2006 Diffuse Knapweed Biological Control Monitoring Summary

Introduction

Diffuse knapweed (*Centaurea diffusa*) is one of the noxious weeds at the Rocky Flats Site (Site). A variety of control methods have been used to control diffuse knapweed at the Site, including biological, mechanical, and chemical control methods. Biological control measures are low cost, have a low impact to surrounding habitat and non-target vegetation, and may provide long lasting effects. The effectiveness of biological controls measured for 6 years on diffuse knapweed populations at the Site is evaluated in this report.

Various species of biological control insects have been released on Site for control of diffuse knapweed, including the Lesser knapweed flower weevil (*Larinus minutus*), Blunt knapweed flower weevil (*L. obtusus*), Banded gall fly (*Urophora affinis*), and UV knapweed seed head fly (*U. quadrifasciata*), all of which cause damage to the seeds of the knapweed. The other diffuse knapweed biocontrol insects that have been released and documented on Site (*Cyphocleonus achates* and *Sphenoptera yugoslavica)* cause damage to either roots or stems of the plant.

Objectives of the study include:

- Evaluate changes in pre and post-treatment diffuse knapweed cover and density at the release locations through time.
- Document visually, through photo monitoring, changes in diffuse knapweed populations at the release locations.
- Using flowerhead and seed counts, evaluate biocontrol insect impacts on diffuse knapweed seed production.

Methods

Location of Study Site

The Site is located in rural Jefferson County, Colorado, 16 miles northwest of Denver. The Site covers approximately 6,266 acres and adjoins undeveloped rangelands that are encroached by housing developments on the northeast and southeast. To the north, east, and west, public open-space lands border much of the Site. On the western edge of the Site there are industrial sand and gravel mining activities, and some small potential sites for industrial use.

Herbicide applications for diffuse knapweed control at the Site have focused primarily on upland areas away from wetlands and riparian habitats, as the latter provides habitat for the Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*), a federally listed threatened species. In the areas where the use of herbicide applications was not feasible or approved until recently; infestations of diffuse knapweed have continued to increase in the drainage bottoms at the Site. Therefore, *L. minutus* were released in the drainage bottoms with the goal that the biocontrol insects would help control the diffuse knapweed at these locations and would eventually spread to the upland areas. The original releases were located in areas with high diffuse knapweed abundance. Figure 1 shows the release locations of the *L. minutus* weevils. The biocontrol insects at release sites one through five were released in 2001. Insects at release site six were released in 2002. Release location number five (label shown in grey on Figure 1) was removed from the study in 2004 because the area was treated with herbicides.

Density and Cover

Density and cover measurements have been made annually when the diffuse knapweed at the Site has reached its maximum growth and flowering. At each of the release locations (Figure 1), a total of ten 1 meter-square (m^2) quadrats were randomly located along lines radiating from the release point. Locations were determined using random aspects and distances from a center flag placed at the release location. Distances consisted of whole numbers and were paced off from the center flag. At each release location, the maximum distance used did not exceed the boundaries of the knapweed infestation and did not exceed 20 meters from the center flag. Placement of the quadrat was done such that one side of the quadrat was roughly centered on, and perpendicular to the line paced from the center flag.

Within each of the quadrats, cover was visually estimated by cover class (0 = 0%, 1 = <5%; 2 = 6-25%; 3 = 26-50%; 4 = 51-75%; 5 = >75%). Cover was estimated for plants that had a canopy within the quadrat frame, regardless of whether they were rooted within the quadrat frame. Diffuse knapweed density was also counted as the number of adult, reproducing plants rooted within the quadrat frame. No counts of diffuse knapweed seedlings or rosettes were made.

Flowerhead Counts

To determine the number of flowerheads per plant at each of the study sites, ten diffuse knapweed plants were collected annually at each of the release locations. Plants from the 2006 growing season were collected on September 19, 2006, from outside a 25-meter radius circle centered on the release point in order to prevent impacts to the cover and density aspect of the study, and to minimize effects to seed availability near the release location.

Average-sized plants were randomly selected per release site. Each plant was clipped at the base of the main stem and the number of flowerheads were counted. This number was used in the calculation for the number of seeds per square meter at each release site.

In previous years, the number of infected and un-infected flowerheads were counted by a visual inspection. This method was discontinued in 2004 because it did not accurately show the actual number of infected and uninfected flowerheads (K-H 2004).

Seed Count per Flowerhead

To determine the average number of seeds per flower at each study site, flowerheads were collected from each release site. Flowerheads were collected on September 19, 2006, from plants selected at random outside a 25-meter radius circle centered on the release point. Collections were made outside the 25-meter radius to prevent impacts to the cover and density aspect of the study, and to minimize effects to seed availability near the release location. In 2006, a total of 500 flowerheads were collected.

Each flowerhead was cut open, and the number of mature, viable seeds in each flowerhead was counted (Figure 2). Evidence of insect damage was documented based on internal and external evidence from the flowerheads.

For flowerheads that had evidence of insect damage, the species of insect and the number of insects present in each flowerhead were recorded. The following criteria were used for determining which species of insect caused the damage:

***Larinus* sp** (includes *L. minutus* as well as *L. obtusus*, Figure 3, Figure 4): the presence of adult weevils; the presence of a large gall that took up the majority of the flowerhead; the presence of an exit hole.

Urophora affinis (Figure 5): the presence of a hard, teardrop-shaped gall with a larva inside.

Urophora quadrifasciata: the presence of a white larva in the flowerhead not inside a hard gall. The flowerhead usually looked like an uninfected flowerhead (Figure 2) with a larva nestled between the papus.

Unknown insect (Figure 6): evidence of some insect damage, but without the above-mentioned signs. The flowerheads usually had some detritus at the bottom of the flowerhead, with evidence of seed consumption.

Data Analysis

Data were entered into an electronic database and quality checked before analysis was conducted. Data were summarized for individual release locations and for all locations combined. Density count data were summarized as the mean number of stems per square meter. Cover was summarized using the midpoints of each cover class. Descriptive comparisons were made between cover, density, flowerhead and seed counts between years to examine potential change over time. The overall/grand means were calculated using original raw data (not group means), except where indicated. Statistical analysis of the results was conducted using a One Way Analysis of Variance (ANOVA) when assumptions of normality and equal variance were met. When significant differences were found ($p < 0.05$) a Holm-Sidak post-hoc test was performed to determine which groups were different. When assumptions of normality were not met a Kruskal-Wallis One Way Analysis of Variance on Ranks was performed. If significant differences were found either the Dunn's Method or the Tukey post-hoc test was performed to determine significant differences between rank sums. Post-hoc non-parametric test choice was determined based on equal or non-equal sample sizes (SigmaStat 2004).

Photo-documentation

Photographs at established photopoints were taken to visually document vegetation growth in the area of the release locations. Photographs were taken in the four cardinal directions from the center flag, and from a location looking back to the overall infestation, in order to visually document the level of infestation at the release locations. Photographs of the release sites are compared from year to year, to evaluate visual changes in the diffuse knapweed population. A full series of the photographs comparing vegetation from 2001 to 2006 is available in Appendix A on the CD-ROM.

Results

Cover and Density

Release location LM5 was dropped from sampling in 2004 because the area was treated with herbicides. Location LM6 was established in 2002, therefore, there are no cover or density data prior to 2002 for this location. When making comparisons between years for the density and cover data, the mean includes data from all locations sampled during a specific year.

The overall mean cover of diffuse knapweed plants at the five locations sampled in 2006 was 0.6%. For years 2001 through 2005 the mean cover for all locations sampled in that specific year was 21.4%, 12.7%, 15.4%, 13.0% and 12.8% respectively (Table 1). Cover for 2006 was significantly lower than 2001 and 2003 ($p = 0.009$).

Percent cover of diffuse knapweed decreased in 2006 at all locations from the previous years 2001 to 2005 (Figure 7).

There was no significant difference found when comparing the 2006 cover results between the five biocontrol locations.

The overall mean density of diffuse knapweed plants at the five locations sampled in 2006 was 0.6 plants per square meter. From 2001 through 2005, the mean density for all locations sampled in each specific year was 9.1, 7.1, 3.0, 4.9 and 12.2 plants per square meter respectively (Table 2). Density was significantly lower in 2006 than in 2005 ($p = 0.012$). In 2006, the overall mean density was the lowest it had been since the initiation of the study.

In past years, the density data have fluctuated (with the density at some sample locations increasing, while at others decreasing). In 2005 densities at all individual locations increased from 2004, but decreased again in 2006 (Figure 8).

Comparing only the 2006 density results between the five locations, there were no significant differences found.

Flowerhead Counts

The mean number of flowerheads per plant at all five locations monitored in 2006 was 169 flowerheads per plant. From 2001 through 2005, the mean number of flowerheads per plant has been 153, 135, 288, 454, and 251, respectively (Table 3).

The mean number of flowerheads per plant doubled from 2002 to 2003 (statistically significant increase, $p = 0.002$), then increased again from 2003 to 2004 (no statistical significance). The year 2004 was significantly higher in flowerhead counts compared to 2001 and 2002 ($p = 0.002$). In 2005, the mean number of flowerheads per plant decreased to about half the number in 2004 (statistically significant decrease, $p = 0.040$). The mean number of flowerheads per plant in 2006 was slightly higher than at the beginning of the study in 2001, but less than 2003 through 2005 (Figure 9).

