Journal of Chemical Ecology, Vol. 19, No. 1, 1993

IMPACT OF ACIDIC DEPOSITION ON *Encelia farinosa* GRAY (COMPOSITAE: ASTERACEAE) AND FEEDING PREFERENCES OF *Trirhabda geminata* HORN (COLEOPTERA: CHRYSOMELIDAE)

T.D. PAINE,* R.A. REDAK, and J.T. TRUMBLE

Department of Entomology
University of California
Riverside, California 92521

(Received March 26, 1992; accepted August 31, 1992)

Abstract—Container grown *Encelia farinosa* were exposed to three 3-hr episodes of acidic fog (pH 2.5) typical of events in southern California. Adults and larvae of the specialist leaf-feeding herbivore, *Trirhabda geminata*, preferred to feed on the acidic-treated foliage compared to control fogged (pH 6.3–6.5) foliage. Previous feeding damage on the plants did not affect feeding preference. The acidic-fogged foliage was significantly higher in total nitrogen and soluble protein but not different from control-treated tissue in water content. Stress on native populations of this drought-deciduous shrub caused by atmospheric pollutants may also result in altered feeding ecology of the beetle.

Key Words—*Encelia farinosa*, Asteraceae, *Trirhabda geminata*, Coleoptera, Chrysomelidae, feeding preferences, acidic deposition, plant stress.

INTRODUCTION

The impact of urban expansion on agricultural and natural ecosystems can be direct (e.g., the development of land for urban uses) or indirect (e.g., the effect of pollutants from urban sources on plants and their herbivores). The southwestern United States continues to be one of the fastest growing population centers in the country. An increase in the number of individuals is accompanied by increases in industries and automobiles. Consequently, there has been an increase in anthropogenic air pollution, including ozone, NO_x, SO_x, peroxyace-

---

*To whom correspondence should be addressed.

0098-0331/93/0100-0097$07.00/0 © 1993 Plenum Publishing Corporation

tyl nitrate (PAN), and acidic deposition. Unlike the sulfate acidic rains typical in eastern North America, the more common form of wet acidic deposition in southern California is fog containing both nitric acid and sulfuric acid (Johnson and Siccama, 1983; Granett and Musselman, 1984). These fog events typically have durations of 4–12 hr with a pH of 2.0–3.0, although events of pH 1.7 have been recorded (Hoffman, 1984).

The acidic fogs that typically occur during the winter and early spring have the potential to damage both agricultural crops (Musselman and McCool, 1989) and native plant communities. *Encelia farinosa* Gray (Compositae: Asteraceae) is a dominant drought-deciduous component of the creosote bush scrub and coastal sage scrub plant communities of arid southern California, southern Nevada, central Arizona, and Baja California (Munz and Keck, 1968; Shreve and Wiggins, 1964; Wisdom, 1988). New foliage is flushed following the annual winter rains. Plants can retain foliage throughout the year, but will defoliate if summer drought stress becomes severe.

Trirhabda geminata Horn (Coleoptera: Chrysomelidae) is the primary insect herbivore on *E. farinosa* foliage (Wisdom, 1985). Estivating eggs hatch following winter rains, and the developing larvae feed on the newly flushed leaves (Redak et al., unpublished). Young larvae skeletonize the upper leaf surface, but mature larvae consume all but the main veins. Adults also feed on the leaves. Mating occurs on the host plant, but eggs are laid in the soil and litter at the base of the plant. There is normally a single generation each year, but rare early summer rains may trigger a second generation (Paine, personal observation). Large local populations of the beetle can defoliate plants over a considerable area.

The objective of this study was to determine if acidic fogs common to southern California affect host plant quality and feeding preference of herbivorous insects. The *T. geminata*/*E. farinosa* system is an excellent model to study the influence of anthropogenic pollution (acidic fogs) on foliage quality, in part because both adults and larvae feed on plant tissue directly exposed to the pollutant. In addition, the system can be used to investigate the potential role of prior insect feeding as a predisposing stress on the leaf tissue. The results have potential implications for the impact of urban expansion on endangered natural and agricultural systems in the southwestern United States.

