Postdispersal Weed Seed Predation Is Affected by Experimental Substrate

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A standard method for evaluating weed seed predation is needed to facilitate generalizations across studies. Identification of general trends could allow practical recommendations for enhancing weed seed predation in agricultural systems. The objective of this study was to compare the commonly used sandpaper and soil substrate methods for offering weed seeds when assessing seed predation rates. Invertebrate seed predators and associated weed seed predation levels were measured in June to July, August, and September of 2005 and 2006 within a conventionally managed corn system. Seed predation levels of three common weed species, velvetleaf, giant foxtail, and common lambsquarters, were estimated using feeding trials in which 40 seeds of each species were offered over a 48 h period using the two substrates. Exclosures were used to distinguish total predation from predation by invertebrates alone. In addition, we investigated the use of geospatial analysis to estimate spatial autocorrelation of invertebrate populations and seed removal rates. Results suggest caution in using synthetic substrates, such as sandpaper, when assessing seed predation, especially when investigating small-seeded species (< 1 mg seed^{-1}) or when seed predators are predominantly invertebrates. By contrast, predation of the larger-seeded species, velvetleaf, was less affected by substrate, perhaps because of removal predominately by vertebrates. One way to overcome problems with the sandpaper substrate method is for studies to include some soil substrate samples for on-site calibration of the sandpaper substrate. If necessary, data could then be corrected by multiplying by the ratio of soil substrate measured-predation rate to sandpaper measured-predation rate. Spatial autocorrelation explained between 6 and 9% of the variation in giant foxtail and common lambsquarters removal rates attributed to invertebrates alone. Researchers should, therefore, be careful not to neglect the impact of clustered invertebrate populations and associated seed removal rates.

Nomenclature: Common lambsquarters, *Chenopodium album* L. CHEAL; giant foxtail, *Setaria faberi* Herrm. SETFA; velvetleaf, *Abutilon theophrasti* Medik. ABUTH; corn, *Zea mays* L.

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Generalist invertebrates that feed on weed seeds can be an important broad-spectrum form of biological weed control affecting weed population dynamics in natural and agronomic systems (Crawley 1990; Cromar et al. 1999). Seed predation can result in lower seed density (Davis and Liebman 2003; Davis et al. 2003; Gonzales-Andujar and Fernandez-Quintanilla 1991; Jordan et al. 1995; Landis et al. 2005; Liebman et al. 2003) and changes in weed community composition (Carroll and Risch 1984; Tooley and Brust 2002).

Postdispersal seed predation is difficult to measure, and distinguishing the portions of predation that can be attributed to invertebrates vs. vertebrates is even more difficult. Both within and across studies, the taxa of principal seed predators have varied, with vertebrates showing more consistent predation rates over the growing season when they are present (Westerman et al. 2003). By contrast, invertebrates exhibit more variable predation rates, perhaps related to species-specific phenology (Marino et al. 1997; Westerman et al. 2003). Multiple studies indicate that invertebrates can be the dominant weed seed predators in agricultural systems (Brust and House 1998; Cromar et al. 1999; Gallandt et al. 2005; Honek et al. 2003). Current research focuses on understanding which farm management practices foster beneficial invertebrates and lead to seed predation rates that reduce weed populations.

The ongoing discussion surrounding the relative importance of invertebrates and vertebrates as seed predators in agroecosystems and the types of management that may enhance seed predation is partly due to the difficulty of synthesizing results from the numerous published seed predation studies into a coherent meta-analysis. Pooling inferences about seed predation is difficult because past work has encompassed a wide array of agroecological environments and management practices. Past research has, nonetheless, shown that the relative abundance and impact of seed predator guilds vary among habitats (Hulme 1998; Menalled et al. 2000), and they can be affected by the type of crop management used (Carmona and Landis 1999; Gallandt et al. 2005). Point estimates of seed predation do not accurately estimate the cumulative numbers of weed seed removed by predators because seed predation is affected by several temporally variable factors not easily assessed, including seed demand by predators, microsite predator density, the timing of seed dispersal, and seed residence time on the soil surface (Westerman et al. 2006).

