Environmental Entomology
Pest Management (Non-target Impacts)

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Spatial Overlap Between Milkweed (*Asclepias spp.*) and Pyrethroid Treatments for Mosquito Control

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Abstract

Risk assessment requires both toxicity and exposure information. A recent study showed some pyrethroids used for mosquito control can cause nontarget mortality to monarch butterfly (*Danaus plexippus* L.) larvae and adult females exposed to treated plants (Oberhauser et al. 2006). In this study we assessed spatial distribution of the monarch's host plant, milkweed (*Asclepias spp.*), in the 7-county Minneapolis-St. Paul region, and examined likely spatial overlap with areas treated by the Metropolitan Mosquito Control District. A sample of 16-hectare (40-acre) units drawn from urban, suburban and rural strata were visually inspected and location of common milkweed (*A. syriaca*) patches and # ramets/patch recorded, along with boundaries of the portion of the area searched. Paths for permethrin barrier treatments and resmethrin fog applications were digitized and buffers applied representing the possible area receiving sufficient dose to affect monarch larvae (based on previous work). A total of 2465 milkweed patches (29,592 ramets) were found in the 860 hectare observed, with an average density of 2.2 ramets/ m^2. Urban areas tended to have fewer and smaller patches. Milkweed density was not significantly different within exposed areas, so proportion of total area exposed estimated proportion of milkweed exposed. In 2001-2005 permethrin exposure area represented 0.22% to 0.52% of metro-area land considered undeveloped, agricultural, residential, or park/golf, and resmethrin or sumithrin fog exposure area ranged from 3% to 6%. Thus we expect that, although treatment might adversely affect localized individuals, effect on monarch butterflies at a population level will be minimal.

Key Words: Non-target impacts, Monarch butterfly, *Danaus plexippus*, Permethrin, Resmethrin, Pyrethroid, Mosquito control, Spatial, Milkweed, *Asclepias*

As part of a continuing effort to evaluate nontarget effects of mosquito control practices, we have done a spatial assessment of adult mosquito control insecticide use by the Metropolitan Mosquito Control District (MMCD), which operates in the Minneapolis/St. Paul 7-county area in Minnesota. Recent work on permethrin toxicity to monarch butterflies (*Danaus plexippus* L.) showed that 0.1095 kg AI/h barrier treatments (0.0977 lb AI/acre) applied with a power backpack to prevent mosquito activity can cause mortality for monarch larvae feeding on treated host plants (*Asclepias spp.*) up to 21 d later (Oberhauser et al. 2006). Additional work on the toxicity of 0.0039 kg per hectare (0.0035 lb AI/acre) resmethrin ULV fog treatments to larvae on caged plants suggested this treatment can also cause mortality at the time of exposure, but plants exposed to sunlight in the treatment area were not toxic to larvae feeding on them after 24 h (MMCD 2005 Operational Review p. 57).

An important part of examining environmental risk is evaluating the likelihood of exposure. For example, previous work on possible effects of transgenic *Bacillus thuringiensis* (Bt) corn (*Zea mays* L.) on monarchs included extensive studies of the chance of larvae encountering toxic doses of pollen (Sears et al. 2001), and determined that risk to monarch populations from pollen from current commercial hybrids is negligible despite the significant mortality found in larvae exposed to one hybrid variety. In a similar approach, we evaluated risk to monarch populations from permethrin and other mosquito adulticides in the Minneapolis/St. Paul metropolitan area by estimating the proportion of the larval host plant, milkweed (*Asclepias spp.*), that is exposed to potentially toxic doses. Although previous work describes the importance of agricultural and nonagricultural areas in milkweed distribution in rural landscapes (Oberhauser et al.

2001), it does not include urban and suburban landscapes that make up the majority of areas where mosquito control is likely to be done.

Milkweed abundance is generally described by observing ramets (stalks), although many stalks may come from one root system. Abundance has been described as density (ramets/m^2, Oberhauser et al. 2001) or as patch size (area encompassed by contiguous stems, single plant=1 m^2, Hartzler and Buhler 2000). Standard sampling used in agricultural or roadside settings has involved randomly selecting either a 2 m x 100 m transect and counting ramets along the transect (Oberhauser et al. 2001) or selecting a 50 m x 100 m sampling area and counting ramets within the area (Hartzler and Buhler 2000). The sampling plans used had an emphasis on agricultural fields and adjacent areas. Oberhauser et al. (2001) calculated the relative contribution of each habitat to area populations based on land use and/or land cover data.

