HERBICIDE EFFECTS ON HOST PLANTS OF KARNER BLUE BUTTERFLY AND ON BUTTERFLY DEVELOPMENT FROM EGG TO ADULT

Edward Sucoff, Thomas Nichols and Ehr-Yang Lu

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ABSTRACT

Two studies examined the compatibility between herbicide release of red or jack pine plantations in Wisconsin and the maintenance of Karner blue butterfly (*Lycaeides melissa samuelis*), a federally listed endangered species. Wisconsin has some of the few remaining viable metapopulations of Karner blue butterfly, hereafter abbreviated as KBB. The habitats favored by KBB are also the sites well suited for the red pine (*Pinus resinosa*)and jack pine (*Pinus banksiana*) plantations that supply the socioeconomically important forest industries. The survival and rapid growth of the pine seedling frequently requires removing vegetative vegetation with herbicides that incidentally also may affect the food sources for KBB.

In order to document the effects of herbicides, we examined how herbicides altered wild lupine (*Lupinus perennis*), the sole food source for KBB larvae, and 14 major nectaring plants, that supply the nectar on which KBB adults depend. Simulated operational levels of Accord® (glyphosate), Accord+Oust® (sulfometuron methyl), and Accord+Garlon4® (triclopyr), all with the adjuvant EntryII®, were sprayed on 350m^2 plots located in seedling red or jack pine stands. Before and for up to three years after spraying, we observed the percent-cover and number of flowering stems of the selected species. A total of 891 measurement-plots were involved.

The results clearly documented that lupine cover and flowering were not inhibited by herbicide treatments and may have been modestly stimulated. In contrast, the percent-cover and flowering of most, but not all, nectaring plants, were reduced the first year after herbicide application. Many species began to regain cover by the second year after spraying. Most, however, had not returned to prespray levels when observations ended two or three years after spraying. The numbers of flowering stems recovered more rapidly and completely than percent-cover. First year responses to herbicides varied among species from total mortality to significant increases. Among herbicides, Accord+Oust had the most negative effects on percent-cover. Late August applications of herbicides had more negative effects on nectaring plants than did early September applications. At this time, the effects of herbicides on KBB food sources cannot be related to changes in KBB populations.

The second study examined the effects on the development of KBB when these same herbicide formulations as well as Garlon4 were sprayed directly on the eggs of Karner blue butterfly. Operational concentrations of Garlon4, Accord or Accord+Oust, all with EntryII, did not affect egg development, but egg hatching was significantly lowered in eggs completely drenched with Accord+Garlon4. However, even for this formulation, the calculated reduction in adults would be less than 3.6% under operational field spraying. None of the herbicides significantly influenced the percent of larvae that formed pupae, or the percentage of pupae that produced adults.

KEY WORDS: Karner blue butterfly, egg, herbicides, larvae, nectaring plants, lupine, red pine, glyphosate, sulfometuron, triclopyr.

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1.0 Introduction

In 1992, the Karner blue butterfly (*Lycaeides melissa samuelis*), hereafter abbreviated as KBB, was listed as a Federally Endangered Species under the Endangered Species Act (US Fish and Wildlife Service 1992). The listing focused attention on the few remaining areas that contained healthy metapopulations of KBB. The pine-oak (*Pinus banksiana-Quercus* sp.) savannah of central Wisconsin is one such area, where sandy soils and open overstory provide the environment required for lupine (*Lupinus perennis*), the leaves of which are the only organic food for KBB larvae. The sites also support the nectaring species (Table 1), plants that provide the nectar on which adult KBB forage.

Historically in central Wisconsin, the open conditions needed by KBB were maintained by fire and large herbivores. Today, the major disturbances creating and/or maintaining open sites on these soils include rights-of way, soil disturbances, controlled or natural fires and forestry practices, particularly harvesting. The same sites that are prime habitat for the Karner blue butterfly are also favored for the growth of red pine and jack pine trees that supply wood to the economically and socially important forest industry of Wisconsin. Therefore, it is important to insure that forest practices are compatible with the conservation of KBB. The relation between KBB conservation and various forest practices, including herbicide use, has been addressed in the *Habitat Conservation Plan (HCP) for the Karner Blue Butterfly in Wisconsin* (Wisconsin DNR 1999), an ongoing document developed by HCP partners among whom are the State of Wisconsin, local Wisconsin government units, the United States Fish and Wildlife Service, commercial utilities, and forest industries.

The research in our report was designed to provide scientific documentation to help guide operational decisions regarding the compatibility of herbicide use and the conservation of KBB. In the first of two studies we examined how herbicides affected the food sources of KBB. The effects on abundance of lupine and numerous nectaring plants are presented in Section 2, and the effects on flowering of these plants are presented in Section 3. The second study examined how direct exposure to herbicides affected the development of KBB eggs into adults (Section 4). In conclusion we explored some operational applications of the findings (Section 5).

2.0 Herbicide Effects on the Abundance of Lupine and Nectaring Plants of Karner Blue Butterfly

The study described in this section determined how simulated operational levels of Accord (glyphosate), Oust (sulfometuron methyl), and Garlon4 (triclopyr), applied singly or in mixtures, affected

* the abundance of wild lupine, the only food for larvae of KBB, and
* the abundance of nectaring plants (scientific names in Table 1), plants that provide nectar to adult KBB.

Common wisdom and field observation suggest that herbicides will not inhibit lupine on the sandy soils of central Wisconsin. In early September, the time of operational spraying, most lupine shoots are dead and unable to transport the herbicides to the underground stems that give rise to next year's shoots. Other species, however, including most of the nectaring plants of KBB may be susceptible to herbicides, and these plant populations may be reduced. To evaluate these posited effects, the study screened wild lupine and other nectaring plants for their sensitivity to several operationally used herbicides. The response of lupine and other nectaring plants to herbicide treatments was measured by changes in shoot abundance (this section) and changes in flowering (Section 3.0).

2.1 Methods

2.1.1 Species - Target Plants and Bonus Plants

Throughout its range, KBB has been observed on more than 100 species of nectaring plants, with some preferred more than others (US Fish and Wildlife Service 1997). Seventy-two of these species/genera were found on plots established during this study (Table 1). Fourteen *target species* (Table 1) were given special consideration because they met one and usually both of two criteria. First, they were available in sufficiently large quantities in at least one young red or jack pine plantation that had no KBB. Absence of the butterfly meant that the plantations could be experimentally sprayed. Second, they were reported to be common providers of nectar to KBB in Wisconsin.

Species of nectaring plants, other than the targeted ones, also occurred on most plots. Their response to herbicides provided bonus information not considered in the original design; therefore, they were called *bonus plants*. A bonus plant is any nectaring plant not of the species targeted on that plot. For example, when a plot targeted for flowering spurge also contained horsemint, the horsemint was a bonus plant on that plot. In addition, all of the 58 nectaring species that were never targeted are called bonus plants. Four of these 58 species (goldenrod,

hoary puccoon, wild rose, and yarrow) were numerous enough in each treatment to permit analyzing them with a design similar to that used for target species.

2.1.2 Herbicide Treatments

In 1995 the three treatments were

* Accord: 4.7 L/ha (64 oz/ha) + Entry II: 1.1 L/ha (15 oz/acre)
* Accord: 4.7 L/ha (64 oz/ha) + Oust: 70 g/ha (1 oz/acre) + Entry II: 1.1 L/ha (15 oz/acre)
* Unsprayed Control

In 1996 a fourth treatment was added

* Accord: 1.74 L/ha (24 oz/acre) + Garlon4 1.74 L/ha (24 oz/acre) + Entry II 0.55 L/ha (7.5 oz/acre)

For all treatments, Entry II was the surfactant and the total spray volume was 173 L/ha (18.5 gal/acre). The volume used is intermediate between that of aerial spraying and ground spraying. Spray was delivered with a CO_2-powered backpack sprayer using a boom for the larger treated areas and using a wand for smaller ones. Sites were sprayed between August 23 and September 1, 1995, or between August 29 and September 9, 1996. All sites were sprayed by the same person. On a site, all treatments for each target species were sprayed within a one to two hour period.

2.1.3 Plot Establishment

Each selected site met six criteria. The site contained a commercial stocking of young pine, was free of overstory trees, had sandy soil, contained an ample population of an important nectaring species, had not previously been treated with herbicides and was free of KBB. The difficult task of locating plantations that met all criteria was accomplished with the cooperation of Burnett County, Wisconsin, the Consolidated Paper Company, and the Georgia Pacific Corporation. One site had natural jack pine regeneration, another was a jack pine plantation, and the rest were red pine plantations. One study area was formerly a fuel brake; the other areas were sites recently occupied by mature pine, usually jack pine or pine-oak. Most plantations had been planted between 1991 and 1993, one having been planted in 1985. The plantations were near Adams, Black River Falls, Danbury, Mauston, New Lisbon, Tomahawk, and Wisconsin Rapids, Wisconsin.

Each species was a separate experiment, and with four exceptions each species was examined at only one location (Table 2). Individual locations had one to five target species. With one exception, plots for each target species were established in only one year, either 1995 or 1996; western sunflower plots were established in both 1995 and 1996.

In 1995, the goal for each target species was to have 9 spray-rectangles on one site, 3 per rectangle (Table 2). Each rectangle had 4 measurement plots making a total of 36 measurement plots per target species. To reach this goal, we searched a site to find nine areas sufficiently rich in the targeted species. When such an area was found, a rectangle (commonly 9 m x 39 m) was established. The dimension of the rectangle allowed use of a boom sprayer, a device that gives a more accurate and consistent sprays than a wand sprayer. The only criterion for locating spray-rectangles was their content of the desired species. As a result, on some sites all nine rectangles lay almost side-by-side in a row, while on other sites they were scattered. The last step in plot establishment was to situate four square-shaped 1-m^2 measurement plots in each rectangle. Measurement plots were situated using only one criterion, high abundance of the target species.

The methods used in 1996 were similar to those used in 1995 with these important exceptions. In 1996, the goal was to have a total of 72 measurement plots distributed as follows. There were to be 12 spray-rectangles (3 rectangles for each of 4 treatments) and 6 measurement plots per spray-rectangle. However, on some sites fewer than 72 measurement plots were involved, either because plots were lost over time or because insufficient numbers of target species occurred on the site. Also, in 1996, some species were so widely scattered, that the only way to get enough plots was to use more numerous and smaller spray-rectangles (3 by 4 meters). These were treated with a wand sprayer and contained only one measurement plot. Considering both 1995 and 1996 plots, soil texture, drainage and topographic position appeared uniform within each site, with one exception. In total, 891 plots persisted throughout the study, 216 of the 1995 plots and 675 of the 1996 plots.

2.1.4 Measurement of Shoot Abundance

We used percent-cover as the index of shoot abundance. Percent-cover was visually estimated using the actual ground intercept of the stems and leaves; the open spaces within the crown perimeter were not included. Percent-cover was estimated during the growing season before spraying (1995 or 1996) and for 2 years (1996 plots) or 3 years (1995 plots) after spraying. For both 1995 and 1996 plots, the final measurement was in 1998. Measurements of each target species were made about the same calendar date each year. When combining the data from 1995 and 1996, we called the data collected before spraying “year 0" data. Post-treatment measurements were labeled “years 1, 2 and 3.” As an example, the observations taken during 1996 were year 0 (prespray) measurements for the 1996 plots but year 1 (first year after treatment) measurements for the 1995 plots.

