Journal of Chemical Ecology, Vol. 19, No. 9, 1993

FOLIAR AND FLORAL PYRETHRINS OF *Chrysanthemum cinerariaefolium* ARE NOT INDUCED BY LEAF DAMAGE

IAN T. BALDWIN,* MICHAEL J. KARB, and PATRICK CALLAHAN

Department of Biological Sciences
SUNY at Buffalo
Buffalo, New York 14260-1300

(Received February 19, 1993; accepted April 20, 1993)

Abstract—Pyrethrins are a class of potent insecticides produced by *Chrysanthemum cinerariaefolium*. Simulated herbivory does not affect concentrations of pyrethrins in damaged and undamaged expanding and fully expanded leaves, or flowers of greenhouse or field-grown plants.

Key Words—Induced defense, *Chrysanthemum cinerariaefolium*, pyrethrins, leaf damage.

INTRODUCTION

In at least some plant species, examples of secondary compounds that increase in response to leaf damage come from every major biosynthetic class of secondary metabolites (Baldwin, 1993; Rosenthal and Berenbaum, 1991; Tallamy and Raupp, 1991). Thus, few generalizations can be made about how inducible a class of metabolites will turn out to be. Many secondary metabolites are synthesized in specialized tissue types that are active only during particular periods of leaf ontogeny and thus their inducibility may be morphologically constrained. For example, many terpenoids are stored in secretory idioblasts, laticifers, and glandular trichomes—cell types active early in leaf development—which may constrain induced responses to the next leaves produced after damage (Gershenzon and Croteau, 1991). However, plant development and organization is known for its plasticity, so it should not be surprising to find biochemical plasticity in differentiated tissues and to find that some tissues are more plastic

*To whom correspondence should be addressed.

0098-0331/93/0900-2081$07.00/0

than others. For example, Nitao (1988) found no evidence for induction in total or specific furanocoumarins in the fruits of wild parsnip, but Zangerl (1990) and Zangerl and Berenbaum (1990) found dramatic evidence for furanocoumarin induction in leaf tissues in the same genotypes of the same species.

The leaves and flowers of *Chrysanthemum cinerariaefolium* (Trev.) contain the potent insecticides, pyrethrins. The highest concentration of the six esters that constitute the natural pyrethrins are found in the flower heads, where they are localized in secretory canals and oil glands of the achenes (Brewer, 1973). The concentration of pyrethrins is also highly correlated with the number of oil glands in the leaves (Zito et al., 1983). Here we examine the effects of leaf damage on the pyrethrin concentrations of damaged and undamaged leaves and flowers of greenhouse and field-grown plants.

METHODS AND MATERIALS

Plant Growth and Sampling. C. cinerariaefolium were grown from seed obtained from the Park Seed Co. (Greenwood, South Carolina; lot #2925) in 25-cm pots with Cornell mix A (Boodley and Sheldrake, 1977) supplemented with 5 g of Osmocote 14–14–14 fertilizer in a glasshouse under supplemental lighting from 400-W sodium vapor lamps for 13 hr/day. Greenhouse experiments were conducted with 2-year-old plants. Four vigorously growing vegetative plants of similar dimensions were sampled for pyrethrins in both expanding and fully expanded leaves. After the initial sampling, half of each plant was cut with scissors, removing the top 3 cm of all leaves on the damaged half of the plant. The cutting removed approximately 24% of the plants' leaf mass. Expanding and fully expanded leaves were sampled on both the damaged and undamaged halves of each plant at the times indicated in Figure 1. Fifteen 0.12-cm^2 leaf disks (one disk from the between-vein portion of the lamina of 15 different leaves) were removed with a cork borer, pooled by leaf developmental stage, and extracted immediately in absolute methanol. Each of the sampled leaves was resampled at each sampling time. At the last sampling, additional samples of leaf disks were removed, weighed, dried at 50°C, and reweighed.

