Hindawi Publishing Corporation
International Journal of Ecology
Volume 2012, Article ID 846546, 6 pages
doi:10.1155/2012/846546

Research Article

Behavioural Studies of *Harpalus rufipes* De Geer: an Important Weed Seed Predator in Northeastern US Agroecosystems

Sara Harrison and Eric R. Gallandt

Department of Plant, Soil, and Environmental Sciences, University of Maine, 5722 Deering Hall, Orono, ME 04469-5722, USA

Correspondence should be addressed to Eric R. Gallandt, gallandt@maine.edu

Received 5 August 2011; Accepted 2 December 2011

Academic Editor: Andrew Denham

Harpalus rufipes, a member of the Carabidae, is the most common granivorous invertebrate in Maine agroecosystems. While previous research demonstrated a positive correlation between *H. rufipes* activity-density and weed seed predation, little is known about the behaviour of this seed predator. We conducted mesocosm experiments to examine seed burial, soil surface conditions, and seed mass effects while tracking *H. rufipes* movement using a video camera, capture card, and EthoVision software. *H. rufipes* showed a preference ($P < 0.001$), for seeds on the soil surface compared to those half or fully buried. Species with larger seeds were preferred, but *Amaranthus retroflexus*, which had the smallest seeds, had the highest feeding efficiency (i.e., seeds eaten per distance travelled). Undisturbed soil resulted in highest predation rates, presumably because seeds were easier to detect relative to disturbed soil. Video-tracking measurements of duration within areas of particular seeds, and efficiency of seed predation, indicate that *H. rufipes* behaviour is prey dependent.

1. Introduction

Seed predation is increasingly viewed as a critical component of multitactic, "Many Little Hammers," weed management strategies [1–4], even an ecosystem service that can be considered at a national scale [5]. Our work in organically managed vegetable-cover cropping systems indicated that invertebrates were responsible for the majority of predation estimated using feeding assays: 73% averaged over two field studies [6]. Pitfall trapping in these experiments indicated that the predominant invertebrate seed predator was *Harpalus rufipes* Degeer, a member of the Carabidae, a particularly well-studied taxa with wide geographic distribution and notable services to agroecosystems [7].

H. rufipes was introduced to North America from Europe in 1937. The first reports came from Topsfield, Maine in 1966 [8]. The beetle is now abundant throughout Canada, including New Foundland, Quebec, Nova Scotia, and New Brunswick and, in the USA, from Maine through to Connecticut [9].

Adult beetles are a dull black, with an elongated oval body and reddish legs. They vary from 1.25–1.6 cm in length [9] and are very mobile [10]. *H. rufipes* overwinters as both larvae and adults, the overwintered adults becoming active towards the beginning of May, with their densities peaking by the end of June [11]. They are most active at night, and cache seeds in burrows beneath plant residue [9]. Vegetation and plant residues may offer a favourable microclimate, and perhaps protection from hyperpredators. In the aforementioned vegetable-cover cropping systems experiment, *H. rufipes* activity-density was greater in plots with vegetation compared to areas recently tilled [6]. This preference and/or increased movement was subsequently confirmed using mark-recapture experiments; *H. rufipes* released in fallow plots (bare soil) were more likely to move to densely vegetated cover crop plots [12]. These studies offered correlative evidence of *H. rufipes* role as a seed predator [6], and laboratory choice tests conducted with seeds in Petri dishes confirmed that weed seeds are eaten (e.g., [9, 13]). However, little is known regarding the behaviour of

H. rufipes prey seeking of weed seeds in a more realistic soil environment, where seeds may be partially or fully buried and surface conditions vary. This is due, at least in part, to the nocturnal habit of *H. rufipes*, as well as the challenge of observing these relatively small animals in the field.

Here, we used a computerized video tracking and movement analysis system to test the following hypotheses.

(1) *H. rufipes* feeding rate and feeding efficiency are affected by seed burial, with maximal predation occurring when seeds reside on the soil surface, intermediate rates with partially buried seed, and very low or no predation observed when seeds are buried.

(2) In choice feeding experiments, *H. rufipes* will prefer larger seeds as they offer more resource for the feeding energy expended, and beetles will spend more time searching for preferred seeds, and these will be consumed faster than less preferred seeds.