Temporal distribution of monarch eggs in the upper Midwest usually includes two peaks, one starting in late May and one in mid to late July (MLMP 2006; Prysby and Oberhauser 2004), with continuous presence of monarch eggs or larvae from late May through late August. Timing of mosquito adulticide treatments done by MMCD varies from year to year based on rainfall patterns and subsequent mosquito production, but the most frequent period for adulticide application is June and July (MMCD 2006, p. 32), and applications often continue through August (MMCD 2005, p.36). Thus treatments often take place at times when monarch larvae are present on plants, making spatial overlap an important factor in determining exposure.

Materials and Methods

Sampling Design. Sampling followed a stratified nested design. The first-order unit for randomization was Public Land Survey "sections" (approximately 259 hectare (1 mile$^{2)}$), which provides a general grid for the Minneapolis./St. Paul 7-county area and is used by MMCD in recording treatments. The universe included 3000 square miles (777,000 hectares) in Anoka, Carver, Dakota, Hennepin, Ramsey and Washington counties in Minnesota. Each section was categorized using two levels of stratification: 1. Treatment history in 2004-2005: containing at least one treatment in both years, containing treatments one of the years only, or none, and 2. Development level: sections outside the area of highest population density (outlined by MMCD's "Priority 1" boundary) were considered rural, sections within the two core cities or first-ring suburbs considered urban, and remaining areas suburban (Figure 1). Table 1 shows the possible units in each of these nine stratum combinations; an equal number of sections was selected from each combination (non-proportional) using a random method.

Each selected section was further divided into 16 quarter-quarter ("q-q") sections (16.2 hectare (40 acres), about 8 city blocks). Treatment records were used to determine which q-q contained treatments, and the number of q-q with treatments was recorded for each section. A random sample of two q-q containing treatments and two untreated q-q (if available) was drawn from each section for detailed examination. If a section did not include any treatments, only two q-q were examined.

Milkweed Observations and Sampling Unit. Of the several species of milkweed present in the Minneapolis/St. Paul metropolitan area, we focused on observing common milkweed, *Asclepias syriaca*. When other species were encountered and reliably identified they were included in the data. Observations were done by teams of two to four observers trained to spot milkweed and record locations. Observers were tested before data collection began. Data collection was done between Sept. 8 and Oct. 3, 2005.

Preliminary surveys showed that in urban areas milkweed patches were often rare and small, so we chose to both count ramets and estimate patch size. In large patches, three 1- m^2 samples were chosen randomly and counted to estimate total number of ramets in the patch.

Transects were not practical in urban/suburban settings, so we used a “walk” in which the observer recorded all occurrences of milkweed and estimated the size of the patch and number of ramets. Because milkweed was generally easy to spot from a distance, area observed included any space that could be examined visually by an observer, generally walking on a street, alley, field lane or open area. The path of the walk and location of each milkweed patch found was recorded using GPS receivers (Garmin 12 or Garmin E-trex, estimated accuracy +/- 10 m). Walk paths were planned to survey as much of the chosen quarter-quarter section as could be done in about 2-3 person-hrs. Areas excluded from search included open water, wetland interiors (common milkweed is considered unlikely to occur in wetlands [Reed 1988]), densely shaded woodlot interiors more than 6 m from edge, large parking lots with continuous pavement, short mowed turf where milkweed plants would be to hard to find, agricultural field interiors (beyond visible edge) where inspection might cause crop damage, and fenced or restricted areas or areas that could not be safely accessed. The initial direction walked in each block was chosen using a random method. An attempt was made to include different land use types if present within the sampling block. Observers generally were not aware of what portion of each q-q block had been treated for mosquitoes.

At the end of the walk the observers marked actual observed area on aerial photos. Observed area ranged from 13 to 87 % of the q-q block (median 32%). Density was calculated as the number of ramets/observed area (m^2) in the sampling block, and average patch size was calculated from data on individual patches.

Mosquito Adulticide Exposed Area. MMCD staff members record approximate adulticide application treatment paths on paper maps at the time of treatment. For sections chosen for detailed analysis, these paths were digitized and a buffer applied (Fig. 2) to approximate the area that could have been exposed to adulticide at doses high enough to potentially affect monarch larvae (Oberhouser et al. 2006; MMCD 2005 p. 57). A buffer of 7.6 m (25 ft) was added on both sides of recorded treatment paths for permethrin barrier treatments applied by personal backpack. A buffer of 76.2 m (250 ft) was added on both sides of paths for ULV fog treatments using either resmethrin or sumithrin adulticides, applied by truck-mounted, ATV-mounted, or hand-held cold fog units. Although the actual area affected by treatments (especially fog) depends on wind direction and speed, we did not attempt to adjust the direction or distance of the buffer area based on that data, so the area actually exposed was probably less than the estimated exposure area used.

