality data collected estimate the host-plant quality at the time the insects were placed on their host plants. The simultaneous impact of acidic fogging and herbivory upon host-plant suitability is the subject of a subsequent study (R.A. Redak *et al.*, unpubl.).

RESULTS

Experiment A: impact of acidic fog on plant foliage quality and photosynthesis

The overall impact of acidic fogging on plant foliage quality was minimal. Relative to control fogs, the acidic fog treatment did not significantly impact the foliar concentrations of water, soluble protein, encecalin, euparin, farinosin, or total chromenes (Table 1). Parameters estimating foliage quality were strongly influenced by time following treatment applications. Foliar concentrations of water and soluble protein declined significantly with time following treatment applications (Table 1, Fig. 1), while concentrations of farinosin, encecalin, and total chromenes increased significantly with time following treatment applications (Table 1, Fig. 2 and Fig. 3(B)). The concentration of euparin dropped sharply within the first week following fog treatments and then stabilized thereafter (Table 1, Fig. 3(A)). There was a significant interaction between acidic fog treatment and time following treatment for the foliar concentration of soluble protein. Initially, acidic-fog treated plants exhibited increasing levels of soluble protein through to the fifteenth day after fog treatment application. Following day 15, acidic-fogged plants displayed an overall decrease in concentration of soluble proteins. Control-fogged plants exhibited a steady decline in soluble protein levels throughout the experimental period (Fig. 1(B)). For all other foliage quality variables, there were no other significant interactions between fog treatment and time.

Acidic fogging significantly increased leaf CO_2 assimilation rates by 10–21%, depending on the time since fog treatment application. For 3, 7, and 21 days following treatment application, plants subjected to acidic fogging exhibited significantly greater rates of CO_2 assimilation relative to plants subjected to control fogging (Table 2). Stomatal resistance and internal leaf CO_2 concentration were not significantly different between control-fogged and acidic-fogged plants throughout the duration of the experiment (Table 2).

Experiment B: impact of acidic fog on insect growth

Insects feeding on acidic-fog treated plants gained approximately 33% more biomass than insects feeding on control-fogged treated plants (mean mg $\pm$ SEM: 2.98 ± 0.14 versus 2.23 ± 0.14, respectively, Table 1, Fig. 4(A)). While biomass gain for both groups of insects appeared to increase through to day 7 after fog application and decrease thereafter, there was no significant effect of time after fog treatment on insect biomass gain. That is, beetles gained relatively the same amount of biomass from feeding on plants treated with fogs 3, 7, 14, 21, and 28 days prior to the initiation of insect feeding bioassays. There was no significant interaction effect on insect biomass gain between fog treatment and the time since treatment (Table 1).

Table 1. ANOVA results for the effects of acidic fog and time since treatment application upon foliage quality

Dependent variable	*F*, *p*, df for fog treatment	*F*, *p*, df for time treatment	*F*, *p*, df for fog by time interaction
Plant responses			
Water	0.02, 0.8838, 1,228	78.26, 0.0001, 5,228	0.58, 0.7120, 5,228
Soluble protein	0.35, 0.5532, 1,228	46.51, 0.0001, 5,228	10.62, 0.0001, 5,228
Encecalin	1.81, 0.1802, 1,228	37.00, 0.0001, 5,228	1.75, 0.1245, 5,228
Euparin	0.26, 0.6105, 1,228	6.64, 0.0001, 5,228	0.60, 0.6986, 5,228
Farinosin	0.04, 0.8502, 1,228	22.64, 0.0001, 5,228	1.65, 0.1467, 5,228
Total chromenes	2.01, 0.1577, 1,228	39.58, 0.0001, 5,228	2.06, 0.0714, 5,228
Insect responses			
Biomass gain	15.32, 0.0001, 1,264	2.02, 0.0765, 5,264	0.33, 0.8921, 5,264
Growth rate	12.97, 0.0004, 1,264	3.64, 0.0033, 5,264	0.30, 0.9150, 5,264