METHODS AND MATERIALS

Acidic (pH 2.5, reagent-grade nitric and sulfuric acids at a 2.5:1 ratio, v/v in distilled water) or control (pH 6.3–6.5) fog treatments (Dercks et al., 1990) were applied for 3 hr every other day (three total applications) to 1-year-old *E. farinosa* grown in 4-liter containers. Ionic components typical of southern

California acidic fogs (Waldman et al., 1982; Musselman and McCool, 1989) were also added to the treatment solutions. Plants were treated inside 1-m^3 plastic fogging chambers (Musselman et al., 1985) set up in a greenhouse room (22–26°C, 300 $\mu E/m^2/sec$ light), then transferred to a shaded greenhouse room to dry. Acidic and control treatments were alternated in individual chambers on different days to minimize any chamber effect. Following the final fog application, the plants were left in the greenhouse for seven days before use in the bioassay tests or harvested for chemical analyses.

Feeding preferences based on the amount of leaf tissue consumed by adult or larval *T. geminata* were assessed in paper cup arenas (9 cm diameter) containing disks (2 cm^2) of leaf tissue (Jermy et al., 1968; Yoshida and Parrella, 1991). The disks were cut from the young fully expanded leaves with a cork borer seven days after the final treatment with either acidic or control fogs (40 plants per treatment). Plants were individually numbered; assignment of leaf disks cut from these plants to arenas (20 arena replicates per test) was by random draw with replacement. Leaf disks from the same plants were used in more than one arena (no plant was drawn more than twice), but because of the randomization, the same combination of plants was never repeated. Disk size was measured with a leaf area meter (LI 3000; Li-cor, Inc., Lincoln, Nebraska), and two disks of each treatment were randomly positioned within a circle around the bottom of the arena. Moist blotter paper covered with Saran screening lined the bottom of the feeding arena, and the test disks were held in place with map pins. Tight-fitting translucent lids covered each arena. Adult and third-instar larval *T. geminata* were field-collected from endemic populations on the University of California, Riverside campus. Feeding preferences of the two insect life stages were separately tested. Two individuals were placed in the arena and allowed to feed for 48 hr at ambient laboratory light and temperature conditions. The feeding interval did not allow the insects to consume more than 50% of any treatment type. Consumption of more than 50% of any treatment could bias the preference evaluation (Jermy et al., 1968; Jones and Coleman, 1988b). Disk areas were again measured after the feeding exposure to determine the area of tissue consumed. Mean values for consumption of the two acid-treatment or two control disks was determined within arenas. If there had been no feeding within the arena, the replicate was disgarded. Differences between treatments ($P < 0.05$) were determined using two-tailed Wilcoxon signed-rank tests, an approach utilizing a nonparametric paired comparison with arena as the replicate (Sokal and Rohlf, 1981).

In addition to testing the effect of acidic deposition on feeding preference, the effect of previous feeding damage on subsequent preference was also tested. A set of 40 plants each for acid and control fog treatments was exposed to feeding larvae seven days before treatment with the fogs. Leaves exhibiting previous feeding damage were included in the bioassay in addition to leaves from plants that had not been previously damaged. Differences between treatments were determined as previously described.

An additional set of plants, 10 receiving the acidic deposition treatment and 10 receiving the control fog treatment, was used for chemical analyses of the foliage. Leaves were removed seven days after the third fogging treatment. Percent water content was calculated from leaf fresh and dry weights [(fresh weight − dry weight)/fresh weight] (×100). Tissue not analyzed immediately was stored in an ultracold freezer (−65°C) for later use. Total nitrogen composition (percent) was determined (10 plants/treatment, four tissue samples/plant) using standard microkjeldahl techniques (McKenzie and Wallace, 1954) as modified by Trumble and Hare (1989). Soluble protein content (milligrams per gram) representative of more easily assimilated nitrogen was assessed (10 plants/treatment, four tissue samples/plant) with the Bradford (1976) reagent using the technique of Jones et al. (1988). Difference in water content between treatments was determined using Student's t test (Sokal and Rohlf, 1981). Differences between treatments in total nitrogen and soluble protein were determined with General Linear Models procedure (SAS Institute, 1988) using type III sums of squares with samples nested within plants as the error term.