Between locations in 2006, location LM2 had significantly higher flowerhead counts compared to LM1, LM3, and LM6 ($p < 0.001$).

Seed Counts/ Insect Evidence

The average number of seeds per flowerhead (of the 500 flowerheads examined) across all five locations monitored in 2006 was 4.5 seeds per flowerhead, which is the highest number since seeds counts were initiated in 2002. From 2002 through 2005 the mean number of seeds per flowerhead was 0.91, 0.55, 3.08, and 0.58, respectively (Table 4, Figure 10). Seed production per flowerhead was significantly higher in 2006 ($p < 0.001$) compared to all the other years in which seed counts were performed.

There was no significant difference in number of seeds per flowerhead between locations for 2006.

In 2006, the percent of flowerheads with evidence of insect damage was 19% (97 flowerheads out of 500 total). This is down from a high of 93% observed in 2003 (Table 5), which was significantly higher ($p < 0.001$) that year compared to the years 2002-2005. Percent of flowerheads with insect damage was significantly lower ($p < 0.001$) in 2006 compared to all the other years of the study (Figure 11).

The average number of seeds per flowerhead for flowerheads with insect evidence and for flowerheads without insect evidence was also calculated (Table 6). In all five years, the overall number of seeds per flowerhead and flowerheads with insect damage, was statistically lower than for those showing no insect damage (2002 = $p<0.05$, 2003 through 2006 $p<0.001$).

Table 7 shows the overall percentages of flowerheads in the following categories: no evidence of biocontrol insects, evidence of *Larinus* spp., evidence of *U. affinis*, evidence of *U. quadrifasciata*, and evidence of an unknown insect between the years 2004−2006. Evidence of multiple insect species damage to a flowerhead was found in three flowerheads in 2004 and eight flowerheads in 2005.

In 2004, the percent of flowerheads with evidence of the two species of *Urophera* and an unknown insect were about the same with about 11−12 percent each. Flowerheads with evidence of *Larinus* damage had the highest percentage, with 36%, and the remaining 31% of flowerheads showed no evidence of any biocontrol insect (out of 500 flowerheads collected). In 2005, the number of flowerheads with no evidence of biocontrol damage declined to 23%, as did the percentage of flowerheads with *Larinus* evidence (27%) and *U. quadrufasciata* evidence (5%). The only increases in percentages were in flowerheads with evidence of *U. affinis*, from 11% to 16%, and in flowerheads with evidence of an unknown insect. The increase in unknown insect percentage was the largest change of all categories, a three-fold increase from 11% in 2004 to 31% in 2005. In 2006 flowerheads with no insect evidence increased to 80% and *Larinus* and *U. affinis* evidence dropped to only 3% percent each. Unknown insect evidence was just 2%. These percentages were the lowest for those insect categories thus far during the study. Evidence of *U. quadrifasciata* increased to the 2004 level of 12% (Figure 12).

Total Seed Production

Using knapweed plant density, the average number of flowerheads per plant and the average number of seeds per flowerhead, diffuse knapweed seed production per square meter was calculated (Table 8). From 2002 to 2003, the average seed production decreased from 892 to 473 seeds/m^2. The overall seed production then increased to 7,433 seeds/m^2, an almost 16-fold increase in 2004. In 2005, the overall seed production once again decreased to 2,363 seeds/m^2, still several times higher than the seed production at the time when the study began. In 2006 the overall seed production decreased to 784 seeds/m^2 (Figure 13). The data show large within site and annual variation in total seed production. As a result, only the increase in the grand mean from 2003 to 2004 is statistically significant (p = 0.027).

Photo-documentation

Six years of photographs taken to visually document changes in diffuse knapweed populations are available in Appendix A on the CD-ROM. The photographs were taken yearly at each release location. The photos show a substantial decrease of diffuse knapweed cover from 2001 to 2003 (sage green colored plants). The photos also show a resurgence in knapweed cover in 2004 and 2005, and subsequent decrease in 2006. Quantitative data from the sites also show a decrease in knapweed cover from 2001 to 2003; an increase in 2004 and 2005; and a decrease in 2006. Visual documentation is an effective tool in showing diffuse knapweed cover changes over time.

Discussion

In general, the average cover of diffuse knapweed has declined at the release locations since the *L. minutus* were released. The greatest decline occurred initially the year after the releases were made and in 2006. Diffuse knapweed density also declined initially, but has not followed the pattern of the cover. Instead it began increasing in 2004 and continued to rise in 2005 to the point where it was above the level of when the study began. In 2006 both density along with cover declined to the lowest levels during the study.

There appears to be a correlation of the diffuse knapweed density data with the previous year's precipitation (November to October; Figure 14). Diffuse knapweed is a winter annual, often germinating in the fall, overwintering as a rosette, then bolting and flowering the following summer. In years with higher moisture, greater numbers of seeds germinate in the fall and survive through the following summer to be counted as adult plants (density counts are based only on the adults). Likewise, years with lower moisture show the opposite effect of decreased germination and density. From 2002-2003 density decreased with lower precipitation (11.99 inches and 13.16 inches, respectively), compared to 15.34 inches in 2001. In 2004 and 2005 precipitation increased to 22.83 inches and 19.35 inches, respectively, and knapweed density increased. In 2006 precipitation decreased to 10.65 inches and diffuse knapweed density once again decreased.

A comparison of cover versus precipitation suggests that cover generally follows the precipitation amounts for the same year (i.e., cover is higher with higher precipitation and vice-versa; Figure 14). This pattern was not maintained in 2004 and 2005, when cover did not increase with additional precipitation. In 2006 however, cover decreased once again with a decrease in precipitation.

One possible explanation for the 2004 and 2005 cover results was that the abundance of biocontrol insects had reached a level where they were starting to impact the diffuse knapweed plants. Not only do *Larinus* weevils destroy seed in the larval form, but they also forage on leaves and stems of the plant as adults. *Cyphocleonus achates* and *Sphenoptera yugoslavica* are two other biocontrol insects that stress knapweed plants by affecting the root systems. In 2006 precipitation may have played a greater role in the decrease of cover and density than the biocontrol insects.

Flowerhead counts from 2002−2006 also appear to correlate well with annual precipitation received. With increased precipitation the number of flowerheads produced per plant increased and vice-versa (Figure 15). From 2002−2005 the number of seeds produced per flowerhead also followed precipitation amounts, for both flowerheads with and without insect damage. Then in 2006 overall seed production increased and was significantly higher compared to all the other years of the study (Figure 14).

Even though for 2006 the seed production results do not coincide with the results of other studies regarding seed production and precipitation (Seastedt et al. 2003, 2005 and Schirman 1981), the increase in seed production may be due to the lower percent of flowerheads with insect damage. In 2006, the percentage of flowerheads with insect damage was significantly lower when compared to flowerheads with insect damage for all other years. The average number of seeds found in flowerheads with insect damage in 2006 was the highest number for the study. In addition, evidence of *Larinus* spp., *U. affinis* and unknown insects in flowerheads were lower in 2006 than for other years (Figure 16).

Seastedt et al. 2005 found that when *Larinus* weevil abundance is high, seed production is low, but when *Larinus* weevil abundance is moderate to low, seed production can vary from low to high. Also, *Larinus* weevils appear to lay few eggs in stressed plants, which could have been the case for 2006 as the November-October precipitation was the lowest since the study began.

Despite the fact that seed production per flowerhead was significantly higher in 2006 than in any other year, the average number of seeds per square meter was the second lowest during the study.

It typically takes 4−6 years after initial releases for the population levels of biocontrol insects to reach levels where they begin to have some effect on the target species populations. While the reduction in seed production is not as great as the biocontrol release study to the north of the Site on Boulder County Open Space land (Seastedt et al. 2003), the data show that the biocontrol insects appear to be having an affect on reducing knapweed cover and overall seed production at the Rocky Flats release locations as well.

The fact that the biocontrol insects have had some effectiveness in reducing the seed production of the diffuse knapweed at the Site is an important step towards controlling this annual species which reproduces by seed. The use of biocontrol insects alone will not likely completely control diffuse knapweed at the Site or most locations along the Front Range where it is problematic. However, biocontrol insects are one of several control methods for invasive species that can be used in conjunction with other control methods, or when other methods are inappropriate or not feasible.

Summary

Biological control insects have had an effect on diffuse knapweed at the Site. Overall reduction in diffuse knapweed cover and seeds per square meter has been observed. It is also apparent that climate (specifically precipitation) has a large influence in the annual variation of different measures of diffuse knapweed characteristics. Future observations will continue to evaluate the long-term effectiveness of biological controls on diffuse knapweed abundance at the Site.

