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EFFECTS OF INSECTICIDE SPRAYING ON PLANT REPRODUCTIVE SUCCESS IN NATIVE MIXED GRASS PRAIRIE OF WESTERN NORTH DAKOTA

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Introduction

Large areas of western rangeland are frequently sprayed with insecticides as a management tactic for the control of grasshoppers. There is widespread concern over potential deleterious side-effects of these sprays on non-target organisms. Because large-scale sprayings typically involve broad spectrum insecticides, they not only kill targeted grasshoppers, but also may eliminate numerous other insects. The most important of these insects to plant populations are native pollinators. Concern has arisen that a significant reduction in pollinators might negatively affect plant reproductive success when an insect vector is required for pollination. For small plant populations, even a slight reduction in potential pollinators could have dramatic adverse effects, causing diminished seed set, increased levels of inbreeding, general reductions in population fitness and possible extinction. There also exists the possibility that certain pollinator populations may be overabundant, and reductions in number of individuals would have little or no effect on plant reproductive success.

Several plant species in western North Dakota were chosen during the summer of 1992 for preliminary assessment of the consequences of grasshopper spray programs on plant reproductive success. Populations of these plants were studied on rangelands used as demonstration sites for the Grasshopper Integrated Pest Management (GHIPM) project. For each plant, the breeding system was studied to confirm whether an insect vector was necessary for sexual reproduction. Direct censussing of pollinator populations was not done due to the difficulties involved in getting accurate population estimates. The main objective of this project for the first study season (1992) was to establish whether differences in seed set occurred between sprayed areas, non-sprayed areas, and areas along the periphery of sprayed zones.

Materials and Methods

Site Selection:

The Medora spray block was selected for field study in 1992. This area lies approximately six miles south of Medora, North Dakota, covering approximately 47,000 acres of federal and private rangeland. Six sites were originally chosen either within or surrounding this block. A last minute change in spray boundaries left the following sites: two control sites approximately four miles outside of the spray boundary (Control 1 and 2); one peripheral site 1/4 mile outside the spray boundary (Periphery 2); and one site 1 mile within the spray area (Spray 1). Criteria used to select sites included forb presence and abundance, distance to spray boundary (either within or outside sprayed area), limited cattle grazing, and site accessibility. All sites were on federal land. The Medora block was aerially sprayed with Sevin-4-Oil (carbaryl) on July 1-2, 1992.

Plant Selection:

Three plant species were selected for examination: *Calylophus serrulatus* Nutt. (Onagraceae), *Melilotis officinalis* L. (Fabaceae) and *Campanula rotundifolia* L. (Campanulaceae). This preliminary report will focus only on *C. serrulatus.* Data analyses are not yet complete for the other two species.

Calylophus serrulatus (Onagraceae) is a common prairie perennial. Plants range from 4-20 cm in height and have several small yellow flowers (1-2 cm in diameter) that are open during the day. Each flower has one stigma and eight stamens. Flowers are open for two to three days. Multiple seeds are formed within a slender capsule.

Breeding Experiment:

The breeding study site was located 2 miles outside of the Medora spray block on land managed by the North Dakota State University Experimental Station (permission granted by Jim Nelson, Dickinson). Twenty-one *C. serrulatus* plants were chosen, each with three buds. One bud was used for each of the following treatments: 1) unmanipulated (control), 2) enclosed within a bag of fine mesh, but otherwise unmanipulated to test for automatic self pollination (autogamy), and 3) also covered with a bag to exclude insect visitors, and pollinated by hand with pollen from a flower on another plant no less than 3 m away (to test for outcrossing). To inhibit seed predators, the flowers, buds, and seed heads were sprayed with Malathion every four days and after a rain. The bags were left in place until flowers had completely closed. Seed heads were collected in early autumn and viable seeds were weighed and counted in the laboratory. Seed viability was determined by the thickness of the seed and the presence of any endosperm.