RESULTS

Adult beetles consumed significantly more leaf tissue (Wilcoxon signed rank $Z = -2.512$, $N = 14$, $P = 0.0120$) from acidic-fog-treated plants than from the control-fogged plants (Figure 1). Because there were no differences in larval preferences among acidic-fogged leaves that had been previously damaged by feeding or were uninjured (Wilcoxon signed rank $Z = -1.161$, $N = 20$, $P = 0.2458$), or among the same two damage classes of control-treated plants (Wilcoxon signed rank $Z = -0.756$, $N = 20$, $P = 0.4496$) (Figure 2), larval consumption data from the two damage classes within each fogging treatment were combined to test for differences between fog treatments. Larval feeding preference followed the same pattern as adults. There was a significant preference (Wilcoxon signed rank $Z = -2.767$, $N = 40$, $P = 0.0057$) for feeding on acidic-fogged leaves compared to the controls (Figure 3).

There were no significant differences ($t = -0.2060$, $df = 17$, $P = 0.8391$) in moisture content (percent) of acid-fogged ($\bar{x} = 75.00$, SE $= 0.882$, $N = 10$) or control-fogged ($\bar{x} = 74.78$, SE $= 0.572$, $N = 9$) leaves. However, there were differences in the amounts of nitrogen between treatments. Total nitrogen (percent) was significantly higher (analysis of variance, $F = 5.98$, $df = 1, 18$, $P = 0.0213$) in acidic-fogged leaves ($\bar{x} = 2.33$, SE $= 0.152$, $N = 10$) than control leaves ($\bar{x} = 1.94$, SE $= 0.160$, $N = 9$). Similarly, soluble protein (milligrams per gram) was also significantly higher (analysis of variance, $F = 9.51$, $df = 1, 18$, $P = 0.0064$) in acidic-fogged leaves ($\bar{x} = 4.96$, SE $= 0.371$, $N = 10$) than control leaves ($\bar{x} = 3.94$, SE $= 0.319$, $N = 10$).

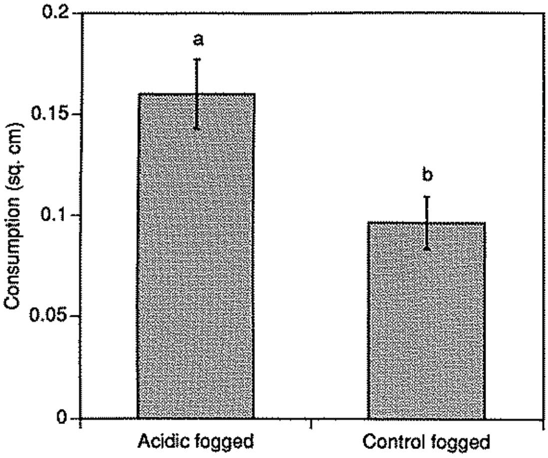

FIG. 1. Amount of acidic or control fogged *Encelia farinosa* tissue consumed by adult *Trirhabda geminata* in a leaf-disk feeding bioassay. Standard errors shown by lines through bars. Significant differences ($P = 0.0120$) indicated by different lowercase letters.

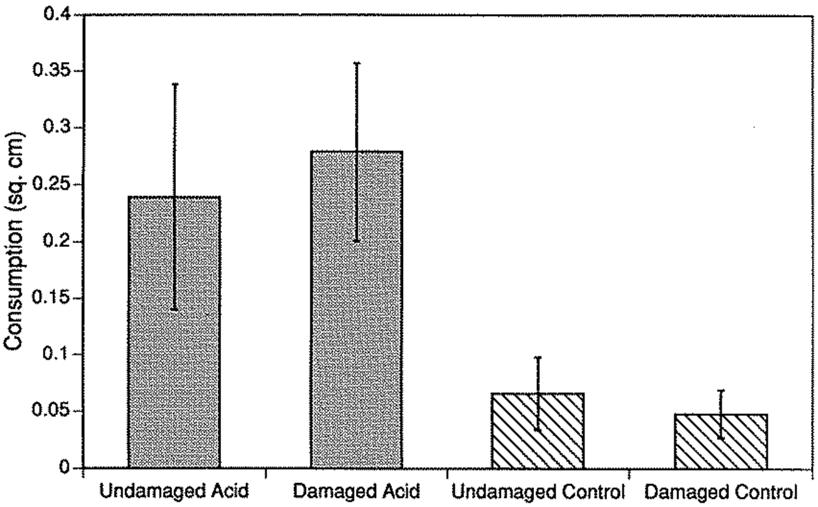

FIG. 2. Amount of tissue consumed by larval *Trirhabda geminata* from undamaged plants or plants damaged by prior insect feeding in a leaf-disk feeding bioassay. Standard errors shown by lines through bars. There were no significant differences between damage classes for either acidic fogged ($P = 0.2458$) or control fogged ($P = 0.4496$) plants.