The estimate of exposed area used in the final comparison of the total amount of treated area vs. amount of likely habitat was calculated using the total volume of material applied multiplied by the dose of application (dose given above, see also MMCD 2005 Operational Review p. 70). This was expected to give a more accurate estimate of the size of the area exposed than would the buffered treatment path, although it would not provide the specific location treated.

Spatial Analysis. GPS data describing walk paths and patch location points were uploaded and transferred into MapInfo® geographic information system (GIS) software. Observed area boundaries and treatment path paper records were digitized in the same software using recent orthoimagery (Metro Council 2005) as a background, treatment path buffers calculated and applied, and area calculated for each spatial object. Milkweed patch points in each q-q block were coded by whether or not they fell within a treatment path buffer area. Density of milkweed in treated and untreated areas was then calculated for each q-q block based on counts in observed area treated or untreated.

Statistical Analyses. Overall density (ramets/ m^2) in the portions of observed areas that overlapped buffered treatment paths was compared with density in untreated observed areas in the same section using paired sample t-tests in Systat®. Analysis of variance for comparing Urban/Suburban/Rural distributions was also done using Systat®. Stratum weights were applied as appropriate.

The final estimate of the proportion of milkweed treated was done by comparing overall area of adulticide treatments with total metropolitan area. For this analysis the estimate of area treated with adulticide was calculated based on MMCD's records of adulticide use and dose. This may have somewhat overestimated area receiving treatment, since an area could receive treatment more than once. The area estimate for total metropolitan area was reduced to approximate the area likely to provide milkweed habitat by subtracting open water, 4-lane highways, industrial/retail developments and the airport using 2005 Land Use information (Metropolitan Council 2005, see complete metadata at http://www.datafinder.org/metadata/landuse_2005.htm). This data set used broad categories (Table 2); for example, agricultural areas were not split by roadside, crop, and pasture, and residential areas did not subtract pavement or building footprints. However, it provided complete data for the entire metropolitan area, unlike more detailed land cover data. Subtracting highways, industrial/retail developments and airports may have resulted in an underestimation of possible habitat, as opportunistic milkweed can sometimes be found in these areas.

Results

Milkweed Presence and Density. We examined a total of 147 q-q section blocks in 45 sections, with a total observed area of 860 hectare (2125 acres). Observations were made between Sept. 8, 2005 and Oct. 3, 2005. A total of 2465 patches of milkweed were found, composed of 29,592 ramets, covering 20,911 m^2, with an average density within patch of 2.2 ramets/ m^2.

Urban areas had marginally fewer patches per hectare examined than did suburban or rural areas, although this difference was not significant at the 0.05 level of confidence (ANOVA, F=2.60, 41 df, p=0.086) (Fig. 3), while rural and suburban areas had similar numbers, with least squares means of about 3.9. Patch size did not vary significantly by stratum (Fig. 4; ANOVA, F=2.35, 41 df, p=0.11). Exceptional sites, including one rural area with a very high number of patches, and one suburban-industrial area with a very large patch, were excluded from their respective analyses as outliers.

[Add more detail? esp. regarding land use/land cover in ag areas?]

Not all of the chosen 30 sections with treatments ended up having overlap of estimated exposure areas and observed area. For ULV fog treatments, 19 sections had observed areas that overlapped with treatment path buffers, and for permethrin barrier treatments 14 sections had overlap. Milkweed density in the portion of a section's observed area that overlapped permethrin estimated exposure areas was not significantly different from density in untreated observed areas, as measured by ramets/ observed m^2 (paired t-test, t= 1.343, 13 df, p=0.2022; mean density in exposed area 0.00380 vs. untreated mean density 0.00138). Density within estimated exposure areas for resmethrin or sumithrin ULV fog treatment was also not significantly different (paired t-test, t=-0.441, 20 df, p=0.664; mean density in exposed area 0.00167 vs. untreated mean density 0.00188 ramets/ m^2).

Given that milkweed density was not significantly different in treated and untreated areas, an estimate of milkweed exposure based on proportion of total area treated was considered reasonable. MMCD adulticide treatments in 2001-2005 ranged from 20,234 to 36,422 hectares (50,000 to 90,000 acres) per year. This represents between 3% and 6% of total land area in likely habitat. Most of this total was resmethrin or sumithrin fog treatments; permethrin barrier treatments were estimated to affect between 0.22% and 0.52% of metropolitan habitat (Fig. 6). As some areas received more than one application, this dose-based calculation probably overestimated total area exposed.