Plant Reproductive Success Experiment:

At each of the five study sites in this experiment, 40 plants of *C. serrulatus*, each with at least three buds, were flagged and censussed repeatedly. One flower on each plant that had finished flowering before spraying was marked with Testor's paint. A second flower that opened during the 7-day period immediately following the spray was also marked. Finally, a flower that opened eight or more days after spraying was marked on each plant. These flowers were then censussed every 2-3 days, and notes taken on their fruit development. All seed heads were collected in early autumn. Viable seed counts and weights were taken in the laboratory. Unfortunately, differing numbers of seed heads and flowers survived at each site, making intended statistical analyses inappropriate. We have therefore limited our analyses in this report to simple Chi-square tests and one-way analyses of variance (ANOVAs).

Results

Breeding Experiment:

The percentage of flowers setting fruit did not differ among breeding treatments ($X^2_{-05,2}$=2.72, p>0.05). Similarly, a one-way analysis of variance of average weight per seed revealed no significant differences among treatments (F_2=.43, p>0.05). However, the number of viable seeds per seed head did differ significantly among treatments (F_2=3.47, p<0.05), with selfed flowers producing significantly fewer viable seeds than did crossed or control flowers. The means and sample sizes for viable seed number and average weight per viable seed are given in Table 1.

Plant Reproductive Success Experiment:

Chi-square analyses were performed to test whether there were significant differences among sites in the percentage of flowers producing viable seeds. Separate analyses were performed for flowers opening in the time periods BEFORE, DURING and AFTER spraying. Flowers opening before and after the spray application differed significantly among sites in their success in setting seed (BEFORE: X^2_4=25.4, $p<0.05$; AFTER: X^2_4=28.0, $p<0.05$). This was not true, however, of flowers opening during the spray period (X^2_4=.683, $p>0.05$). Figure 1 shows that Spray 1 and Periphery 1 had very high values before the spray, and Periphery 2 had the lowest. In the AFTER time period, the percentage of flowers setting viable seed in Periphery 1 exceeded that of the BEFORE time period. This was the only site to do so. In comparing the remaining sites, Spray 1 and Control 2 had similar percentages as did Control 1 and Periphery 2. The DURING percentages remained fairly constant.

Similar results were obtained in one-way ANOVAs for number of viable seeds per seed head and the average weight per viable seed among sites during the three periods (Table 2). Both variables differed significantly among sites for BEFORE and AFTER flowers. Neither variable differed significantly for DURING flowers (Table 2).

The percentage of flowers setting viable seed were generally greatest before spraying at all sites with the exception of Periphery 1 (Figure 1). The Spray 1 and Control 2 sites also exhibited a tendency to have higher percentage of flowers setting viable seed in the AFTER treatment as opposed to the DURING treatment, but not to such a great extent as the Periphery 1 site. A contingency table analysis combining site and time treatments gave significant results for site (X^2_4=33.9, $p<0.05$), time (X^2_2=23.5, $p<0.05$), and their interaction (X^2_4=34.1, $p<0.05$) with regards to number of viable seeds per seed head.

To test whether increased sample sizes would reveal more clear cut trends concerning the impact of spraying, results for flowers from the two control sites were lumped together and contrasted with results for flowers from the spray site and the site directly on the spray border (Periphery 2) (Figure 2). Periphery 1 was excluded from this test because of its distance from the spray boundary. The spray group had a greater percentage of seed set before the spray was applied than did the control group, but a smaller percentage thereafter. The spray group also exhibited a 37% reduction in total viable seed set after spraying versus and 18% reduction in the control group. A Chi-square analysis for treatment and time between these two groups, however, showed only time to be significant (X^2_2=14.87,

$p<0.05$).

Results and Discussion

Breeding Experiment:

The aim of the breeding experiment was to assess whether pollinators were required for successful sexual reproduction of *C. serrulatus*. The results of these tests suggest that this may be the case.

While many member of the Onagraceae outcross, the lack of spatial or temporal separation of anthers and stigma and the short flowering time may facilitate autogamy. The lack of significant difference among treatments in the number of flowers producing seed, implies that plants are partially self-compatible and that autogamy takes place in *C. serrulatus*. However, those flowers allowed to self, produced significantly less seed than those artificially cross pollinated or allowed open access to native pollinators. (Table 1). This decreased reproductive success may be due to self pollination, indicating that increased fitness can be obtained with cross-pollination. Similar numbers of viable seeds were produced by crossed and control flowers (Table 1), suggesting that seed production may not be pollinator limited. Unfortunately, the small sample sizes do not permit definite conclusions at this time.