2.1.5 Data Analysis: Percent-Cover of Target Species

Fourteen experiments, one for each of 14 target species, examined the effects of herbicides on the percent-cover of each species. For most species, treatment effects were analyzed using two statistical designs. One used the measurement plot as the experimental unit, and the other used the spray-rectangle as the experimental unit.

When the measurement plot was the experimental unit, we used a simple one-factor ANOVA to assess statistical differences among treatments for most species. In 1995 experiments, the usual design was 3 treatments x 12 plots (replicates) per treatment. In 1996 the usual design was 4 treatments x 18 plots per treatment. For species occurring on more than one site (Table 2), a two-way ANOVA was employed. For example, lupine was studied on three sites and the design was 3 sites x 4 treatments x 18 plots per treatment. When the P-value of the F-test was less than 0.075, an unprotected LSD test (α= 0.05) was used to compare means.

When the spray-rectangle was the experimental unit, the ANOVA contained three treatments x three spray-rectangles (replicates) per treatment for the 1995 experiments, and four treatments x three spray-rectangles for the 1996 experiments. When the P-value of the F-test was less than 0.075, an unprotected LSD test (α= 0.05) was used to compare means. Butterfly weed, common cinquefoil, raspberry, and rough-fruited cinquefoil were not analyzed using spray-rectangles because they had fewer than three rectangles per treatment, or their rectangles contained only one or two plots.

Four of the bonus species (goldenrod, hoary puccoon, wild rose, and yarrow) occurred on enough plots before treatment to justify a comparison of their treatment differences (Table 2). These were analyzed using a two-way ANOVA with site as the block. As an example, goldenrod had 14 sites, 3 treatments (control, Accord, and Accord+Oust), and 31 to 49 plots per treatment. All statistical analyses were conducted using JMP version 3.16.2 of the SAS Institute Inc. (1989-1996).

2.1.6 Data Analyses: Percent-Cover of Bonus Species

Three data sets were created to examine the overall effect of the herbicides on the percent-cover of the 72 bonus species. The measurement plot was the experimental unit. These data sets included all 216 1995 plots and 675 of the 1996 plots. A two-way ANOVA, with sites as blocks and plots as replicates, was used to analyze differences among treatments for all three data sets. The 1995 data set included 6 sites, 3 treatments, and 12 plots per treatment per site. The 1996 data set included 13 sites, 4 treatments, and 5 to 18 plots per treatment per site. The 1995 +1996 data set included 19 sites, 3 treatments, and 5 to 18 plots per treatment per site. The datum entered for each plot was the sum of the percent-cover of all bonus species on that plot. For example, if a plot had two bonus species, species Y with 10% cover and species Z with 4% cover, the entered datum would be 14%. The reported percent-cover for a treatment was the sum of percent-covers from all plots in a treatment divided by the number of plots in that treatment, including those plots that contained no bonus species.

2.2 Results and Discussion

The study objective was to determine how simulated operational levels of herbicides affected the abundance of naturally grown nectaring plants of KBB, with special emphasis on 14 species including lupine. Herbicide effects were measured by the difference between control and treated plots. Differences between control and herbicide treatments were assessed in four ways, so as to allow a more thorough interpretation of the results than could be obtained from using only the actual percent-cover measurements. All assessments involved tracking the same set of plots for a two- or three-year period.

Assessment 1. The *actual percent-cover* for each of 18 nectaring species (14 target and 4 bonus species) before and up to three years after spraying (Table 3 and Figure 1). This assessment assumed that all plots of a target species had identical cover before treatment.

Assessment 2. *Percent of original cover* or *normalized percent-cover* (Table 4). This method accounted for differences in pre-spray cover by normalizing, for each plot, the cover after treatment by the cover present before treatment. The formula used was:

Normalized cover = 100 x (percent-cover in year X) / (percent-cover in year 0)

Assessment 3. The *herbicide effect index (HEI)*. This index (Table 5) was calculated for each species and each year as:

HEI = (normalized percent-cover of herbicide treatment H) minus
(normalized percent-cover of the control treatment that same year).

The HEI accounts for changes in normalized cover that would have occurred in the treated plots had they not been treated. It assumes the normalized cover in all plots would have been the same had there been no treatments. For example, if in year 1, the normalized cover in the control plots was 80% and the normalized cover in the Accord plot was 35%, then the HEI would equal -45%, calculated from (35%- 80%).

Assessment 4. *Composite of herbicide effects with all bonus species combined.* This method shows the overall effects of herbicides on bonus species without reference to individual species (Table 6).

Unless otherwise indicated, the results are presented and discussed using the measurement plot as the experimental unit. As shown in Tables 3 and 4, the probability for treatment effects using measurement plots were generally similar to those using spray-rectangles. However, the probabilities were almost always lower when analyzed with spray-rectangles.

2.2.1 Response to Herbicides of Percent-Cover in Lupine

The percent-cover of lupine did not differ significantly among treatments during the first two years after spraying (Table 3 and Figure 1). Neither did the normalized percent-cover differ among treatments except in year 1, when lupine cover was significantly higher in the Accord+Garlon4 treatment than in other treatments (Table 4). In the second year, lupine was more abundant (but not significantly) in herbicide-treated plots than in the control (Tables 3-4). Lupine may have benefitted when herbicides suppressed sedges and other competitors for light and soil resources. Regardless of treatment, percent-cover of lupine increased with time (Table 4). In a preliminary experiment on two sites, Accord and Accord+Oust did not significantly affect lupine cover measured one year after spraying (unpublished data of authors).

2.2.2 Response to Herbicides of Percent-Cover in 18 Nectaring Species

The percent-cover of each of 18 nectaring species (14 target including lupine and 4 bonus) was measured in separate experiments before and up to three years after treatment with herbicides. One year after spraying, percent-cover of most nectaring species had been reduced by at least two herbicide formulations (Tables 3-5, Figure 1). Out of 44 combinations of herbicide treatment by species, 29 resulted in significantly lower percent-cover (11 with Accord, 14 with Accord +Oust, and 4 with Accord+Garlon4) (Table 4). Of these 29, 25 had HEI index values more negative than -50% (Table 5). Although most species/herbicide combinations reduced percent-cover, in four cases cover was significantly higher the year after herbicide application: hoary alyssum with Accord, horsemint with Accord, horsemint with Accord+Garlon4, and lupine with Accord+Garlon4.

Modest recovery of nectaring species were noted in year 2. Six fewer herbicide/species combinations were significantly lower than the controls. However, the remaining 23 combinations all had HEI more negative than -50%. The new shoots originated from both seeds and pre-existing underground stems or roots. Further recovery of affected species was observed in the third year on 1995 plots (Tables 4 and 5).

2.2.3 Response of Bonus Species to Herbicides

The presence of bonus species on some plots in all treatments provided the opportunity to approximate the effects of herbicides on the combined array of 72 KBB nectaring species: 58 nontarget nectaring species and the 14 targeted nectaring species when they occurred as bonus species. Although not a random sample of the vegetation, these plots had been selected without regard to the initial cover of the bonus species. Therefore, they provided useful insight into the overall effects of herbicides on nectaring species. Table 6 reveals the combined response of the 72 nectaring species to herbicides. The results from the bonus species were similar to those from target species (Tables 3-5), in part because the targeted species often qualified as bonus species (Section 2.1.1). For the combined bonus species, as with the target species, percent-cover was

most reduced in year 1, was reduced more in 1995 plots than in 1996 plots and was most suppressed by the Accord+Oust treatment. These topics are discussed in later sections.

The combined data, however, mask that the herbicides changed the composition of nectaring species. For example, Accord almost eliminated blackberry while at the same time increasing hoary alyssum and horsemint. Large changes in species composition frequently occur following herbicide application (Sucoff and Snow 1994). The interpretation of Table 6 also needs to be tempered because some of the listed bonus species, while used by KBB elsewhere in North America, have yet to be evaluated as food sources for adult KBB in Wisconsin.

2.2.4 Responses Differed Between Plots Established in 1995 and 1996

The difference in cover between 1995 plots and 1996 plots was not evaluated with formal statistics because neither species nor sites were matched across years. However, anecdotal evidence supports the conclusion that herbicides reduced plant cover more on plots sprayed in 1995 than on plots sprayed in 1996 (Tables 3-5). For example in year 1, Accord+Oust significantly reduced normalized percent-cover of all target species on 1995 plots but reduced only five of nine species on 1996 plots (Table 4). As another example, Accord reduced the percent-cover of bonus plants to 23% of the adjusted control in 1995 plots, but to only 68% if the adjusted control in 1996 plots. A third example is provided by western sunflower, the only species sprayed both years with the same herbicide, Accord+Oust. The Accord+Oust plots sprayed in 1995 had no western sunflower in years 1 and 2, while the 1996 plots had normalized cover of 26% and 66%, respectively (Table 4).

Different species were sprayed in 1995 and 1996, but we do not think that this can account for all or even most of the difference between years. As already mentioned the sunflower and many bonus species were sprayed in both years but were affected more by the 1995 application. We think that a major additional factor is the timing of treatment as related to plant senescence. At the time of spraying, many species, including western sunflower, were less senescent in 1995 than in 1996. Accord, at least, becomes less toxic as senescence progresses. Because herbicide effectiveness varied with year of spraying, and different species were studied in different years, tolerance ranking of species should only be done within year of plot establishment.

2.2.5 Comparing Toxicity of Herbicides

Species were not equally sensitive to all herbicide formulations. For example, hoary alyssum was stimulated by Accord and inhibited by Accord+Oust. For almost all species in year 1, Accord+Oust had the lowest percent-cover among all treatments (Tables 3 and 4). Accord+Oust was also the most toxic treatment when evaluated using bonus species (Table 6). By year 2, percent-cover differed less among herbicide treatments (Tables 3-6).

2.2.6 Differences Between Simulated and Operational Spraying

Our experiment did not create conditions identical to those in operational spraying, a caveat to consider in applying the results to field situations. Possible differences caused by timing of sprays were already mentioned. Also, although herbicide dosage did not differ between the experimental and operational spraying, operational spraying will have smaller droplet size and less complete area-wide coverage. Finally, seed and vegetative propagules would more rapidly recolonize our relatively small rectangular plots than they would multi-hectare areas sprayed in normal operations.

2.3 Summary of Results Related to Percent-Cover

The percent-cover of lupine was either unaffected or modestly stimulated by all three herbicide formulations (Accord, Accord+Oust and Accord+Garlon4). In contrast, one year after spraying one or more of these herbicides markedly reduced the percent-cover of most, but not all, targeted nectaring species. Although some species began to regain cover during the second year after herbicide application, most targeted species had not recovered by the second or third year after spraying. Herbicides varied in their effect on a particular species, but in general Accord+Oust reduced cover more that Accord or Accord+Garlon4. Plants were damaged more by the 1995 spray than by the 1996 spray, probably because the 1996 plants were more senescent at the time of spraying. Nectaring species which were not targeted generally responded to herbicides in the same way as the targeted species.