Discussion

Milkweed Density. Estimated patch area coverage from Iowa (Hartzler and Buhler 2000) ranged from 14-30 m^2/ha in pastures and agricultural fields, up to 100-169 m^2/ha in roadsides and waterways, and over 200 m^2/ha conservation reserve program (CRP) lands. Our estimate of patch area coverage in rural areas was 34 m^2/h, which included crops, roadsides and other habitats. Our estimate for suburban areas, about 12 m^2/ha, was similar to that in pastures and soybean fields. However our estimates of numbers of patches per hectare were generally higher than those found by Hartzler and Buhler: we found about 3.8 patches/ha overall in rural and suburban areas and 2.0 patches/ha in urban areas, compared with Iowa data of 4.8 in roadsides, about 2 in waterways and other areas, and <1 in corn, soybean, pasture and CRP.

Density as ramets/m^2 was reported in Oberhauser et al. 2001 as ranging from 0.003/ m^2-0.004/ m^2 in corn and other agricultural habitat in Maryland, to 0.285-1.052/ m^2 in corn and adjacent nonagricultural habitat in MN. Our study found mean densities in observed areas of about 0.002/ m^2, similar to the Maryland data, which was apparently collected by similar means (count total milkweeds, measure area by aerial maps) as opposed to examining a sample of m^2 quadrats.

Risk Assessment. Risk to a non-target population is the combined probability of exposure to a toxic agent and the toxic effect of this agent (US EPA 1998). Barrier applications of permethrin for mosquito control can kill exposed monarch butterfly larvae up to 21 d post-application, and adults are killed if exposed to recently-treated leaves (Oberhauser et al. 2006). Monarch larvae and adults suffer some mortality if they are within 76.2 m (250 ft) of ULV treatments of resmethrin, although the extent of this mortality varies with wind speed and direction, and the harmful effects disappear within 24 h post-application (MMCD 2005). Thus, toxic effects of adult mosquito control treatments on exposed monarchs are potentially high.

While it is likely that mosquito adulticide treatments will cause monarch mortality in small areas, our findings suggest the population-level risk of exposure to permethrin applied as a barrier treatment for adult mosquito control in the Minneapolis/St. Paul area is low; approximately 0.2 to 0.5% of potential monarch habitat is affected by this treatment. Actual risk for reproductive adult females might be higher as they move through the habitat depositing eggs. Risk of exposure to ULV fog treatments using resmethrin or sumithrin is slightly higher; approximately 3 to 5% of the potential habitat is affected by these treatments. However, given the low persistence of these formulations (MMCD 2005), adult monarch movement is less likely to increase the proportion exposed, and adult monarch activity is usually low during the evening hours when these treatments are usually applied.

Acknowledgements

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Table 1. Number of sections in each stratum combination. Most sections have area of 1 square mile (259 hectare); some sections are smaller if on edge of a survey grid boundary or if split for reference by MMCD at city boundaries. In sections containing treatments not all of the section was treated; actual area treated varied.

	Rural	Suburban	Urban	Total	Treatment stratum as % of total
No treatments	1091	472	170	1733	54%
Some treatment in both 2004 & 2005	239	500	77	816	25%
Some treatment in one of 2004 or 2005	257	342	66	665	21%
Total	1587	1314	313	3214	
% of sections in stratum with no treatments both years	69%	36%	54%		

Figure Legends

Fig. 1. Minneapolis/St. Paul 7-county metropolitan area, showing rural/suburban/urban stratum boundaries and treatment status for square-mile sections.