This study addresses the second major source of variation between seed predation studies, namely, the variety of experimental substrates used to assess seed removal rates. Choice by experimenters of seed predation substrate methodology is justifiably based on convenience, given that most often research focuses on comparisons of treatments. The five most common substrate methods currently used are distinguished from one another based on whether they employ (1) a natural or synthetic substrate; (2) an adhesive material, such as glue or tape, to temporarily hold seeds to the substrate; and (3) the relative difficulty of processing samples (Table 1).

The most comprehensive investigation to date of the impact of experimental substrate on seed removal rates is Gallandt (2005). This study summarized the diversity of seed predation assays in use and compared different experimental substrates in the field to determine methodological effects on seed predation. During a 3-d period in July, seeds of six common weed species were offered using three to six different substrates in a 2-ha red clover (*Trifolium pratense* L.) field near Stillwater, ME. Predation rates in this study varied with seed

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Table 1. Overview of the five most common seed predation substrate focusing on setup and processing time and approximation of the substrate to resemble field conditions.

Substrate	Pad	Tape	Sandpaper	Sand	Soil
Setup	Scouring pad topped with seeds	Double-sided tape adheres seeds; dusted with soil	Aerosol glue adheres seeds; dusted with soil	Seeds placed on sand in dish flush w/ ground	Seeds dusted on field soil in dish flush with ground
Time	Very fast no lab setup	Setup and processing relatively fast	Setup and processing relatively fast	Slower setup, variable processing	Slower setup, very long processing
Pros	Easy setup and processing, reusable	Closer to field surface than pads	Less synthetic; no adhesive impact	Natural; less invertebrate avoidance	Natural; minimal impact on invertebrates
Cons	Small seeds lost in pad	Tape traps invertebrates & has scent	Small seeds adhere too tightly	Sand causes increased seed searching	Processing time nearly prohibitive
Substrate	All synthetic	Synthetic + soil	Synthetic + soil	All sand	All soil
Adhesive	None	Tape	Aerosol glue	None	None
	Faster processing → Slower processing				
	Substrate unlike field conditions ← Substrate closer to field conditions				

presentation substrate. In particular, invertebrate predation, estimated using vertebrate exclosures, showed variation potentially related to the differential appearance of weed seeds on different media. Overall, seeds were more difficult for invertebrate predators to find against a soil background (whether using the soil substrate or in methods that dust soil over various substrates) compared with other substrates. Species by substrate predation rates also depended on seed type. These clear weed species by substrate interactions, even in this small-scale study, indicate that more comprehensive research is needed to determine the most suitable substrates for assessing seed predation.

Although concerns about the effect of specific substrates on animal behavior in seed predation studies may be less important when seeds of multiple species are offered (Brust and House 1988; Cromar et al. 1999; Menalled et al. 2000; Westerman et al. 2003), recent work has demonstrated that predator behavior can be substantially affected by the manner in which seeds are presented (Gallandt 2005; O'Rourke et al. 2006). This impact on behavior suggests that published predation rates in agroecosystems may be overestimating or underestimating the seed removal capabilities of taxa that are drawn to or avoid certain experimental substrate setups.

The objective of this 2-yr field study was to assess the relative impact of sandpaper substrate vs. soil substrate on predator behavior and seed removal rates. The soil substrate method was chosen because it is the closest approximation to natural field conditions. Although it requires extensive field installation time and several hours of laboratory processing per sample to recover seeds, it probably has the least effect on seed predator behavior and thus comes closest to estimating natural predation rates. The sandpaper substrate method was selected from among the time- and cost-effective seed predation measurement methods because the final soil dusted surface appears to approximate the surface of an agricultural field.

We also used geospatial analysis to estimate spatial autocorrelation of invertebrate populations and seed removal rates. We hypothesized that spatially autocorrelated seed removal rates might be observed because of clustering of invertebrate seed predator populations. Clustered invertebrate populations can be observed because of various environmental and behavioral factors, including favorable microsites or preferred conditions in the field margins or interior and because of gregarious invertebrate behavior (e.g., during breeding). To our knowledge, no previous seed predation research in agroecosystems has employed geospatial analysis for assessing invertebrate activity density and associated seed removal. Spatial clustering (or skewness) can easily be overlooked using traditional statistical analysis, adding to the difficulty of generalizing findings across different studies.