(3) Substrate condition will affect predation rates, with reduced predation occurring following disturbance, compared to undisturbed, relatively smooth soil surface conditions.

2. Methods

Experiments were conducted using EthoVision, a computerised video tracking and movement analysis system [14] that incorporates software able to analyse movement of a variety of species, thus allowing the acquisition of an insight into animal behaviour otherwise difficult to obtain. Animals may be tracked longer periods of time than if the research were to be carried out by observation [15]. The system uses a frame grabber card (Picolo) that digitises an analog signal provided from an overhead camera thus the computer can read the output as a set of pixels. Arena boundaries are defined by accurately tracing the outline of the arena on the tracking screen. Zones can then be defined and outlined within this arena boundary. The software is calibrated to the dimensions of the given arena so that subsequent position data can be converted from the pixel output into actual measurements in terms of centimetres. Tracking is determined by a grey-scale detection method, that is, brightness, therefore, the greater the colour differentiation between the animal and the substrate background, the more reliable the tracking. For each sample, the arena is scanned and the position of the tracked animal is recorded; normally tracks are analysed at a rate of 6 samples per second. The resultant x-y coordinates, and time, are used to calculate the movement pattern during the trial period [16]. Calculations are performed on a series of frames to derive the output set of quantitative descriptors of the tracked animal's movements [17].

Preliminary tracking experiments compared both sand and a soil substrate, but because results were similar, sand was chosen over soil as the tracking process was easier to set up due to the greater difference in colour between *H. rufipes* beetles and sand. The substrate was moistened at the beginning of each new trial to ensure the beetles were not stressed through dry conditions.

All experiments were carried out at night as *H. rufipes* is nocturnal. In preliminary trials, beetles were found not to predate during the day and only remained static in one corner of the arena. To enable tracking at night time, a black light (15 W; BioQuip Products, Rancho Dominguez, CA, USA), suspended horizontally 1 m above the arena, was used to illuminate the arena, and a small dot of white neon paint was placed on the back of the individual being tracked.

Adult *H. rufipes* were collected from a local field site in September, 2005, by pitfall trapping. They were maintained in 48 by 30 by 10 cm deep polyethylene containers with 3-4 cm of moist sand and weedy plant debris to provide habitat. The experimental colony was maintained at 4°C. Individual adult *H. rufipes* were removed from the colony, placed in a Petri dish with moist filter paper, and acclimated to room temperatures (20°C) for four days prior to use in an experiment. All experiments were carried out using 5 individuals in the arena, but solely tracking one throughout; the tracked beetle was different in each experiment. To initiate an experiment, beetles were released in the center of the arena, regardless of the within-arena zone characteristics. Preliminary tracking experiments demonstrated that beetle density had a large effect on behaviour, with considerably greater movement of a tracked individual if conspecific neighbors were present (data not shown). Although the EthoVision system can track multiple individuals if marked with unique colors, our requirement for tracking at night under a black light prevented us from taking advantage of this feature.

Wild mustard (*Sinapis arvensis* L.; 175 mg 100 seeds^{-1}) was used for studies of seed burial and substrate disturbance; yellow foxtail (*Setaria glauca* L.; 164 mg 100 seeds^{-1}) and redroot pigweed (*Amaranthus retroflexus* L.; 33 mg 100 seeds^{-1}) were included when investigating weed seed preferences. Seeds were collected in the fall of 2005 at the University of Maine Rogers Farm, Stillwater, Maine, by gentle shaking from mature mother plants; they were air dried and stored at 20°C until use. Sixteen seeds were subjected to predation; using fewer seeds ran the risk of all seeds being predated.

