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Precipitation and the Interaction of Seedhead Biological Control Insects for Spotted Knapweed in the Rocky Mountain Front Range

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Abstract

Biological control of spotted knapweed (*Centaurea stoebe*) is an ongoing topic of research that holds ecological importance due to the extent to which knapweed has spread across the Western United States. Using herbivores (insects) as biological controls of spotted knapweed can be successful at reducing plant density in certain habitats. However, the exact relationship among the insects, knapweed, and abiotic factors affecting knapweed growth such as precipitation, remains uncertain. Through collection and dissection of knapweed seedheads, I estimated densities of two insects, *Larinus minutus* and *Urophora affinis*, as well as the average number of seeds produced by seedheads of spotted knapweed at a long-term study site in the Front Range of Colorado The data I collected were then added to a data set collected at this site extending back to 2002, which represents the longest data set of its kind presently available. After inclusion of my data, I found a positive trend between precipitation and seed production that differed from the findings of previous work. Insect presence decreased the number of seeds per seedhead, and this effect was independent of the amount of precipitation. I also found that the interaction of the two species of insects in the seedheads negatively affected the individual effectiveness of each insect, although not significantly, which supports previous research. My findings will benefit model predictions for knapweed spread and inform land management decisions about spotted knapweed infestations in the Rocky Mountain Front Range.

Introduction

Spotted knapweed (*Centaurea stoebe*) is a highly invasive plant in North America that was imported from Eastern Europe sometime in the late 19th century. This invasive species spread rapidly across nearly every state and developed a reputation as the "wicked weed of the west" for its ability to outcompete native plants and create monocultures (Alper 2004). These monocultures collectively cover millions of acres and cause substantial losses in agricultural production, and burden land managers with the cost of herbicides and manual removal to control infestations (Alper 2004). Not only do species within this genus of knapweeds cause monetary loss to property owners, they also have the potential to change soil biogeochemistry (LeJeune and Seastedt, 2001), and degrade native habitats (Wright & Kelsey, 1997). Migrating elk populations in Montana have been observed changing their migration routes to avoid knapweed-infested landscapes (Alper 2004). It was previously believed that knapweed utilized the chemical (-) catechin to inhibit the growth of native plants, through an effect known as allelopathic inhibition, (Callaway & Ridenour 2004), however, more recent work has shown that there is not a strong allelopathic effect under most soil conditions (Duke et al. 2009; Blair et al. 2005, 2006, 2009; Tharayil et al. 2008). More likely, the competitive advantage of knapweed is due to its deep tap root, high seed production, and organic compounds present in the plant tissue causing a bitter taste that can reduce its use as forage by some livestock and native fauna (Olson and Kelsey, 1997). Spotted knapweed is a short-lived perennial, and tends to grow rapidly when both moisture (Sheley et al. 1998) and soil nitrogen (Knochel et al. 2010) are abundant.

Plant distributions are determined by many factors, but arguably none more so than precipitation (Bradley et al 2009). Changes in climate and precipitation patterns are, therefore, important to consider when predicting how populations of invasive species will change. Not only could a change in climate cause a habitat to become totally unsuitable for a species and lead to its extirpation, but a less dramatic change could alter the competitive balance between invasive and native species, such that the habitat becomes less invasible and the introduced species persist but in much lower densities.

Biological controls are another way of how the competitive balance can be shifted between invasive and native plants. Numerous insects have been released as biological controls in North America in attempts to control spotted knapweed and the closely related diffuse knapweed (*Centaurea diffusa*). There are several reasons to release more than one biological control insect at a time. The insects will have varying effectiveness depending on the new environment. A native predator could target one of the insects when it is introduced, or the knapweed may thrive in a new environment in which the insects cannot sustain a population. There is also the idea, known as the cumulative stress hypothesis (Stephens et al. 2013), that multiple different species attacking the plant will result in higher overall mortality as small amounts of damage add up. Having the plant stressed by multiple sources - insects, competition and grazing - appears to be most effective at reducing density over time (Muller-Scharer and Schroeder, 1993). It has also been noted that using more than one insect is most effective when these insects attack different parts of the plant, for example a seedhead weevil and a root weevil (Stephens et al. 2013). In the Front Range of Colorado, there are five biological control species established : two gall flies (*Urophora affinis* and *quadrifasciata*), one seedhead weevil (*Larinus minutus*), and two root-boring beetles (*Cyphocleonus achates, Sphenoptera jugoslavica*).