3.0 Herbicide Effects on Flowering of Nectaring Plants for Karner Blue Butterflies

This section evaluated how herbicides affected the flowering of nectaring plants, including lupine. Data was collected from the same plots used to determine how herbicides affected percent-cover of nectaring plants (see Section 2.0).

3.1 Methods

3.1.1 Species, Herbicide Treatments and Plot Establishment.

The same plots that were used to study percent-cover of lupine and other nectaring species were used to study flowering in those species. Therefore, the previously described methods for Species (Section 2.1.1), Herbicide Treatment (2.1.2) and Plot Establishment (Section 2.1.3) are not repeated here.

3.1.2 Measurement of Flowering

We estimated flowering by counting the number of flower-bearing (flowering) stems of the one target species and any bonus species on each plot. A single stem was defined as a shoot system

that originated from a single stem at the soil surface. A stem emerging from the soil surface was counted as one stem whatever the eventual number of branches or flowers it supported. We did not correlate total number of flowers with number of flowering stems. The term "flower" referred to a reproductive structure from the time the petal color was visible in the bud until all of the petals had browned or dropped.

Depending on the year, flowering stems were counted during the first and/or second flights of KBB. In Wisconsin, KBB has two complete generations each year. The adult flight of the first generation peaks from late May to late June depending on weather; the second-generation flight peaks from late July to early August. In Wisconsin the total flight period of KBB ranges from 87 to 107 days depending on the year (Swengel and Swengel 1996). Some plants flower only during one KBB flight, and some during both (Table 1).

Counting of flower-bearing stems was added to the study in July 1996. This addition enabled us to measure second flight flowers before the 1996 plots were treated and one year after the 1995 plots had been treated. No useable flowering data was collected in 1997. In 1998, first and second flight data were collected on both 1995 and 1996 plots. The following list summarizes when flowering of target species was observed:

1995 plots, first flight — 3 years after treatments (Table 7).
1995 plots, second flight — 1 and 3 years after spraying (Table 8).
1996 plots, first flight — 2 years after spraying (Table 7)
1996 plots. second flight — before and 2 years after spraying (Table 9)

For bonus species, the number of flowering stems was counted during both flights in 1998; i.e., 3 years (1995 plots) or 2 years(1996 plots) after treatment (Table 10).

3.1.3 Data Analysis

We used 14 experiments of similar design to examine separately the effects of herbicides on each of the 14 target species. The design was the same as that used to assess percent-cover and is described in Section 2.1.5. The number of flowering stems in bonus species was analyzed using the same ANOVA procedures employed for percent-cover (Section 2.1.6). As with percent-cover, the designs alternatively used measurement plots or spray-rectangles as the experimental units

In addition, bonus species were evaluated by percent frequency. To calculate frequency (percent stocking), the number of plots that contained at least one flowering stem of any bonus plant was divided by the total number of plots in the sample (216 in 1995 plots, 675 in 1996 plots). Then, this number was multiplied by 100. Frequency also was determined based on the number of plots that contained at least three flowering stems of any bonus plants. The species of bonus plant was not a variable in analysis. We used a Chi-squared test ($\alpha = 0.05$) to decide if frequency was affected by herbicide treatments.

3.2 Results and Discussion

Unless otherwise indicated, results are presented and discussed using the measurement plot as the experimental unit. The probability values for treatment effect were usually similar regardless of whether measurement plots or spray-rectangles were used as the experimental unit (Tables 7-9).

3.2.1 Flowering of Target Species During the First Flight of Karner Blue Butterfly

Flowering information gathered during the flight of the first generation (first flight) of KBB is presented separately by year of plot establishment. Because nectaring species flower at different times of the year, and different species occurred in 1995 plots than in 1996 plots, only 8 of the 14 target species flowered during the first flight and these were divided between the 1995 and 1996 plots. On plots established in 1995, first flight data were available for three species and only during the third year after treatment (Table 7).

In interpreting the flower stem data we attributed differences among treatments to herbicide effects. This was easily done for 1996 plots, since the number of flowering stems before spraying did not differ significantly among treatments (Table 9). However, we did not measure flowering before spraying the 1995 plots and, therefore, only assumed that all treatments within a species had identical flowering before treatment. This assumption is supported by the absence of differences in flowering among treatments in prespray measurements of 1996 plots. As further support, before spraying there were no treatment differences in percent-cover for 12 of the 14 target species (Table 3).

In 1995 and 1996 plots, herbicide effects on flowering varied with species. At one extreme, blackberry sprayed with Accord or Accord+Oust in 1995 still had no flowers in 1998 (Table 7), a consequence of the almost complete absence of blackberry in the first year after treatment (Table 3). In contrast, 3 years after treatment, flowering of orange hawkweed and hoary alyssum did not differ significantly between control and treated plots (Table 7). Variability within treatment was often high; for example, although hoary alyssum had four times more flowering stems in the Accord and Accord+Oust treatment than in the controls, no statistically significant differences occurred among treatments.

During the first flight, flowering stems on 1996 plots were measured only during the second year after treatment (Table 7). At that time, flowering did not differ among treatments for lupine or lyre-leaved rockcress. For raspberry, however, the control plots had significantly more flowering stems than any herbicide treatment. With common cinquefoil, the control and Accord+Garlon4 treatments had significantly more flowering stems than other treatments.

3.2.2 Flowering of Target Species During the Second Flight of Karner Blue Butterfly

During the flight of the second generation of KBB (second flight), nine nectaring species were flowering, five on 1995 plots (Table 8) and five on 1996 plots (Table 9); Western sunflowers flowered on both sites and butterfly weed did not flower.

On 1995 plots, one year after application, herbicide treatments significantly reduced flowering to levels below the controls in four species: flowering spurge, lead plant, orange hawkweed, and western sunflower (Table 8). At most, there were 17% as many flowering stems as in the control plots. Flowering of hoary alyssum, in contrast, was significantly higher in the Accord treatment, although significantly reduced in the Accord+Oust treatment. In 1995 plots, herbicide treated plots gained flowering stems between the first and third years, while control plots, except for hoary alyssum, lost them (Table 8). In the third year after treatment, Accord and control treatments did not differ significantly. However, compared to the control, the Accord treatment had fewer flowering stems of flowering spurge and western sunflower and four times more flowering stems of hoary alyssum. Accord+Oust, however, significantly reduced flowering of western sunflower and flowering spurge. In year 3, although values did not differ significantly, the Accord and Accord+Oust treatments of hoary alyssum had three to four times more flowering stems than did the controls. Neither leadplant nor orange hawkweed had sufficient flowering stems to justify analyzing or interpreting their year 3 data for herbicide effects.

On plots established in 1996, flowering during the second generation flight was measured before (year 0) and 2 years (year 2) after treatment (Table 9). In general, the 1996 applications reduced flowering less than did the 1995 applications. Probable reasons for the difference were discussed in Section 2.2.4. In 1996, before herbicide application, no significant differences existed among treatments in any target species. During the second year after treatment (year 2), the number of flowering stems on the control plots was about the same or lower than those in other treatments, but differences were significant in only two cases: Accord plots of Black-eyed Susan and Accord+Oust plots of horsemint had significantly more flowering stems than the corresponding controls.

3.2.3 Flowering Stems of Bonus Species

Treatments also significantly affected flowering of bonus species (Table 10). In absolute numbers and usually in statistical significance, the Accord treatment had or shared title to the most flowering stems in both 1995 and 1996 plots during both flights. Controls and the Accord + Oust treatments had the fewest flowering stems except that controls were similar to Accord during the first flight in 1995 plots. Accord+Garlon4 plots were intermediate.

During the first flight of year 2 (1996 plots) and year 3 (1995 plots), the control and Accord treatments often had more flowering stems, often significantly, than the Accord+Oust treatment. During the second flight of year 2 (1996 plots) and year 3 (1995 plots), the Accord and

Accord+Garlon4 treatments (only in 1996) always had as many or more flowering stems than the control or Accord+Oust treatments. As with the abundance data for percent-cover (Section 2.2.3), herbicides changed both the composition and the abundance of flowering stems. Herbicides also affected the frequency of flowering stems of bonus species (Table 10). The general patterns for frequency effects were similar but not identical to those for number of flowering stems.

3.2.4 Relationship Between Percent-Cover and Number of Flower-Bearing Stems

Because the flowering data were less complete than cover data, we explored the possibility of using herbicide effects on percent-cover to predict herbicide effects on flowering. In the first approach, we ran separate linear regressions for each species and time of measurement using the experimental plot as the experimental unit. Each of these regressions pooled, regardless of treatment, the relevant measurement plots where we had both percent-cover and flowering stems. Twenty-nine data sets contained enough flowering stems to regress against percent-cover. The regression coefficients were all positive and they were significant in 15 cases, but only five data sets produced R^2 values above 0.50. Given these outcomes, we chose not to predict flowering stems from regressions based on percent-cover.

In another approach to estimate flowering from percent-cover, we compared for each species how treatments ranked in percent-cover with how they ranked in flowering stems. For 1995 plots, rankings were compared for year 1 (second KBB flight) and year 3 (both KBB flights). For 1996 plots, comparisons were for year 0 (second flight) and year 2 (both flights). Comparisons were not made when the numbers of flowering stems were low in all treatments.

For 1995 plots one year after spraying, the statistical rankings for normalized percent-cover (Table 4) were either identical or very similar to the statistical rankings for number of flowering stems (Table 8). For example, for both percent-cover and flowering stems, the LSD ranking of means for hoary alyssum was “b” for controls, ”a” for Accord, and”c” for Accord+Oust (Tables 4 and 8). In year 3, treatment rankings in percent-cover and number of flowering stems matched only for blackberry while still being similar for flowering spurge and sunflower.

In 1996 plots, before treatment, neither flowering stems (Table 9) or percent-cover (Table 3) differed among treatments except in western sunflower, where Accord+Garlon4 plots had significantly more cover. Before treatment, the Accord+Garlon4 treatment had 85% more flowering stems than the other treatments, but the P_{plot} was 0.30. In 1996 plots, 2 years after treatment, statistical rankings between cover and flowering were identical for horsemint and raspberry, and very similar for common cinquefoil and black-eyed Susan.

Briefly summarized, rankings of percent-cover and number of flowering stems usually matched before treatment (1996 plots) and 1 year after treatment (1995 plots). Cover and flowering of

some species also matched 2 years (1996 species) or 3 years (1995 species) after treatment. From this matching we inferred that the rankings in percent-cover in year 1 on 1996 plots (Table 4) could be used to rank how herbicides affected flowering on those same plots in year 1. Using this approach we inferred that the flowering stems of black-eyed Susan, common cinquefoil, lyre-leaved rock cress, raspberry, and western sunflower were reduced by at least one herbicide in year 1 (Table 11). We also inferred that flowering stems of horsemint and lupine were increased significantly by one herbicide in year 1. Table 11 summarizes the inferred as well as the statistically observed effects of herbicides on the flowering of nectaring plants.

3.3 Summary of Results

In the year following treatment, the occurrence of flowering stems was suppressed in most target species by Accord, Accord+Oust and Accord+Garlon4. By the second (1996 plots) or third (1995 plots) year after treatment, the negative effects of herbicides on flowering had lessened, and treated plots often had more flowering stems than controls. Recovery was slower when the plants showed more inhibition during the first year after spraying. Two or three years after treatment, the bonus plants had at least as many flowering stems in the Accord and Accord+Garlon4 treatments as in the controls.