The test arena was a 48 by 30 by 10 cm deep polyethylene tray filled with 4 cm of moistened sand. Zones were defined as follows: Zone 1 was the whole arena; Zone 2 was outside the feeding zone; Zone 3 was the feeding zone which measured 5 by 8 cm. An undisturbed substrate was present, and 16 wild mustard seeds were placed in a 1 by 1 cm grid formation in Zone 3. The trial was then repeated to simulate cultivation by scoring in four parallel ridges to a depth of 1 cm, and again with foliage placed over the top of the seeds in Zone 3 to simulate ground cover. Seed burial treatments included seeds placed either on the surface, half buried, or completely buried (1-mm deep). In the seed preference studies, four zones (6 × 20 cm), one for each weed species, and a control (no seeds added) were included; zones were separated from each other, and the arena sides, by 5 cm. The nature of the sand substrate resulted in an apparently uniform arena condition following placement of the seeds. In these experiments, 20 seeds were placed in each feeding zone, in a grid formation; species were randomly assigned to

individual feeding zones. Seeds were counted this time after 5 hours of the time period and then after 10 hours when the trial finished. The disturbance regimes were all carried out at all three seed exposure levels: fully exposed, half buried, and fully buried. Trials were conducted over consecutive nights during February through April, 2006.

ANOVA (SPSS 14.0) was used to test for effects of seed burial, seed species, and disturbance, on predation rate, predation efficiency, and residence in particular arena zones. Means were separated using Fisher's protected least significant difference.

3. Results

3.1. Seed Exposure. Both feeding rate, that is, seeds consumed per hour, and feeding efficiency, defined as amount of seed eaten per unit time per distance travelled, decreased with increasing seed burial (Figure 1).

3.2. Seed Preference. H. rufipes spent considerable time travelling the perimeter of the arena (Figure 2(a)), but when in the central area, more time was spent in yellow foxtail and wild mustard Zones (Figure 2(b)), meaning that the beetle entered these feeding zones more regularly or remained there to a greater extent after entering, compared to the redroot pigweed or empty feeding zones. Consistent with this observation, wild mustard predation was greater than redroot pigweed, with yellow foxtail intermediate (Figure 3). Although yellow foxtail and wild mustard exhibited the highest feeding rates, redroot pigweed showed the highest feeding efficiency, $P = 0.008$ (Figure 4).

3.3. Soil Disturbance. In the undisturbed trials, the levels of seed exposure followed the same pattern as described earlier with the most seeds being eaten when on the surface and the least when buried. Out of the three disturbance regimes, the most seeds were eaten on undisturbed sites. The cultivated and ground cover disturbances were not found to be significantly different (Figure 5). Predation of half-buried and fully buried seeds were not significantly different from each other on both cultivated and foliage-covered soils. When the seed husks were collected at the end of the trial periods on cultivated regimes, a high proportion of them were found to be within the ridges (data not shown).

4. Discussion

Carabids are considered important agents in the natural control of weeds. Consistent with our first hypothesis, both the feeding rate and feeding efficiency were highest when the seed was on the surface, compared to half buried or fully buried, suggesting that burial, either by farmers' management or through natural mechanisms [18], will greatly reduce predation losses, perhaps to an even greater extent than observed in natural ecosystems [19]. Earlier crop sowing [20], and no-till fall cover cropping [6] have been proposed as techniques that farmers could employ to extend the surface residence time of weed seeds, thereby maximizing potential predation losses.

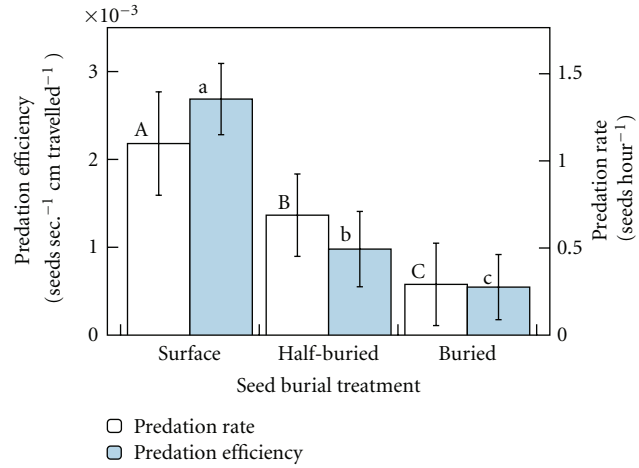

Figure 1: Seed burial effects on *H. rufipes* predation efficiency and rate (mean and SE). Seeds were placed on the soil surface, buried to half their longest dimension, or buried with 1 mm of sand. Bars with different upper case (predation rate) or lower case (predation efficiency) letters are significantly different ($P < 0.05$).