References

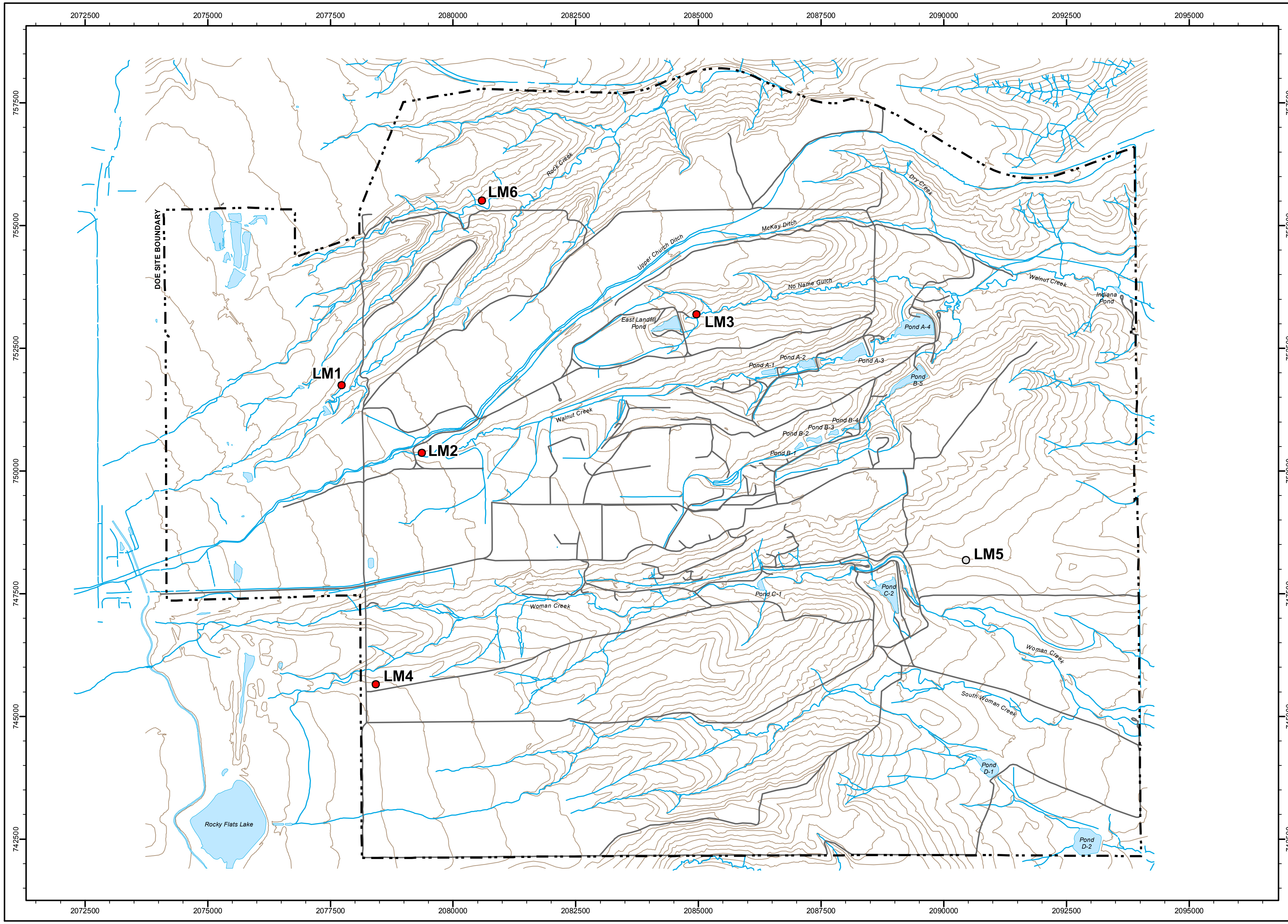
K-H, 2004. 2003 Annual ecology report for the Rocky Flats Environmental Technology Site. Kaiser-Hill Company, LLC, Rocky Flats Environmental Technology Site, Golden, Colorado.

Seastedt, T.R., N. Gregory and D. Buckner, 2003. Effect of biocontrol insects on diffuse knapweed (*Centaurea diffusa*) in a Colorado grassland. Weed Science 51:237-245.

Seastedt, T.R, K.N. Suding,and R.D. LeJeune, 2005. Understanding invasions: the rise and fall of diffuse knapweed (*Centaurea diffusa*) in North America. Inderjit (ed.), Invasive plants: ecological and agricultural aspects. Birkhauser Verlag, Switzerland.

Schirman, R, 1981. Seed production and spring seedling establishment of diffuse and spotted knapweed. Journal of range management 34(1):45-47.

SigmaStat, 2004. SigmaStat Advisory Statistics for Scientists, Version 3.1.1. Systat Software, Inc., Richmond, California.



2006 Biocontrol Study Locations at the Rocky Flats Site

Figure 1

LEGEND

- ● *Larinus minutus* biocontrol study location
- ○ *Larinus minutus* biocontrol study location not monitored in 2006
- Site boundary
- Road
- Stream, ditch, or other drainage feature
- Lake or pond
- Topographic contour (20-foot interval)

N

SCALE IN FEET

1,000 500 0 1,000 2,000

STATE PLANE COORDINATE SYSTEM
COLORADO CENTRAL ZONE
NORTH AMERICAN DATUM OF 1927

U.S. DEPARTMENT OF ENERGY GRAND JUNCTION, COLORADO	Work Performed by S.M. Stoller Corporation Under DOE Contract No. DE-AC01-02GJ79491
DATE PREPARED: January 29, 2007	FILENAME: S0296900-01

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Figure 2: Diffuse Knapweed flowerhead with no evidence of biocontrol insects. The phyllaries were pulled backwards and the seeds were pushed out from between the Pappus.

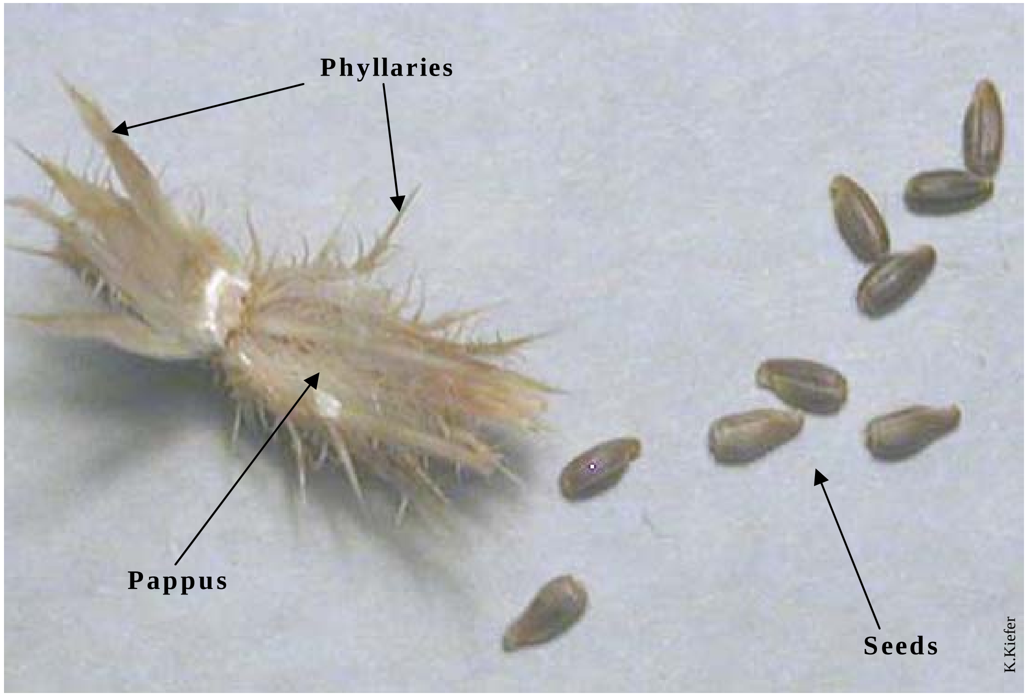


Figure 3: Larinus minutus weevil in a Diffuse Knapweed flowerhead. The flowerheads were cut lengthwise. In figure (a), the weevil is still inside its gall in the flowerhead. Note the “plug” (top of the corolla) that sat on top of the gall prior to cutting.