Herbicides varied in toxicity. Based on measured and estimated values, Accord+Oust reduced flowering stems more than did other herbicides. Furthermore, species varied in their sensitivity to herbicides. Flowering stems in some species (e.g., lupine) were never significantly reduced by herbicides, some (e.g., horsemint) were increased, and most were significantly reduced by at least one herbicide treatment. Herbicide application reduced the number of flowering stems more in 1995 plots than in 1996 plots, probably because the plants were less senescent during the 1995 treatments. The statistical rankings of percent-cover very closely matched the statistical rankings of flowering stems for the first year after treatment in the 1995 plots. The rankings did not match as well during the second and third years after treatment.

4.0 Effect of Exposing Eggs to Herbicides on the Development of Karner Blue Butterfly

The previous two sections (2.0 and 3.0) examined the effects of herbicides on food sources of KBB. This section examined if herbicides directly sprayed on eggs interfered with insect development. In late August and early September, the time of herbicide spraying, KBB exists almost exclusively in the egg stage. The objective was to determine if the development of KBB from egg into adult is altered by spraying eggs with three herbicides used singly or in combinations: glyphosate (Accord), sulfmeturon methyl(Oust), and triclopyr (Garlon4). Insect development was measured with six criteria: percent of eggs that hatch, percent of hatched eggs

that pupate, percent of pupae that emerge as adults, percent of eggs that produced adults, rate of development, and size of pupae.

4.1 Methods

Experiments with similar objectives and methods were started in 1995 and 1996. All taking, handling and returning of butterflies were authorized by and were in accord with United States Fish and Wildlife Service Subpermit 95-42 A1.

4.1.1 Egg Collection and Pretreatment Storage

The experiments were conducted near Stevens Point, Wisconsin. Eggs were collected, eggs incubated, and larvae hatched using methods described by Lane and Welch (1994). In 1995, 30 female KBB were collected in mid-August and kept in oviposition cages with honey water and natural nectaring stems. Natural egg-laying surfaces of grass and lupine were provided. Following oviposition, the butterflies were released, and the eggs were transferred to small, perforated plastic cups, two eggs per cup. The cups were exposed to natural photoperiods and natural lighting from a north-facing window. Relative humidity in the cup was always above ambient and commonly above 90%. Methods used in 1996 were similar to those used in 1995 with these exceptions: eggs were collected from 49 females; during oviposition, natural photoperiods were provided by sodium vapor lamps and fluorescent lamps; and only one egg was placed in each cup.

4.1.2 Herbicide Treatments

The concentrations of herbicides in the 1995 and 1996 treatments are given in Table 12. In 1995, the concentrations were about one-half of those used in field applications because the volume sprayed (133 L/ha or 18.5 gallons/ac) was about twice the volume sprayed operationally. The higher volume insured better coverage, but even at 18.5 gallons per acre, the spray droplets covered only about 2/3 of the water-sensitive paper (Ciba-Geigy). In 1996, both the concentration and volume of spray were increased in order to obtain a worse case scenario. Concentrations were doubled to reach the level used in field mixtures, and the volume was adjusted to guarantee drenching of the eggs. Drenching was verified by microscopic examination of eggs and by 100% coverage of the water-indicating paper. In both years, eggs were sprayed at the same time with a CO_2 backpack sprayer calibrated to deliver known volumes of fine droplet size. Eggs were sprayed on August 23, 1995 and September 15, 1996.

4.1.3 Postspray Handling and Overwintering Techniques

After the herbicide had dried on the eggs, we transferred them back to the small plastic cups and kept them in the previously described conditions. In late October 1995 the eggs were placed out-of-doors using three overwintering methods, one for each replicate. The first method, that of Van Luven (1994, 1994a), resulted in few larvae and the data from this replicate were discarded. The second method was developed by Lane and Welch (1994). First, we transferred 20 eggs of each

treatment into 10 petri dishes, two eggs per dish. The 10 dishes in each treatment were then put in a shoe box and the four boxes, one with each treatment, were placed in an insulated shelter out-of-doors. The shelter was a wooden box, perched just above the ground. It was covered with a 1.6 m x 1.6 m x 5 cm thick blanket of straw sandwiched between an inner and outer layer of microfoam. The ends of the blanket rested on the ground with the middle portion perched upon the boxes. The third overwintering method was like the second except that bubble wrap was used instead of straw.

Eggs were handled in 1996 the same as in 1995 with these three changes. Just before placing the eggs outside in 1996, the eggs were rinsed with water to simulate the action of rain in removing herbicide. Also, in 1996, only one egg was placed in each petri dish and all three replicates overwintered beneath blankets of microfoam and straw.

4.1.4 Measurements

Beginning in early April 1996 and 1997, we observed each egg every two days and recorded dates of hatching, pupation, and adult emergence. Upon hatching, an egg was moved indoors. While in their fourth instar, the larvae were moved to 225-cm^3 waxed cartons covered by fine-meshed netting. From the time of hatching until the time of pupation, larvae were fed fresh lupine leaves and water. Adults were released upon emergence. For a random subsample of eight pupae per treatment, pupal weight and length of the wing case were measured as indices of larval size.

4.1.5 Data Analysis

In the 1995 experiment, we used logistic regression to analyze hatching of eggs, with each egg classed as "hatched" or "not hatched." The analysis involved four herbicide treatments and two overwintering techniques, with 20 observations (eggs) per herbicide per overwintering technique. In addition, percent hatching was compared among the four herbicide treatments using the Chi-square test. In both tests, statistical significance between herbicides was accepted at α=0.05. We did not analyze other response variables for the reasons described in Section 4.2.

The 1996 experiment involved four herbicide treatments, each treatment being replicated three times. Each replicate contained 23 eggs, and the percent occurrence of an event or the average value of a measurement was based on these 23 eggs. All data, including pupal size, were analyzed using analysis of variance, with replicates as experimental units. When the P-value of an F-test was less than 0.075, the Least Significant Difference (LSD, α=0.05) test was used to rank the means among treatments.

4.2 Results and Discussion

In 1995, herbicides did not significantly affect the amount or rate of egg hatching (Table 13). Eighty percent of the KBB eggs in the control hatched, while hatching in herbicide treatments ranged from 88% to 75%. The effects of herbicides on development of larvae into pupae could

not be evaluated because most of the larvae, including those in the control, died before pupation. The suspected cause of mortality was the commercially grown lupine fed to the early instars. Postmortem inquiry disclosed that the greenhouse had treated the lupine with a systemic insecticide one week before it was fed to the larvae.

In 1996, we were able to evaluate the development of KBB from hatching through adult emergence (Tables 14 and 15). The percentages of eggs that became adults did not differ significantly and were similar among the control, Accord, and Accord+Oust treatments. Significantly fewer adults, however, appeared in the Accord+Garlon4 treatment, 52% of eggs compared to 67% for the control. The difference was largely accounted for by reduced hatching, 68% in Accord+Garlon4 treatment compared to 82% in the controls. All of the treatments had very similar percentages of pupal formation (80% to 89%) and adult emergence (97% to 100%). Rates of development did not differ significantly among treatments (Table 15). As regards pupal size, wing case length did not vary significantly with treatment. Pupal weight was highest, although not significantly, in the Accord+Garlon4 treatment (Table 15), possibly because this treatment had the highest proportion of female pupae.

Data from both 1995 and 1996 experiments indicated that neither Accord nor Accord+Oust significantly reduced the hatching of KBB eggs (Tables 13 and 14). The 1996 data also showed that neither Accord nor Accord+Oust affected pupation of larvae, emergence of adults, the size of pupae or the rate of pupal formation (Table 14 and 15). Since EntryII was the surfactant used in these treatments, we concluded that EntryII did not inhibit the development of KBB from eggs into adults. Other surfactants could be more or less effective in transporting herbicides into the egg, or in inducing direct surfactant toxicity.

The results for treatments involving Garlon4 are less easily interpreted. In 1996, when the data were normalized to 100%, eggs treated with Accord+Garlon4 produced 22% fewer adults than did the water-treated controls (P= 0.024) (Table 14). Most of this difference was the result of reduced hatching in the Accord+Garlon4 treatment (P=0.097). The inhibition cannot be attributed to the Accord component because the Accord treatment did not affect hatching at twice the concentration present in Accord+Garlon4. Similarly, Garlon4, alone, did not reduce egg hatching in the 1995 experiment (Table 12) at a concentration 25% higher than that occurring in the Accord+Garlon4 mixture (Table 12). However, Garlon4 toxicity cannot be ruled out, because the chance of all eggs receiving some spray was higher in 1996 than in 1995. As described in Section 4.1.2, the 1996 eggs were drenched in herbicide while some eggs in the lower volume spray of 1995 might not have received a droplet. Perhaps Accord and Garlon4 may have interacted synergistically. Viewing the limited evidence, we remain uncertain about the toxicity of Accord+Garlon4.

We are confident, however, that damage caused by any herbicide in the 1996 experimental conditions is greater than that which would occur in the field using operational volumes of spray. This is because *Effect = Toxicity x Exposure*, and the chances of an egg being exposed to herbicide are small in operational aerial spraying. For example, at the high end of coverage in aerial spraying, there would be about 20 droplets, each 1 mm in diameter, per cm^2 of surface (Michael Newton, Oregon State University, personal communication). Assuming no overlap, these droplets would wet only 16% of the area with herbicide. Because the KBB egg is only 0.7 mm in diameter, we assumed that only 16 percent of the eggs would receive any spray. In the Accord+Garlon4 treatment, the reduction in hatching was 22%, meaning that in field conditions the reduction would be at most 3.5% (0.16 x 22%). This figure is higher than what might occur in the field since the calculation does not consider that a sizeable proportion of eggs would be shielded from herbicides by leaves, stems, and litter.

4.3 Summary of Results

Operational concentrations of Accord with EntryII or Accord+Oust with EntryII did not adversely affect the development of KBB eggs into adults. This was true even when the eggs were drenched with spray. However, eggs completely covered with operational levels of Accord+Garlon4 and EntryII produced significantly fewer adults than did eggs sprayed with water or other herbicides. The results involving Accord+Garlon4 are inconclusive because drenching with higher concentrations of Accord and less than complete coverage with higher concentrations of Garlon4 had no significant effect on egg hatching. No herbicide formulation significantly affected pupation or adult emergence.

The results from these studies cannot be directly transferred to the field because the chance of an egg receiving herbicide in the field is much less than in our experiments. In our 1996 experiment, all eggs were completely covered with herbicide. In contrast, by one conservative calculation, at most only 16% of naturally laid eggs would be exposed to herbicides during operational spraying. This means that the Accord+Garlon4 treatment would reduce adult production by no more than 3.5%.

5.0 Management Considerations

This research was part of a larger effort to examine the compatibility between using herbicides to release red pine plantations and the maintenance of KBB. Our experiments focused mainly on how simulated operational spraying of herbicides altered the food sources of KBB. In this section we examine some possible management implications of the results in light of the literature and field experience.