Since some damage-induced chemical responses are inhibited in pot-bound plants (Baldwin, 1988; Karban et al., 1989), a second experiment was conducted on plants transplanted to a field plot where root growth would remain unrestricted. Two-year-old greenhouse-grown plants were transplanted into a rototilled field plot in Amherst, New York, on June 5 with a between-plant spacing of 50 cm. On August 17, ten flowering plants growing in the center row of the plot were selected for the experiment. Every other plant was cut with scissors as previously described and five to six fully expanded undamaged leaves were removed from each plant at the times indicated in Figure 2, placed on ice, and

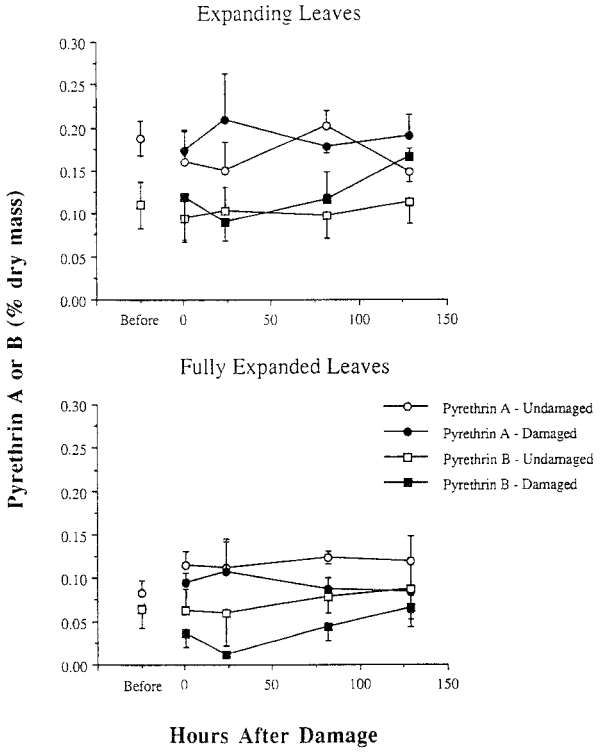

FIG. 1. Mean pyrethrin A and B (±SEM) concentrations for expanding and fully expanded leaves of four greenhouse-grown *C. cinerariaefolium* plants. After initial sampling (designated as before), the top 3 cm of half of each plant was cut with scissors. Expanding and fully expanded leaves were then resampled on both the damaged and undamaged halves of each plant at 0.75, 24, 82, and 129 hr. Pyrethrin A represents total pyrethrin I, jasmolin I, and cinerin I amounts, and pyrethrin B represents total pyrethrin II, jasmolin II, and cinerin II amounts.

returned to the laboratory for pyrethrin extraction. Leaves were ground to a powder in liquid nitrogen. A portion (200–700 mg) of the leaf material was weighed (to 0.1 mg) and extracted in absolute methanol. Another portion of leaf material was weighed, dried at 50°C, and reweighed for percent leaf dry mass measures. Flowers (1–3/plant/sampling) with at least the ray florets expanded but not yet completed anthesis were removed from each plant at the beginning and the end of the experiment, placed on ice, and returned to the laboratory for pyrethrin analysis. Flowers were also extracted in absolute methanol.

Pyrethrin Extraction and Quantification. Pyrethrin leaf and flower extracts

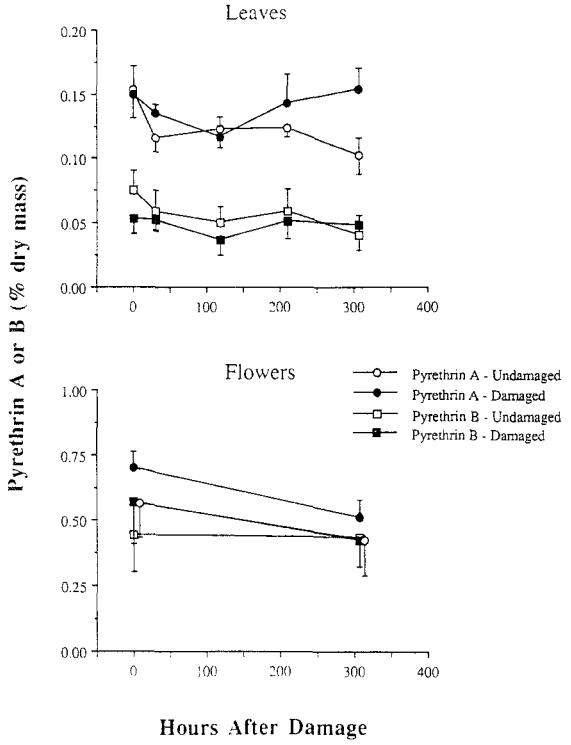

FIG. 2. Mean pyrethrin A or B (±SEM) concentrations of leaves or flowers from five undamaged and five damaged *C. cinerariaefolium* plants grown in a field plot in Amherst, New York. Plants were damaged at time 0.