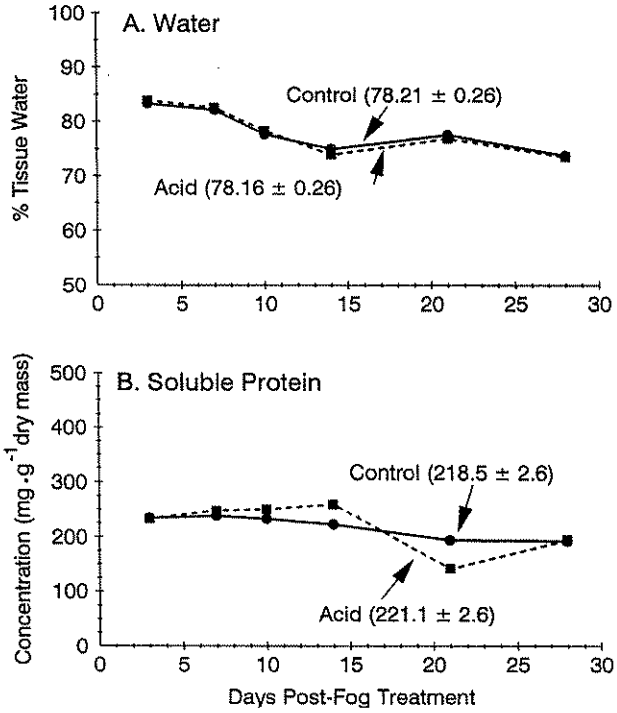

Fig. 1. Effect of acidic fog upon *E. farinosa* foliage quality with respect to insect nutrition. (A) Foliar percent water. (B) Foliar concentrations of soluble protein. Plotted values are means; errors bars representing 1 standard error are less than the width of a graphed point. See Table 1 for statistical analysis.

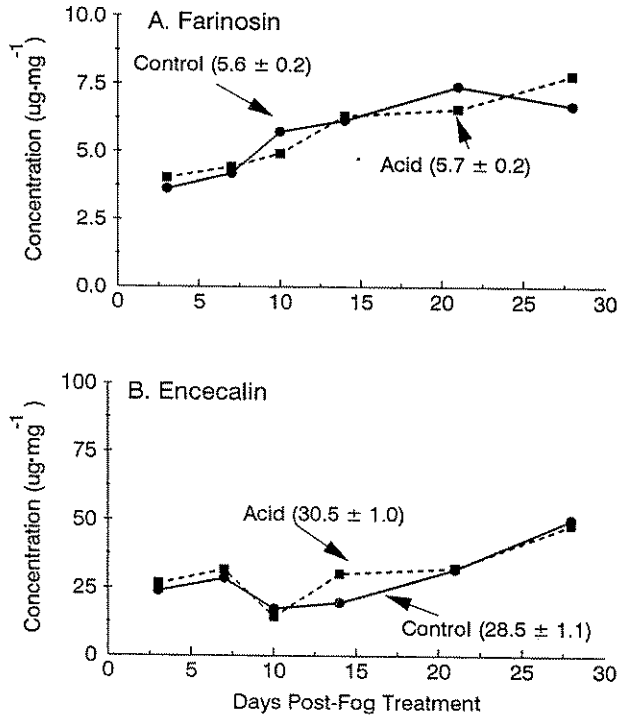

Fig. 2. Effect of acidic fog upon *E. farinosa* foliage quality with respect to known plant defensive compounds. (A) Foliar concentrations of farinosin. (B) Foliar concentrations of encecalin. Plotted values are means; errors bars representing 1 standard error are less than the width of a graphed point. See Table 1 for statistical analysis.

Beetle growth rate was 31% greater on plants treated with acidic fog compared with beetles feeding on plants treated with control fogs (mean mg gain per mg of insect per day ±SEM: 0.21±0.01 versus 0.16±0.01, respectively, Table 1, Fig. 4(B)). Regardless of fog treatments, beetles growing on plants treated with fog 7 and 10 days previous to insect bioassays grew significantly faster than any other group of insects feeding on acidic-fogged plants (*posteriori* analysis using Ryan's Q-test following a significant analysis of variance, Table 1). As with biomass gain, there was no significant interaction effect on insect growth rate between fog treatment and time since treatment (Table 1).

DISCUSSION

Exposure to acidic fog alters *E. farinosa* host-plant status such that *T. geminata* larvae feeding on previously exposed plants gain significantly more biomass and grow significantly faster for a minimum of 4 weeks following the exposure. The changes responsible in *E. farinosa* foliage for this increase in host quality with acidic fogging are not related to known plant defensive compounds in *E. farinosa* nor due to increased nutritional quality of the foliage. Although all of the estimated foliage quality variables changed similarly through time, none of these estimates were affected by acidic fogging. (Figs 1–3). The temporal change in foliar concentrations of plant defensive chemicals, soluble protein, and water is quite similar to earlier published data showing the temporal variation that occurs with tissue aging in *E. farinosa* (Wisdom & Rodriguez, 1982, 1983).