5.1 Herbicides and Lupine

Lupine is the one plant species that is absolutely required for the survival of KBB and, within limits, the size of the KBB population increases with the density of lupine (Savignano 1994; Herms 1996; Lane 1999). Our research clearly documented that simulated operational applications of Accord, Accord+Oust, and Accord+Garlon4 applied in late August and early September had no direct negative effects on the vegetative growth and flowering of lupine in subsequent years. These results suggest that lupine sensitivity to herbicides does not need be considered in deciding whether to use these herbicides on most sites.

In fact, there was indication that herbicides may have stimulated lupine, possibly by removing the competitors of pine. However, the removal of competitors may occasionally have undesired indirect effects on the relation between lupine and KBB when the plants that shade lupine are damaged. Lupine can senesce very early when all shade is removed and the weather is droughty (Maxwell and Givnish 1996, Lane 1999), raising the hypothetical possibility that lupine would be unavailable for the second brood in dry years. Lane (1999), however, found that lupine senescence was not a major cause of mortality. As regards quality, Lupine growing beneath partially to completely closed tree canopies produces the highest quality food to KBB larvae (Grundel et al. 1998; Lane 1999). This has led to untested speculation that herbicide-induced reduction of shade might lower food quality of lupine.

5.2 Herbicides and Nectaring Plants

This paragraph briefly restates the results and the remainder of the section examines their potential management significance. In our experiments, the three herbicide formulations reduced the vegetative cover of most, but not all, nectaring plants during the first growing season after application. Except in the Accord+ Oust treatments, no species was completely eliminated. Although recovery began during the second year after application, for a number of species, percent-cover remained lower in the treated plots after the second (1996 plots) or third year (1995 plots) after herbicide application. Herbicides also reduced the number of flowering stems the first year after spraying, but recovery was more rapid. By the second or third year after application, many treatment/species combinations did not significantly differ from the controls.

Because nectaring plants are important to adult KBB, one could hypothesize that herbicide reduction of nectaring plants would limit the size of KBB populations, particularly the first year after spraying. Unfortunately, our experiment could not assess this hypothesis because the treatments were restricted to locations that had no pretreatment KBB, and the spray-rectangles were surrounded by untreated vegetation. Lacking direct evidence for effects on KBB, we searched the literature for evidence linking the quantity of nectaring species to KBB population size. Maxwell (1988) stated that the abundance of KBB was strongly correlated to the frequency of nectaring plants but provided no data. Maxwell (personal communication) is preparing a manuscript on this relationship. Decline of KBB in New Hampshire was attributed in part to lack

of nectaring species (Helmboldt and Amaral 1994). The literature review of the biology of KBB (USFWS 1997) cites four additional studies that suggest or correlate KBB populations with the amount of nectaring plants. The review also cites two studies that found no correlations. In short the literature does not provide quantitative response curves that would allow us to predict how the herbicide effects would quantitatively alter the size of KBB populations. One such response curve may soon be available from a current Wisconsin study using operational spraying of Accord+Garlon4 in pine plantations that contain KBB populations (Kit Hart, personal communication).

Despite their limitations, our results reinforce and refine previously suggested ways to make KBB conservation more compatible with forest management for wood production. Examples are given here, and Lane(1999) suggested additional possibilities.

Timing of sprays. At present, when used to release red pine plantations, Accord must be sprayed late enough to avoid damaging the red pine shoots, and early enough to kill the competing vegetation. This study suggests that KBB will benefit if Accord herbicides are applied toward the end of that window of opportunity. Of course, factors such as equipment availability make it difficult to schedule application at the optimum time.

Selection of Herbicide formulation. Accord+Oust reduced vegetative growth and flowering of nectaring plants more than did Accord or Accord+Garlon4. This finding suggests that Accord+Oust should not be used on sites where Accord or Accord+Garlon4 can give equally effective release of red pine. Where grass and sedge are the predominant competitors, both pines and nectaring species could benefit from using a herbicide toxic to grasses and sedges, assuming a herbicide label could be obtained. As regards toxicity to eggs, the equivocal result that Accord+Garlon4 may reduce egg hatching up to 3.5% in operational spraying should be verified if this possible effect becomes a practical consideration.

Different herbicide treatments for different sites. Sites exist where both nectaring species and pines need release from the same competitor. For one example, when sedge or grass is the major competitor, removal of the monocots with a selective herbicide would benefit both pine and nectaring species. This may require a new label or a special label extension for a grass killer. A second example is similar to the first but would involve the removal of dense shrubs.

Intentionally incomplete coverage of sprayed area. Vegetative competition is not uniform throughout some pine plantations. If a plantation contains large inclusions that have only light competition to pine growth, consider the costs and benefits of intentionally avoid that area when spraying. Conversely, if an entire area seems to depend on blackberry and raspberry as the major nectar source for first generation KBB results, perhaps these inclusions should not be sprayed if that would permit release of the rest of the area. Woody *Rubus* sp. is

slow to recover from herbicides. Other scenarios for minimizing the impacts of herbicide use on KBB populations were discussed by Lane (1997).

5.3 Other Potential Effects of Herbicides

This study only examined food sources. Herbicides, however, could also positively or negatively affect roosting sites, ant populations, predators and other factors that influence viability of KBB populations.

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Table 1. Nectaring species of Karner blue butterfly occurring on experimental plots

Common Name	Scientific Name	Time of Flowering[1]	Location[2]
Target species			
Black-eyed Susan	*Rudbeckia serotina*	Second	WI
Blackberry	*Rubus allegheniensis*	First	WI
Butterfly weed	*Asclepias tuberosa*	Second	WI
Common cinquefoil	*Potentilla simplex*	Both	WI
Flowering spurge	*Euphorbia corollata*	Second	WI
Hoary alyssum	*Berteroa incana*	Both	WI
Horsemint	*Monarda punctata*	Second	WI
Lead plant	*Amorpha canescens*	Second	WI
Lupine	*Lupinus perennis*	Both	WI
Lyre-leaved rock cress	*Arabis lyrata*	Both	WI
Orange hawkweed	*Hieracium aurantiacum*	Both	WI
Raspberry	*Rubus strigosus*	First	WI
Rough-fruited cinquefoil	*Potentilla recta*	Both	WI
Western sunflower	*Helianthus occidentalis*	Second	WI
Other species			
Aster sp.	*Aster sp.*	Second	WI
Bastard toad flax	*Commandra umbellata*	First	MI
Bed straw	*Gallium sp.*	Second	WI
Bindweed	*Polygonum sp*	Second	WI
Bird foot violet	*Viola pedata*	First	WI
Blueberry	*Vaccinium angustifolium*	First	NY
Butter and eggs	*Linaria vulgaris*	Second	WI
Cinquefoil	*Potentilla tridentata*	First	MI
Common fleabane	*Erigeron philadelphicus*	Second	WI
Cylindrical blazing star	*Liatris cylindracea*	Second	WI
Cynthia	*Krigia biflora*	Both	WI
Daisy fleabane	*Erigeron annuus*	Second	WI
Evening primrose	*Oenothera biennis*	Second	WI
Field hawkweed	*Hieracium pratense*	Both	WI
Goldenrod	*Solidago sp.*	Second	WI
Harebell	*Campanula rotundifolia*	Second	WI
Hawkweed	*Hieracium sp.*	Both	WI

Table 1 Continued

Common Name	Scientific Name	Time of Flowering	Location
Hoary puccoon	*Lithospermum canescens*	Both	WI
Horseweed	*Erigeron canadensis*	Second	WI
Juneberry	*Amelanchier sp.*	First	ONT
Kalm's lobelia	*Lobelia kalmii*	Second	WI
Lance leaf loosestrife	*Lysimachia hybrida*	Second	WI
Large-leaved aster	*Aster macrophyllus*	Second	WI
long-leaved houstonia	*Houstonia longifolia*	Both	WI
Loosestrife	*Lysimachia quadrifolia*	Second	WI
Milkweed	*Asclepias syriaca*	Second	WI
Mint	*Monarda sp.*	Second	
Northern bed straw	*Gallium boreale*	Second	WI
Panicle hawkweed	*Hieracium paniculatum*	Both	WI
Phlox	*Phlox pilosa*	Both	IN
Polygala	*Polygala sp.*	Second	WI
Red clover	*Trifolium pratense*	Second	WI
Rough blazing star	*Liatris aspera*	Second	WI
Round-headed bushclover	*Lespedeza capitata*	Second	WI
Rubus	*Rubus sp.*	First	WI
Sheep sorrel	*Rumex acetosella*	First	WI
Show aster	*Aster spectabilis*	Second	WI
Spiderwort	*Tradescantia virginiana*	Second	MI
Spotted knapweed	*Centaurea maculosa*	Both	WI
Spreading blackberry	*Rubus sp.*	First	WI
Spreading dogbane	*Apocynum androsaemifolium*	Second	NH
St. John’s wort	*Hypericum perforatum*	Second	MI
Star flower Solomon seal	*Smilacina stellata*	Both	WI
Steeple bush	*Spiraea tomentosa*	Second	WI
Tall cinquefoil	*Potentilla arguta*	First	MI
Thimbleweed	*Anemone virginiana*	First	WI
Tickseed	*Coreopsis sp.*	Second	WI
Upland aster	*Aster ptarmicoides*	Second	WI
Violet	*Viola sp.*	First	
Strawberry	*Fragaria vesca*	First	WI
Sweet everlasting	*Gnaphalium obtusifolium*	Second	WI

Table 1. Continued

Common Name	Scientific Name	Time of Flowering	Location
Wild bergamot	*Monarda fistulosa*	Second	IN
Wild geranium	*Geranium sp.*	First	ONT
Wild rose	*Rosa sp.*	Second	WI
Willow	*Salix sp.*	First	
Wood betony	*Pedicularis canadensis*	First	WI
Woodland sunflower	*Helianthus divericatus*	Second	WI
Yarrow	*Achillia milliflorum*	Both	WI

1 Time of flowering is during first, second, or both flights of Karner blue butterflies. In Wisconsin from 1989 through 1995, first flight activity peaked between late May and late June; second flight activity peaked between early July and early August (Swengel and Swengel 1996)

2 Location where feeding was observed. IN= Indiana, MI= Michigan, NH= New Hampshire, NY= New York, ONT= Ontario, WI= Wisconsin.

