Improvement of the Residual Activity of a Cucurbitacin-based Adult Corn Rootworm (Coleoptera: Chrysomelidae) Insecticide[1]

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J. Entomol. Sci. 36(4): 416-425 (October 2001)

Abstract Areawide management of the corn rootworm, *Diabrotica* spp., focuses on managing adult beetles to decrease egg deposition. The primary management tool for this approach is a commercial bait composed of a feeding stimulant (cucurbitacin), inert ingredients, and a small quantity of insecticide. Cucurbitacin-based baits have had poor residual insecticidal activity that decreases efficacy and economic practicality. These studies were designed to extend the residual activity of 2 commercial bait formulations, Slam Prader® and Slam SD® (MicroFlo Co., Lakeland, FL), by adding materials as a tank-mixed adjuvant or as a part of the formulation to prevent wash-off by rain. Initial assays with Slam Prader identified low pH gluten and sodium lignate to have the greatest potential to provide rainfastness with a trend for less residual activity with higher concentrations (>2% of spray volume) of adjuvant. An additional assay demonstrated that low pH gluten was effective for resisting wash-off by natural rain when added to the Slam SD as a formulation ingredient. Large-plot experiments conducted over 2 yrs in growers' fields in northwestern Illinois failed to show a statistically significant advantage of gluten and lignin additives. For both years, all bait treatments provided adequate management of the adult corn rootworm for 3 wks after application. Based on sticky trap counts, insecticide treated plots maintained beetle populations below the economic threshold of 5 corn rootworms/trap/day and significantly below the corn rootworm density in the untreated plots.

Key Words Cucurbitacin, corn rootworm, adjuvant, formulation, insecticidal bait, natural enemies

Areawide management of the corn rootworm, *Diabrotica* spp. (Coleoptera: Chrysomelidae), focuses on managing adult beetles to decrease egg deposition and thus decrease the number of future larvae in continuous-corn cropping systems. This management strategy is based on monitoring beetle populations and making insecticide applications only when the number of beetles exceeds the economic threshold. The primary management tool is a commercial bait that is a combination of cucurbitacins, inert ingredients, and a small quantity of insecticide. Cucurbitacins are extremely bitter triterpenoids found mainly in plant members of the Cucurbitaceae family; although feeding deterrents for many phytophagous insects, they stimulate corn rootworm beetles to feed uncontrollably (Metcalf and Lampman 1989). Therefore,

[1]Received 23 June 2000; accepted for publication 05 February 2001.

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only a low insecticide concentration is required in the bait, which may reduce the impact of its use on non-target organisms and beneficial insects. Currently, there is only one commercially available bait that uses this concept for insect management. There are two formulations of this bait. One has been used extensively but is expensive to manufacture; the other, a spray-dried formulation, is cheaper to manufacture but was withdrawn from the market in part due to its short residual activity.

In general, the cucurbitacin-based adulticides have a limited residual life: a dry formulation only maintained efficacy for 1 to 2 wk (Lance and Sutter 1992), and both dry and liquid bait formulations had to be replaced every 5 to 7 d to maintain effectiveness (Brust and Foster 1995). It is possible to improve coverage and resist wash-off of the bait with the addition of adjuvants to the tank mix or by making improved formulations. Various materials have been tested in our laboratory and found effective in increasing the residual life of insect pathogens. In particular, gluten and lignin adjuvants can increase rainfastness (Behle et al. 1997, Shasha et al. 1998). The main objective of the research reported herein was to determine the most efficacious additives and concentrations of each additive to decrease wash-off and extend the residual life of bait formulations. In addition, the effect of the experimental treatments on natural enemies was evaluated.