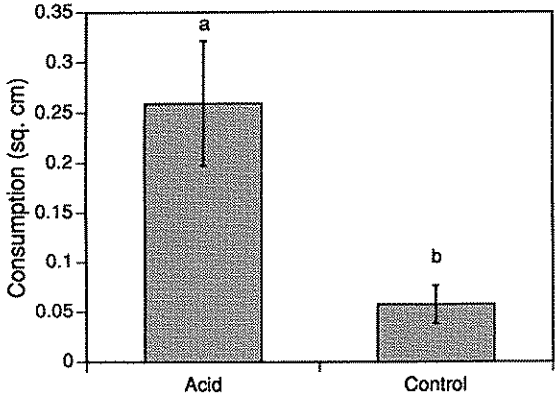

FIG. 3. Amount of acidic or control fogged *Encelia farinosa* tissue consumed by larval *Trirhabda geminata* in a leaf-disk feeding bioassay. Standard errors shown by lines through bars. Significant differences ($P = 0.0057$) indicated by different lowercase letters.

DISCUSSION

In previous studies of *T. geminata* feeding on *E. farinosa*, Wisdom (1985) demonstrated that the larvae move around on plants and aggregate to feed on particular plants or plant parts. He suggested that the larval feeding pattern was related to variation in chemical composition of plant tissues. *Encelia farinosa* shows high levels of seasonal, individual, and population variation in the concentrations of a sesquiterpene lactone, farinosin, and two chromenes, encecalin and euparin, and it has been suggested that the high concentrations found in leaves of some populations are maintained in response to herbivore pressure (Wisdom and Rodriguez, 1982). In fact, *T. geminata* develop at slower rates on diets containing high concentrations of these secondary chemicals and consequently may suffer higher rates of parasitism because of prolonged development time (Wisdom, 1985, 1988).

Wisdom and Rodriguez (1983) found that encecalin and euparin concentrations were greater in a population of *E. farinosa* subject to herbivory by *T. geminata* as compared to a population without significant herbivory, but nitrogen content did not differ between populations. In feeding preference trials, the larvae were unable to distinguish between diets containing high or low concentrations of the secondary chemicals but did prefer to feed on leaves with high nitrogen contents (Wisdom, 1985). Thus, although the sesquiterpene lactone and chromenes may affect the survivorship of the leaf beetle through delayed development and the action of natural enemies (Wisdom, 1985, 1988), differences in nitrogen content rather than secondary chemicals appear to affect the feeding preferences of the beetle. Acidic deposition increases both total nitrogen and soluble protein in treated leaves. Although variation in plant chemistry may result in aggregated feeding patterns and prolonged insect development on foliage characterized by high concentrations of the sesquiterpene lactone and chromenes under endemic conditions, acidic deposition on plants growing adjacent to urban environments may result in greater levels of foliar nitrogen and thus alter the nature of the interactions between herbivore and host. Specifically, if preference for treated foliage leads to increased herbivory in areas exposed to acidic fogs, plant distributions and local or regional ecologies may be altered.

Preference of a chrysomelid leaf beetle for host foliage exposed to an atmospheric pollutant is not limited to the *T. geminata/E. farinosa*/acid deposition system (Hughes et al., 1982; Endress and Post, 1985). Jones and Coleman (1988a) found that adults and larvae of *Plagiodera versicolora* Laich. preferred to feed on leaf disks cut from *Populus deltoides* Bartr. that had been treated with two 5-hr ozone (0.20 ppm) fumigations. Although both adults and larvae consumed more ozone-fumigated tissue, adult fecundity was lower than in those insects feeding on foliage that had not received the ozone exposure (Coleman and Jones, 1988). The investigators suggested that the ozone treatment reduced the quality of the host. In contrast, acidic deposition results in an increase in foliar nitrogen in *E. farinosa*. Not only do *T. geminata* adults and larvae prefer to feed on the treated leaf tissue, but larvae feeding on treated plants are significantly larger than those feeding on control plants [larval biomass gain: acid $\bar{x} = 2.98 \pm 0.14$ mg, control $\bar{x} = 2.23 \pm 0.14$ mg (unpublished data)].