Materials and Methods

Field Site and Test Species. The study was conducted at the Robert B. Musgrave Research Farm of Cornell University near Aurora, NY (42°44′N, 76°39′W). Soil is a moderately well-drained Lima silt loam (fine-loamy, mixed, mesic Glossoboric Hapludalf). A randomized block design of three treatments replicated five times was used with plots measuring 51 by 46 m. Seed predation substrate comparisons were conducted in the last 2 yr of a larger 4-yr investigation of corn rootworm *Diabrotica* spp. (Coleoptera: Chrysomelidae) control techniques. Treatments consisted of (1) a Cry3Bb *Bacillus thuringiensis* (Bt) hybrid, Monsanto Yieldguard rootworm control (MON863); (2) a non-Bt isoline, treated with the broad spectrum insecticide, tefluthrin,[1] which was applied at planting at a rate of 388 ml per 1,000 m of row; and (3) a control consisting of the non-Bt isoline with no corn rootworm control. Treatments are referred to as Bt, tefluthrin, and control, respectively. Corn seed was treated with the fungicide captan[2] (*N*-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide) at the label rate of 2.3 ml kg^{-1} of seed. A range of 6 to 12 corn rows buffered plots from one another and from the edges of the field to limit edge effects. The field was managed as described above for 2 yr before the start of the study.

Given that Bt endotoxin has a greater effect on the larval stage of beetles than on adult populations, the Bt produced in the first year might have a proportionally larger impact on the coleopteran larvae that hatch and mature in the field during the first growing season or winter (depending on a species reproductive phenology). Adult activity densities of carabids and other beetles would, therefore, likely not be affected by Bt toxins until the second season when the mortality in the larval population would impact the adult density. Insecticides, by contrast, have a significantly deleterious effect on both adult and larval populations in the first year (Brust et al. 1986; Lesiewicz et al. 1984; Noordhuis et al. 2001; Reed et al. 1992). Thus, initiating the seed predation study after several years of management helped to mitigate ephemeral differences of insecticide and Cry3Bb Bt toxins on adult carabid activity densities and, therefore, on any associated weed seed predation.

Seed predation rates were determined for three annual weeds: common lambsquarters, giant foxtail, and velvetleaf. These are three of the most common and troublesome agricultural weeds in domestic corn production (Bridges and Baumann 1992; Hartzler et al. 1993; Singer et al. 2000). Seeds were collected on site at the Robert Musgrave Research Farm in 2003 and 2004 and stored in paper bags at 3.5 C. The wide range in average seed size of these three species (7.3 mg for velvetleaf, 1.6 mg for giant foxtail, and 0.70 mg for common lambsquarters) was helpful for attracting different weed seed predators as well as for determining possible interaction effects between seed size and substrate.

The seed density of each weed species offered on either the sandpaper or soil substrate was 1,000 seeds m^{-2}, with a combined seed density of 3,000 seeds m^{-2}, levels typical of temperate cropping systems with moderate weed seed production. The suitability of the seed density used was confirmed by on-site sampling of the surface-available seedbank (mean, 3,200 seeds m^{-2}; SD, 2,300 seeds m^{-2}). The experimental seed density was less than or about equal to those used in other seed predation experiments (Brust and House 1988; Cardina et al. 1996; Cromar et al. 1999) and was far lower than the combined density of 25,211 seeds m^{-2} used by Gallandt (2005). Using a seed density typical of our system avoided density-dependent inflation of predation rates (Cardina et al. 1996; Cromar et al. 1999).

Invertebrate Pitfalls. Within each of the 15 plots, locations for eight pitfall traps were selected randomly. Pitfall traps consisted of a buried plastic cup (9 by 12 cm) with the upper rim flush with ground level and a smaller inner cup (7.5 by 4.5 cm) serving as a lining for easy specimen removal. Pitfall traps were opened for a 24 h wk^{-1} period, from planting to harvest (May to September). These pitfalls were filled with 1 : 1 ethylene glycol mixed with water. After this period, arthropods were stored in capped pitfall cups at −17 C in ethylene glycol, awaiting processing. Arthropods were removed from the freezer, washed with water to remove ethylene glycol, identified, and stored in vials containing 70% ethanol. Once monthly when weather conditions were suitable, six randomly selected pitfalls per plot were assigned.