Investigating the same plant–insect association earlier, the present authors found that applications of acidic fog did alter *E. farinosa* foliage quality (increased concentrations of nitrogen and soluble protein), with responses in insect feeding parameters of *T. geminata* (i.e. increased levels of larval and adult consumption) similar to those found here (Paine *et al.*, 1993). The differences between the earlier studies and the results presented here may be due, in part, to methodological differences (use of adults and older larvae in Paine *et al.*, 1993) and variability in plant response to acidic fogging. Paine *et al.* (1993) only performed a single insect bioassay of fog-treated host plants 7 days after a series of fog applications. In the current study, insect responses to acid treatments were examined six different times following treatment applications covering a period of 4 weeks. The present results indicate that the responses of plant foliage quality to acidic fogging are variable over time (Figs 1–3), and multiple assays are required to confirm any trends found for a particular post-fog period. Thus, while previous studies (Paine *et al.*, 1993) suggested that changes in soluble protein or water content were possible causes of the observed *T. geminata* response, present results indicate that soluble protein and water are probably not the primary causal factors.

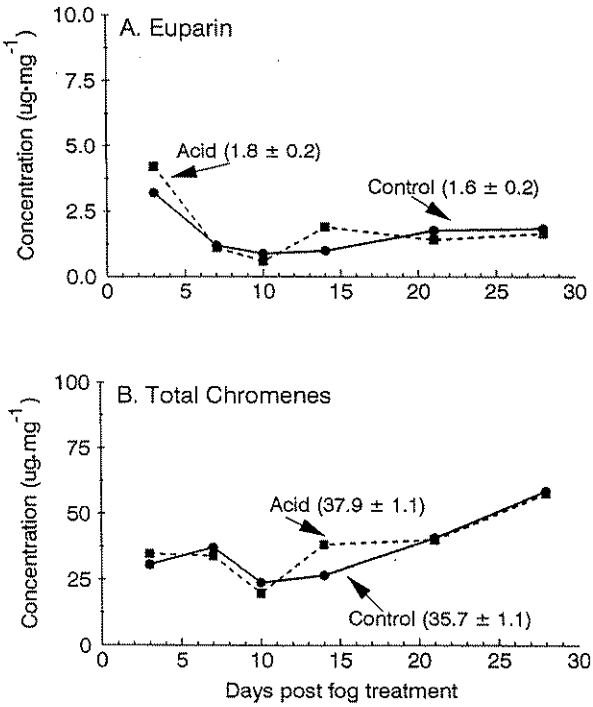

Fig. 3. Effect of acidic fog upon *E. farinosa* foliage quality with respect to known plant defensive compounds. (A) Foliar concentrations of euparin. (B) Foliar concentrations of total chromenes. Plotted values are means; errors bars representing 1 standard error are less than the width of a graphed point. See Table 1 for statistical analysis.

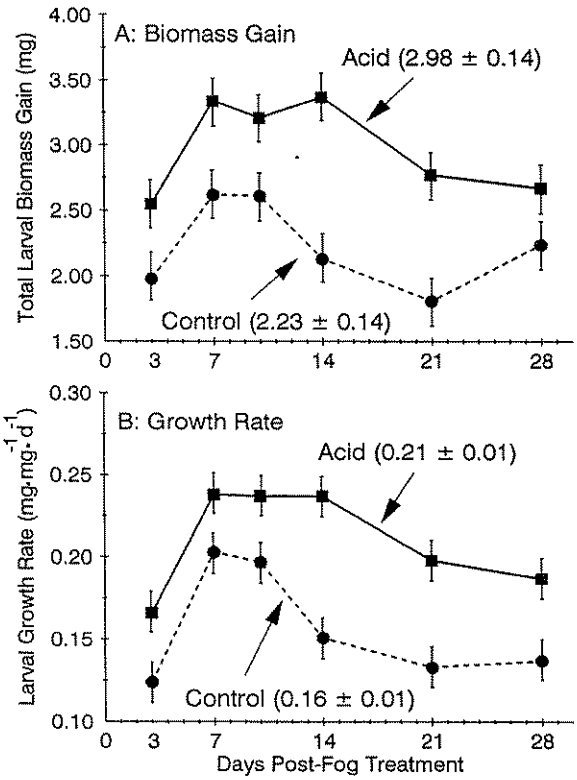

Fig. 4. Effect of acidic fog upon *T. geminata* performance. (A) Biomass gain. (B) Relative growth rate. Plotted values are means; vertical bars are 1 standard error of the mean. See Table 1 for statistical analysis.