The results demonstrate that the acidic fogs typical of southern California can alter the primary nutritive value of *E. farinosa* foliage. Both adults and larvae of *T. geminata* prefer to feed on leaves exposed to the acidic fogs. While arena bioassays on excised tissue may not completely reflect the natural system (Barnes, 1963; Jones and Coleman, 1988b; Risch, 1985), the results presented in this study are supported by increases in *T. geminata* larval growth rates (acid $\bar{x} = 0.21 \pm 0.01$ mg/mg/day, control $\bar{x} = 0.16 \pm 0.01$ mg/mg/day) and larval biomass gains in whole plant no-choice tests (Redak, unpublished data). Previous studies in other systems have described the physical changes in leaf tissue, increased stomatal resistance with a decrease in CO_2 assimilation, and changes in leaf chemistry following acidic fog events (Musselman and McCool, 1989; Trumble and Hare, 1989; Dercks et al., 1990; Trumble and Walker, 1991). Many of these studies examined the impact on agricultural plants, and, consequently, effects of anthropogenic pollution on agricultural productivity have been relatively well documented (Takemoto et al., 1988a, b; Musselman and McCool, 1989; McCool et al., 1990). Less well understood are the subtle impacts of these pollutants on noncultivated plant–insect systems. Although there is increasing interest in preserving tracts of open space, massive development of urban areas may indirectly have a great impact on the remaining remnants of the fragile arid ecosystems by altering the interactions among plants, herbivores, and, potentially, their natural enemies.

Acknowledgments—We thank C.C. Hanlon, M.K. Malinoski, A. Gonzales, and D. Messore for their assistance with the bioassays and chemical analyses. Critical reviews of the manuscript by S. Bertram, P. Luft, L. Hanks, T. Grasswitz, J. Gould, and C. Hanlon are appreciated.

REFERENCES

BARNES, O.L. 1963. Food-plant tests with the differential grasshopper. *J. Econ. Entomol.* 56:396–399.

BRADFORD, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein binding. *Anal. Biochem.* 72:248–254.

COLEMAN, J.S., and JONES, C.G. 1988. Plant stress and insect performance: Cottonwood, ozone and a leaf beetle. *Oecologia* 76:57–61.

DERCKS, W., TRUMBLE, J.T., and WINTER, C. 1990. Impact of atmospheric pollution on linear furanocoumarin content in celery. *J. Chem. Ecol.* 16:443–454.

ENDRESS, A.G., and POST, S.L. 1985. Altered feeding preference of Mexican bean beetle, *Epilachna varivestis*, for ozonated soybean foliage. *Environ. Pollut.* 39:9–16.

GRANETT, A.L., and MUSSELMAN, R.C. 1984. Simulated acidic fog injures lettuce. *Atmos. Environ.* 18:887–890.

HOFFMAN, M.R. 1984. Comment on acidic fog. *Environ. Sci. Technol.* 18:61–64.

HUGHES, P.R., POTTER, J.E., and WEINSTEIN, L.H. 1982. Effects of air pollution on plant-insect interactions: Reactions of the Mexican bean beetle to sulfur dioxide fumigated pinto beans. *J. Environ. Qual.* 2:365–368.

JERMY, T., HANSON, F.E., and DETHIER, V.G. 1968. Induction of specific food preference in lepidopterous larvae. *Entomol. Exp. Appl.* 11:211–230.

JOHNSON, A.H., and SICCAMA, T.G. 1983. Acid deposition and forest decline. *Environ. Sci. Technol.* 17:299–304.

JONES, C.G., and COLEMAN, J.S. 1988a. Plant stress and insect behavior: Cottonwood, ozone and the feeding and oviposition preference of a beetle. *Oecologia* 76:51–56.