While previous studies using information from this data set have focused on all five of the established biological control insects in the Front Range (Knochel and Seastedt 2009, 2010; Knochel et al 2010 a, b; Wooley et al 2011), the present study focuses specifically on the interaction of insects that consume plant materials in the knapweed seedheads, i.e., *U. affinis* and *L. minutus*. A study in Montana showed that competition with a native grass and the release of different biological controls caused an over-compensatory response in knapweed (Ridenour & Callaway, 2003). An over-compensatory response is when a plant reacts to damage by regrowing more biomass than was lost from the damage. However, releasing two root weevils (*C. achates, S. jugoslavica*), two gall flies (*U. affinis* and *quadrifasciata*), and the seedhead weevil (*L. minutus*) in Colorado reduced the reproductive output, size, and densities of invasive plants at this site in previous years (Knochel and Seastedt 2009, 2010; Knochel et al 2010 a, b; Wooley et al 2011). The consensus from these findings is that the biological controls have the potential to be effective in the management of spotted knapweed, but that information is lacking on how these effects vary across a large range of climatic conditions. Maines et al. (2013a) modeled knapweed population dynamics and noted that both reproduction (seed production) and survivorship needed to be reduced in order to control spotted knapweed populations . Empirical data to inform that model were, however, limited. With additional knowledge about the interaction between climate and biological controls, decisions about how to manage lands invaded by spotted knapweed can be informed in a context-specific manner, and more targeted management approaches can be taken rather than using a general approach that may not work in certain places.

Information on spotted knapweed seed production and seedhead insect impacts have been obtained from a site in the Front Range since 2002 (Seastedt et al. 2007; Maines et al. 2013b), allowing for a deeper understanding of trends that datasets collected over a shorter time period cannot answer. The available 11-year data set combined with my own studies at this site allowed me to address several questions: 1) How does precipitation affect spotted knapweed in both the presence and absence of biological controls? 2) How does the presence of *U. affinis* affect the ability of *L. minutus* to decrease viable seed production in a seedhead? 3) How does a native herbivore affect the ability of *U. affinis* and *L. minutus* to control spotted knapweed? The results from the present study will allow for predictions to be made of how spotted knapweed populations may change in density over coming decades both with and without biological controls added to the system.

Methods

Spotted knapweed samples for this study were collected from Spruce Gulch, located on private land northwest of Boulder in Lefthand canyon (approx. 40° 07′ N, 105° 18′ W; elev 1910-2070 m). Average annual precipitation over the past 15 years is 52.7 cm. Precipitation data for this study were gathered from the NOAA site's database of monthly precipitation for Boulder Colorado (NOAA 2015). The site contains an infestation of spotted knapweed primarily focused within and around an area that was burned in 1988, with portions of the area burned again in 2003. Approximately half of this area is in a flat meadow several miles up a small canyon with a stream, Spruce Creek, flowing through it. The knapweed has formed thick stands (monocultures) in some areas around the meadow and upstream as well as having colonized the surrounding hillsides. A more detailed description of this site can be found in Knochel and Seastedt (2009). The samples collected during the 2014 growing season for the present study were taken at three different sites near this meadow, one within the flat portion of the meadow and two others upstream several hundred meters on north-facing slopes. Previous sampling by

Seastedt et al. (2007) and Knochel et al. (2010) showed that landscape aspects such as slope and direction of exposure had a modest impact on attack rates of the insects. In the present study, the emphasis was on yearly abundances and the relationship of knapweed, insects, and precipitation rather than landscape aspect. Samples collected in the 2015 season were from two sites in the meadow and one from a site lower in the canyon detailed in Seastedt et al. (2007). In previous years samples were taken from these and other nearby slopes directly adjacent to the meadow and from various spots further down the canyon. However, the knapweed at most of these sites had either been removed mechanically or scoured by a flood resulting from an extreme precipitation event in September of 2013.