In the simulation models of Westerman et al. [18], sensitivity analyses revealed that predation was affected more by seed availability then seed demand. Further, it has been widely reported that burial reduces, and sometimes eliminates, seed predation [21]. It is important to note that datasets supporting this conclusion are often from forest or grassland ecosystems, where rodents are the predominant seed predators [22, 23]. There are surprisingly few published datasets focused on invertebrate seed predation responses to seed burial, however, evidence suggests that burial may not always prevent seed predation. White et al. [24] conducted controlled environment predation assays with velvetleaf (*Abutilon theophrasti* L. Merr.), redroot pigweed, and giant foxtail (*Setaria faberi* L.), both on the soil surface and buried at a depth of 0.5 or 1.0 cm. Burial reduced predation by *Amare aenea* and *A. sanctaecrucis*, but, interestingly, *Harpalus pensylvanicus* predation was mostly unaffected by burial. The different responses between *H. rufipes* in our experiments, and these results with *H. pensylvanicus*, indicate that further studies of seed burial are likely warranted, at least for invertebrate predators.

Consistent with our second hypothesis, *H. rufipes* predated more seeds from the yellow foxtail and wild mustard feeding zones, the two largest species tested. *H. rufipes* also spent more time in these two zones and entered these zones more frequently than the redroot pigweed zone. Because the four feeding zones were placed across the arena, we interpret the frequency in areas to reflect the beetle's preference for these species. However, feeding efficiency (seeds consumed per unit time per distance travelled) was greater for the smaller seeds of redroot pigweed. While we expected predation efficiency to be regulated by preference, or perhaps ease of detecting larger seeds, this result suggests that seed size and shape affects ease of consumption. Redroot pigweed was the smallest of the three species used in these experiments; wild mustard and yellow foxtail are larger but comparatively

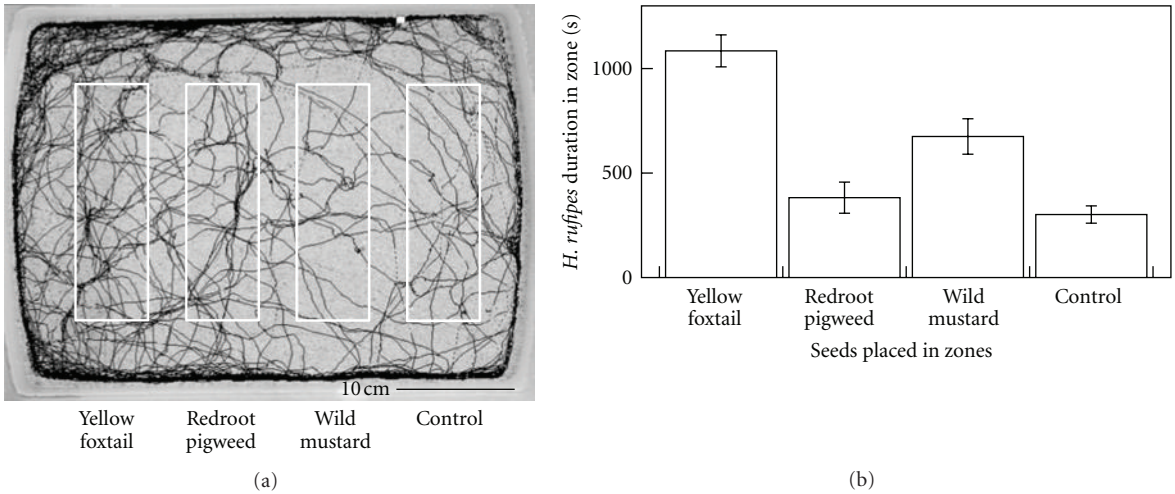

Figure 2: *H. rufipes* tracks from representative choice-feeding assay (a); note, this demonstrates movement but not residence time with a particular zone. Photo shows 48 by 30 arena and the four zones within the arena in which various seeds were placed. As evident in this image, *H. rufipes* spent considerable tracked time in the arena perimeter, outside the defined, seed-containing and control zones in the central area of the arena. Also shown is the total duration of tracked beetle within the zone (mean and SE), in seconds (b).