Evaluation of Seed Predation Levels. Seed predation was measured once monthly from June to September during a 48-h sampling period, beginning between 9:00 A.M. and 11:00 A.M. Eastern Daylight Time. Two soil and four sandpaper substrates were used per plot. This unbalanced design was used because a greater number of soil substrate samples would have been impractical to process. To avoid seed loss during rainfall events, sampling was carried out when the weather forecast called for less than 20% probability of rain. Samples were discarded if any precipitation occurred during the 48-h sampling period to avoid the possibility of rain splashing seeds out of buried sampling trays. These precipitation restrictions ultimately resulted in a total of three sampling periods in each year (i.e., June to July, August, and September).

To quantify the portion of target seeds removed that could be attributed to vertebrates rather than ground-dwelling arthropods, vertebrate exclosures were placed over half of each type of seed predation setup. The vertebrate exclosures consisted of a 30 by 30 by 10 cm cap constructed from 0.5-cm wire mesh. This allowed the passage of beetles and other invertebrates but not small mammals. In preliminary work, these vertebrate exclosures did not reduce or otherwise change foraging behavior for invertebrates (data not shown).

To calculate experimental error associated with sample handling, a 1.5 by 1.5 mm window screen was affixed over some vertebrate exclosures to deny all predators entry. At each sampling period, these total exclosures were placed over 10 sandpaper predation substrate setups and 10 soil substrate predation setups. Samples were processed to determine the percentage of seed loss associated with handling error rather than predation. This was particularly important to determine for the soil substrate method because recovery of seeds required a multistep process involving several sample transfers, elutriation, and handling by several investigators.

Soil Substrate Method. Soil was collected from the Mt. Pleasant Research Farm Facility of Cornell University, 10 km east of Ithaca, NY, and was a Mardin silt loam, which did not contain seeds of the three target weed species or other common agricultural weeds. Soil was placed in square trays buried flush with the ground. These consisted of a 20 by 20 cm chlorinated polyvinyl chloride (CPVC) frame, 2 cm deep, with 0.5 by 0.5 mm nylon window screen hot glued to the frame. The permeable bottom allowed soil moisture in the tray to equilibrate with the surrounding soil overnight. Care was taken to ensure a continuous substrate surface between the buried trays and the surrounding field. Seeds were dusted on the soil in the trays. Soil in trays was left in the field for 48 h and then carefully removed and placed in bags sewn from 0.5 by 0.5 mm nylon window screen. Bags were immediately transported to the laboratory and dried at 40 C for at least 24 h. This procedure ensured that none of the target weed seeds germinated in the bags. Weed seeds present in the dried soil were extracted using a high-volume hydraulic elutriator, dried, sorted, and counted to determine the percentage of seeds removed.

Sandpaper Substrate Method. Sandpaper with maroon/brown backing[3] was cut into 23 by 14 cm rectangles. Adhesive[4] was sprayed lightly and evenly over the surface of each card for 2 s at a distance of approximately 30 cm. Forty seeds of each of the three target weed species were placed evenly over the sandpaper surface while avoiding the perimeters. A small glass jar was gently rolled over the card, to increase surface area contact of the large velvetleaf seeds with the adhesive. A fine layer of seed-free soil was then dusted over the entire card surface using a large chefs' canister with a perforated lid designed for dusting spices. After 30 min, the adhesive was dry, and cards were carefully placed in plastic bags. This protocol is slightly modified from O'Rourke et al. (2006) and Westerman et al. (2003). After sampling, the sandpaper cards were bagged. Seeds were removed from the cards, sieved to separate species, and counted.

Data Analysis. Seed predation rates were recoded into ordinal variables and subjected to polytomous universal model (PLUM) ordinal regression with a negative log–log or probit link function (determined by dependent variable distribution) using SPSS for Windows.[5] The PLUM ordinal regression procedure in SPSS also produced both Cox and Snell and the Nagelkerke pseudo-R^2 measures to estimate percentage of variation in the dependent variable explained by the model. Statistical significance was accepted at the 0.05 α level.