were kept in the dark at −5°C until analysis. When fresh-frozen leaf material was spiked with 5–100 μg of total pyrethrin, greater than 95% of the added amount was recovered in a single methanolic extraction when the concentration of leaf material was less than 90 mg/ml. Extracts were centrifuged to remove debris before HPLC analysis. Dilutions of a commercial standard (Premium Pyrocide; McLaughlin, Gormely, King Co., Minneapolis, Minnesota) containing 10.98% pyrethrin A and 8.92% pyrethrin B were used as an external standard for the quantification. Pyrethrin A represents a mixture of pyrethrin I, cinerin I, and jasmolin I, whereas pyrethrin B represents a mixture of pyrethrin II, cinerin II, and jasmolin II. Pyrethrin A and B were separated using a reverse-phase HPLC on a Versapack C-18 (250 × 4.1 mm; 10 μm) column (Alltech, Avondale, Pennsylvania) with an LC-18 Pellicular Packing (Supelco, State College, Pennsylvania) containing a guard column and an isocratic mobile phase consisting of 77% aqueous methanol. At a 1.3 ml/min flow rate, pyrethrins A

and B had retention times of 14 and 7 min, respectively. Pyrethins were detected by their absorbance at 254 nm and peak areas were integrated with an HP 3392 integrator. Leaf and flower extracts had peaks that coeluted with the pyrethrins A and B standards; these coeluting peaks were collected separately from the HPLC, rotoevaporated to dryness, taken up in 100 μl of acetonitrile, and subjected to GC-MS analysis (50-m × 0.20-mm HP-5 column, cool on-column injection onto a 1-m × 0.53-mm precolumn, 80–250°C temperature ramp at 10°C/min, 70 eV EI with an HP 5971 Mass Selective Detector). The pyrethrin A HPLC peak contained well-resolved cinerin I, jasmolin I, and pyrethrin I peaks and their mass spectra matched those in the Wiley 138K Mass Spectra Database. The pyrethrin B HPLC peak contained a well-resolved cinerin II peak and broad jasmolin II and pyrethrin II peaks and their mass spectra matched those reported by Kawano et al. (1974). Due to the notorious thermal instability of these esters, we did not attempt to quantify the relative amounts of pyrethrin, cinerin, and jasmolin in the pyrethrin A and B peaks by GC-MS. However, using the straight-phase HPLC technique of Mourot et al. (1978), we separated the pyrethrins, cinerins, and jasmolins in the pyrethrin A and B peaks from the analytical standard, and a pooled flower and leaf extract of damaged and undamaged field-grown plants. In all three samples, pyrethrins I and II represented more than 90% of pyrethrin A and B peak areas, respectively. In expressing the pyrethrin A and B values as percentages of leaf and flower mass, we are assuming that the relative ratios of the three esters in each HPLC peak were not strongly influenced by damage. Given that the molar extinction coefficients of the two cinerins and jasmolins are very similar (ϵ_{229nm} = 21,000–28,000) and that pyrethin I and II represent the majority of the total pyrethrins in both our analytical standard and our plant extracts, we feel that this assumption is justified.

Statistical Methods. Repeated-measures one-way ANOVAs with damage as the main effect were used to analyze the pyrethrin estimates of both field and greenhouse experiments. Analysis was performed with the MGLH ANOVA module from Systat Inc. (Evanston, Illinois).

RESULTS AND DISCUSSION

Pyrethrin concentrations in *C. cinerariaefolium* were not affected by damage (Figures 1 and 2). No statistically significant differences were found in the pyrethrin concentrations of leaves from damaged and undamaged plants for both the greenhouse experiment (pyrethrin A: $F_{1,9} = 0.003$, $P = 0.954$; pyrethrin B: $F_{1,9} = 0.024$, $P = 0.880$) and the field experiment (pyrethrin A: $F_{1,8} = 1.117$, $P = 0.321$; pyrethrin B: $F_{1,8} = 0.270$, $P = 0.617$). Likewise, the effect of leaf damage on flower pyrethrin concentrations was not significant (pyrethrin

A: $F_{1,8} = 0.580$, $P = 0.468$; pyrethrin B: $F_{1,8} = 0.112$, $P = 0.746$). Expanding leaves had significantly higher pyrethrin A and B concentrations than did fully expanded leaves (pyrethrin A: $F_{1,9} = 6.201$, $P = 0.035$; pyrethrin B: $F_{1,9} = 5.541$, $P = 0.043$), which may reflect a higher density of pyrethrin secretory structures in young leaves (Zito et al., 1983).