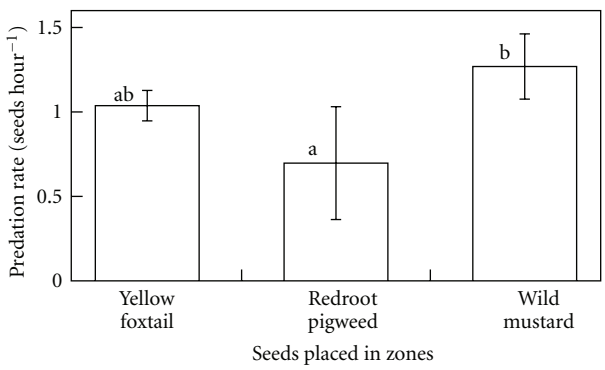

Figure 3: Predation rate for seed of three weed species, yellow foxtail, redroot pigweed, and wild mustard over 10 hour trial periods (mean and SE). Bars with different letters are significantly different ($P < 0.05$).

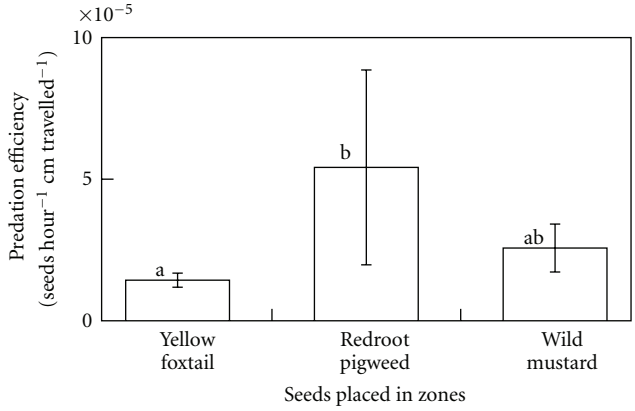

Figure 4: Predation efficiency for seed of three weed species, yellow foxtail, redroot pigweed, and wild mustard, over 10-hour trial periods (mean and SE). Bars with different letters are significantly different ($P < 0.05$).

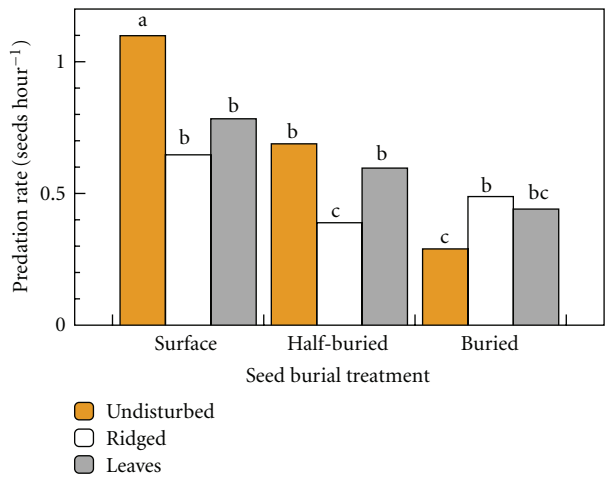

Figure 5: Effects of seed burial and disturbance regime on predation rate. Bars with different letters are significantly different ($P < 0.05$).

similar in mass. Such a preference has been reported for other important carabid seed predators. Harrison et al. [25] found *H. pensylvanicus* preferred the smaller and smoother seeds of yellow foxtail and smooth pigweed (*Amaranthus hybridus* L.) over giant ragweed (*Ambrosia trifida* L.), which has a dispersal unit comprised of a single achene with fused, hardened bracts. Elsewhere, species of *Harpalini* have been described as "generalist" predators, even when individual species were compared to similarly sized species of another carabid genus, *Zabrini* [26].

Predation was greatest for surface seeds on an undisturbed substrate (Figure 5). However, when seed husks were collected at the end of each trial, they were more dispersed in the disturbed treatment, especially being found in the

actual ridges, suggesting that the beetles may have preferred feeding in ridges when given the choice. This suggests the beetles would take a seed, remove it from the feeding zone, and predate on it whilst in the ridge, possibly enjoying the protection a ridge may provide to such a small animal.