Ordinal categories for seed removal of each weed species were coded as 0, no measurable predation (0 to 3 seeds missing); 1, moderate seed predation (4 to 10 seeds removed or 1 to 28% removal); and 2, extensive seed predation (10+ seeds removed or 31 to 100% removal). Ordinal categories were determined based on frequency distributions of seed removal of velvetleaf, giant foxtail, and common lambsquarters. Frequency distributions of velvetleaf and giant foxtail removal were bimodal. Local maxima had distinct variances and skewness, with one maximum peaking between 1 and 28% and the other peaking at 31 to 100% seeds removed. The frequency distribution of common lambsquarters was unimodal, with mean peaking between 1 and 28% seeds removed. The distribution for common lambsquarters lacked the second peak of extensive seed predation observed in the larger seeded species. Ordinal coding and ordinal regression was preferred over ANOVA with an arcsine transformation because the substantial number of samples with 0% seed removal violated assumptions of ANOVA.

Seed loss from processing and other sources of experimental error varied by substrate and by size of weed species. The range across substrates and seed types included an error of $\pm$ 1 seed for large-seeded velvetleaf recovered from the sandpaper substrate to an error of $\pm$ 3 seeds associated with the small-seeded common lambsquarters from the soil substrate. This experimental error is reflected in ordinal category coding, with seed loss of $<$ 3 seeds counted as no predation, so that moderate seed predation of samples ranged from 4 to 10 seeds removed (1 to 28% removal).

A final dependent variable was constructed by adding the ordinal variables from all three weed species into one number. This allowed ranking of total seed predation from 0 to 6, such that samples with extensive seed removal (category 2) for all three seed species were coded as 6 (i.e., 2 + 2 + 2) and those samples, for example, with extensive seed predation on only one species and no seed predation on the other two were ranked as 2 (i.e., 2 + 0 + 0). Because predation rates on the three species were not independent, this pooled variable indicates relative total rates of predation per sample. Given the spatial variability of seeds and seed removal, this was useful in identifying predation hotspots in the field. A high score could indicate thorough, voracious, or gregarious invertebrate populations or the presence of a vertebrate predator.

Spatial Analysis. Geospatial analyses of invertebrate activity density and seed removal rates were conducted using the Manifold[6] System 6.50 to map spatial autocorrelation onto satellite images with added global positioning system (GPS) locations of plot boundaries and sampling locations. We used *z* values of predation rate or invertebrate counts applied over x and y coordinates of latitude and longitude of sample locations to test for significant clustering. Significance was determined using CrimeStat,[7] to compute the Morans' *I*, a standard index of autocorrelation that varies from -1 (indicating clustering) to 1 (indicating dispersion). A computed *Z* value was used to determine the significance of the Morans' *I* index. CrimeStat was also used to create weighted triangulated ellipses of the mean and standard deviation applied to Manifold maps to illustrate significant spatial autocorrelation.

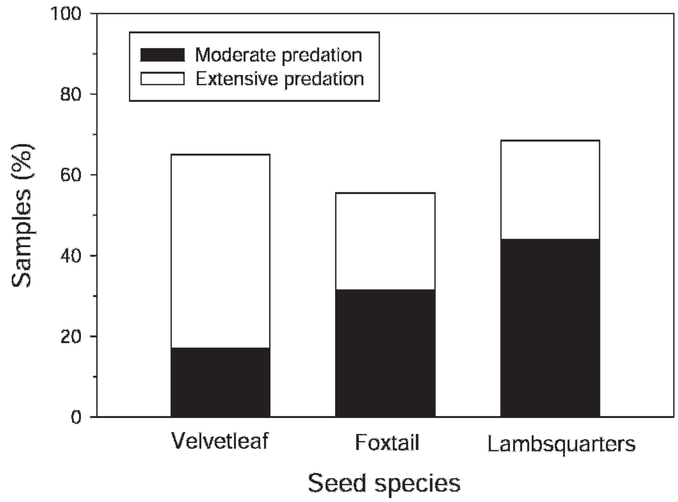

Figure 1. Percentage of samples from 2005 and 2006 showing velvetleaf, giant foxtail, and lambsquarters predation. Moderate predation refers to 1 to 10 seeds removed in 48 h, or 1 to 28% removal, extensive predation is 10 to 40 seeds removed in 48 h, or 31 to 100% removal.