Other studies also have demonstrated a plant-mediated effect of acidic fog upon insect herbivores. In the lima bean (*Phaseolus lunatus* L.) and *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) system, Trumble and Hare (1989) showed that when plants were treated with acidic fogs (pH 3.0), the foliar concentration of total nitrogen increased to a level that presumably resulted in enhanced insect growth and consumption rates. Treatment applications of fogs with lower pH values (2.5, 2.0), while altering bean foliage quality (increased concentrations of total nitrogen, soluble protein, and various amino acids), did not significantly alter insect growth or consumption (Trumble & Hare, 1989). Differences between the study presented here and that of Trumble and Hare (1989) may be due to different experimental systems employed (*T. ni* and *P. vulgaris* versus *T. geminata* and *E. farinosa*) as well as different treatment methodology (different pH of fogs used, different treatment exposure times). It is interesting to note that, where Trumble and Hare used a similar pH value for treatment fogs as those used here, they did not demonstrate a significant impact on *T. ni* biology (Trumble & Hare, 1989).

A few generalizations can be made with respect to the overall impacts of nitrogen-based acidic deposition on phytophagous insects. A majority of studies (30 out of 44) show a similar positive effect on insect herbivores as that shown here (e.g. increased biomass gains, growth and consumption rates, survival, density; see Heliövaara and Väisänen, 1993, pp. 73–74 and 80). Many of these studies correlate these effects with acidic deposition-induced increases in host-plant quality (increased levels of foliar nitrogen or soluble protein). Those studies that have differentiated between plant-mediated and direct effects of acidic deposition on insect herbivores have found that the effects are plant-mediated. Terrestrial

Table 2. Photosynthetic capacity (mean ±1 standard error) of *Encelia farinosa* exposed to acidic and control fogs

Time after exposure (days)	pH of fog	CO_2 assimilation rate ($mg\,s^{-1}\,m^{-2}$)	Stomatal resistance ($s\,cm^{-1}$)	Internal CO_2 concentration (ppm)
3	5.60	13.098 ± 0.655^{A}	0.660 ± 0.041^{A}	362.355 ± 4.592^{A}
	2.75	14.605 ± 0.467^{B}	0.693 ± 0.060^{A}	354.545 ± 6.479^{A}
7	5.60	13.193 ± 0.613^{A}	0.733 ± 0.070^{A}	346.725 ± 5.388^{A}
	2.75	15.052 ± 0.626^{B}	0.705 ± 0.083^{A}	341.370 ± 6.487^{A}
21	5.60	10.368 ± 0.475^{A}	0.886 ± 0.055^{A}	348.388 ± 3.379^{A}
	2.75	12.581 ± 0.641^{B}	0.856 ± 0.067^{A}	338.557 ± 6.283^{A}

Values within columns and time after exposure are significantly different if followed by different superscripts (*t*-test, $p < 0.05$).

arthropods are relatively resistant to direct acidic deposition (Gunnarsson & Johnsson, 1989; Heliövaara *et al.*, 1992; Redak *et al.*, 1995).

Clearly, acidic fogging does not affect the known plant defensive compounds of *E. farinosa* measured here (Figs 2 and 3) nor the nutritional quality (as defined by soluble nitrogen and water) of the plant as host for *T. geminata* (Fig. 1). Whether acidic fogging influences unknown and unmeasured plant defensive chemicals (e.g. tannins, terpenes) or other insect nutrients (e.g. soluble carbohydrates, soluble amino acids) is not known and needs to be determined. Given the elevated CO_2 assimilation rates, it is possible that acidic fogging is significantly altering the overall metabolism of the plants such that *T. geminata* exhibits greater performance on acid-fogged plants. The exact mechanisms that are responsible for this increased insect performance remain unknown.

In conclusion, it should be noted that direct long-term impact of acidic fog on herbivorous insect populations (i.e. impact on insect fitness over several generations) was not determined and is not known. Furthermore, the impact of acidic fog, either direct or plant-mediated, on such parameters as adult host-plant choice, oviposition, mating, and reproduction is also unknown. As acidic fogs affect significant areas of coastal North America and Europe, including areas of agricultural and natural biotic importance, further studies investigating both the direct and plant-mediated impact of acidic-fog upon plant–insect interactions are warranted.

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