Collections were taken of mature knapweed seed heads, evidenced by the presence of senesced flower petals about to fall out of the seed head. At each of the three sites, between 35 and 40 seedheads were randomly collected by tossing a stick/rock and taking one seed head from whichever plant was hit by or was closest to where the stick/rock landed. Only one seedhead was collected from each plant. Seedheads were brought back to the laboratory, where about 30 seedheads from each site were randomly selected and dissected. Seeds and insects were counted using either a dissection scope or a hand lens. Upon dissection, both the number of viable seeds as well as the number of *L. minutus*, and *U. affinis* were quantified. Viable seeds were defined as those that appeared healthy (smooth/unshriveled, dark in color) and had not been chewed on by *L. minutus* larvae. *L. minutus* were identified by life stage at the time of dissection. The different life stages used were larvae, pupae, adult, and "gone",(presence of chewed seeds but no insect). Often, there was an obvious exit hole from the seed head through which the *L. minutus* had apparently exited, although some of those recorded as "gone" may have been preyed upon by predaceous insects prior to maturation. Each year the first field seedhead collections were conducted at the beginning of August and then once for each subsequent two week period for at least one month. This process continued until plants senescenced and there were not enough unopened seedheads to collect. This resulted in an average of 430 seedheads collected each year with a range from 252 to 1189 for the complete data set.

No quantitative measurements of herbivory were made, but qualitative observations indicated the extent of seedhead and foliage damage generated particularly by *L. minutus*, but also by generalist feeders such as grasshoppers. Measurements of other biological controls of knapweed present at the site were occasionally made, but those results are not reported here.

Annual mean values were calculated for seeds, *L. minutus*, and *U. affinis*, and analyzed with the CORR procedure in SAS to obtain Pearson product-moment correlation. The means were also analyzed with the GLM (General Linear Model) procedure in SAS, which is an ANOVA that better handles variables that have varying numbers of samples.

Results

Spotted knapweed seed production per seedhead was variable throughout the years, with significantly different levels of seed production in many consecutive years (Figure 1a). *Larinus minutus* and *Urophora affinis* densities were also variable throughout the time period investigated, although *L. minutus* showed more consistency between consecutive years and inconsistencies can be more easily explained by other environmental factors (Figure 1b and 1c).

Negative correlations were observed between seed production and the presence of *L. minutus* (r= -0.305, p<0.0001, n=6026), *U. affinis* (r= -0.1524, p<0.0001, n=6026), and that of both insects (r=-0.2723, p<0.0001, n=6026). There was also a negative correlation between *L. minutus* and *U. affinis* presence (r= -0.1167 p<0.0001, n=6026) (Table 1). In addition to these correlations, *L. minutus* significantly decreased seed counts when the yearly effects were averaged (ANOVA, F=12.45, p=0.0009). However, *U. affinis* had no significant effect (ANOVA, F=2.53 p=0.1178) on seeds or *L. minutus*' effect on seeds (ANOVA, F=2.1, p= 0.1531) (Table 2). Presence of *L. minutus* in a seed head decreased seed production by 50.7% and presence of *U. affinis*, decreased seed production by 26.5%, which was, however, not a significant effect (Table 2).

In addition to herbivory by adult *L. minutus* on the knapweed, a local grasshopper was first observed consuming knapweed in 2012. There was increased herbivory in 2013, so much so that many knapweed plants were almost completely defoliated (Seastedt 2015). This phenomenon occurred again in 2014 with a similar intensity with all seedheads at the lower monitoring sites and many at the upper sites being consumed. While still present in 2015, the grasshoppers caused much less damage to the knapweed compared to the previous two years.

Comparison of precipitation and seed counts revealed a trend for spotted knapweed of higher seed production with increased precipitation (Figure 2). This trend existed both in the presence and absence of insects (Figure 3). Interestingly, the trend was not linear for higher amounts of precipitation. In a previously established trend from this same data set the predicted number of seeds produced per seedhead in 2014 and 2015 was estimated to be 6.93 and 5.01 respectively. However the actual numbers of seeds produced were 12.5 (181% higher) and 12.7 (253% higher).

I found that across all years and precipitation levels observed, presence of insects (either *L. minutus* or *U. affinis*) apparently served to decrease the number of seeds produced (Figures 3,4,5). Previously a comparison was done of how presence and absence of insects in a seedhead affected the production of seeds based on precipitation. It found that not only did the presence of insects decrease the absolute number of seeds produced per seedhead, it also reduced the slope of the trend between precipitation and seed production. In other words, more precipitation with insects present resulted in a smaller increase in seed production than in the absence of insects. I found that by including the years of 2014 and 2015, the differences in slopes of the two curves disappeared and the only remaining effect was the reduction of seeds associated with insect presence. This result is also apparent in Figure 5, which shows that for a small range of precipitation levels there is large variation in the decrease in seed production associated with insect presence. There was no pattern for insect effectiveness at seed reduction throughout the years (Figure 6a). The two years with much lower insect effectiveness, 2004 and 2014, were marked by significantly lower *L. minutus* densities than the other years (Figure 1b).