Plant Reproductive Success Experiment:

Significant differences among sites occurred in one-way ANOVAs BEFORE and AFTER treatments in numbers and weights of viable seeds per seed head. However, these differences did not conform to a priori hypothesis. Sites were predicted not to differ significantly before spraying, reflecting similar pollinator abundance and diversity. After spraying, seed set differences were expected to be significant as a result of pollinator reduction in the spray sites. Unfortunately, our results did not support this. Results show significant differences among sites before and after the spray, but not during. One possibility for this display is that pollinator communities vary within both space and time, creating large differences within a small area (Eckhart, 1992). Because of initial disparity between sites, both in insect and plant populations, comparisons of results might be weakened, showing no particular patterns.

The contingency table analyses for the percentage of flowers that set viable seed indicated significant within and among treatment differences. These percentages were lower in DURING and AFTER time periods that in the

BEFORE (with one exception), but this variation was not particularly linked to spraying as hypothesized. Instead, these differences may simply reflect diminished growth and reproduction that many plants show later in their growing season (Brunet, 1990; Montalvo, 1992). Also, changing environmental conditions over the course of the summer may increase the relative importance of favorable microhabitats. Such microhabitats may have remained particulary favorable at the Periphery 1 site late in the season. Seasonal changes in pollinator abundances and plant communities may also contribute to noticed variability. There are also many factors that could result in no seed being set. These range from plant damage or destruction, to premature seed dehiscence, to lack of fertilization. All of these were present in varying amounts at each site. Pooling data for sites (sprayed vs. control groups) eliminated the significant interaction between sites and time in the percentage of flowers setting seed (Figure 2). Simple ratios showed the spray group had a reduction 19% greater than the control group when comparing BEFORE and AFTER flowering success, indicating a possible negative effect of spraying on seed production of *C. serrulatus*. The larger sample sizes in this analysis may have resulted in decreased variance associated with differences between sites.

Summary

Overall, our data does not support the hypothesis that insecticidal spraying for grasshoppers reduces reproductive success in the native prairie plant *C. serrulatus*. However, these preliminary experiments show some possible, though weak, trends in this direction. Our test of this hypothesis in 1992 was weakened by small sample sizes and logistical problems in selecting suitable study sites. It is hoped that next year these problems can be remedied.

References Cited

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Eckhard, V.M. 1992. Spatio-temporal variation in abundance and variation in foraging behavior of the pollinators of gynodioecious *Phacelia linearis* (Hydrophyllaceae). Oikos 64:573-586.

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Figure 1. Percentage of Calylophus serrulatus flowers that set viable seed.