Materials and Methods

Field/laboratory assays. Field/laboratory assays were conducted in 1997 and 1998 to determine the most effective lignin and gluten additives and their concentrations to resist wash-off when added to two commercial bait formulations, Slam Prader® and Slam SD® (MicroFlo Co., Lakeland, FL). In 1997, two assays were conducted to test the additives, used as adjuvants, to resist wash-off of Slam Prader by simulated rain. Hybrid field corn (Golden Harvest 2344) was planted on 29 April in a 10 × 20 m plot at the USDA-ARS National Center for Agricultural Utilization Research in Peoria, IL. On 14 July (Assay 1) and 22 July (Assay 2), experiments were conducted in four rows located in the center of the 20-row plot. A randomized block design was used with each row a replicate, and treatments were randomly assigned to the first fully expanded leaf from the top of the each plant within the row. A 40-mm diam circle was drawn on each leaf to delineate the area to be treated. Treatments were prepared by adding the adjuvants to suspensions of Slam Prader that contained 45 mg of bait/ml H_2O. The additives included low pH gluten, high pH gluten, urea with gluten (Assay 1 only), sodium lignate, potassium lignate, and Windbrake® (Riverside/Terra Corp., Sioux City, IA) (Table 1). Gluten treatments were tested at 2 and 4% w/v concentrations (% w/v = gram weight adjuvant per 100 ml final volume spray) in the Assay 1 and at 0.5 and 1% w/v in Assay 2. Lignin adjuvants were tested at 1 and 2% w/v in Assay 1 and 0.5 and 1% w/v in Assay 2. Windbrake was used at the recommended rate of 0.1% v/v for all experiments (Hoffmann et al. 1996a). For both experiments, Slam Prader without adjuvants was tested with and without exposure to simulated rainfall and the control was a no treatment. All treatments of Slam Prader were stirred prior to application of 250 µl, which was pipetted onto the previously marked circles. Treatments were spread evenly within the circle with a glass rod and were allowed to dry overnight for the Assay 1 and for 3 d for the Assay 2. Leaf circles containing Slam Prader without adjuvants and without exposure to simulated rainfall were cut from the leaf with a cork borer and refrigerated. Two overhead sprinklers were used to apply ≈5 cm of water in the first assay and ≈5 and 10 cm of water in the

Table 1. Ingredients of non-commercial adjuvants added to Slam to increase rainfastness in assays and large-scale plot research

Designation	Ingredients
Low pH Gluten	Wheat Gluten, Citric Acid (5:1, wt/wt)
High pH Gluten	Wheat Gluten, Sodium Triphosphate (10:1 wt/wt)
Gluten/Urea	Wheat Gluten, Urea (1:1, wt/wt)
Sodium Lignate	PC-1307 Kraft Lignin, Calcium Chloride (10:1 wt/wt)
Potassium Lignate	Kraft Lignin, Calcium Chloride (10:1 wt/wt)

second assay. After drying for 1 h, the leaf circles were cut as before and placed individually on moistened filter paper in 100 × 50-mm Petri dish cages. Five adult western corn rootworms, *Diabrotica virgifera virgifera* LeConte, were placed in each dish along with a water-moistened, 3-cm cotton wick. The beetles were provided by USDA–ARS Northern Grain Insect Research Laboratory, Brooking, SD, and were chilled to 5°C prior to placement in the dishes. Any beetles that did not recover within 30 min of the transfer were replaced. The number of dead beetles was counted after 24 h.

One assay was set up in 1998 to test the rainfastness of the Slam SD formulation with and without gluten additives. The additives were mixed with Slam SD prior to the addition of water. The experiment was conducted similarly to those in 1997 but with the following differences: the bait treatments were applied as 5 drops (25 µl per drop) with a pipette to the corn leaves to better simulate field applications; natural rain rather than simulated rain from irrigation sprinklers was used to test the treatments; and a no rain sample was collected for each formulation treatment, not only the Slam SD treatments. The six treatments were Slam SD without adjuvants, Slam SD with low pH gluten at 0.5 and 1% w/v and Windbrake (0.1% v/v), Slam Prader without additives, and an untreated control. The treatments were applied on 17 June. Leaf samples for the no rain treatments were collected after drying and were placed individually in cages and refrigerated until assayed. On 18 June, 0.63 cm of rain fell within 1 h, and the rain treatment samples were collected on 19 June and placed in cages. All rain and no rain treatments were assayed against 10 western corn rootworm beetles per leaf disk and five leaf disks per treatment. The number of dead beetles was counted after 24 h as before. Percentage mortality data for all assays were analyzed using a nonparametric method that first ranks each data point within each replicate separately and then analyzes the ranks as if they are observed responses using ANOVA (Eskridge 1995). Treatment means were separated using least significant difference (LSD) at the $P = 0.05$ level (SAS Institute Inc. 1995).

Large-scale field experiments. The 1997 large-scale research was conducted in cooperating growers' fields of continuous corn in northwestern Illinois (Whiteside Co.). The treatment plots within each field were rectangular in shape and approximately 8 ha in size. In each of four fields, six treatments were randomly assigned to plots: control (no treatment), Slam Prader at a recommended rate of 0.57 kg/ha, Slam mixed with each of three adjuvants (low pH gluten, sodium lignate, and potassium lignate at 1% wt/vol.) and Windbrake at 0.1% v/v (Hoffmann et al. 1996a). Adult beetle emergence and sex ratio were monitored using whole plant counts of adult beetles to time the application of treatments; the treatments were applied on 31 July at a spray rate of 9.5 L/ha. A J164A AgCat, flying at 175 kph with a swath width of 15 m, was equipped with 12 D6 nozzles without screens at 0° deflection to apply the desired quantity and droplet size, i.e., relatively large droplets with diameters within the 500 to 700 μ range (Hoffmann et al. 1996b).