JONES, C.G., and COLEMAN, J.S. 1988b. Leaf disk size and insect feeding preference: Implications for assays and studies on induction of plant defenses. *Environ. Exp. Appl.* 47:167–172.

JONES, C.G., HARE, J.D., and COMPTON, S.J. 1988. Measuring plant protein with the Bradford assay: I. Evaluation and a standard method. *J. Chem. Ecol.* 15:979–992.

MCCOOL, P.M., MUSSELMAN, R.C., and STERRETT, J.L. 1990. Injury of three flower crops from simulated acidic fog. *Plant Dis.* 74:310–312.

MCKENZIE, H.A., and WALLACE, H.S. 1954. The Kjeldahl determination of nitrogen: A critical study of digestion conditions, temperature, catalyst, and oxidizing agent. *Aust. J. Chem.* 7:55–70.

MUNZ, P.A., and KECK, D.D. 1968. A California Flora and Supplement. University of California Press, Los Angeles.

MUSSELMAN, R.C., and MCCOOL, P.M. 1989. Effects of acidic fog on productivity of celery and lettuce and impact on incidence and severity of diseases. *Ann. Appl. Biol.* 114:559–565.

MUSSELMAN, R.C., STERRETT, J.L., and GRANETT, A.L. 1985. A portable fogging apparatus for field or greenhouse use. *HortScience* 20:1127–1129.

RISCH, S.J. 1985. Effects of induced chemical changes on interpretation of feeding preference trials. *Entomol. Exp. Appl.* 39:81–84.

SAS INSTITUTE. 1988. SAS User's Guide: Statistics. SAS Institute, Cary, North Carolina.

SHREVE, F., and WIGGINS, I.L. 1964. Vegetation and Flora of the Sonoran Desert. Stanford University Press, Stanford, California.

SOKAL, R.R., and ROHLF, F.J. 1981. Biometry, 2nd ed. Freeman and Co., New York.

TAKEMOTO, B.K., BYTNEROWICZ, A., and OLSZYK, D.M. 1988a. Depression of photosynthesis, growth, and yield in field-grown green pepper (*Capsicum annuum* L.) exposed to acidic fog and ambient ozone. *Plant Physiol.* 88:477–482.

TAKEMOTO, B.K., OLSZYK, D.M., JOHNSON, A.G., and PARADA, C.R. 1988b. Yield responses of field-grown crops to acidic fog and ambient ozone. *J. Environ. Qual.* 17:192–197.

TRUMBLE, J.T., and HARE, J.D. 1989. Acidic fog-induced changes in host-plant suitability. *J. Chem. Ecol.* 15:2379–2390.

TRUMBLE, J.T., and WALKER, G.P. 1991. Acute effects of acidic fog on photosynthetic activity and morphology of *Phaseolus lunatus. HortScience* 20:1531–1534.

WALDMAN, J.M., MUNGER, J.W., JACOB, D.J., FLAGEN, R.C., MORGAN, J.J., and HOFFMAN, M.R. 1982. Chemical composition of acid fog. *Science* 218:677–680.

WISDOM, C.S. 1985. Use of chemical variation and predation as plant defenses by *Encelia farinosa* against a specialist herbivore. *J. Chem. Ecol.* 11:1553–1565.

WISDOM, C.S. 1988. Comparisons of insect use and chemical defense patterns of two Sonoran desert shrubs, pp. 36–49, *in* R.G. Zahary (ed.). Desert Ecology 1986: A Research Symposium. Southern California Academy of Sciences and the Southern California Desert Studies Consortium.

WISDOM, C.S., and RODRIGUEZ, E. 1982. Quantitative variation of the sesquiterpene lactones and chromenes of *Encelia farinosa. Biochem. Syst. Ecol.* 10:43–48.

WISDOM, C.S., and RODRIGUEZ, E. 1983. Seasonal age-specific measurements of the sesquiterpene lactones and chromenes of *Encelia farinosa. Biochem. Syst. Ecol.* 11:345–352.

YOSHIDA, H.A., and PARRELLA, M.P. 1991. Chrysanthemum cultivar preferences exhibited by *Spodoptera exigua* (Lepidoptera: Noctuidae). *Environ. Entomol.* 20:160–165.