Table 2. Numbers of sites (locations), treatments, and plots established for each target species and common bonus species

Species	Sites	Treatments	Plots /treatment	Total plots	Year established
Target species					
Blackberry	1	3	12	36	95
Black-eyed Susan	2	4	6 and 18[1]	96	96
Butterfly weed	1	3	5 to 11[2]	30	96
Common cinquefoil	1	4	6	24	96
Flowering spurge	1	3	12	36	95
Hoary alyssum	1	3	12	36	95
Horsemint	2	4	6 and 18[1]	95	96
Leadplant	1	3	12	36	95
Lupine	3	4	54	216	96
Lyre-leaved rock cress	1	4	18	72	96
Orange hawkweed	1	3	12	36	95
Raspberry	1	4	8 to 12[2]	44	96
Rough-fruited cinquefoil	1	4	12	48	96
Western sunflower (1995)	1	3	12	36	95
Western sunflower (1996)	1	3	14 to 18[2]	50	96
Bonus species					
Goldenrod	14	3	31 to 49[2]	123	95 & 96
Hoary puccoon	13	3	16 to 29[2]	68	95 & 96
Wild rose	10	3	32 to 41[2]	106	95 & 96
Yarrow	11	3	50 to 58[2]	164	95 & 96

1 six on one site and eighteen on the other

2 minimum and maximum number of plots depending on treatment

Table 3. Percent-cover before and up to 3 years after treatment of 14 target species and 4 bonus species

Species and Treatment	Before spraying	Year after herbicide spray		
		Year 1	Year 2	Year 3
	Species sprayed in 1995			
Blackberry				
Control	19.2	20.0a[1]	30.4a	23.8a
Accord	16.7	0.3b	2.5b	4.5b
Accord+Oust	17.5	0.0b	0.7b	1.8b
P plot[2]	0.5865	<0.0001	<0.0001	<0.0001
P rect[3]	0.6904	<0.0001	<0.0001	0.0002
Flowering spurge				
Control	6.8	6.7a	6.8a	2.3a
Accord	6.2	1.6b	2.5b	2.5a
Accord+Oust	7.1	2.6b	2.0b	0.9b
P plot	0.5564	<0.0001	0.0003	0.0063
P rect	0.5949	0.0091	0.0037	0.0227
Hoary alyssum				
Control	3.4	2.0b	0.5b	0.4
Accord	3.7	4.7a	6.6a	0.6
Accord+Oust	3.3	0.1c	0.7b	0.5
P plot	0.6868	<0.0001	<0.0001	0.1291
P rect	0.7251	0.0195	<0.0001	0.1517
Leadplant				
Control	13.1	10.9a	10.3a	12.2a
Accord	13.5	0.3b	2.5b	4.3b
Accord+Oust	12.8	0.5b	3.1b	6.8ab
P plot	0.9337	<0.0001	0.0013	0.0703
P rect	0.9506	0.0076	0.0266	0.1296
Orange hawkweed				
Control	7.5	8.9a	11.3a	8.4a
Accord	5.3	3.3b	4.0b	2.7b
Accord+Oust	5.4	2.6b	6.3b	7.0ab
P plot	0.0951	0.0021	0.0002	0.0426
P rect	0.2668	0.0009	0.0224	0.3279

Table 3. Continued

Species and Treatment	Before spraying	Year after herbicide spray		
		Year 1	Year 2	Year 3
Western sunflower				
Control	8.9	10.9a	10.3a	10.5a
Accord	7.6	0.8b	2.2b	2.3b
Accord+Oust	7.0	0.0b	0.0b	0.4b
P plot	0.1241	<0.0001	<0.0001	<0.0001
P rect	0.2166	0.0018	0.0030	0.0028
Species sprayed in 1996				
Black-eyed Susan				
Control	2.3	1.4a	0.8b	
Accord	2.7	0.8ab	2.4a	
Accord+Oust	2.9	0.3b	2.2a	
Accord+Garlon4	2.3	0.4b	0.8b	
P plot	0.1347	0.0091	0.0063	
P rect	0.3563	0.0778	0.0233	
Butterfly weed				
Control	4.0	1.5	3.1	
Accord	4.6	0.6	1.0	
Accord+Oust	4.4	0.4	0.9	
Accord+Garlon4	3.9	0.7	2.0	
P plot	0.8927	0.6287	0.2045	
P rect	---	---	---	
Common cinquefoil				
Control	4.7	7.2a	7.3b	
Accord	3.7	0.2b	0.2c	
Accord+Oust	6.2	0.0b	0.4c	
Accord+Garlon4	4.7	9.2a	15.3a	
P plot	0.1515	<0.0001	<0.0001	
P rect	---	---	---	
Horsemint				
Control	6.8	1.8b	3.6b	
Accord	6.4	6.0a	4.6b	
Accord+Oust	6.2	0.0b	9.7a	
Accord+Garlon4	7.8	6.3a	4.0b	
P plot	0.4771	<0.0001	<0.0001	
P rect	0.6580	0.0003	0.0140	

Table 3. Continued

Species and Treatment	Before spraying	Year after herbicide spray		
		Year 1	Year 2	Year 3
Lupine				
Control	11.0	15.4	14.5	
Accord	10.1	14.1	18.3	
Accord+Oust	10.5	13.5	16.3	
Accord+Garlon4	9.8	16.5	18.2	
P plot	0.6814	0.2623	0.2679	
P rect	0.8099	0.2950	0.4105	
Lyre-leaved rock cress				
Control	1.2	0.7a	0.6ab	
Accord	1.0	0.6a	0.6a	
Accord+Oust	1.1	0.1c	0.7a	
Accord+Garlon4	1.1	0.4b	0.5b	
P plot	0.3144	<0.0001	0.0338	
P rect	0.3300	0.0041	0.6131	
Raspberry				
Control	20.4	22.5a	27.3a	
Accord	25.0	0.7b	0.8b	
Accord+Oust	28.5	0.2b	0.8b	
Accord+Garlon4	23.0	1.3b	3.4b	
P plot	0.2085	<0.0001	<0.0001	
P rect	0.7857	0.0006	0.0785	
Rough-fruited cinquefoil				
Control	2.8	1.8a	1.6ab	
Accord	2.0	0.9ab	0.8b	
Accord+Oust	3.0	0.1b	0.6b	
Accord+Garlon4	3.0	1.3ab	2.4a	
P plot	0.2613	0.0715	0.0706	
P rect	0.6979	0.4221	0.2860	
Western sunflower				
Control	5.2b	9.5a	13.5a	
Accord+Oust	5.5b	1.8b	4.2b	
Accord+Garlon4	7.5a	6.4a	9.9a	
P plot	0.0493	<0.0001	0.0030	
P rect	0.3501	0.0124	0.0082	

Table 3. Continued

Species and Treatment	Before spraying	Year after herbicide spray		
		Year 1	Year 2	Year 3
		Bonus species		
Hoary puccoon				
Control	3.9	4.6a	2.2a	
Accord	2.9	0.4b	0.8b	
Accord+Oust	2.6	0.0b	0.1b	
P plot	0.4312	<0.0001	0.0009	
Goldenrod				
Control	3.4	3.2a	3.0a	
Accord	4.1	1.3b	2.4ab	
Accord+Oust	3.3	0.2b	1.7b	
P plot	0.9162	<0.0001	0.0499	
Wild rose				
Control	4.4	4.5a	4.2a	
Accord	3.9	0.5b	0.6b	
Accord+Oust	3.9	0.3b	0.4b	
P plot	0.5144	<0.0001	<0.0001	
Yarrow				
Control	2.2	2.0a	1.4	
Accord	2.0	1.1ab	2.1	
Accord+Oust	2.0	0.6b	2.1	
P plot	0.5695	<0.0001	0.1623	

1 For each species within each year, treatment values not sharing a letter differ significantly at a=0.05. Analysis based on measurement plots

2 Probability of observed F-value using measurement plot as experimental unit

3 Probability of observed F-value using spray rectangle as experimental unit

Table 4. Percent of pretreatment cover (normalized percent-cover) of 14 target species and 4 bonus species measured for up to three years after treatment.

Species and Treatment		Year after herbicide spray		
		Year 1	**Year 2**	**Year 3**
		Species sprayed in 1995		
Blackberry				
	Control	109a[1]	170a	132a
	Accord	2b	16b	30b
	Accord+Oust	0b	4b	15b
	P plot[2]	<0.0001	<0.0001	<0.0001
	P rect[3]	<0.0001	0.0002	0.0004
Flowering spurge				
	Control	106a	108a	39a
	Accord	28b	42b	45a
	Accord+Oust	44b	29b	13b
	P plot	0.0004	0.0004	0.0175
	P rect	0.0207	0.0023	0.0987
Hoary alyssum				
	Control	67b	14b	14
	Accord	142a	189a	17
	Accord+Oust	3c	29b	19
	P plot	<0.0001	<0.0001	0.6833
	P rect	0.0407	0.0004	0.4643
Leadplant				
	Control	85a	79a	92a
	Accord	2b	16b	26b
	Accord+Oust	3b	27b	62ab
	P plot	<0.0001	0.0016	0.0652
	P rect	0.0050	0.0163	0.0946
Orange hawkweed				
	Control	119a	167a	116a
	Accord	70b	85b	57b
	Accord+Oust	46b	112b	111ab
	P plot	0.0031	0.0107	0.0609
	P rect	0.0493	0.1105	0.2707

Table 4. Continued

Species and Treatment	Year after herbicide spray		
	Year 1	**Year 2**	**Year 3**
Western sunflower			
Control	126a	115a	118a
Accord	10b	28b	28b
Accord+Oust	0b	0b	6b
P plot	<0.0001	<0.0001	<0.0001
P rect	0.0006	0.0025	0.0016
Species sprayed in 1996			
Black-eyed Susan			
Control	73a	56	
Accord	31b	119	
Accord+Oust	12b	80	
Accord+Garlon4	29b	62	
P plot	0.0035	0.1841	
P rect	0.1201	0.3537	
Butterfly weed			
Control	27	84a	
Accord	40	14b	
Accord+Oust	9	15b	
Accord+Garlon4	18	55ab	
P plot	0.5604	0.0179	
Common cinquefoil			
Control	152a	178b	
Accord	6b	6c	
Accord+Oust	0b	6c	
Accord+Garlon4	196a	354a	
P plot	<0.0001	<0.0001	
Horsemint			
Control	29b	62b	
Accord	105a	75b	
Accord+Oust	0b	159a	
Accord+Garlon4	88a	61b	
P plot	<0.0001	<0.0001	
P rect	<0.0001	0.0047	

Table 4. Continued

Species and Treatment	Year after herbicide spray		
	Year 1	**Year 2**	**Year 3**
Lupine			
Control	152b	164	
Accord	148b	191	
Accord+Oust	147b	185	
Accord+Garlon4	184a	212	
P plot	0.0482	0.3282	
P rect	0.2410	0.4701	
Lyre-leaved rock cress			
Control	65a	50ab	
Accord	64a	64a	
Accord+Oust	6c	64a	
Accord+Garlon4	37b	46b	
P plot	<0.0001	0.0278	
P rect	0.0038	0.6054	
Raspberry			
Control	124a	158a	
Accord	3b	3b	
Accord+Oust	1b	3b	
Accord+Garlon4	7b	15b	
P plot	<0.0001	<0.0001	
P rect	0.0060	0.1153	
Rough-fruited cinquefoil			
Control	61	54	
Accord	86	51	
Accord+Oust	8	36	
Accord+Garlon4	42	70	
P plot	0.3769	0.7403	
P rect	0.6722	0.8718	
Western sunflower			
Control	216a	307a	
Accord+Oust	26c	66b	
Accord+Garlon4	95b	148b	
P plot	<0.0001	<0.0001	
P rect	0.0179	0.0059	

Table 4. Continued

Species and Treatment		Year after herbicide spray		
		Year 1	**Year 2**	**Year 3**
		Bonus species		
Hoary puccoon				
	Control	116a	81a	
	Accord	14b	18b	
	Accord+Oust	0b	1b	
	P plot	<0.0001	0.0001	
Goldenrod				
	Control	102a	107	
	Accord	69a	140	
	Accord+Oust	3b	66	
	P plot	<0.0001	0.3036	
Wild rose				
	Control	101a	104a	
	Accord	7b	11b	
	Accord+Oust	12b	19b	
	P plot	<0.0001	<0.0001	
Yarrow				
	Control	119a	78	
	Accord	72b	149	
	Accord+Oust	28c	118	
	P plot	0.0003	0.1862	

1 For each species within each year, treatment values not sharing a letter differ significantly at a=0.05
2 Probability of observed F-value using measurement plot as experimental unit
3 Probability of observed F-value using spray rectangle as experimental unit

Table 5. Herbicide effectiveness index (HEI) for 18 nectaring species and three herbicides. Positive values mean herbicides increased cover; negative values mean herbicides decreased cover.