The number of adult beetles captured on Pherocon AM unbaited traps® (Trece Inc., Salinas, CA) was used to evaluate the treatments (Hein and Tollefson 1985). The traps were placed in the plots prior to application (28 July and removed on 30 July) and the day after application (1 Aug) and exchanged on day 4, 7, 11, 14, 18, 21, 25 and 28 post-application. Ten traps were placed within the center of each treatment plot. The pattern of trap placement consisted of five traps (each wrapped around corn plants above the primary ear, ≈1.2 m above ground) in two lines in each treatment plot with 30 m separating each trap; all traps were at least 30 m from plot edges. The number of northern (*Diabrotica barberi*, Smith & Lawrence) and western corn rootworm beetles and species of adult lady beetles (Coleoptera: Coccinellidae) and green/brown lacewings (Neuroptera: Chrysopidae/Hemerobiidae) were counted and recorded after each trap collection. One plastic rain gauge (12.5 cm) was placed in each field to monitor rainfall during the post-application sampling period. Rainfall was recorded, and gauges were emptied on each sampling date.

In 1998, three fields in Whiteside Co. and one field in Ogle Co. were selected for the experiment, which was conducted the same as in 1997 with respect to experimental design, plot size, and application rates and methods. Six insecticidal treatments were applied on 6 August within each field. The treatments included experimental formulations of Slam SD with gluten at 0.5 and 1% and sodium lignate at 2 and 3%, Slam SD and Slam Prader with Windbrake. A control (no insecticide) treatment was included at each field. The densities of corn rootworm beetles in each plot were monitored using the same number and similar placement of sticky traps as in 1997. Traps were placed in the fields 2 ds prior to insecticide applications and removed before application. Traps were again placed in the field the day after application and exchanged twice per week for the following 3 wks. After traps were collected, counts of corn rootworm, lady beetle and lacewing adults were made. For both years on each sample date, the average number of corn rootworms and natural enemies collected per trap per day was calculated. The data were analyzed by ANOVA as a repeated measure, and treatment means were separated using Tukey-Kramer at the $P = 0.05$ level (SAS Institute 1995). Data on natural enemies (lady beetle and lacewing) were combined in 1997. The data for natural enemies were analyzed separately for each group, and orthogonal contrasts were used to compare the combined insecticidal treatment means with the unreated (control) means for lady beetles and lacewings in 1998.

Results and Discussion

Field/laboratory assays. In 1997, all the additives were tested as adjuvants with Slam Prader to resist wash-off and maintain insecticidal activity. In the first assay after 5 cm of simulated rain, there was no significant difference in residual activity between the two lignin adjuvants or among the three gluten adjuvants (Table 2). The lignin adjuvants maintained insecticidal activity better than gluten adjuvants, causing at least 80% adult western corn rootworm mortality; however, only potassium lignate at

Table 2. First 1997 assay of residual activity of Slam Prader with and without adjuvant combinations measured by percentage mortality of *D. v. virgifera* adults after 5 cm of simulated rainfall

Treatments	% Mortality ± SEM
Slam w/o rain	100 ± 0.00a
K Lignate 1%	100 ± 0.00a
K Lignate 2%	90 ± 0.12ab
Na Lignate 1%	90 ± 0.12ab
Na Lignate 2%	80 ± 0.40abc
Gluten/Urea 2%	75 ± 0.19bcd
LpH Gluten 2%	60 ± 0.16cde
LpH Gluten 4%	59 ± 0.43cde
Slam Prader w/o adj	55 ± 0.30cde
Gluten/Urea 4%	55 ± 0.25cde
Windbrake 0.1%	50 ± 0.20def
HpH Gluten 4%	25 ± 0.10ef
Control (no trt)	10 ± 0.12f

Means are based on 4 replicates. Data were analyzed by nonparametric ANOVA.
Column means with same letters do not differ significantly ($P > 0.05$) by LSD.

1% caused significantly higher mortality than the gluten adjuvants. Only potassium lignate at 1 and 2% and sodium lignate at 1% increased the residual activity when compared with Slam Prader without adjuvants, which maintained 55% of its original activity. There were no significant differences in the activity of the adjuvants with Slam after simulated rain due to the adjuvant concentrations; however, there was a trend for less residual activity with higher concentrations of adjuvant.