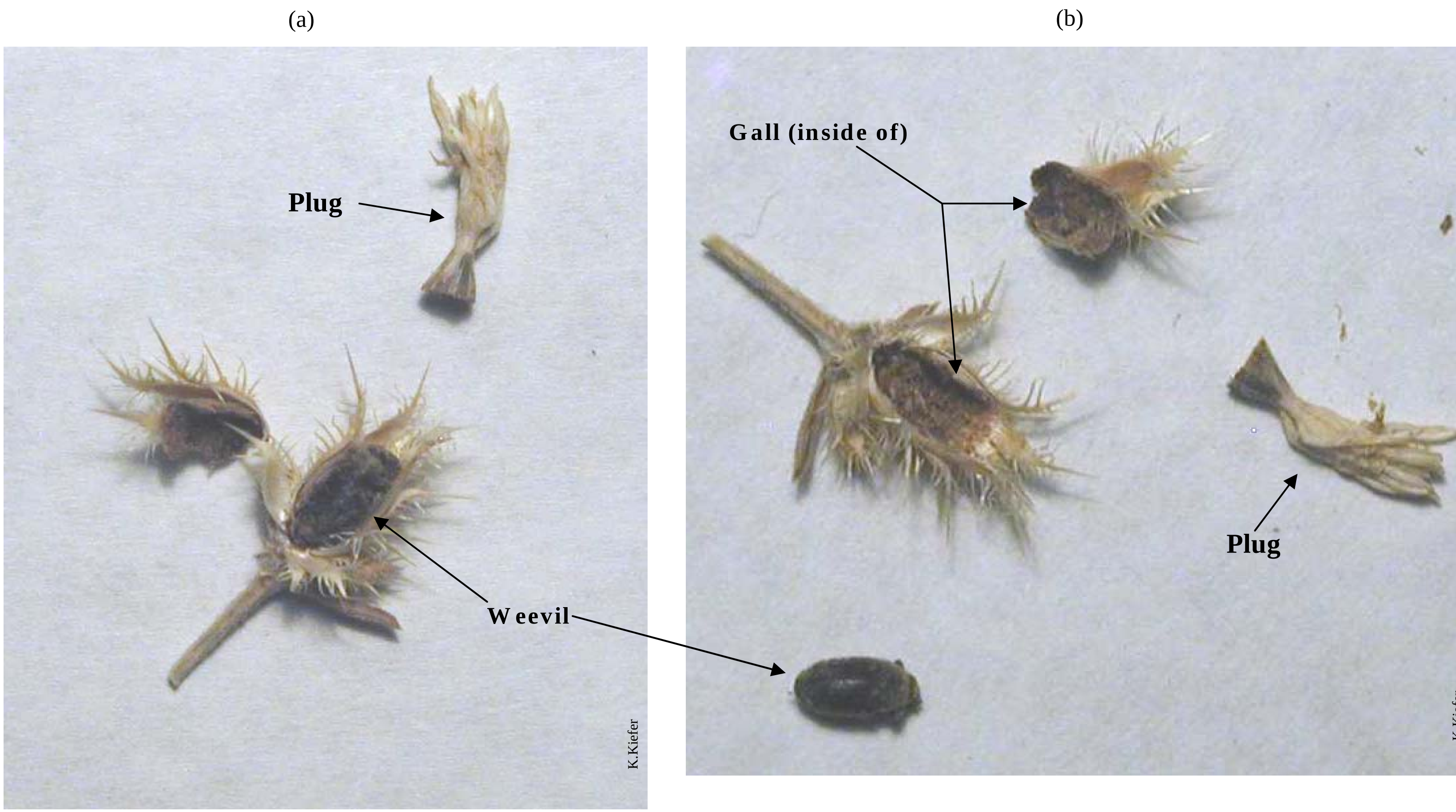


Figure 4 : *Larinus minutus* (a) and *L. obtusus* (b) damage on Knapweed flowerheads.

(a)

Photo by Bob Richard, USDA-APHIS-PPQ
http://mtwow.org/Larinus-minutus-photos.html

(b)

PhotoCredit:
Montana State University Archives
Montana State University
www.forestryimages.org

Figure 5: *Urophora* fly gall and larva. The larva in (a) is still inside the gall. Note the presence of a few seeds.

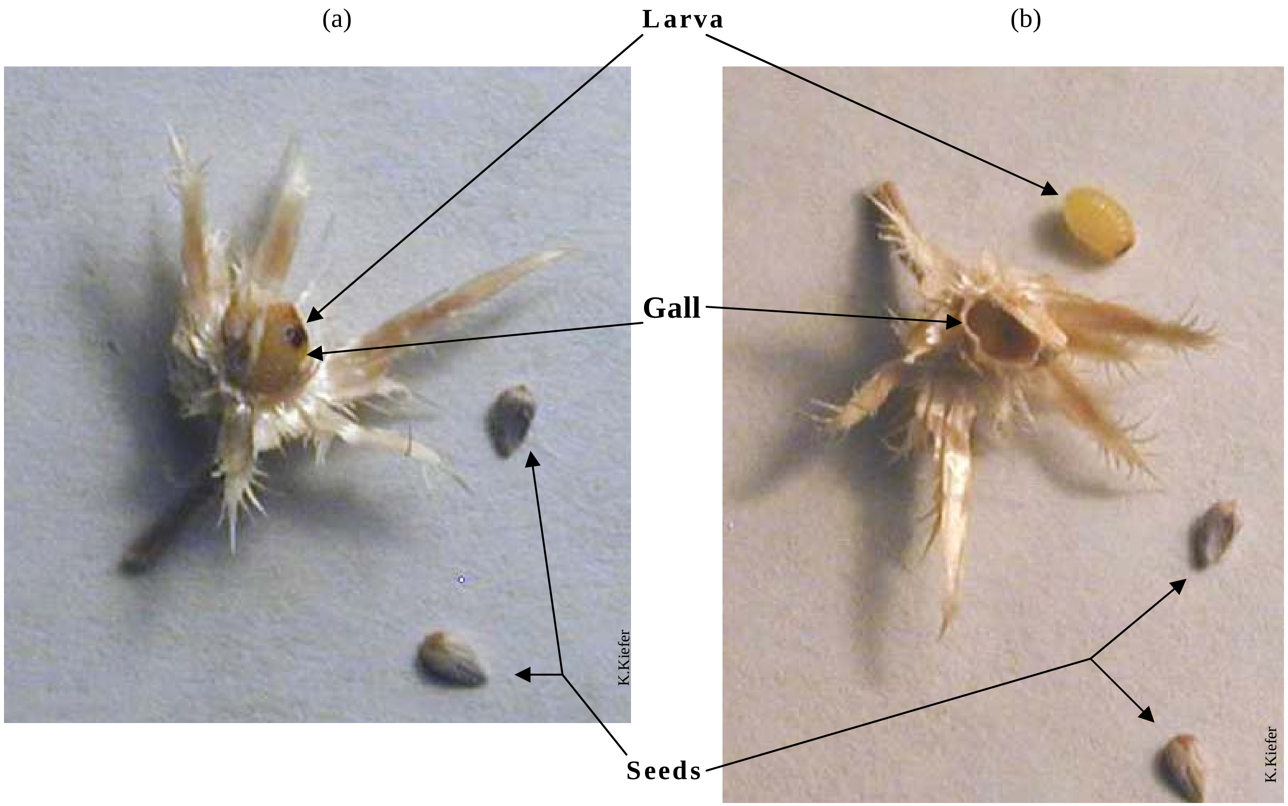


Figure 6: Insect feeding damage on Diffuse Knapweed flowerhead. The flowerhead is cut lengthwise. Note detritus and lack of seeds.