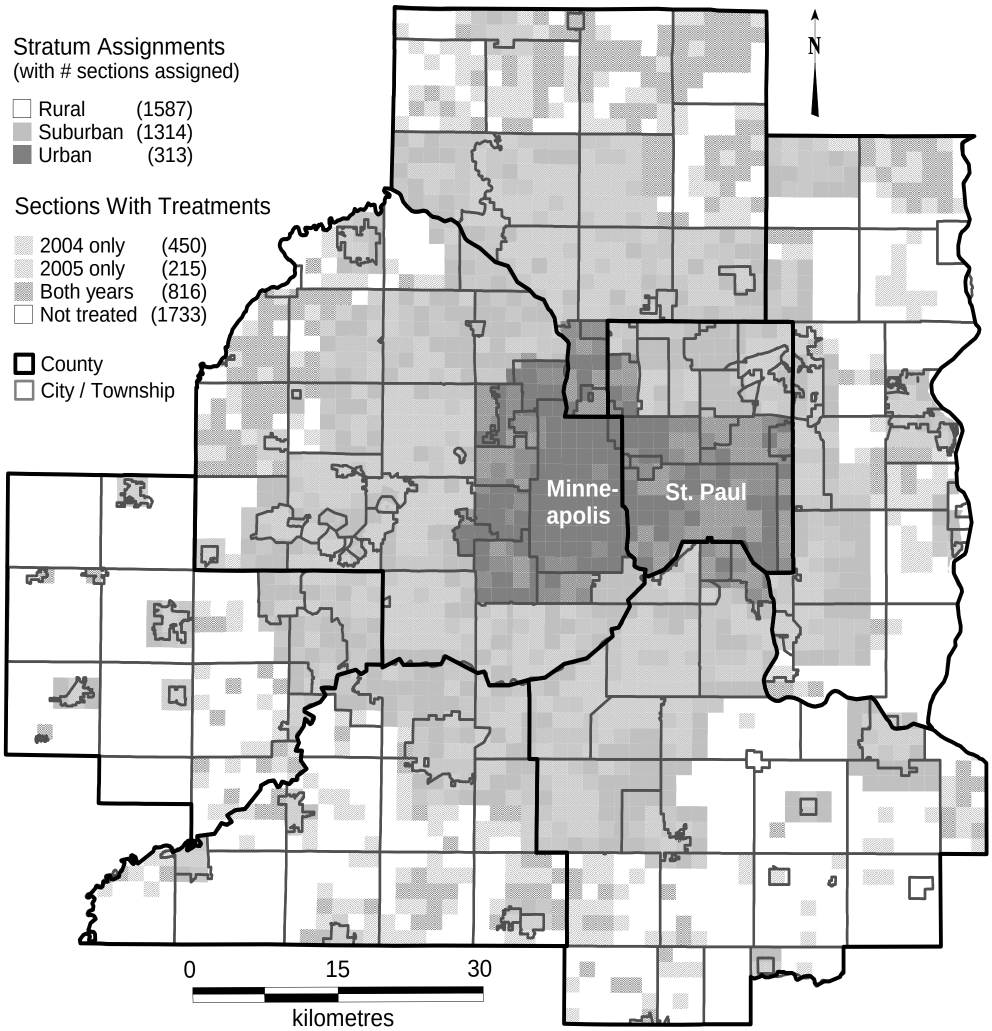
Figure 2. Example of map for a 1-sq-mile section showing observed area (yellow), treatment path and buffer approximating area treated (red), and milkweed patch locations found (pink stars).

Figure 3. Milkweed patches per hectare (box plot, with notches for confidence intervals) for rural, suburban, and urban strata. The highest count in "Rural" was detected as an outlier and removed for analysis of variance.

Figure 4. Milkweed patch area coverage in m^2/hectare (box plot, with notches for confidence intervals) for rural, suburban, and urban strata. The very high count in "Suburban" was removed as an outlier for analysis of variance.

Figure 5. Percent of likely milkweed habitat area in Minneapolis/St. Paul 7-county metropolitan area that was treated with adulticides for mosquito control by MMCD, per year. Likely habitat area defined by land use types.

Figure 1



Stratum Assignments
(with # sections assigned)
Rural (1587)
Suburban (1314)
Urban (313)
Sections With Treatments
2004 only (450)
2005 only (215)
Both years (816)
Not treated (1733)
County
City / Township
N
Minne-
apolis
St. Paul
0
15
30
kilometres