Discussion

The goals of the present study were to determine associations between precipitation and effectiveness of *L. minutus* and *U. affinis* as biological controls for spotted knapweed and to determine if the negative interactions reported for these two insects (Seastedt et al. 2007) were having a significant effect on their roles as biological controls. Additionally, I wanted to see how a native grasshopper affected the biological control insects and the spotted knapweed.

There are several factors that can explain the negative correlations between insects and seed production. Most obviously, the fact that *L. minutus* directly consumes seeds and *U. affinis* draws resources away from the plant such that fewer seeds are produced. There are several possible explanations for the negative correlation seen between *L. minutus* and *U. affinis* co-infestation as well. It has been previously observed for this population that *L. minutus* will sometimes consume *U. affinis* when they co-infest a seedhead (Seastedt et al. 2007). This effect would cause underestimation of co-infestation and overestimation of how many seedheads contained *L. minutus* only. It is also possible that *L. minutus* and *U. affinis* look for different characteristics in seedheads or plants when choosing where to lay their eggs. There is observational evidence at this site for *L. minutus* and *U. affinis* having different preferences for which knapweed seedheads they choose. Predation of *U. affinis* by *L. minutus* may also explain why there is a stronger negative correlation between seeds and *L. minutus* when only *L. minutus* is present. It was expected that, because *L. minutus* and *U. affinis* damage the plant in different ways despite targeting the same part of the plant, their co-infestation would result in an additive effect on seed production, reducing it more together than either individually. It was also expected that the effect of co-infestation would be even greater than added independent effects because the greater level of stress experienced by the plant would limit seed production even more. These ideas, however, do not take into account the predation of *U. affinis* by *L. minutus*, which would cause less time to be spent by the larvae on consumption of seeds, thereby diminishing the effect of *L. minutus* and *U. affinis*. The finding that co-infestation leads to diminished effects by the insects matches with what Stephens et al. (2013) found. The *U. affinis* reduced the number of seeds available for the *L. minutus* to eat, which in turn may facilitate the consumption of *U. affinis* by *L. minutus*

The trend of increased seed production with increased precipitation was expected. When plants are given access to more resources, they will grow larger, grow more quickly and produce more seeds. It is notable that the trend changes when extreme precipitation events and very high rainfall years are included. One possibility is that plants have a threshold of water they require for growth and maintenance, and any water taken up after reaching that threshold is used preferentially for seed production. It is important to note that this trend was calculated without regard to the presence or absence of insects such that there may be confounding factors that would help to explain these differences from what was predicted by the 2002-2013 trend. This trend also covers only a relatively small range of precipitation levels, based on available data. Continued sampling may reveal a more consistent pattern.

The observed effect of the grasshoppers on knapweed and *L. minutus* was very significant. In 2013, there was extensive herbivory on knapweed and very few seedheads were produced, especially on south facing and lower-elevation populations. When eating the seedheads, the grasshoppers were not only eating plant material but the insects developing within as well. This resulted in an apparent decrease in the *L. minutus* population the following year (Figure 1b), which, when combined with the extreme amount of precipitation received during that period, accounted for the spike in seed production. The decrease in *L. minutus* continued into 2015, possibly because of the reduced insect numbers in 2014 and the large number of seedheads produced per plant during the wet 2015 season. If the population of *L. minutus* continues to decline, another release *L. minutus* may be required to maintain a sustainable population at this site. It is possible that the *L. minutus* population is going through a boom bust cycle and will begin increasing again should the grasshopper herbivory not reoccur.

Overall, I found that *L. minutus* and *U. affinis* reduced spotted knapweed seed production by a consistent amount regardless of the amount of precipitation. With future years in the Front Range area predicted to be warmer and drier in the summer (Western Water Assessment, 2014), the threat of spotted knapweed invading native habitats should decrease, especially when biological controls are present that reduce seed production. Support was also found for a negative interaction of *L. minutus* and *U. affinis* when co-infesting a seedhead, although the lack of a significant difference in *L. minutus* effectiveness means that releasing both species on one population will not be detrimental to efforts to control knapweed.