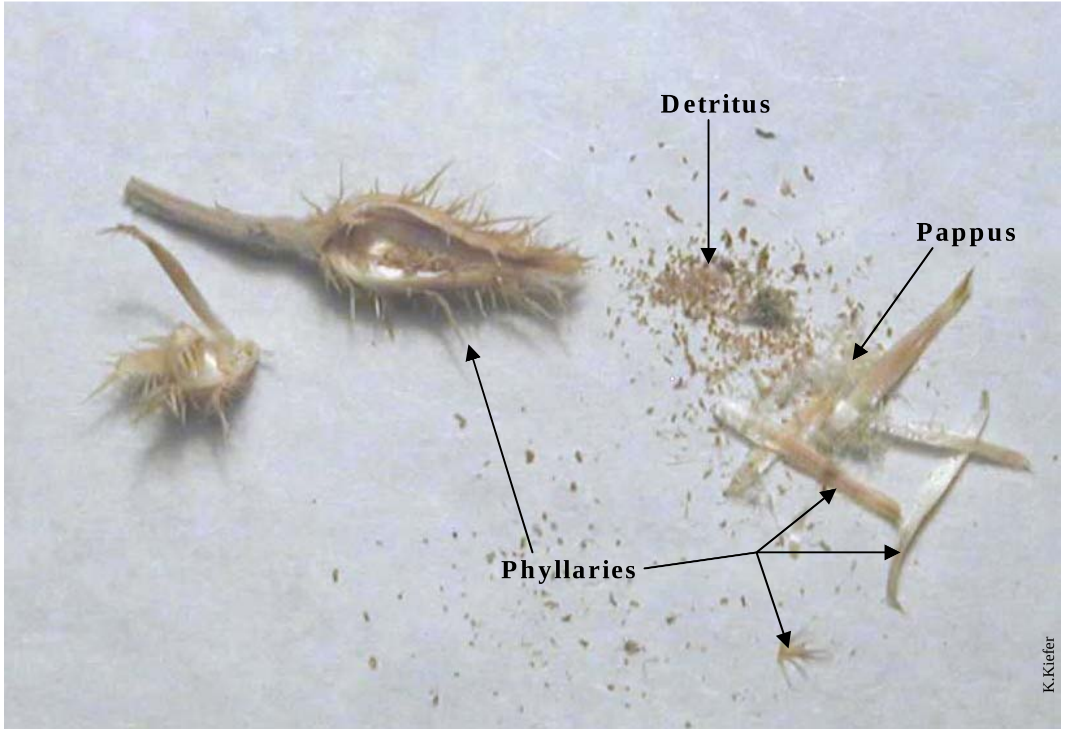


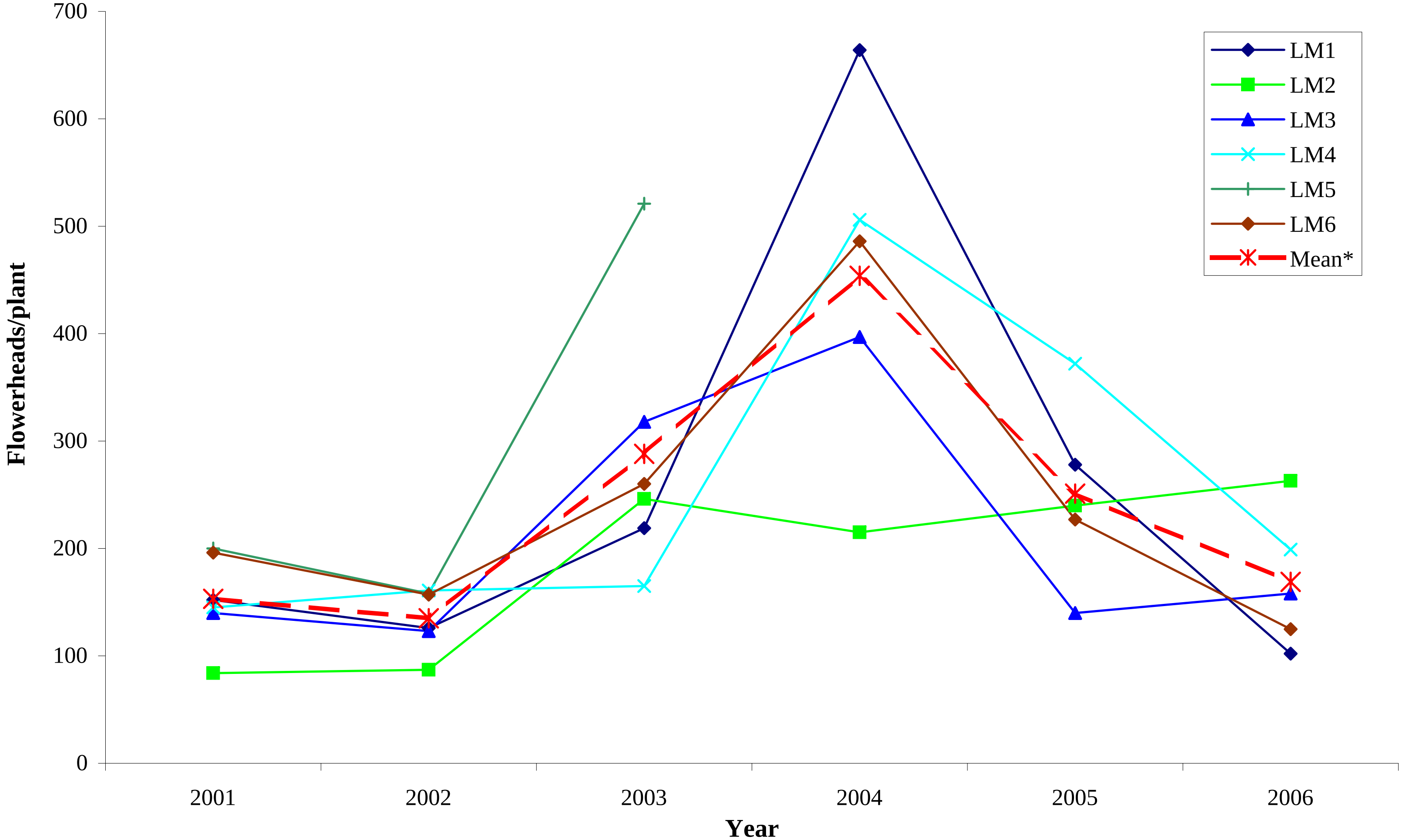
Figure 7: Diffuse Knapweed Cover at Biocontrol Release Locations (2001-2006)

*The mean includes data from all locations sampled during a specific year.

Figure 8: Diffuse Knapweed Density at Biocontrol Release Locations (2001-2006)

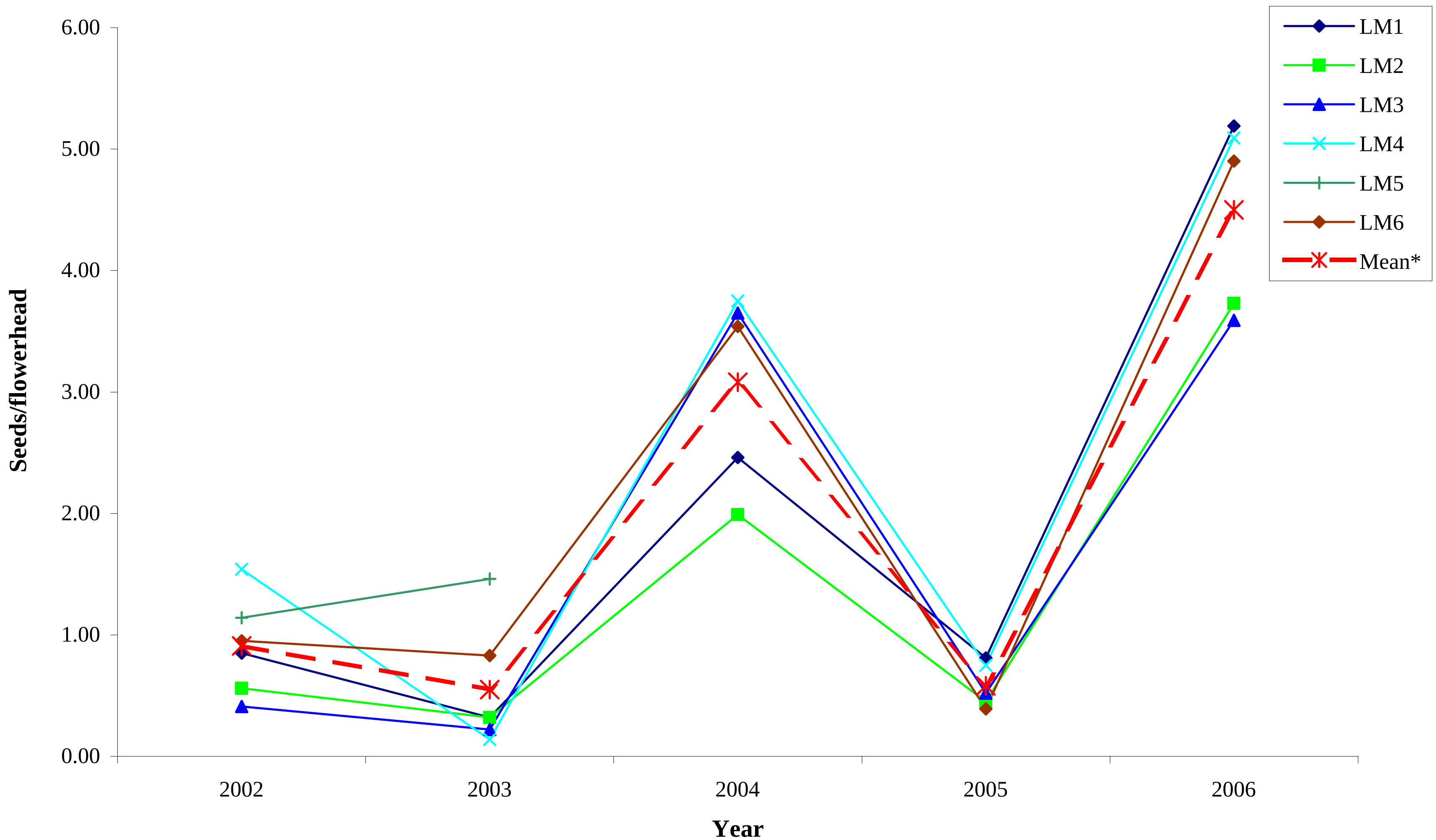
*The mean includes data from all locations sampled during a specific year.

Figure 9: Average Number of Diffuse Knapweed Flowerheads per Plant at Biocontrol Release Locations (2001-2006)