5. Conclusion

H. rufipes predation behaviour was influenced by prey species, seed burial, and soil surface conditions. Although predation assays may be conducted without the specialized equipment, video tracking demonstrated that seed burial similarly affected predation rate (seeds predated per hour) and predation efficiency (predation rate per distance travelled). In contrast, predation rate and efficiency differed for certain weed species; yellow foxtail and redroot pigweed predation rates were not different, but predation efficiency was up to several fold greater for redroot pigweed. While our results support an already large body of literature regarding seed burial and preference effects on seed predation, video tracking offered unique-dependent variables that indicated *H. rufipes* behaviour is prey dependent.

Acknowledgments

The authors thank Dr. Mark Hassall, University of East Anglia, for his invaluable supervision throughout this project, also Tom Molloy, Dan Allalemdjian, and Peter Rogers for help with laboratory, beetle and EthoVision work, and Aubrey Barse for provision of essential equipment. Lastly, they thank an anonymous reviewer who made many detailed and thoughtful criticisms of an earlier version of this paper. The authors have no relation to Noldus Information Technology, the manufacturer of EthoVision, nor the Picolo brand of frame grabbing computer hardware. These trade names and sources have been included to assist a reader in repeating our experiments and should not be considered an endorsement. This is Publication no. 3241 from the Maine Agricultural and Forest Experiment Station.

References

[1] M. Liebman and E. R. Gallandt, "Many little hammers: ecological approaches for management of crop-weed interactions," in *Ecology in Agriculture*, L. E. Jackson, Ed., pp. 291–343, Academic Press, San Diego, Calif, USA, 1997.

[2] E. R. Gallandt, "How can we target the weed seedbank?" *Weed Science*, vol. 54, no. 3, pp. 588–596, 2006.

[3] P. R. Westerman, M. Liebman, F. D. Menalled, A. H. Heggenstaller, R. G. Hartzler, and P. M. Dixon, "Are many little hammers effective? Velvetleaf (*Abutilon theophrasti*) population dynamics in two- and four-year crop rotation systems," *Weed Science*, vol. 53, no. 3, pp. 382–392, 2005.

[4] P. Westerman, C. D. Luijendijk, J. D. A. Wevers, and W. Van Der Werf, "Weed seed predation in a phenologically late crop," *Weed Research*, vol. 51, no. 2, pp. 157–164, 2011.

[5] D. A. Bohan, A. Boursault, D. R. Brooks, and S. Petit, "National-scale regulation of the weed seedbank by carabid predators," *Journal of Applied Ecology*, vol. 48, no. 4, pp. 888–898, 2011.

[6] E. R. Gallandt, T. Molloy, R. P. Lynch, and F. A. Drummond, "Effect of cover-cropping systems on invertebrate seed predation," *Weed Science*, vol. 53, no. 1, pp. 69–76, 2005.

[7] D. Johan Kotze, P. Brandmayr, A. Casale et al., "Forty years of carabid beetle research in Europe—from taxonomy, biology, ecology and population studies to bioindication, habitat assessment and conservation," *ZooKeys*, vol. 100, pp. 55–148, 2011.

[8] G. A. Dunn, "Distribution of *Harpalus rufipes* DeGeer in Canada and United States (Coleoptera: Carabidae)," *Entomological News*, vol. 92, no. 5, pp. 186–188, 1981.

[9] J. Zhang, *Biology of* Harpalus rufipes *DeGeer (Coleoptera: Carabidae) in Maine and dynamics of seed Predation*, M.S. thesis, University of Maine, Orono, Me, USA, 1993.

[10] J. A. Lys and W. Nentwig, "Surface activity of carabid beetles inhabiting cereal fields: seasonal phenology and the influence of farming operations on five abundant species," *Pedobiologia*, vol. 35, no. 3, pp. 129–138, 1991.

[11] J. Zhang, F. A. Drummond, M. Liebman, and A. Hartke, "Phenology and dispersal of *Harpalus rufipes* DeGeer (Coleoptera: Carabidae) in agroecosystems in maine," *Journal of Agricultural and Urban Entomology*, vol. 14, no. 2, pp. 171–186, 1997.