Results and Discussion

Seed Predation Rates. Seed removal rates varied by weed species, but 83% of all seed predation samples over the 2 yr, and 6-mo pooled totals had at least moderate seed predation (1 to 28% removed) on one or more weed species. Predation was greatest on velvetleaf seeds: 47% of all samples showed extensive predation (31 to 100% of seeds removed), and a further 15% of the locations had at least moderate predation (1 to 28% removed; Figure 1). Across all weed species, both year of sampling and treatment (Bt, tefluthrin, and control) had no impact on predation.

Predation Levels and Substrate Method Used. More seed predation occurred using the soil substrate than using the sandpaper substrate ($P < 0.001$) (Figure 2). Instances of extensive seed removal of all three species (an ordinal value of 6) was observed three times more often on soil substrates than

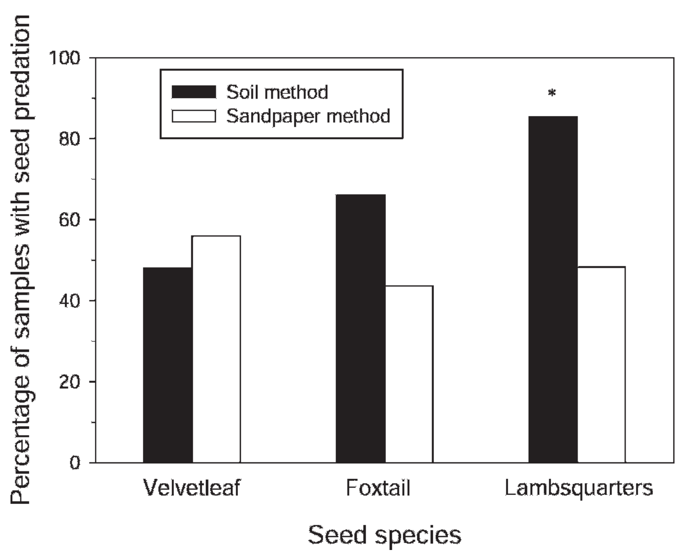

Figure 2. Percentage of samples showing removal of velvetleaf, giant foxtail, and lambsquarters by seed predation substrate in 2005 and 2006. An asterisk (*) indicates significantly more instances of predation using a substrate ($P < 0.01$).

Table 2. Summary of polytomous universal model (PLUM) ordinal regression analysis of velvetleaf, giant foxtail, and common lambsquarters removal rates.

Model effect	Velvetleaf		Giant foxtail		Common lambsquarters	
	Pseudo R^2	P	Pseudo R^2	P	Pseudo R^2	P
	%					
Whole model	19	0.0001	16	0.0001	32	0.02
Substrate	2	0.009	6	0.0001	18	0.0001
Exclosure	8	0.0001	3	0.001	0	0.568
Year	0	0.197	0	0.291	2	0.001
Month	9	0.0001	2	0.02	1	0.347
Spatial	0	0.09	6	0.05	9	0.03

on sandpaper cards. Considering the proportion of predation attributed to invertebrates alone, significantly more soil samples than sandpaper samples had values of 2 to 6, and significantly fewer soil substrate samples had the two lowest predation classes (0 and 1) ($P < 0.001$).

Seed predation substrate explained 10% of the variation in pooled total removal rates ($P < 0.001$) and 8% of the variation in pooled total removal when only invertebrates are considered ($P < 0.001$). However, using ordinal regression of each weed species separately, 18% of the variability in common lambsquarters removal was explained by substrate ($P < 0.001$) (Table 2), which dropped only slightly to 12% when considering common lambsquarters removal attributed to invertebrates alone ($P < 0.001$). The large impact of substrate on common lambsquarters seed removal levels may be due to its predators being primarily invertebrates. Common lambsquarters removal with and without the vertebrate exclosures did not differ significantly (Table 2). Seed predation of common lambsquarters was more likely to occur using the soil substrate than the sandpaper substrate, which may have been due to behavioral changes in invertebrates in response to the unfamiliar surface of the sandpaper. Smaller seeds are more difficult to locate and offer fewer resources per seed. This makes small seeds less-desirable targets for generalist vertebrates. Invertebrates are thus the primary predators of small seeds, especially predispersal specialists (Hulme 1998).