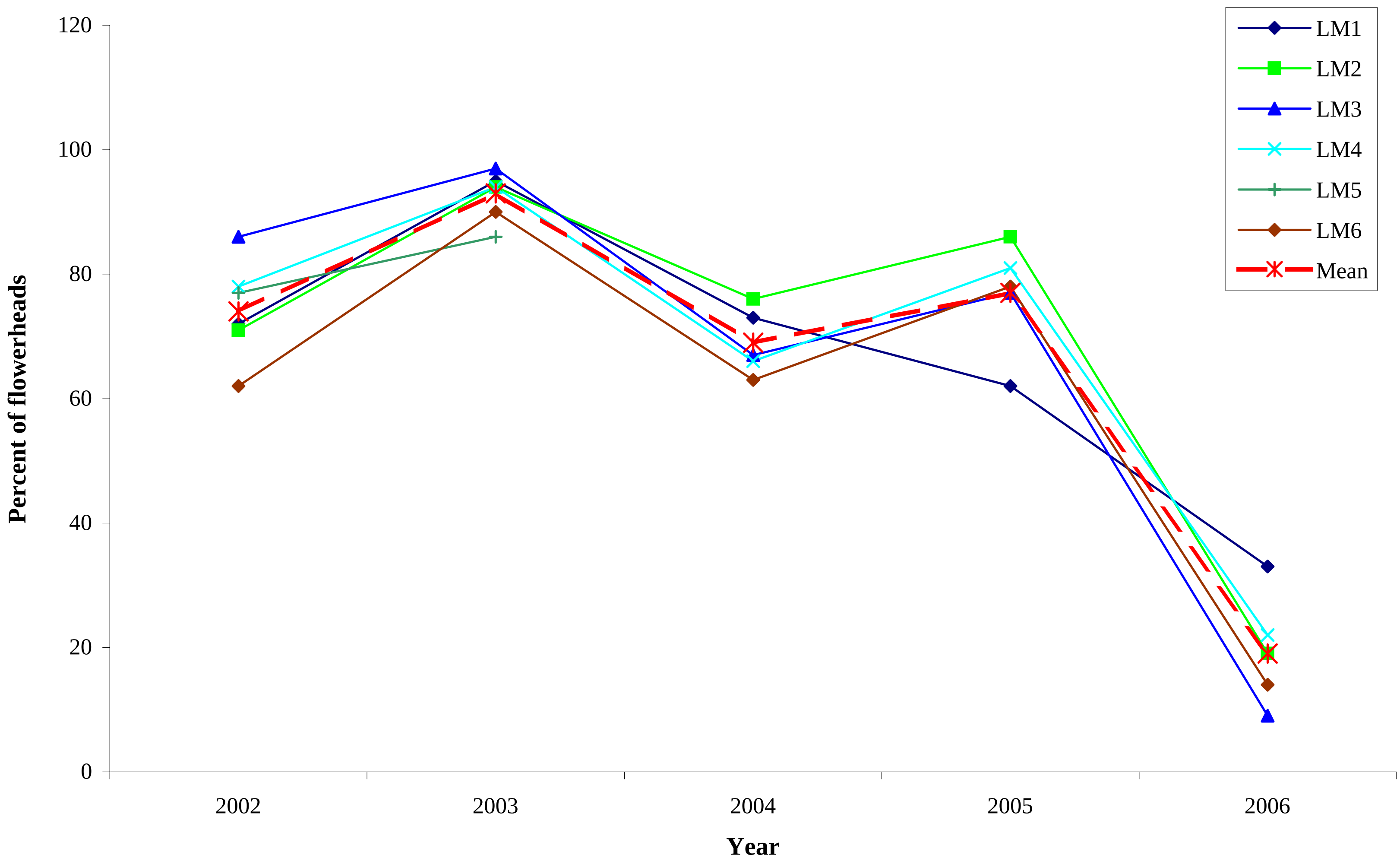
*The mean includes data from all locations sampled during a specific year.

Figure 10: Average Number of Diffuse Knapweed Seeds per Flowerhead at Biocontrol Release Locations (2002-2006)



*The mean includes data from all locations sampled during a specific year.

Figure 11: Percent of Diffuse Knapweed Flowerheads with Evidence of Insect Damage at Biocontrol Release Locations (2002-2006)



*The mean includes data from all locations sampled during a specific year.

Figure 12: Percent of Diffuse Knapweed Flowerheads Divided into Insect Damage Categories at Biocontrol Release Locations (2004-2006)

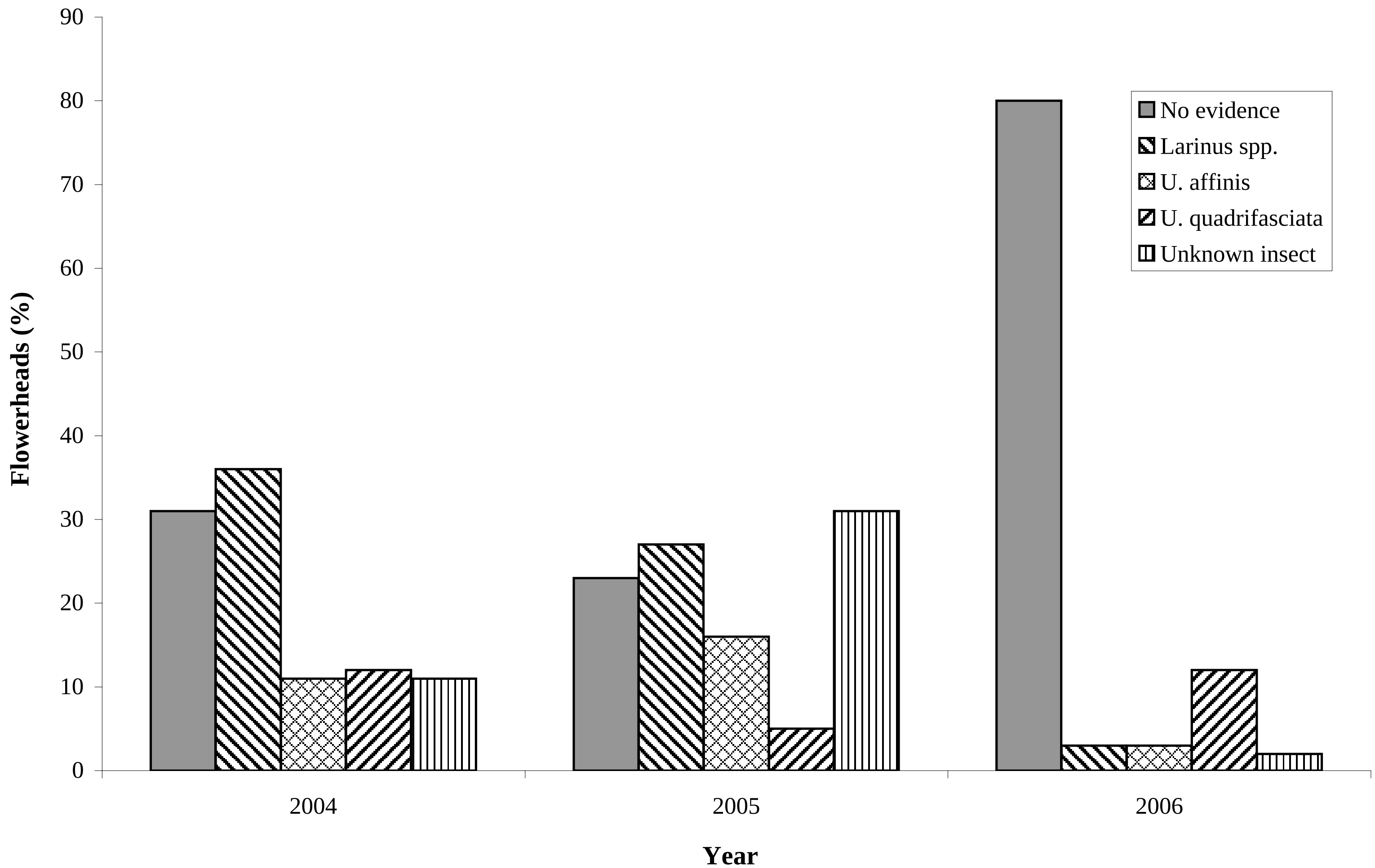
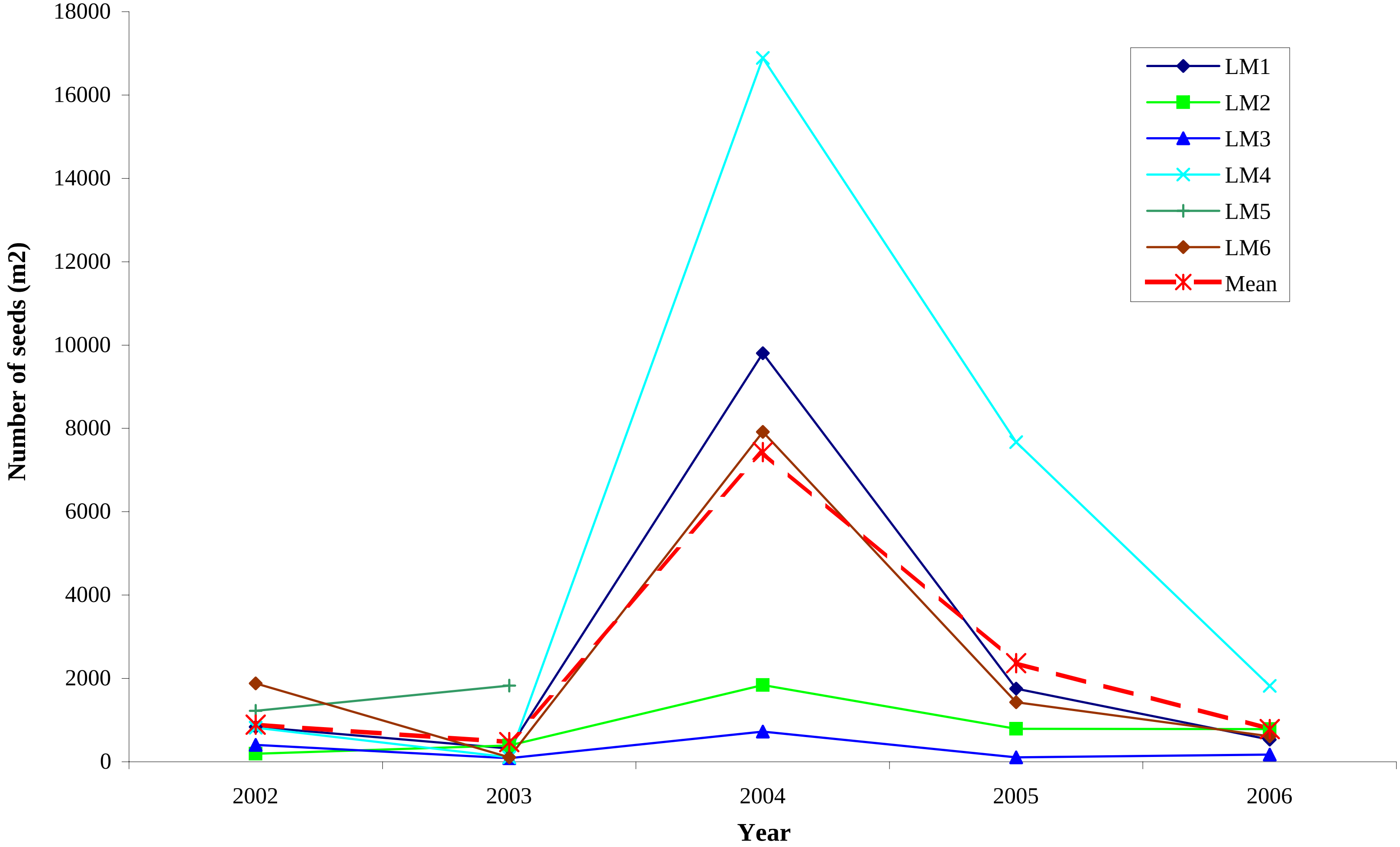