Species and Treatment	Year after herbicide spray		
	1	2	3
Accord-1995 plots			
Blackberry	-107 R[1]	-154 R	-102 R
Flowering spurge	-78 R	-66 R	6[2]
Hoary alyssum	75 I[3]	175 I	2
Leadplant	-83 R	-63 R	-66 R
Orange hawkweed	-49 R	-82 R	-59 R
Western sunflower	-116 R	-87 R	-89 R
Accord-1996 plots			
Black-eyed Susan	-42 R	64	
Butterfly weed	13	-70 R	
Common cinquefoil	-146 R	-172 R	
Horsemint	76 I	14	
Lupine	-4	27	
Lyre-leaved rock cress	-1	14	
Raspberry	-121 R	-155 R	
Rough-fruited cinquefoil	25	-2	
Accord-Bonus species (1995 & 1996)			
Hoary puccoon	-102 R	-62 R	
Goldenrod	-32	33	
Wild rose	-94 R	-94 R	
Yarrow	-46 R	70 I	
Accord+Oust-1995 plots			
Blackberry	-109 R	-166 R	-116 R
Flowering spurge	-62 R	-79 R	-25 R
Hoary alyssum	-64 R	15	4
Leadplant	-82 R	-52 R	-29
Orange hawkweed	-73 R	-55 R	-5
Western sunflower	-126 R	-115 R	-112 R

Table 5. Continued

Species and Treatment	Year after herbicide spray		
	1	2	3
Accord+Oust-1996 plots			
Black-eyed Susan	-61 R	24	
Butterfly weed	-18	-69 R	
Common cinquefoil	-152 R	-172 R	
Horsemint	-29	97	
Lupine	-5	21	
Lyre-leaved rock cress	-59 R	14	
Raspberry	-123 R	-155 R	
Rough-fruited cinquefoil	-53	-17	
Western sunflower	-190 R	-241 R	
Accord+Oust-Bonus species (1995 & 1996)			
Hoary puccoon	-116 R	-79 R	
Goldenrod	-99 R	-41	
Wild rose	-89 R	-85 R	
Yarrow	-91 R	39	
Accord+Garlon-1996 plots			
Black-eyed Susan	-44 R	7	
Butterfly weed	-9	-29	
Common cinquefoil	44	177 I	
Horsemint	59 I	-1	
Lupine	32 I	49	
Lyre-leaved rock cress	-31 R	-4	
Raspberry	-117 R	-143 R	
Rough-fruited cinquefoil	-19	16	
Western sunflower	-121 R	-159 R	

1 R indicates that herbicides significantly reduced percent cover, the more negative the index value the greater the reduction

2 Absence of an I or R indicates lack of statistical significance between control and treatment

3 "I" indicates that herbicides significantly (a=0.05) increased percent-cover, the more positive the number the greater the increase

Table 6. Effects of herbicides on percent-cover of bonus species grouped by year of plot establishment. Percent-cover for each plot is the sum of all nectaring species except the species targeted on that plot..

Year sprayed and Treatment	Year after herbicide spray				% of Year 0[1]		
	Year 0	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
1995 plots							
Control	9.0	12.1a[3]	12.8a	11.5a	134	142	127
Accord	9.4	2.9b	12.3a	8.2b	31	131	87
Accord+Oust	8.7	1.5b	6.0b	6.1b	17	68	70
Pplot[2]	0.8578	<0.0001	<0.0001	<0.0001			
1996 plots							
Control	10.5a	16.6a	20.0a		158	190	
Accord	7.5b	8.1c	10.5c		108	140	
Accord+Oust	9.1a	4.8d	9.3c		53	102	
Accord+Garlon	7.7b	10.0b	14.0b		130	182	
Pplot	0.0003	<0.0001	<0.0001				
1995+1996 plots							
Control	10.1a	15.3a	17.9a		152	178	
Accord	8.1b	6.5b	11.1b		80	137	
Accord+Oust	9.0ab	3.8c	8.3c		42	92	
Pplot	0.0513	<0.0001	<0.0001				

1 Values for percent of year 0 are based on the averages shown in columns 2-5; they were not analyzed statistically

2 Probability of observed F-value with plot as experimental unit

3 For each spray year and measurement year, treatment values not sharing a letter differ significantly at á=0.05

Table 7. The effects of herbicides on the number of flowering stems/40 m^2 (~0.01 acre) of nectaring species. Measured during first flight of KBB on 1995 and 1996 plots.

Year established and Species	Year after spray	Herbicide treatment				P plot[1]	P rect[2]
		Control	Accord	Accord+ Oust	Accord+ Garlon4		
1995 plots							
Blackberry	3	44a[3]	0b	0b	---	0.0002	0.0835
Hoary alyssum	3	36	156	160	---	0.1478	0.2915
Orange hawkweed	3	256	188	296	---	0.7205	0.8827
1996 plots							
Common cinquefoil	2	464a	8b	0b	756a	0.0017	---
Lupine	2	448	552	632	560	0.2375	0.3350
Lyre-leaved rock cress	2	124	112	208	164	0.1581	0.4735
Raspberry	2	96a	0b	0b	8b	0.0008	---

1 Probability of observed F-value using measurement plot as experimental unit

2 Probability of observed F-value using spray rectangle as experimental unit

3 For each species, treatment values not sharing a letter differ significantly at α=0.05. Analysis based on measurement plots

Table 8. The effects of herbicides on numbers of flowering stems/40 m^2 (~0.01 acre) of nectaring species of KBB in 1995 plots. Measured 1 and 3 years after treatment. Measured during second flight of KBB.

Species	Year after spray	Herbicide treatment			P plot[1]	P rect[2]
		Control	Accord	Accord+Oust		
Flowering spurge	1	600a[3]	80b	56b	<0.0001	0.0019
	3	356a	244ab	68b	0.0253	0.0051
Hoary alyssum	1	84b	192a	12c	<0.0001	0.0359
	3	92	396	268	0.1554	0.2531
Leadplant	1	76a	0b	0b	0.0004	0.0795
	3	---[4]	---	---	---	---
Orange hawkweed	1	48a	8b	0b	0.0426	0.0005
	3	---	---	---	---	---
Western sunflower	1	728a	0b	0b	<0.0001	<0.0001
	3	52a	20ab	4b	0.0218	0.1543

1. Probability of observed F-value using measurement plot as experimental unit
2. Probability of observed F-value using spray rectangle as experimental unit
3. For each species within each year, treatment values not sharing a letter differ significantly at α=0.05 Analysis based on measurement plot
4. Insufficient data or unsuitable analysis

Table 9. The effects of herbicides on numbers of flowering stems/40 m^2 (~0.01 acre) of nectaring species in 1996 plots. Measured during second flight of KBB.

Species	Year after spray	Herbicide treatment				P plot[1]	P rect[2]
		Control	Accord	Accord+ Oust	Accord+ Garlon4		
Black-eyed Susan	0	120	168	148	112	0.1436	0.1610
	2	28b[3]	132a	56b	44b	0.0340	0.0177
Horsemint	0	536	600	732	524	0.2850	0.6738
	2	172b	288b	808a	260b	<0.0001	0.0103
Lyre-leaved rock cress	0	28	12	20	28	0.5881	0.5150
	2	---[4]	---	---	---	---	
Rough-fruited cinquefoil	0	60	48	72	52	0.6528	0.8794
	2	---	---	---	---	---	
Western sunflower	0	104	---	104	192	0.2951	0.3103
	2	56	---	64	52	0.9308	0.9432

1. Probability of observed F-value using measurement plot as experimental unit
2. Probability of observed F-value using spray rectangle as experimental unit
3. For each species within each year, treatment values not sharing a letter differ significantly at α=0.05 Analysis based on measurement plot

Table 10. The effects of herbicides on the number and frequency of flowering stems of bonus species. Measured 2 or 3 years after treatment.

Year established (Flight)	Years after treatment	Herbicide treatment				P plot
		Control	Accord	Accord+Oust	Accord+Garlon4	
		Numbers of Flowering stems / 40m^2				F-test
1995 plot (1st)	3	162ab[1]	177a	100b	---[2]	0.0711
1995 plot (2nd)	3	96b	247a	104b	---	<0.0001
1996 plot (1st)	2	355bc	616a	202c	443b	<0.0001
1996 plot (2nd)	2	130c	386a	272b	354a	<0.0001
		Percent of plots with ≥ one flower stem				X^2- test
1995 plot (1st)	3	43	46	36	---	0.3384
1995 plot (2nd)	3	25b	42a	39a	---	0.0272
1996 plot (1st)	2	59a	64a	43b	59a	0.0170
1996 plot (2nd)	2	45b	69a	55b	66a	0.0019
		Percent of plots with ≥ three flower stems				X^2- test
1995 plot (1st)	3	24a	28a	14b	---	0.0483
1995 plot (2nd)	3	13b	26a	11b	---	0.0084
1996 plot (1st)	2	37a	50a	24b	44a	0.0013
1996 plot (2nd)	2	19b	43a	39a	47a	0.0002

1 For each flight within each year of establishment, treatment values not sharing a letter differ significantly at α=0.05

2 No data

Table 11. Measured and inferred effects of herbicides on the number of flowering stems of KBB nectaring plants. Inferred effects 1 year after treatment are derived from rankings of percent cover (Table 4). No inferences were made for year 2 (1996 plots) or year 3 (1995 plots) after treatment.

"I" means the treatment significantly increased flowering stems relative to the controls.

"N" means that no significant difference was observed or predicted.

"R" means the treatment significantly reduced flowering stems relative to the controls.

Subscript "in" means that an increase (Iin) or decrease (Rin) was inferred from percent-cover.

- - - means insufficient data available for analysis.

Year established and Species	1 year after treatment			2 or 3 years after treatment		
	Accord	**Accord +Oust**	**Accord +Garlon4**	**Accord**	**Accord +Oust**	**Accord +Garlon4**
1995 plots						
Blackberry	--	--	--	R	R	--
Flowering spurge	R	R	--	N	R	--
Hoary alyssum	I	R	--	N	N	--
Leadplant	R	R	--	--	--	--
Orange hawkweed	R	R	--	--	--	--
Western sunflower	R	R	--	N	R	--
Bonus plants- 1st flight	--	--	--	N	N	N
Bonus plants- 2nd flight	--	--	--	I	N	I
1996 Plots						
Western sunflower	--	Rin	Rin	--	N	N
Black-eyed Susan	Rin	Rin	Rin	I	N	N
Common cinquefoil	Rin	Rin	N	R	R	N
Horsemint	Iin	N	Iin	N	I	N
Lupine	N	N	Iin	N	N	N
Lyre-leaved rock cress	N	Rin	Iin	--	--	--
Raspberry	Rin	Rin	Rin	R	R	R
Rough-fruited cinquefoil	N	N	N	--	--	--
Bonus plants - 1st flight	--	--	--	I	N	N
Bonus plants- 2nd flight	--	--	--	I	N	I

Table 12. Herbicides applied to Karner blue butterfly eggs in 1995 and 1996 experiments.