The only significant predictors of common lambsquarters predation were the substrate used (Figure 2) and sampling month, with greater predation earlier in the season. Inclusion of invertebrate counts as covariates resulted in no significant association between any invertebrate species and seed removal levels; however, slugs (Stylommatophora: *Deroceras* spp., *Arion* spp.) were positively correlated with higher common lambsquarters predation levels during the wet June to July months. Although slugs may be important seed predators of common lambsquarters, slug populations cannot explain August and September common lambsquarters removal levels.

In contrast with common lambsquarters, 2% of the variation in velvetleaf seed removal by all predators was explained by the substrate used ($P < 0.01$) (Table 2). Similarly, 2% of variation in removal attributed to invertebrates alone was explained by substrate ($P < 0.05$). Extensive velvetleaf removal occurred 78% more often with vertebrate access, and instances of no predation were almost twice as likely when vertebrates were excluded. A total of 8% of the variation in velvetleaf seed removal could be attributed to the presence or absence of vertebrate exclosures ($P < 0.001$). These results indicate that vertebrates were predominantly responsible for extensive velvetleaf removal. Although vertebrates drove extensive velvetleaf predation, pitfall counts of the carabid beetle (*Pterostichus melanarius* Illiger) were positively correlated with the component of velvetleaf predation that was attributable to invertebrates ($P < 0.001$). *Pterostichus melanarius* is a common introduced ground beetle, known to be an important predator of invertebrates and weed seedlings in annual cropping systems (Thomas et al. 1998).

Unlike the early season removal associated with common lambsquarters, velvetleaf seed predation rates increased steadily during the growing season. Significant velvetleaf predation increased by 2.3 times with each successive month of the growing season, with month explaining 8% of the variation in velvetleaf removal ($P < 0.0001$). Removal of velvetleaf seeds appeared to show an all-or-nothing seed predation pattern, with 203 instances of no predation decreasing to 79 instances of moderate predation, and increasing again to 250 instances of extensive predation. This all-or-nothing approach may be indicative of larger predators (e.g., mice and birds), which can easily consume or disperse 15 or more seeds from one location. This distribution, combined with the impact of exclosures on removal rates, indicates that the most consistent predators of velvetleaf in our system were likely vertebrates. Given the impact of vertebrates on this species, the observed month effect may be due to the higher weed seed rain later in the growing season, which creates abundant resources known to attract vertebrate populations and drive up seed predation rates (Westerman et al. 2003).

The substrate used explained 6% of the variation in giant foxtail removal ($P < 0.001$), with seed removal more likely to occur using the soil than the sandpaper substrate. Although 6% of the variation in removal of giant foxtail by all predators was explained by substrate ($P < 0.001$) (Table 2), the variation explained by substrate dropped to 2% when vertebrates were excluded ($P < 0.05$). This indicates that both vertebrate and invertebrate predators of giant foxtail were affected by substrate used. Moreover, there were significantly higher instances of no predation by either vertebrates or invertebrates on the sandpaper substrate ($P < 0.001$). Giant foxtail predation in our system was not as dramatically correlated to the soil substrate as common lambsquarters removal. However, the similar effect of substrate on seed removal indicates that common lambsquarters and giant foxtail may share the same invertebrate predators in our system or that the sandpaper substrate may similarly alter invertebrate giant foxtail predator behavior. Vertebrate access was correlated with a greater likelihood of extensive giant foxtail seed removal, explaining 4% of the variation in extensive removal rates ($P < 0.001$). Although vertebrate predation of velvetleaf was unaffected by the substrate used, the higher rates of giant foxtail removal by vertebrates using the soil substrate indicate possible shifts in vertebrate feeding behavior in the presence of the sandpaper substrate. Invertebrates appear to be responsible for instances of moderate giant foxtail removal because vertebrate access explained only 1% of the variation in moderate removal ($P < 0.05$). Month had a small but significant impact on removal, with slightly more giant foxtail predation later in the season ($P < 0.001$).