[12] A. F. Shearin, S. C. Reberg-Horton, and E. R. Gallandt, "Cover crop effects on the activity-density of the weed seed predator *Harpalus rufipes* (Coleoptera: Carabidae)," *Weed Science*, vol. 56, no. 3, pp. 442–450, 2008.

[13] A. Hartke, F. A. Drummond, and M. Liebman, "Seed feeding, seed caching, and burrowing behaviors of *Harpalus rufipes* de geer larvae (Coleoptera: Carabidae) in the maine potato agroecosystem," *Biological Control*, vol. 13, no. 2, pp. 91–100, 1998.

[14] L. P. J. J. Noldus, A. J. Spink, and R. A. J. Tegelenbosch, "Computerised video tracking, movement analysis and behaviour recognition in insects," *Computers and Electronics in Agriculture*, vol. 35, no. 2-3, pp. 201–227, 2002.

[15] L. P. J. J. Noldus, A. J. Spink, and R. A. J. Tegelenbosch, "EthoVision: a versatile video tracking system for automation of behavioral experiments," *Behavior Research Methods, Instruments, and Computers*, vol. 33, no. 3, pp. 398–414, 2001.

[16] A. Walton, A. Branham, D. M. Gash, and R. Grondin, "Automated video analysis of age-related motor deficits in monkeys using EthoVision," *Neurobiology of Aging*, vol. 27, no. 10, pp. 1477–1483, 2006.

[17] A. J. Spink, R. A. J. Tegelenbosch, M. O. S. Buma, and L. P. J. J. Noldus, "The EthoVision video tracking system—a tool for behavioral phenotyping of transgenic mice," *Physiology and Behavior*, vol. 73, no. 5, pp. 731–744, 2001.

[18] P. R. Westerman, M. Liebman, A. H. Heggenstaller, and F. Forcella, "Integrating measurements of seed availability and removal to estimate weed seed losses due to predation," *Weed Science*, vol. 54, no. 3, pp. 566–574, 2006.

[19] P. E. Hulme and T. Borelli, "Variability in post-dispersal seed predation in deciduous woodland: relative importance of location, seed species, burial and density," *Plant Ecology*, vol. 145, no. 1, pp. 149–156, 1999.

[20] P. R. Westerman, A. Hofman, L. E. M. Vet, and W. Van Der Werf, "Relative importance of vertebrates and invertebrates in epigeaic weed seed predation in organic cereal fields," *Agriculture, Ecosystems and Environment*, vol. 95, no. 2-3, pp. 417–425, 2003.

[21] P. E. Hulme, "Post-dispersal seed predation: consequences for plant demography and evolution," *Perspectives in Plant Ecology, Evolution and Systematics*, vol. 1, no. 1, pp. 32–46, 1998.

[22] P. E. Hulme and J. Kollmann, "Seed predator guilds, spatial variation in post-dispersal seed predation, and potential effects on plant demography: a temperate perspective," in *Seed Fate. Predation, Dispersal and Seedling Establishment*, P. Forget, J. Lambert, and P. Hulme, Eds., pp. 9–30, CAB International, Wallingford, UK, 2005.

[23] P. E. Hulme, "Post-dispersal seed predation in grassland: its magnitude and sources of variation," *Journal of Ecology*, vol. 82, no. 3, pp. 645–652, 1994.

[24] S. S. White, K. A. Renner, F. D. Menalled, and D. A. Landis, "Feeding preferences of weed seed predators and effect on weed emergence," *Weed Science*, vol. 55, no. 6, pp. 606–612, 2007.

[25] S. K. Harrison, E. E. Regnier, and J. T. Schmoll, "Postdispersal predation of giant ragweed (*Ambrosia trifida*) seed in no-tillage corn," *Weed Science*, vol. 51, no. 6, pp. 955–964, 2003.

[26] A. Honek, Z. Martinkova, P. Saska, and S. Pekar, "Size and taxonomic constraints determine the seed preferences of Carabidae (Coleoptera)," *Basic and Applied Ecology*, vol. 8, no. 4, pp. 343–353, 2007.