Figure 13: Average Number of Diffuse Knapweed Seeds per Square Meter at Biocontrol Release Locations (2002-2006)



*The mean includes data from all locations sampled during a specific year.

Figure 14: Rocky Flats Precipitation versus Diffuse Knapweed Cover, Density and Number of Seeds/flowerhead (2001-2006)

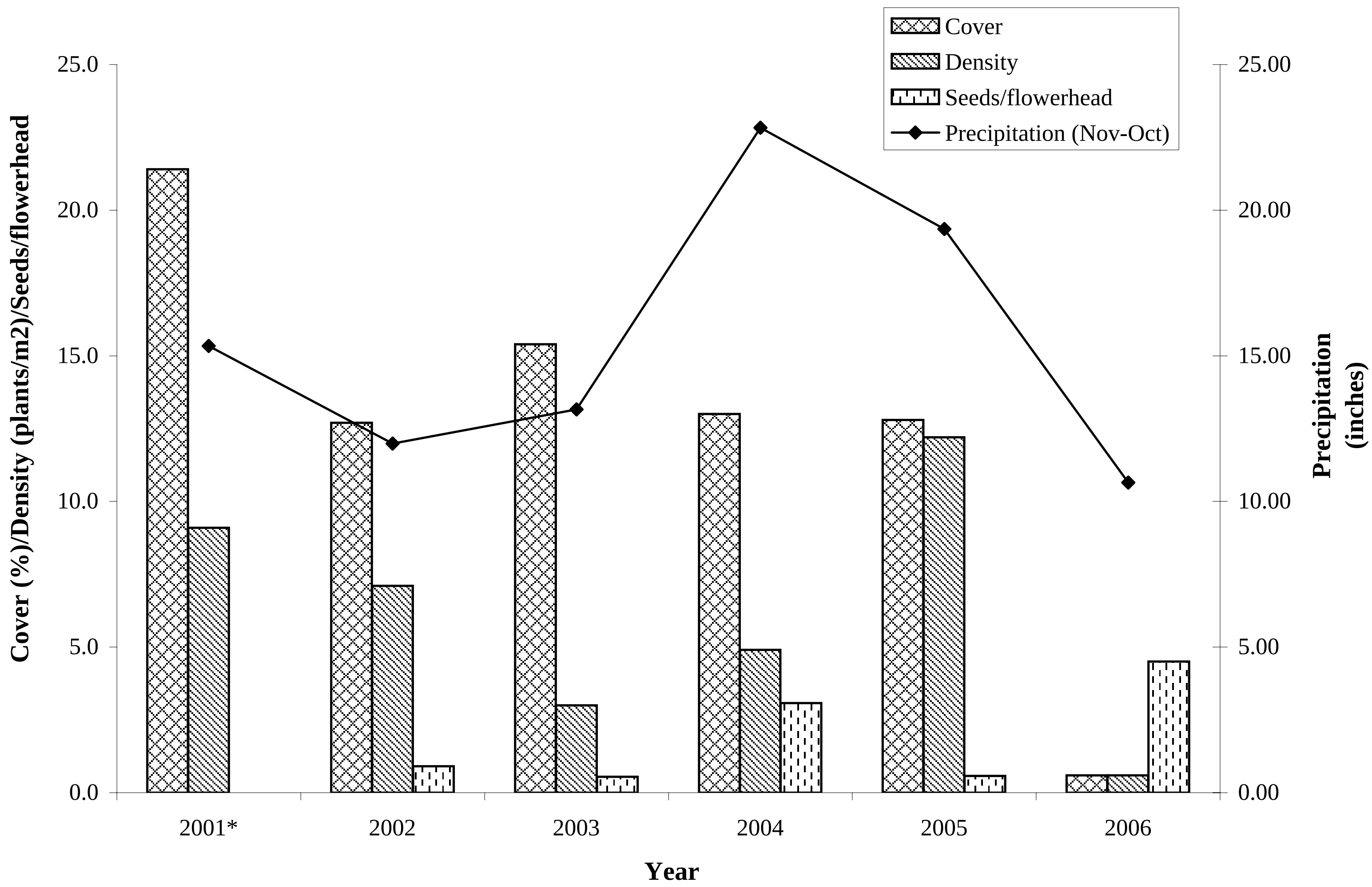


Figure 15: Rocky Flats Precipitation and Mean Number of Flowerheads/plant of Diffuse Knapweed at Biocontrol Release Locations (2001-2006)

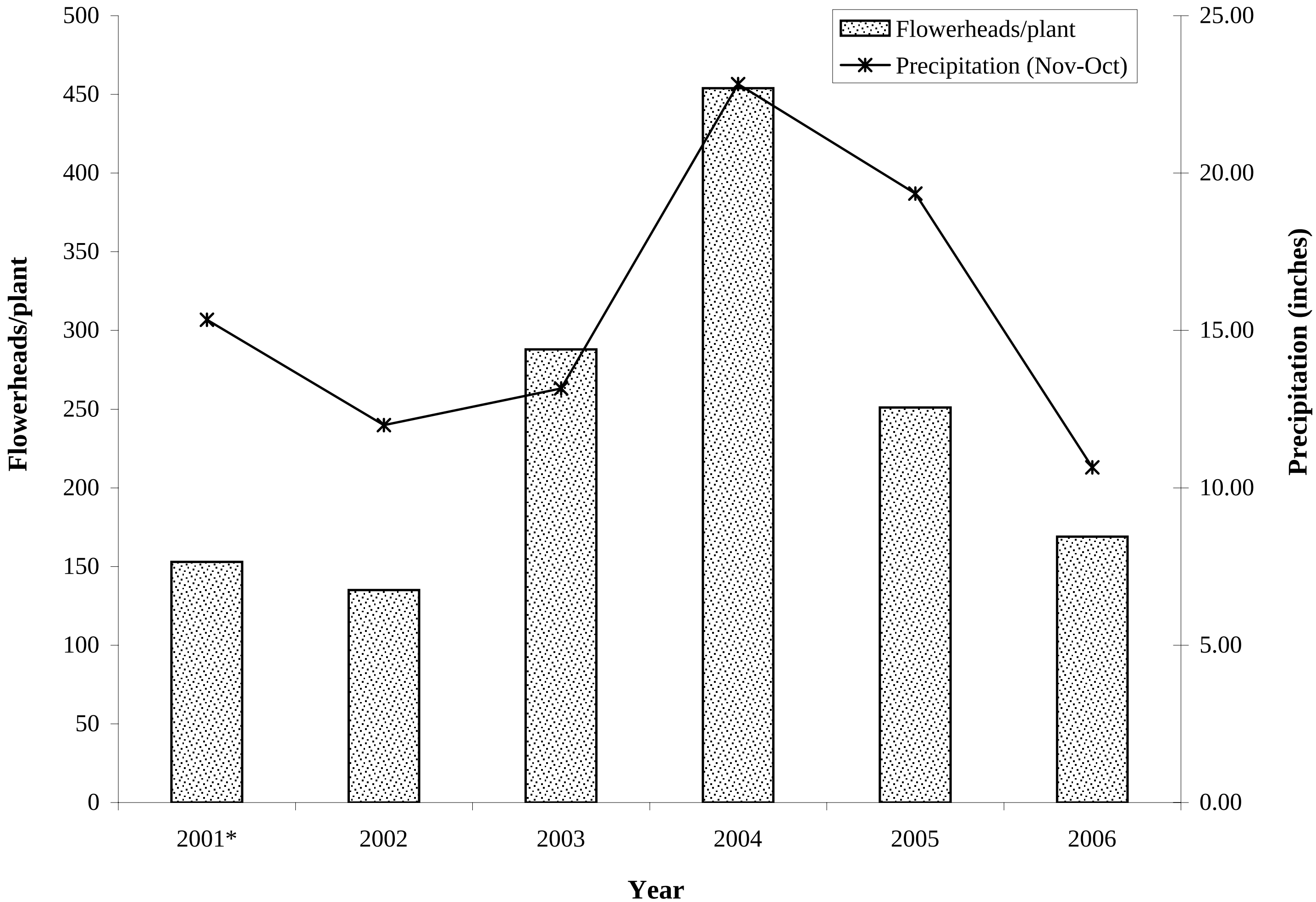


Figure 16: Percent of Diffuse Knapweed Flowerheads Divided into Insect Damage Categories and Mean Number of Seeds/flowerhead with and without Insect Damage at Biocontrol Release Locations (2004-2006)