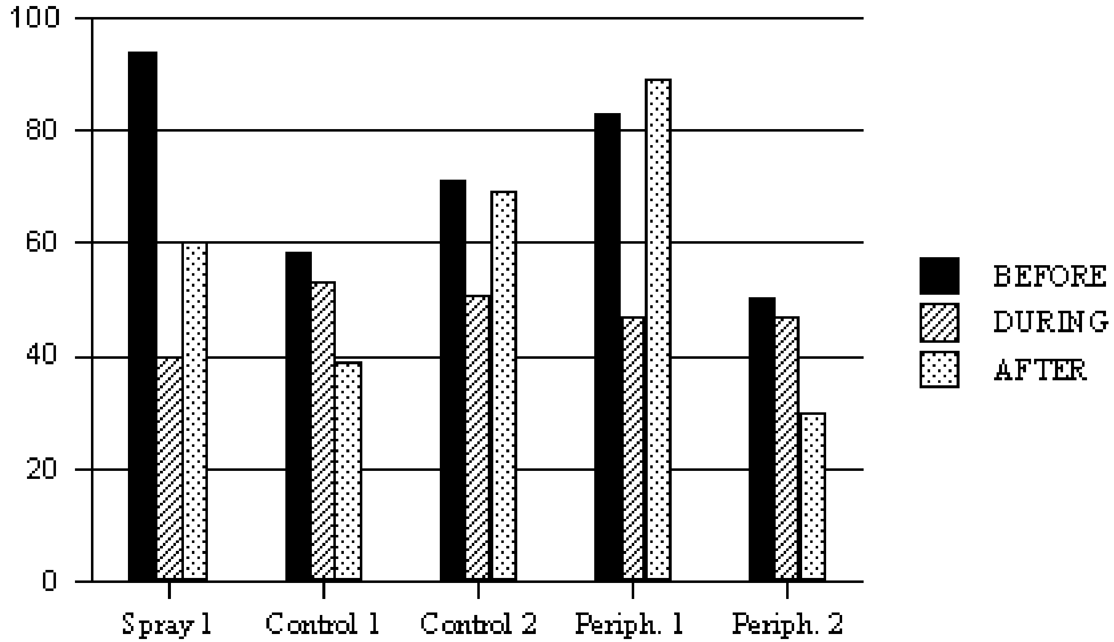

Fig 2. Combined percentage of Calylophus serrulatus flowers that set viable s

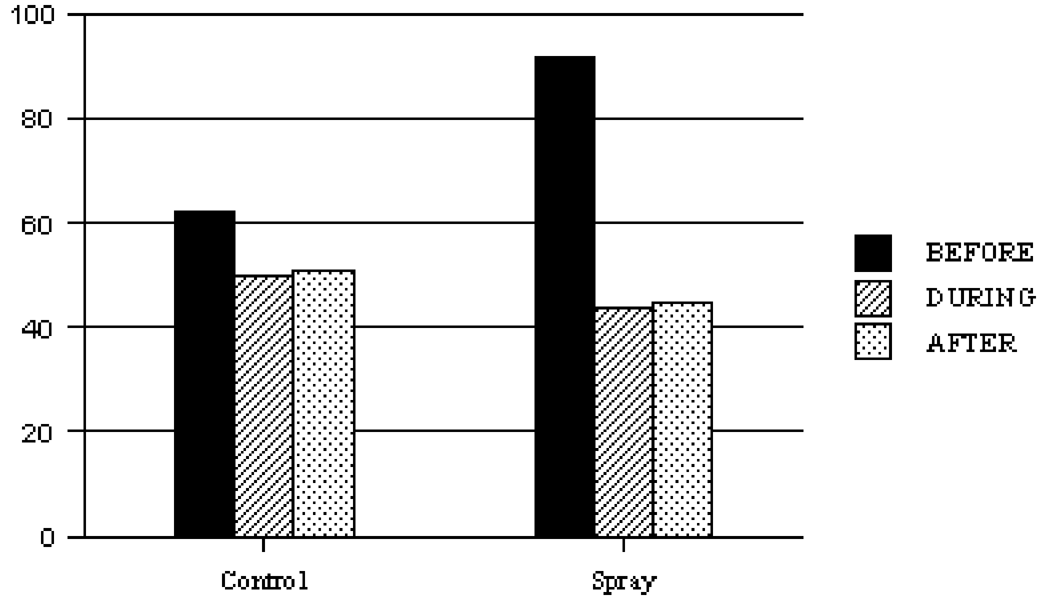

Table 1. Mean number of viable seeds per seed head (± sd) and mean average weight per viable seed (± sd) in the breeding experiment. n=number of fruit collected containing one or more viable seeds.				
	N	**n**	**Viable seed**	**Average weight per viable seed (mg)**
Control	21	12	12.00±8.77	0.19±0.077
Selfed	21	8	5.87±3.44	0.21±0.045
Crossed	21	7	14.40±4.50	0.20±0.027

Table 2. One-way ANOVAs testing the effect of flowering time (in relation to spraying) on number of viable seeds per seed head and the average weight per viable seed across all sites.				
Time		**df**	**F**	**p value**
Before	Seed number	4	2.65	0.0362
Before	Ave. wt./seed	4	14.08	0.0001
During	Seed number	4	1.80	0.1374
During	Ave. wt./seed	4	1.69	0.1602
After	Seed number	4	3.73	0.0079
After	Ave. wt./seed	4	4.44	0.028

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