Foliar and floral pyrethrins are constitutively produced and their biosynthesis is not induced within 13 days of damage. The amount of pyrethrins produced in a leaf may be developmentally constrained by the number of pyrethrin secretory canals or glands produced early in leaf ontogeny. Although the morphological "packaging" of secondary metabolite synthesis and storage may constrain inducibility for pyrethrins, the production of other terpenoid secondary metabolites, such as the oleoresins in *Pinus pinaster* (Walter et al., 1989, Marpeau et al. 1989), are not similarly constrained. The lack of inducibility of pyrethrins may have ecological explanations; for example, the increased pyrethrin concentration above the constitutive level may not deter potential herbivores. Understanding the patterns of inducibility among secondary metabolites may provide important insight into the roles these compounds play in plants.

Acknowledgments—This research is supported by the National Science Foundation grant BSR-9157258, a small grant from the Health in Housing Center, and a generous grant from the Hewlett-Packard University Equipment Grants Program. We thank Z. Rosaldo and J. Kingsley for superb technical assistance, W. Gullickson for the generous donation of analytical standards of pyrethins and important *C. cinerariaefolium* references, and E. Wheeler for editorial assistance. Two anonymous reviewers substantially improved the manuscript.

REFERENCES

BALDWIN, I.T. 1988. Damage-induced alkaloids in tobacco: Pot-bound plants are not inducible. *J. Chem. Ecol.* 14:1113–1120.

BALDWIN, I.T. 1993. Chemical changes rapidly induced by folivory, *in* E.A. Bernays (ed.). Insect-Plant Interactions. CRC Press, Boca Raton, Florida. In press.

BOODLEY, J.W., and SHELDRAKE, R., JR. 1977. Cornell peatlite mixes for commercial plant growing. Cornell University Information Bulletin Number 43.

BREWER, J.G. 1973. Microhistological examination of the secretory tissue in pyrethrum florets. *Pyrethrum Post* 12:17–22.

GERSHENZON, J., and CROTEAU, R. 1991. Terpenoids, pp. 165–219, *in* G.A. Rosenthal and M.R. Berenbaum (eds.). Herbivores: Their Interactions with Secondary Plant Metabolites. Academic Press, New York.

KARBAN, R., BRODY, A.K., and SCHNATHORST, W.C. 1989. Crowding and a plant's ability to defend itself against herbivores and disease. *Am. Nat.* 134:749–760.

KAWANO, Y., YANAGIHARA, K.H., and BEVENUE, A. 1974. Analytical studies of pyrethrin formulations by gas chromatography: III. Analytical results on insecticidally active components of pyrethrins from various world sources. *J. Chromatogr.* 90:119–128.

MARPEAU, A., WALTER, J., LAUNAY, J., CHARON, J., BARADAT, P., and GLEIZES, M. 1989. Effects of wounds on the terpene content of twigs of maritime pine (*Pinus pinaster* Ait.): II. Changes in the volatile terpene hydrocarbon composition. *Trees* 4:220–226.

MOUROT, J., BOISSEAU, J., and GAYOT, G. 1978. Separations of pyrethrins by high-pressure liquid chromatography. *Anal. Chim. Acta* 97:191–193.

NITAO, J.K. 1988. Artificial defloration and furanocoumarin induction in *Pastinaca sativa* (Umbelliferae). *J. Chem. Ecol.* 14:1515–1522.

ROSENTHAL, G.A., and BERENBAUM, M.R. 1991. Herbivores, Their Interactions with Secondary Plant Metabolites. Academic Press, New York.

TALLAMY, D.W., and RAUPP, M.J. 1991. Phytochemical Induction by Herbivores. John Wiley, New York.

WALTER, J., CHARON, J., MARPEAU, A., and LAUNAY, J. 1989. Effects of wounding on the terpene content of twigs of maritime pine (*Pinus pinaster* Ait.): I. Changes in the concentration of diterpene resin acids and ultrastructural modifications of the resin duct epithelial cells following injury. *Trees* 4:210–219.

ZANGERL, A.R. 1990. Furanocoumarin induction in wild parsnip: Evidence for an induced defense against herbivores. *Ecology*. 71:1926–1932.

ZANGERL, A.R., and BERENBAUM, M.R. 1990. Furanocoumarin induction in wild parsnip: Genetics and populational variation. *Ecology* 71:1933–1940.

ZITO, S.W., ZIEG, R.G., and STABA, E.J. 1983. Distribution of pyrethrins in oil glands and leaf tissue of *Chrysanthemum cinerariaefolium. Planta Med.* 47:205–207.