Figure 2

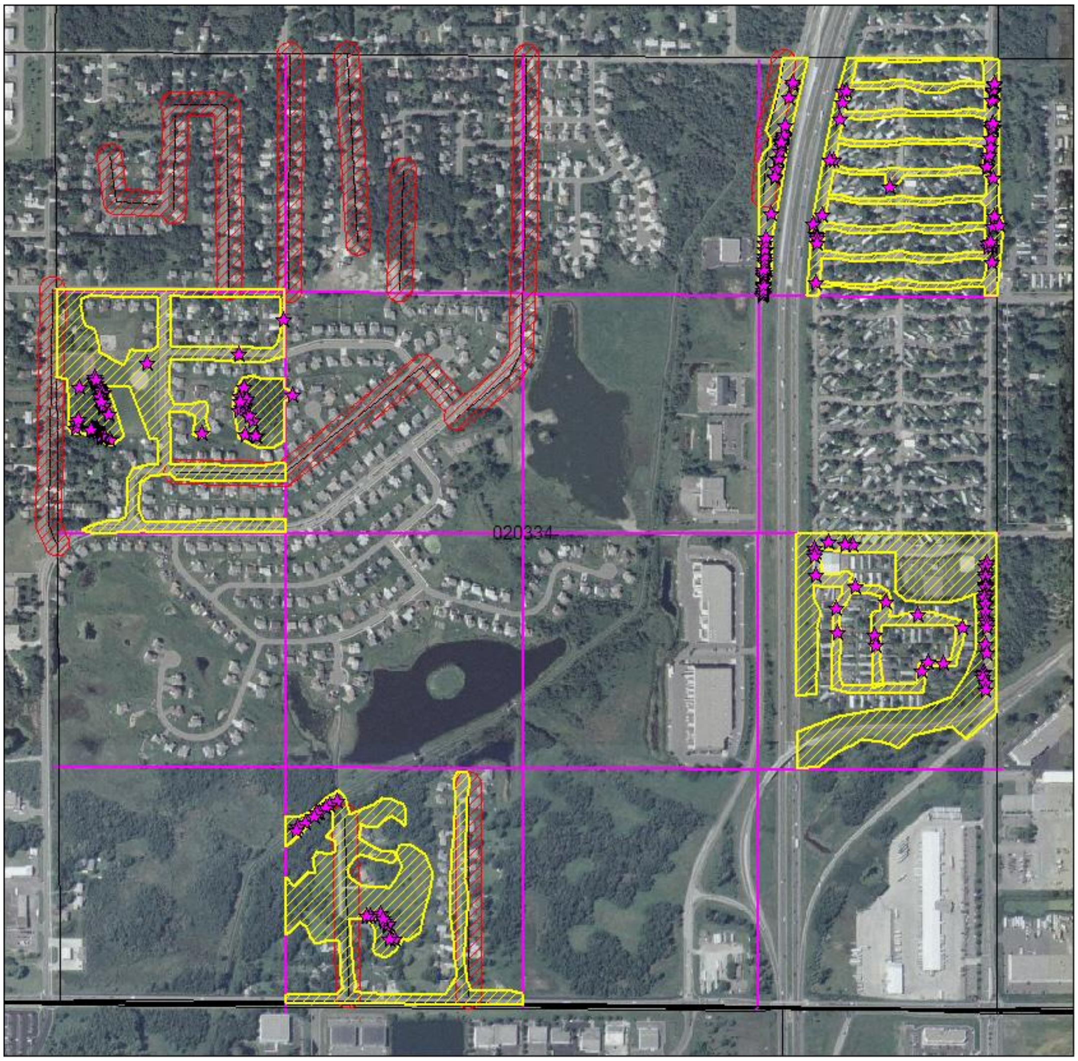
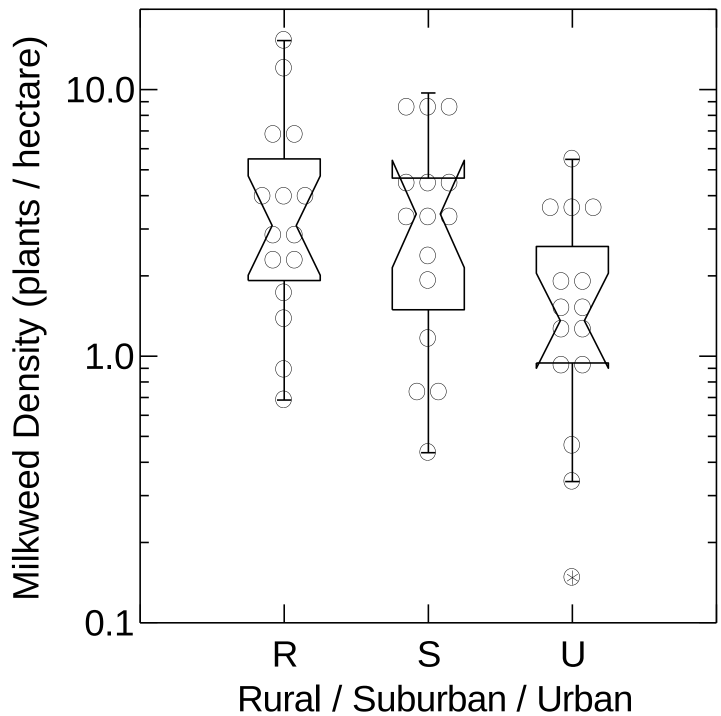
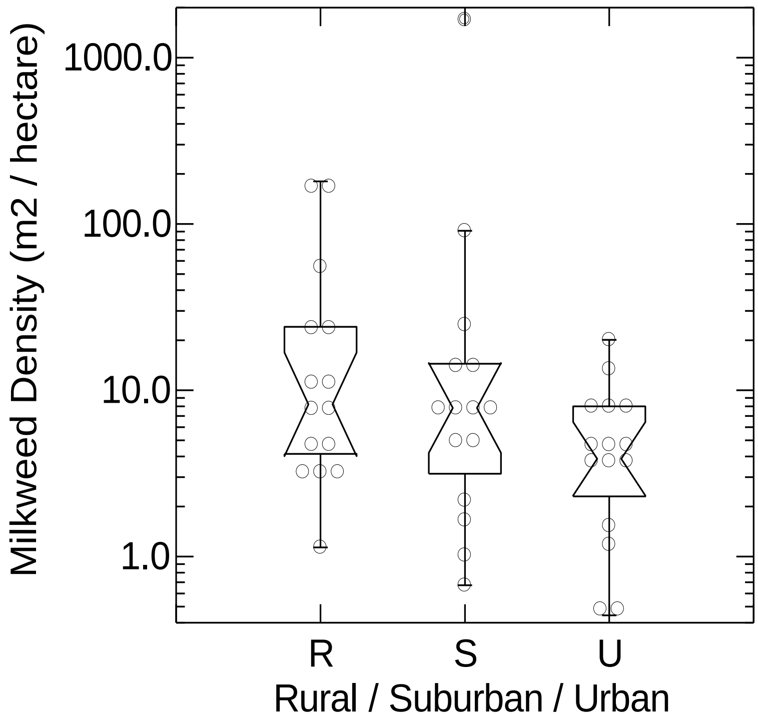