Conclusions

This study supports the conclusions reached in Knochel and Seastedt (2009, 2010), Knochel et al. (2010 a, b) and Wooley et al. (2011) that the biological controls *L. minutus* and *U. affinis* can significantly reduce spotted knapwed seed production. When combined with other management techniques that reduce population densities *L. minutus* and *U. affinis* can be an efficient way to control spotted knapweed infestations. It also supports the simultaneous use of multiple biological controls to manage spotted knapweed populations, rather than choosing only the single most effective insect for each site for fear that indirect effects between the insects will decrease their effectiveness. This makes management a simpler task as it encourages a broader approach to biological control of spotted knapweed. The effect of the native grasshopper showed that, in some cases, it might be possible for native herbivores to control spotted knapweed populations without the aid of an introduced biological control. This scenario would be an ideal solution for dealing with spotted knapweed as it would avoid any indirect effects caused by introducing new insects into the ecosystem. Although this effect was unpredicted and has not been seen elsewhere, it remains an area for future research to determine what caused these grasshoppers to suddenly start consuming knapweed, and what then caused them to decline or find other food resources. The finding that insect effectiveness did not appear to depend on the amount of precipitation means that even in the drier predicted future of the Front Range these insects should continue to serve reliably as biological controls. How this relationship is maintained in other areas with spotted knapweed infestations remains to be seen, but these conclusions are an encouraging sign that these biological controls may work at least in areas with similar precipitation patterns.

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Table 1. Pearsons correlations between seed production and the biological control insects *L. minutus* and *U. affinis* (n=6026).

Variables	Seeds*Larinus	Seeds*Urophora	Seeds*Insects	Larinus*Urophora
Correlation	-0.305	-0.1524	-0.27228	-0.11671
p value	<.0001	<.0001	<.0001	<.0001

Table 2. Mean seedhead production in the presence or absence of insects. Calculated overall mean using yearly means to avoid bias towards years that had more samples.

Insect present	No *L. minutus*	*L. minutus*	No *U. affinis*	*U. affinis*
Seeds/seedhead	6.2401	3.0779	5.3717	3.9463
Significance and percent change	F= 12.45, p=0.0009 % change=-50.7		F=2.53, p=0.1178 % change=-26.5	

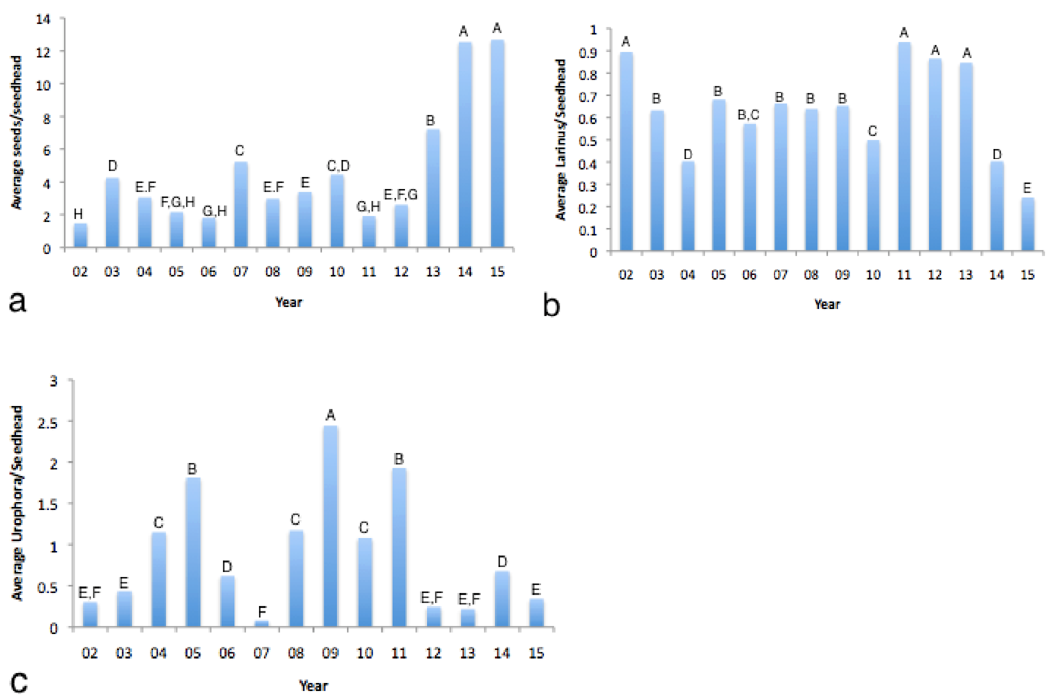

Figure 1. The average number of seeds (a), *L. minutus* (b), and *U. affinis* (c) per seedhead each year. Letters reflect significantly similar and different values within each chart.

