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# Effects of Cadmium on Two Biocontrol Insects and Their Host Weeds

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Alligatorweed [Alternanthera philoxeroides (Mart.) Griseb.] and purple nutsedge (Cyperus rotundus L.) are affected by weed-eating insects. The alligatorweed flea beetle (Agasicles hygrophila Selman & Vogt) is one of three species of insects introduced into the United States from South America for biocontrol of alligatorweed (SPENCER & COULSON 1977). Bactra verutana Zeller, the nutsedge moth (a native insect), is being investigated for its potential when manipulated by mass rearing as a biocontrol agent against purple nutsedge (FRICK 1974, FRICK & GARCIA 1975, FRICK & QUIMBY 1977). Alligatorweed is considered an aquatic weed when it clogs waterways although it has recently been evaluated as a potential biological filter for sewage effluent (WOLVERTON et al. 1975). Purple nutsedge is a major weed pest in croplands around the world (HOLM et al. 1977).

The aquatic habitats and cropland areas may be subject to potential contamination by heavy metals, e.g. Cd (LELAND et al. 1974, WILLIAMS & DAVID 1976) from industrial effluent and impurities in fertilizers, but little is known about heavy metal/-plant/insect interactions. Selenium effects on insects have been reported (HURD-KARRER & POOS 1936). BYERS and ZEIDERS (1976) reported that feeding by the cereal leaf beetle (Dulema melanopus L.) and the frit fly (Oscinella frit L.) was less on reed canary-grass plants spray irrigated with sewage effluent than on plants grown without the irrigation. Other workers (MADDOX & MCCREADY 1975, MADDOX & RHYNE 1975) have studied the effects of mineral-deficient alligatorweed on feeding by the alligatorweed flea beetle.

Our objective was to study the effects of Cd on the growth of alligatorweed and purple nutsedge and on the feeding and fecundity of the alligatorweed flea beetle and the nutsedge moth.

## MATERIALS AND METHODS

Conduct of experiments with alligatorweed and the alligatorweed flea beetle. Alligatorweed, as a source of propagules, was grown in the greenhouse according to methods previously described

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(QUIMBY & KAY 1977). Propagules, three 9-cm long, 2-node stem cuttings, were placed in each of several Erlenmeyer flasks with 250 mL nutrient solution with or without Cd $(NO_3)_2$ at 1 $mg \cdot L^{-1}$Cd. The pH of the nutrient solutions varied between 5.5 to 6.5 during the experimental periods. Distilled water was added daily to replace losses caused by transpiration. Plants were grown without aeration in a growth chamber under 14 h light (700 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 25/20°C day/night temperature. The alligatorweed was grown for 3 wk and then discs (8 mm diameter) were cut with a #4 corkborer from fully expanded leaves for feeding trials. Nutrient solutions were changed (including Cd) at 1-1/2 wk. Alligatorweed was similarly grown for growth comparisons and harvested for total dry weight at 3 wk with five replications per treatment. Leaf samples were held for Cd determination.

No-choice feeding experiments were conducted with the alligatorweed flea beetle in plastic slide top boxes, 2.5 x 2.5 x 6.0 cm. Two discs cut from each of four leaves were placed on moistened filter paper in each box with two males and two females of newly emerged adult beetles. Duplicate leaf-disc samples were dried for 48 h at 70°C in the oven for determination of specific leaf weight. Beetles were transferred to a clean box daily with two fresh leaf discs per surviving beetle. Feeding was determined by measuring the leaf-disc areas before and after each feeding period with a leaf area meter. Areas were converted to weight by the appropriate specific leaf weight. Eggs were removed daily and held in the slide top boxes at room temperature (75°C) to determine viability. Treatments were replicated twice and the experiment was conducted three times.

Freedom-of-choice feeding tests were conducted similarly except leaf discs with and without Cd were alternately pinned to blotter paper in 9-cm diameter petri plates and beetles were transferred to clean petri plates with fresh leaf discs every other day.

<u>Conduct of experiments with purple nutsedge and the nutsedge moth</u>. These experiments were conducted in the greenhouse as previously described (FRICK & QUIMBY 1977). Each nutsedge plant (one plant per pot, 15.5 cm in diameter) was trimmed to three leaves, 10 cm long, and then infested weekly for 4 wk with three first instar larvae per shoot. The pots were nested in 2.5-L cans and the vermiculite medium subirrigated with capillary action of a nylon rope wick extending into the cans. Ten replicates of each treatment were included. Plant material from five replicates was harvested at 28 days, dried at 70°C, weighed, ashed, and analyzed for Cd. The other five replicates were held for 10 additional days to recover moths emerging from the initial infestation. Moths were held in mating/oviposition cages (GARCIA & FRICK 1975), and egg production was determined. Wing lengths of moths were measured as an index of size (MILLER 1977).

Larvae were fed on diet (GARCIA & FRICK 1975) in cups with and without Cd added at five concentrations from 1.12 to 18.0

$\mu g \cdot g^{-1}$ on a dry weight basis. This experiment included 10 larvae per cup, 50 cups per treatment, and the experiment was repeated once. Moths emerging from pupae reared on the treated diet were held in mating/oviposition cages, and egg production was determined.