Spatial Analysis. Spatial analysis of seed predation of common lambsquarters and velvetleaf visualized the higher predation rates in the eastern section of the field. Morans' *I* test, a measure of spatial autocorrelation, had significant *Z* values in the August samples for both common lambsquarters and giant foxtail removal across both years. Activity density for the important seed predator, *Poecilus lucublandus* Say was also significantly spatially autocorrelated and located in the same eastern portion of the field. Inclusion of invertebrate counts as covariates resulted in no significant association between *P. lucublandus* and giant foxtail removal levels ($P = 0.16$), although *P. melanarius, Harpalus rufipes* Degeer, and cricket species were all significantly associated with giant foxtail removal ($P < 0.05$).

The clustering in the eastern portion of the field prompted us to create a simple spatial variable that scored plots 1 to 15, from the northeast to the southwest; and plots 1 to 5, which make up the eastern portion of the field, and these plots were determined to have significantly higher giant foxtail and common lambsquarters predation levels ($P < 0.05$). For giant foxtail, adding this spatial component to the independent variables of predation substrate and vertebrate exclosure increased the pseudo-R^2 value to 15 from 10% (model $P < 0.001$). For lambsquarters this spatial component increased the pseudo-R^2 value to 31 from 22% (model $P < 0.001$). For each one unit increase in plot, moving from the northeast to the southwest, the odds of moderate or extensive predation on giant foxtail or common lambsquarters decreased by a factor of 0.92 ($P < 0.01$). This spatial variability may be due to the eastern third of the field draining less rapidly after rain events and remaining slightly moister even in dry months. The observed increase in giant foxtail and common lambsquarters predation in the southeastern portion of the field may have been related to a spatial environmental component driving activity of important seed predators.

Research Implications. The findings reported here have three important implications. First, predation rates of larger-seeded velvetleaf appeared to be attracting predator guilds predominantly composed of vertebrates, and predation rates for this species were only minimally affected by the substrate. Data from the vertebrate exclosures suggest that the lesser effect of substrate on predation rate of large-seeded species may be due to greater vertebrate predation on these species. In a study primarily interested in vertebrate predation, the sandpaper substrate would, therefore, be an adequate and expedient procedure for assessing velvetleaf seed removal.

Second, researchers should be cautious in using synthetic substrates when assessing seed predation, especially of small-seeded species (< 1 mg seed^{-1}) or when seed predators are predominantly invertebrates. The largest impact of substrate in this study was on common lambsquarters, the species with the smallest seed (0.70 mg) and the only species whose seeds were predominantly removed by invertebrates. However, giant foxtail removal by both invertebrates and vertebrates was more likely using the soil substrate, indicating that the sandpaper substrate underestimates seed removal by vertebrates for some species.

One solution for addressing the impact of substrate on seed removal would be to include a small number of soil substrate samples in each study to evaluate the site- and species-specific impact of the sandpaper substrate on seed removal levels. Once the difference between substrates has been calibrated for a given study system, researchers could then proceed using only the more easily installed and rapidly processed sandpaper substrate. If necessary, data from soil substrate could be used to adjust data from the sandpaper substrate to achieve more realistic seed removal rates.

Third, geospatial analysis of seed predator activity density can help reveal clustering of invertebrate populations that can otherwise skew statistical analysis. In this study, spatial autocorrelation explained between 6 and 9% of the variation in giant foxtail and common lambsquarters removal rates attributed to invertebrates alone ($P < 0.05$). Without investigating the spatial component, the possible impact of invertebrate spatial autocorrelation on seed removal can be masked when it is averaged across the entire system. Researchers should, therefore, be careful not to neglect the impact of clustered invertebrate populations and associated seed removal rates. Fortunately the CrimeStat software used for spatial analysis in this study is available free of cost.

Sources of Materials

[1] Force 3G, Zeneca Agrochemicals, Syngenta Corporation, Wilmington, DE 19803.

[2] Captan, Drexel Chemical Co., Memphis, TN 38113-0327.

[3] Norton 3× fine 150 grit, Saint-Gobain Abrasives, Inc; Worcester, MA 01615-0008.

[4] 3M Brand general purpose spray adhesive, 3M Company, St. Paul, MN 55144-1000.

[5] SPSS for Windows 5, release 7.0. SPSS Inc., Chicago, IL 60606.

[6] Manifold 6.50 enterprise edition, CDA International Ltd., Carson City, NV 89701.

[7] CrimeStat, developed by the National Institute of Justice, Ned Levine and Associates, Houston, TX, crimestat@nedlevine.com.

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