Figure 3.

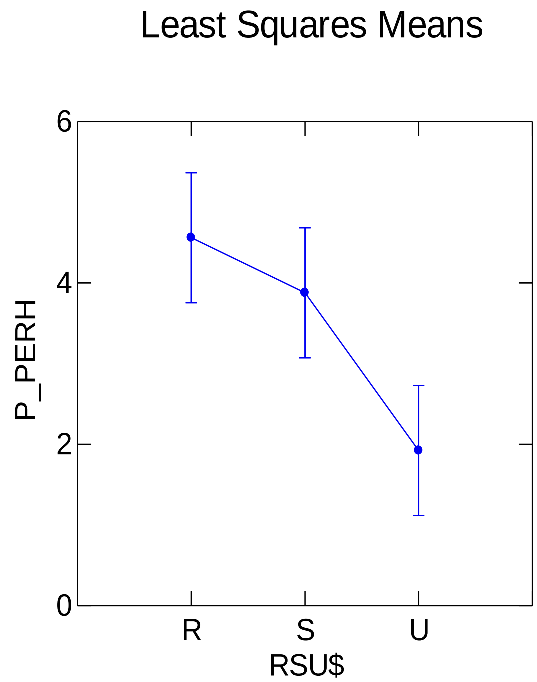


Milkweed Density (plants / hectare)
10.0
1.0
0.1
R
S
U
Rural / Suburban / Urban

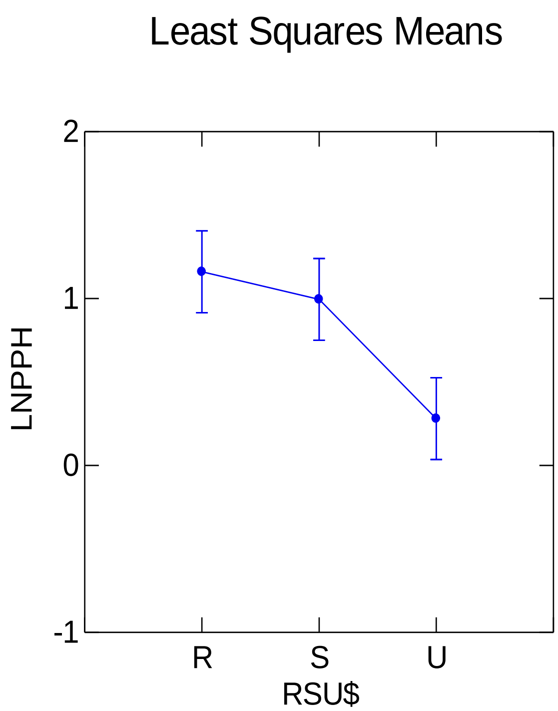
Figure 4.



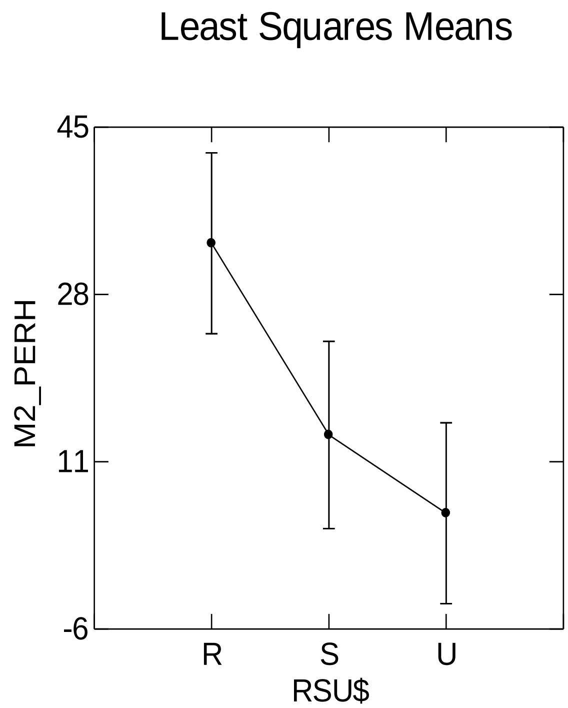
Milkweed Density (m2 / hectare)
1000.0
100.0
10.0
1.0
R
S
U
Rural / Suburban / Urban