Analysis of plant material. Plant material was ground with a Wiley mill to pass through a 40-mesh screen, ashed at 500°C for 3 h, taken up with 20% HCl and analyzed for Cd by atomic absorption spectrophotometry. Recovery of Cd by this method was 94% as determined by standard additions of Cd to plant samples.

## RESULTS AND DISCUSSION

Effects of Cd on alligatorweed. Alligatorweed accumulated 8.7 $\mu g \cdot g^{-1}$ of Cd in a 3-wk period when exposed to 1 $mg \cdot L^{-1}$ Cd added to the nutrient medium; this was a 3-1/3-fold increase over the plants that did not receive added Cd (Table 1). The Cd treatment reduced the total growth of alligatorweed 63% and specific leaf weight 19% in the 3-wk period. The 2.6 $\mu g \cdot g^{-1}$ Cd level found in the plants serving as controls was similar to the mean level of 2.0±.45 (SD) $\mu g \cdot g^{-1}$ Cd found in 27 samples of alligatorweed collected from 15 sites in the lower Mississippi Valley between Pendleton, AR and Ponchatoula, LA (authors' unpublished data). The Cd in the control plants may have originated from contaminants in the chemicals used for the nutrient solutions which were not analyzed for Cd. The Cd content of 8.7 $\mu g \cdot g^{-1}$ for alligatorweed leaves (from plants exposed to added Cd) is within the range of widely differing values (2.5 to 326 $\mu g \cdot g^{-1}$) reported by some authors (TURNER 1973, PAGE *et al*. 1972) for its near relative, beet (*Beta vulgaris* L.), exposed to Cd at the same level (1 $mg \cdot L^{-1}$) in nutrient solution culture.

Effects of Cd on purple nutsedge. Purple nutsedge plants exposed to added Cd at 1 $mg \cdot L^{-1}$ had about 2.8 times higher Cd content than the control plants and about 32% less growth (TABLE 1).

Effects of Cd on the alligatorweed flea beetle. The higher level of Cd in Cd-treated alligatorweed increased mortality and decreased feeding and oviposition in the alligatorweed flea beetle (TABLE 1). The most pronounced effect of Cd on the alligatorweed flea beetle was reduced fecundity. Oviposition was completely eliminated in two of three experiments and greatly reduced in the third to give an overall reduction of 92% (TABLE 1). Mortality was 3.4 times greater for flea beetles fed on Cd-treated alligatorweed than for those fed on untreated plant tissue. In no-choice tests the cumulative feeding decreased steadily over the 10-day feeding period for those beetles feeding on Cd-treated alligatorweed (FIGURE 1); this decline was more pronounced after the first day. The total amount of Cd ingested (on a per beetle basis) after 1 day was twice as high for flea beetles fed on leaf discs

TABLE 1

Effects of cadmium at 1 μg $L^{-1}$ in nutrient solution on growth of alligatorweed and purple nutsedge and on feeding, oviposition, and mortality of the alligatorweed flea beetle and the nutsedge moth.

| Organisms and responses considered | Treatment: Control, no Cd added | Treatment: Cd added at 1 mg $L^{-1}$ | Ratio Cd/no Cd | Significance of t-test for differences |
|---|---|---|---|---|
| A. Alligatorweed/alligatorweed flea beetle | | | | |
| Content of Cd in plant leaves after 3 weeks | 2.6 $\mu g \cdot g^{-1}$ | 8.7 $\mu g \cdot g^{-1}$ | 3.35 | * |
| Growth per plant after 3 weeks | 4.3 g | 1.6 g | 0.37 | * |
| Specific leaf weight after 3 weeks | 5.2 $mg \cdot cm^{-2}$ | 4.2 $mg \cdot cm^{-2}$ | 0.81 | * |
| Feeding per adult beetle, no choice, 10 day trials | 1240 $\mu g \cdot day^{-1}$ | 513 $\mu g \cdot day^{-1}$ | 0.41 | * |
| Feeding per adult beetle, freedom of choice, 10-day trials | 279 $\mu g \cdot day^{-1}$ | 318 $\mu g \cdot day^{-1}$ | 1.14 | ns |
| Oviposition as total eggs per female for 10-day period, no choice trials | 53 eggs | 4 eggs | 0.08 | * |
| 10-day mortality, each mean based on 48 adults, percent dead, no choice trials | 10.4% | 35.4% | 3.40 | * |
| B. Purple nutsedge/nutsedge moth | | | | |
| Content of Cd in plant leaves after 4 weeks | 2.3 $\mu g \cdot g^{-1}$ | 6.5 $\mu g \cdot g^{-1}$ | 2.83 | * |
| Growth per plant after 4 weeks | 6.6 g | 4.5 g | 0.68 | * |
| 4-week no-choice feeding by four weekly infestations of 3 larvae per shoot, percent reduction of plant weight | 91% | 95% | 1.04 | ns |
| Oviposition as total eggs per female until dead | 173 eggs | 161 eggs | 0.93 | ns |
| Moths recovered per 10 plants by 38 days | 15 moths | 15 moths | 1.00 | ns |
| Wing lengths per moth, both sexes | 6.6 mm | 6.5 mm | 0.98 | ns |