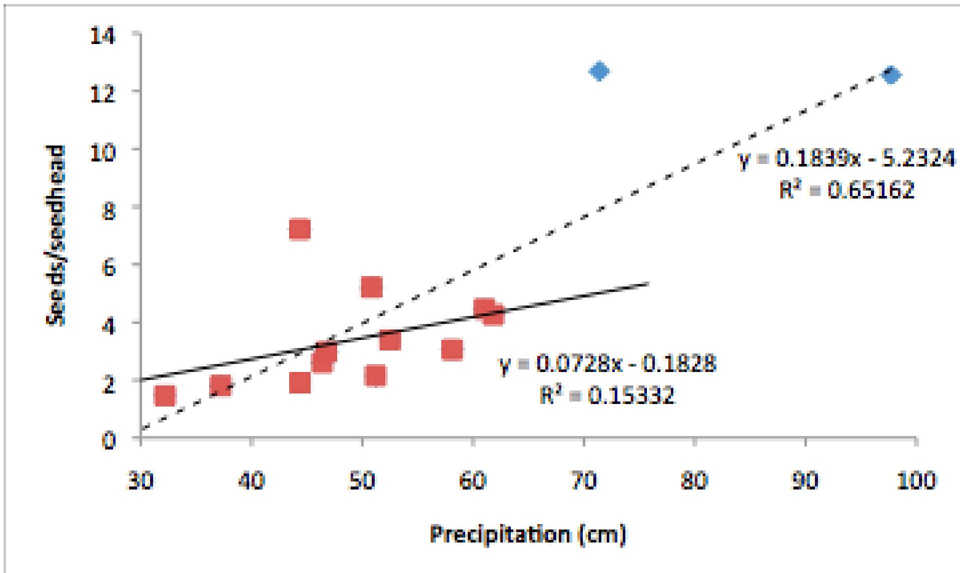

Figure 2. The relationship between seeds produced per seedhead and the amount of precipitation from the preceding September through August. The solid trend line includes the growing seasons from 2002-2013 (data shown in red), while the dashed trend line also includes 2014 and 2015, data points shown in blue. The huge amount of precipitation that fell in the 2014 and 2015 growing seasons caused seed production to increase far more than was expected based on the 2002-13 trend line.

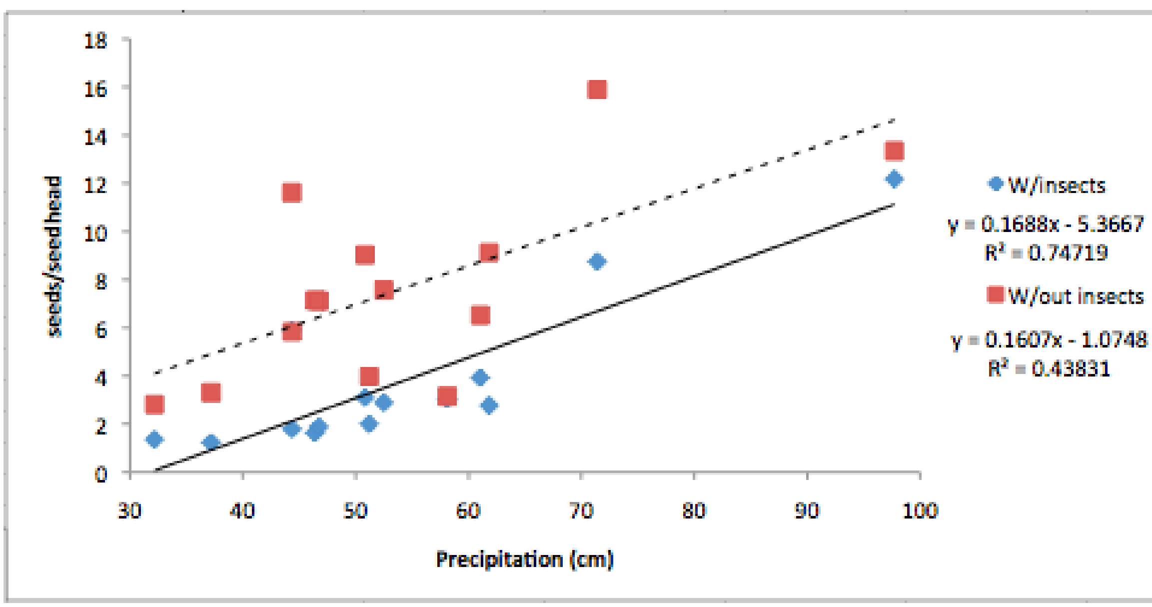

Figure 3. Seed production per seedhead based on precipitation and the presence (blue) or absence (red) of insects (*L. minutus, U. affinis*). The presence of insects decreases seed production on average by 3.85 seeds/seedhead.