Herbicide	Herbicide treatment				
	Control	**Accord**	**Accord+Oust**	**Garlon4**	**Accord+Garlon4**
			1995 application[1]		
Accord *L/ha (oz/acre)*	0	4.7 (64)	4.7 (64)	0	---
Oust *gm/ha (oz/acre)*	0	0	70 (1)	0	---
Garlon4 *L/ha (oz/acre)*	0	0	0	4.7 (64)	---
Entry II *L/ha (oz/acre)*	0	1.1 (15)	1.1 (15)	1.1 (15)	---
			1996 application[2]		
Accord *L/ha (oz/acre*)	0	9.4 (128)	9.4 (128)	---	!.75 (24)
Oust *gm/ha (oz/acre)*	0	0	140 (2)	---	0
Garlon4 *L/ha (oz/acre)*	0	0	0	---	1.75 (24)
Entry II *L/ha (oz/acre)*	0	1.1 (15)	1.1 (15)	---	0.55 (7.5)

1 The 1995 spray volume was 175 L/ha (18.5 gallons/acre)
2 The 1996 spray volume was 390 L/ha (42 gallons/acre)

Table 13. The 1995 experiment. Herbicide effects on developmental success of Karner blue butterfly from eggs to adults. No significant difference among treatments for any parameter at α=0.05.

	Herbicide treatment				P[1]
	Control	**Accord**	**Accord+Oust**	**Garlon4**	
Hatch (% of eggs)	80	75	88	79	0.1339
Days from April 20 to Hatch	9.1	7.1	8.5	8.8	--
Pupae (% of hatched eggs)	24	23	11	26	--
Adults (% of pupae)	92	100	100	88	--

1. Probability of observed chi-square value

Table 14. The 1996 experiment. Herbicide effects on developmental success of Karner blue butterfly from eggs into adults

	Herbicide treatment				P[1]
	Control	Accord	Accord+Oust	Accord+ Garlon4	
Hatch (% of eggs)	82	76	75	68	0.0966
Pupae (% of hatched eggs)	83	84	89	80	0.7896
Adults (% of pupae)	100	100	100	97	0.4411
Adults (% of eggs)	67a[2]	64a	66a	52b	0.0237
Adults (% of control)	100a	95ab	99a	78b	0.0649

1 Probability of observed F-value

2 Within each column, values without a shared letter differ significantly at α=0.05

Table 15 . The 1996 experiment. Effects of three herbicides on pupal size and rates of development of Karner blue butterfly.

	Herbicide treatment				P[1]
	Control	Accord	Accord+ Oust	Accord+ Garlon4	
Days from April 20 to hatch	25	24	22	25	0.3904
Days from hatch to pupation	35	36	38	38	0.3049
Days from pupation to adult emergence	8.4	8.4	8.3	7.7	0.3100
Pupal weight (g)	0.075	0.076	0.081	0.086	0.0918
Wing case length (mm)	6.0	5.9	6.1	6.1	0.3117

1 Probability of observed F-value

2 Within each column, values without a shared letter differ significantly at α=0.05

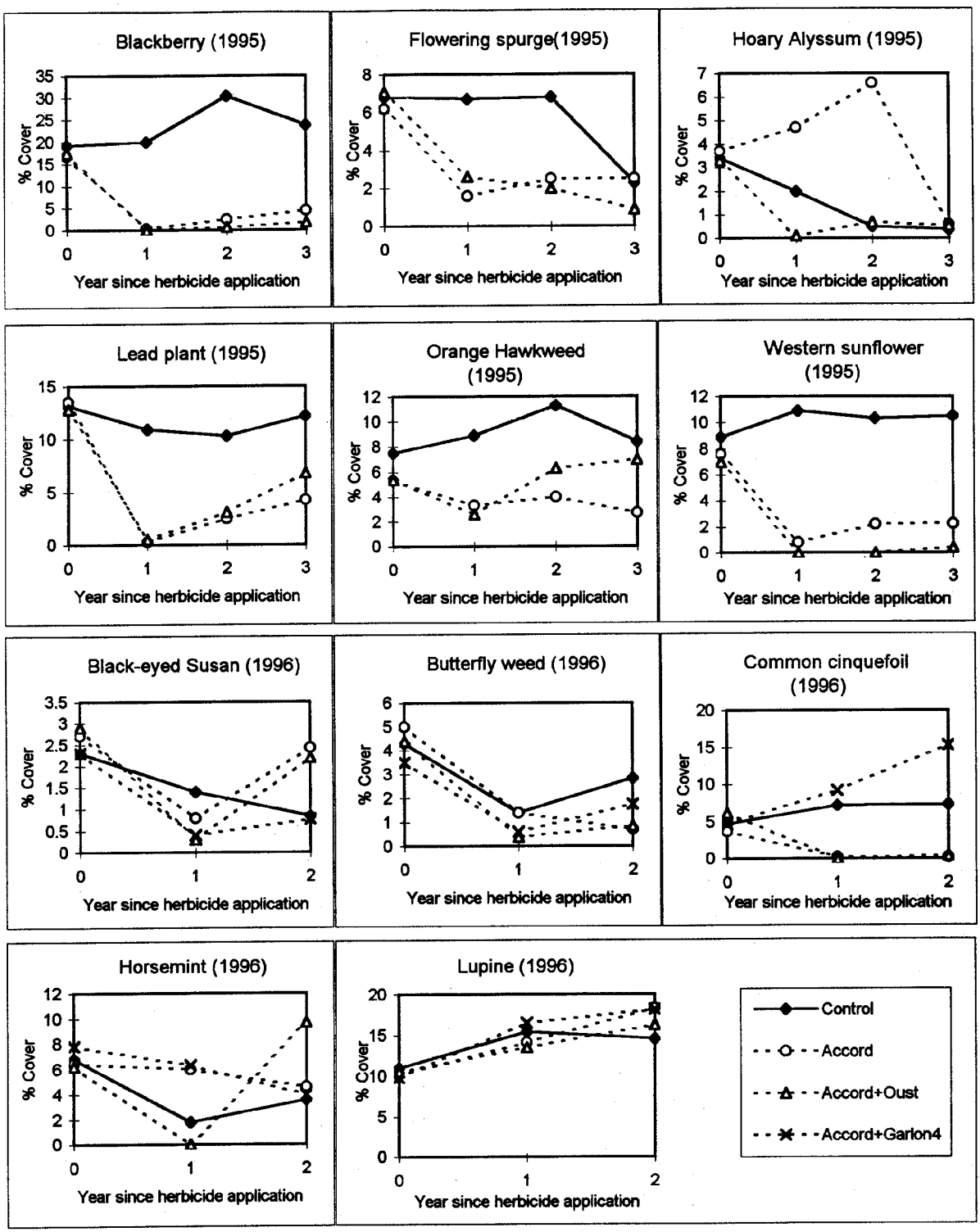

Figure 1. Percent cover of 14 target species and four bonus species one or two years after herbicide spray.

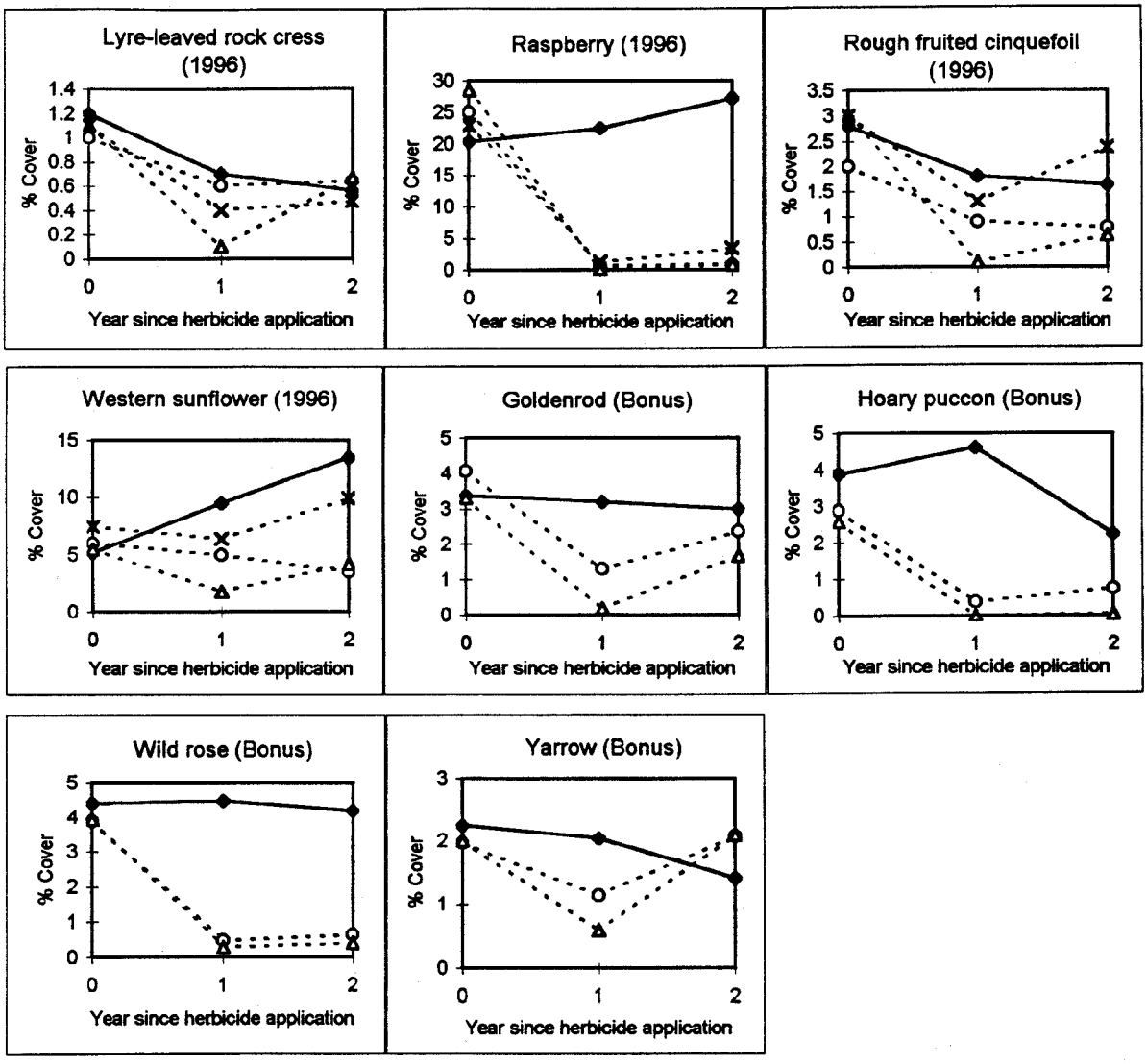

Figure 1. (Continued)