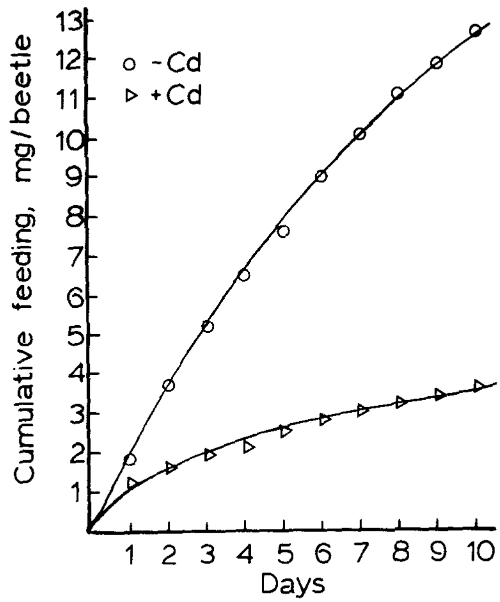


Fig. 1. Cumulative feeding over a 10-day period by the alligatorweed flea beetle on alligatorweed grown with and without Cd added to the nutrient solution.

from plants grown with Cd added to the nutrient solution than for control flea beetles fed on leaf discs from plants grown without added Cd (TABLE 2). However, the total amount of Cd ingested (on a per beetle basis) over the entire 10-day period was slightly higher for those flea beetles feeding on leaf discs from plants grown without Cd added to the nutrient solution than for those feeding on Cd-treated plant material. This apparent anomaly occurred because the control beetles ingested nearly 4 times more host-plant material. However, these control beetles were apparently able to tolerate (possibly through excretion?) the lower concentration of Cd in untreated leaf tissue and could grow and reproduce normally. In contrast, the plant tissue with the higher concentration of Cd was acutely toxic to the flea beetles; those fed on Cd-treated plant tissue ingested more Cd than did the control beetles on the first day. The Cd apparently interfered with normal metabolism early in the 10-day period and retarded the

TABLE 2

Intake of cadmium by alligatorweed flea beetle.

| Treatment | Day | Cumulative feeding (mg/beetle) | | Leaf Cd content (ng/mg) | | Cd, tot. ingested (ng) | | Beetle weight (mg) | | Cd per unit wt (ng/mg) | | Days of feeding (no.) | | Cd, daily intake (ng/mg/day) | Ratio, +Cd/-Cd |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| -Cd | 1 | 1.8 | · | 2.6 | = | 4.7, | ÷ | 2(est) | = | 2.4, | ÷ | 1 | = | 2.4 | |
| +Cd | 1 | 1.2 | | 8.7 | | 10 | | 2(est) | | 5 | | 1 | | 5 | 2.1 |
| -Cd | 10 | 13 | | 2.6 | | 34 | | 2.8[a] | | 12 | | 10 | | 1.2 | |
| +Cd | 10 | 3.6 | | 8.7 | | 31 | | 1.8[b] | | 17 | | 10 | | 1.7 | 1.4 |

[a]Average weight of eight beetles

[b]Average weight of 11 beetles.

subsequent feeding and reproductive rates of the beetles. The final daily intake of Cd, on a beetle weight basis, was 1.4 times greater for beetles fed on Cd-treated alligatorweed than for those fed on untreated plant tissue.

In freedom-of-choice tests, the flea beetles did not discriminate against Cd-treated alligatorweed. Apparently, all beetles fed coequally on leaf discs from both Cd-treated and untreated alligatorweed and were adversely affected by the Cd so that their overall feeding rate was reduced (TABLE 1). We attempted recovery experiments in which flea beetles were transferred after 2 days from Cd-treated to untreated alligatorweed (data not shown). No recovery in feeding rates of beetles was observed during the subsequent 8-day period.

Effects of Cd on the nutsedge moth. Feeding, fecundity, and longevity of the nutsedge moth were unaffected when larvae were fed on plants that had a Cd concentration of 6.5 $\mu g \cdot g^{-1}$. Moreover, when larvae were fed on diet to which Cd had been added in concentrations up to 18.0 $\mu g \cdot g^{-1}$, no adverse effects were observed on larval feeding or growth, emergence of moths from pupae, or reproductive potential of the moths.

## CONCLUSION

Cd added to the nutrient solution at 1 $mg \cdot L^{-1}$ significantly reduced growth of alligatorweed and purple nutsedge. Both plant species increased about threefold in Cd content when exposed to the added Cd over the 3 to 4 wk periods. A significant difference existed between the two insect species in their response to Cd; the alligatorweed flea beetle was very sensitive to Cd levels in the alligatorweed and the nutsedge moth was virtually unaffected by the levels in the purple nutsedge and diet. These results implied that the alligatorweed/alligatorweed flea beetle system might serve as a sensitive bioassay for heavy metal pollution in sewage effluent, particularly since alligatorweed has been considered as a potential biological filter for effluent (WOLVERTON et al. 1975). Other trace elements may be involved in the biogeography of plants and insects and would merit investigation.

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