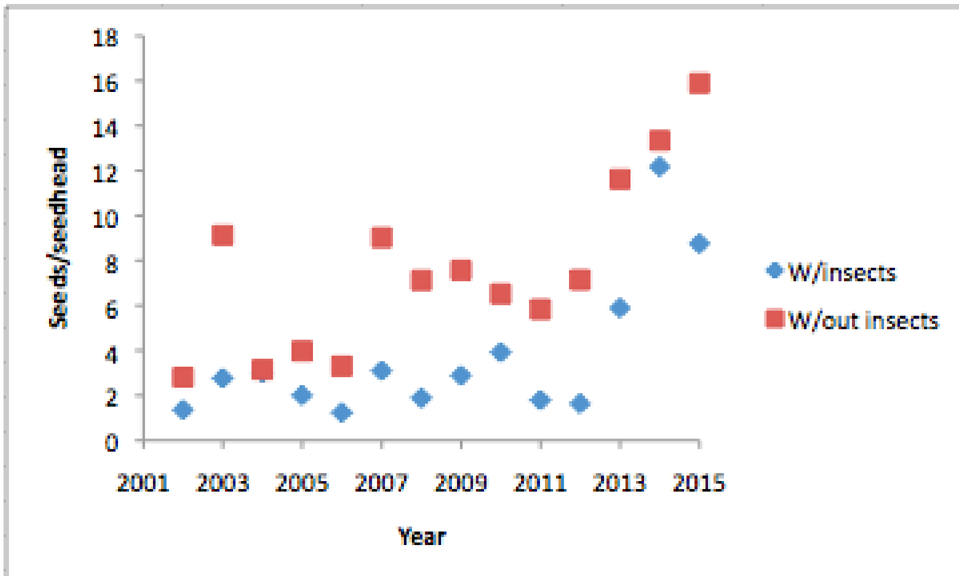

Figure 4. Average seeds/seedhead in the presence and absence of insects each year. In every year the insects caused at least some decrease in the number of seeds, ranging from as little as 0.12 fewer seeds (2004) up to 7.14 fewer seeds (2015).

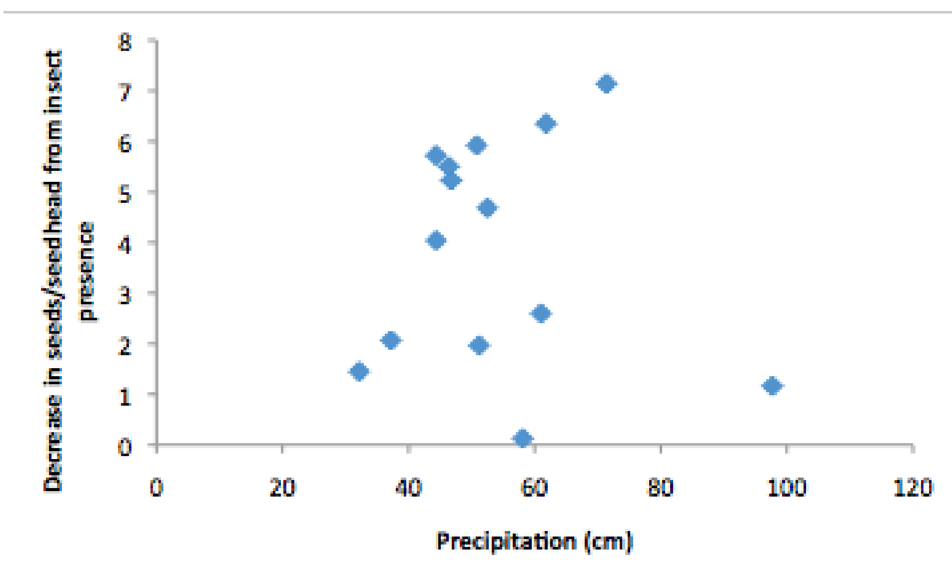

Figure 5. Decrease in seeds/seedhead in the presence of insects compared to the amount of precipitation. There appears to be no relationship between the amount of precipitation and the effectiveness of the insects at reducing seed count.