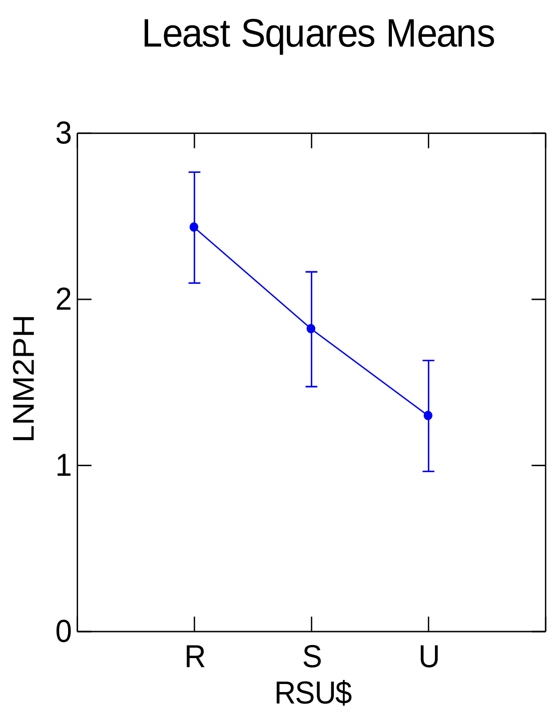
Least Squares Means
6
4
2
0
P_PERH
R
S
U
RSU$



Least Squares Means
2
1
0
-1
LNPPH
R
S
U
RSU$

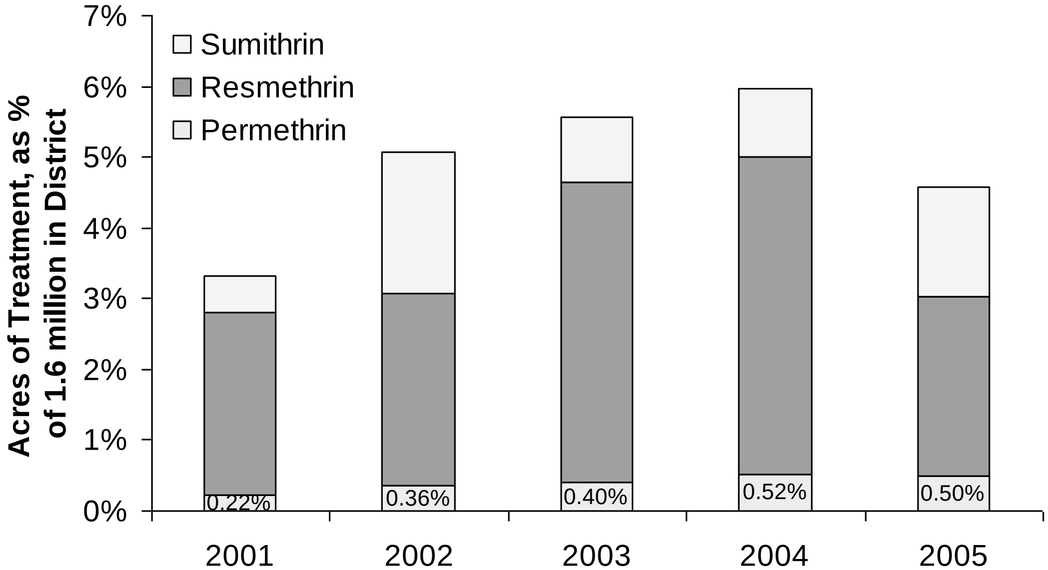


Least Squares Means
45
28
11
-6
M2_PERH
R
S
U
RSU$



Least Squares Means
3
2
1
0
LNM2PH
R
S
U
RSU$

Figure 5



Acres of Treatment, as % of 1.6 million in District
7%
6%
5%
4%
3%
2%
1%
0%
Sumithrin
Resmethrin
Permethrin
0.22%
0.36%
0.40%
0.52%
0.50%
2001
2002
2003
2004
2005