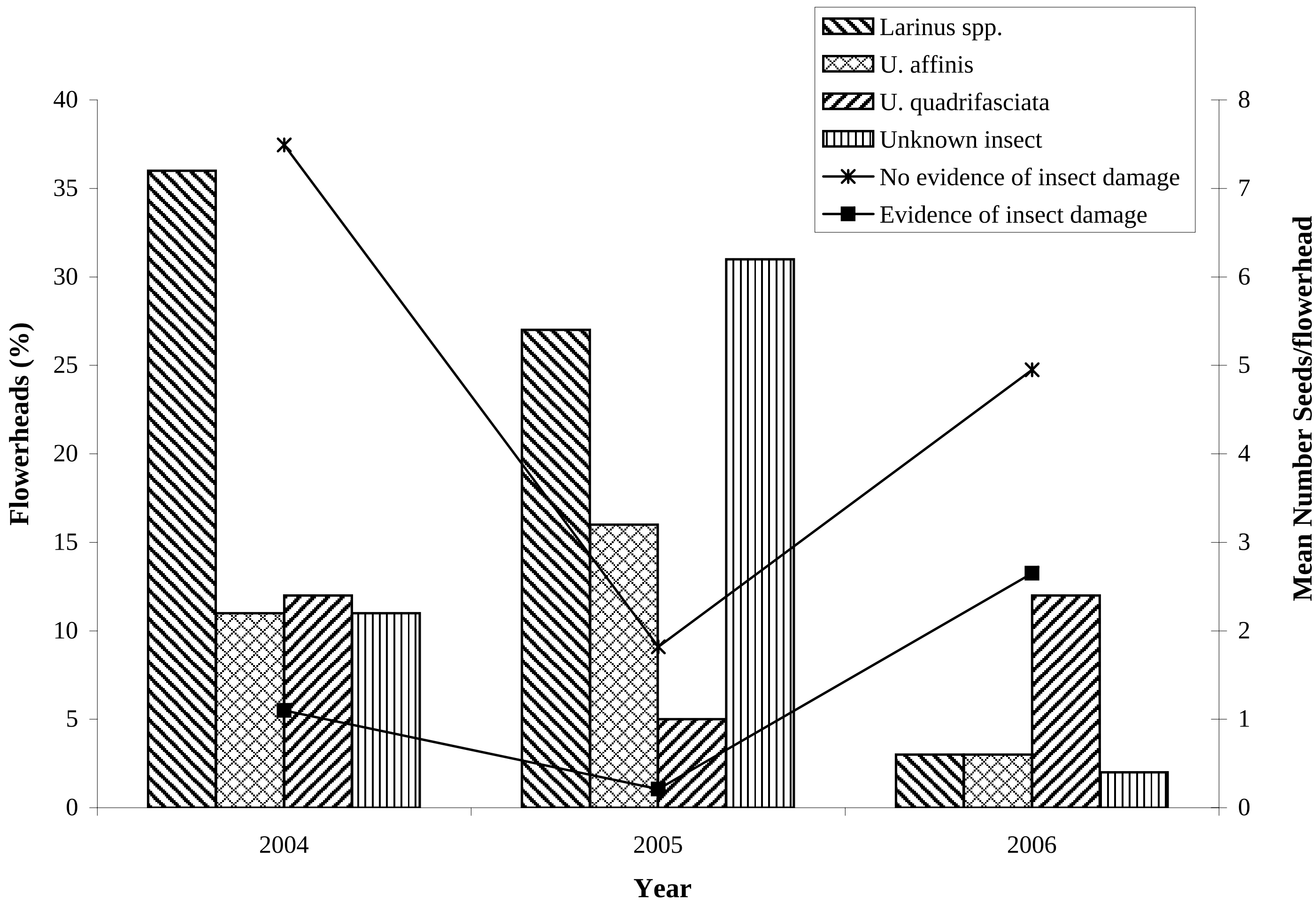


Table 1. Average percent cover of diffuse knapweed at biocontrol release locations (2001-2006).

Location	2001	2002	2003	2004	2005	2006
LM1	33.5	15.9	12.7	14.1	11.7	0
LM2	19.4	6.0	19.2	11.7	6.8	1.5
LM3	21.6	12.3	9.2	1.8	3.3	0.3
LM4	26.1	10.4	22.0	23.8	32.1	1.2
LM5	6.3	14.5	24.1	N/A	N/A	N/A
LM6	N/A	17.1	5.4	13.8	10.1	0
Mean	**21.4**	**12.7**	**15.4**	**13.0**	**12.8**	**0.6**

Table 2. Average density (plants/m$^{2)}$ of diffuse knapweed plants at biocontrol release locations (2001-2006).

Location	2001	2002	2003	2004	2005	2006
LM1	15.8	7.8	4.6	6.0	7.8	0
LM2	14.5	3.9	5.0	4.3	7.3	0.8
LM3	4.4	8.0	1.2	0.5	1.5	0.3
LM4	9.5	3.3	4.5	8.9	27.5	1.8
LM5	1.1	6.8	2.4	N/A	N/A	N/A
LM6	N/A	12.6	0.5	4.6	16.8	0
Mean	**9.1**	**7.1**	**3.0**	**4.9**	**12.2**	**0.6**

Table 3. The average number of flowerheads per plant at biocontrol release locations (2001-2006).

Location	2001	2002	2003	2004	2005	2006
LM1	152	126	219	664	278	102
LM2	84	87	246	215	240	263
LM3	140	123	318	397	140	158
LM4	145	161	165	506	372	199
LM5	200	158	521	N/A	N/A	N/A
LM6	196	157	260	486	227	125
Mean	**153**	**135**	**288**	**454**	**251**	**169**

Table 4. The average number of seeds per flowerhead (out of 500 flowerheads examined) at biocontrol release locations (2002-2006).

Location	2002	2003	2004	2005	2006
LM1	0.85	0.32	2.46	0.81	5.19
LM2	0.56	0.32	1.99	0.45	3.73
LM3	0.41	0.22	3.65	0.52	3.59
LM4	1.54	0.14	3.75	0.75	5.09
LM5	1.14	1.46	N/A	N/A	N/A
LM6	0.95	0.83	3.54	0.39	4.9
Mean	**0.91**	**0.55**	**3.08**	**0.58**	**4.50**

Table 5. Percent of flowerheads (out of 500 flowerheads examined) with evidence of insect damage at biocontrol release locations (2002-2006).

Location	2002	2003	2004	2005	2006
LM1	72	95	73	62	33
LM2	71	94	76	86	19
LM3	86	97	67	77	9
LM4	78	94	66	81	22
LM5	77	86	N/A	N/A	N/A
LM6	62	90	63	78	14
Mean	**74**	**93**	**69**	**77**	**19**

Table 6. The average number of seeds per flowerhead (out of 500 flowerheads examined) at all five release locations, calculated for the following categories: all flowerheads, flowers with evidence of insect damage, and flowerheads with evidence of no insect damage (2002-2006).

Year	Mean # seeds (all)	Mean # seeds (w/evidence)	Mean # seeds (no evidence)
2002	0.91	0.37	2.48
2003	0.55	0.14	5.66
2004	3.08	1.10	7.49
2005	0.58	0.21	1.82
2006	4.5	2.65	4.95

Table 7. The percent of flowerheads (out of 500 examined) at all five release locations with the following flowerhead damage: no evidence of insect damage, evidence of *Larinus* spp., evidence of *U. affinis*, evidence of *U. quadrifasciata*, and evidence of an unknown insect (2004-2006).

Year	No evidence	*Larinus* spp.	*U. affinis*	*U. quadrifasciata*	Unknown insect
2004*	31	36	11	12	11
2005*	23	27	16	5	31
2006	80	3	3	12	2

*The total percentages sum to a slightly higher number than 100, due to the overrepresentation of three flowerheads in 2004 and eight flowerheads in 2005 that showed evidence of damage from multiple insects.

Table 8. The average number of diffuse knapweed seeds per square meter at biocontrol release locations (2002-2006).

Location	2002	2003	2004	2005	2006
LM1	835	322	9801	1756	529
LM2	191	394	1840	788	785
LM3	403	84	725	109	170
LM4	820	104	16888	7673	1823
LM5	1226	1827	N/A	N/A	N/A
LM6	1879	108	7914	1427	613
Mean	**892**	**473**	**7433**	**2363**	**784**