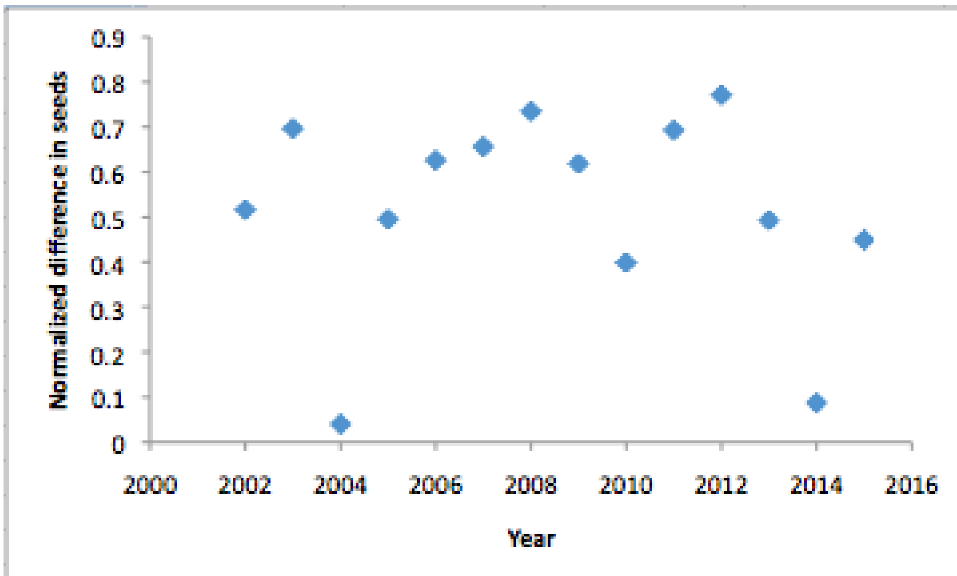

Figure 6a. Differences in seeds/seedhead for presence and absence of insects. Normalized using (# of seeds w/out insects-# of seeds w/insects)/# of seeds w/out insects.

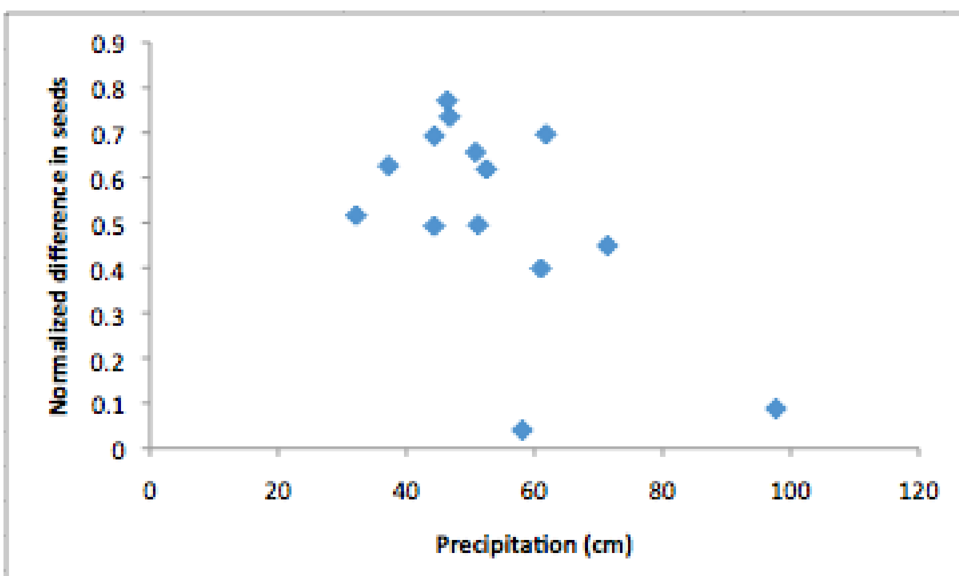

Figure 6b. Differences in seeds/seedhead for presence and absence of insects and annual precipitation. Normalized using (# of seeds w/out insects-# of seeds w/insects)/# of seeds w/out insects.

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