All insecticidal treatments maintained at least 95% residual activity after 5 cm of simulated rain in the second assay. After 10 cm of simulated rain, the residual activity of the insecticidal treatments ranged from 30 to 100% (Table 3). Low pH gluten at 0.5 and 1% maintained the activity of Slam significantly better than potassium lignate at 0.5 and 1% and sodium lignate at 0.5%. Only low pH gluten at 0.5% provided increased residual activity when compared with Slam without adjuvants. In both 1997 experiments, there was no increased rainfastness of Slam from the addition of Windbrake at 0.1%.

One assay in 1998 tested the rainfastness of Slam SD with the addition of gluten as part of the formulation. Beetle mortality ranged from 88 to 96% with no significant differences among the Slam treatments in the first assay without exposure to rain (Table 4). After 0.6 cm of natural rain, percentage beetle mortality for Slam Prader, gluten 1%, and gluten 0.5% treatments after rain was 62%, 60%, and 46%, respectively. Also, Slam SD and Slam SD with Windbrake caused significantly less mortality than the other Slam treatments, which was not significantly different than the un-

Table 3. Second 1997 assay of residual activity of Slam Prader with and without adjuvant combinations measured by percentage mortality of *D. v. virgifera* adults after 10 cm of simulated rainfall

Treatments	% Mortality ± SEM
Slam w/o rain	100 ± 0.00a
LpH Gluten 0.5%	100 ± 0.00a
LpH Gluten 1%	90 ± 0.20ab
Na Lignate 1%	80 ± 0.16bc
LpH Gluten 2%	75 ± 0.25bc
Slam Prader w/o adj	65 ± 0.10cd
K Lignate 1%	60 ± 0.33cd
K Lignate 0.5%	60 ± 0.33cd
Na Lignate 0.5%	40 ± 0.0.23de
Windbrake 0.1%	30 ± 0.12ef
Control (no trt)	5 ± 0.10f

Means are based on 4 replicates. Data were analyzed by nonparametric ANOVA.
Column means with same letters do not differ significantly ($P > 0.05$) by LSD.

Table 4. 1998 Assay of residual activity of Slam formulations measured by percentage mortality of *D. v. virgifera* adults before and after 0.6 cm of natural rainfall

	% Mortality ± SEM	
Treatments	No rain	Rain
Slam Prader	96 ± 0.05a	62 ± 0.18a
Slam SD	95 ± 0.11a	0 ± 0.00d
SD + Gluten (0.5%)	92 ± 0.04a	46 ± 0.15b
SD + Gluten (1.0%)	88 ± 0.08a	60 ± 0.19ab
SD + Windbrake	92 ± 0.08a	10 ± 0.10c
Untreated	2 ± 0.04b	2 ± 0.04d

Means are based on 5 replicates. Data were analyzed by nonparametric ANOVA.
Column means with same letters do not differ significantly ($P > 0.05$) by LSD.

treated control. For both years, the assays indicated the most appropriate additives to be tested in the large-plot experiments.

Large-scale field experiments. In both years, the total number of adult corn rootworms consisted of ≈80 to 90% of western corn rootworms, and the number of corn rootworm beetles within the fields decreased during the sampling periods. In

1997, beetle counts decreased from 15.7 rootworm adults/trap/day prior to applications to 9 rootworm adults/trap/day on 29 Aug in the untreated plots (Fig. 1). Preapplication trap counts of adult corn rootworms ranged from 12.3 to 26.2 per trap per day. On 5 Aug (the first sampling date after treatment application), there were considerable reductions (≈94%) in the number of beetles captured per trap per day. Trap counts per trap per day averaged 1.1 for Slam Prader, 0.7 for Slam Prader with Windbrake, 1.2 for low pH gluten, 2.7 for sodium lignate, and 2.3 for potassium lignate compared with 19.3 in the control plots. Significantly fewer rootworm adults/trap/day were found for each insecticide-treated plot when compared with the untreated plots for the first 6 sampling dates after treatment applications. Trap counts in all Slam treatments were generally below 5 rootworm adults/trap/day through 22 Aug after which there were no significant differences in corn rootworm numbers among any of the experimental treatments. For the entire post-application period, there were significant reductions in number of beetles captured per trap per day between each Slam treated plot and the control.

In 1998, the initial population level of corn rootworms within the experimental fields was considerably lower than in 1997, and beetle densities in the untreated plots steadily declined during the sampling period. Insecticide treatments were applied on

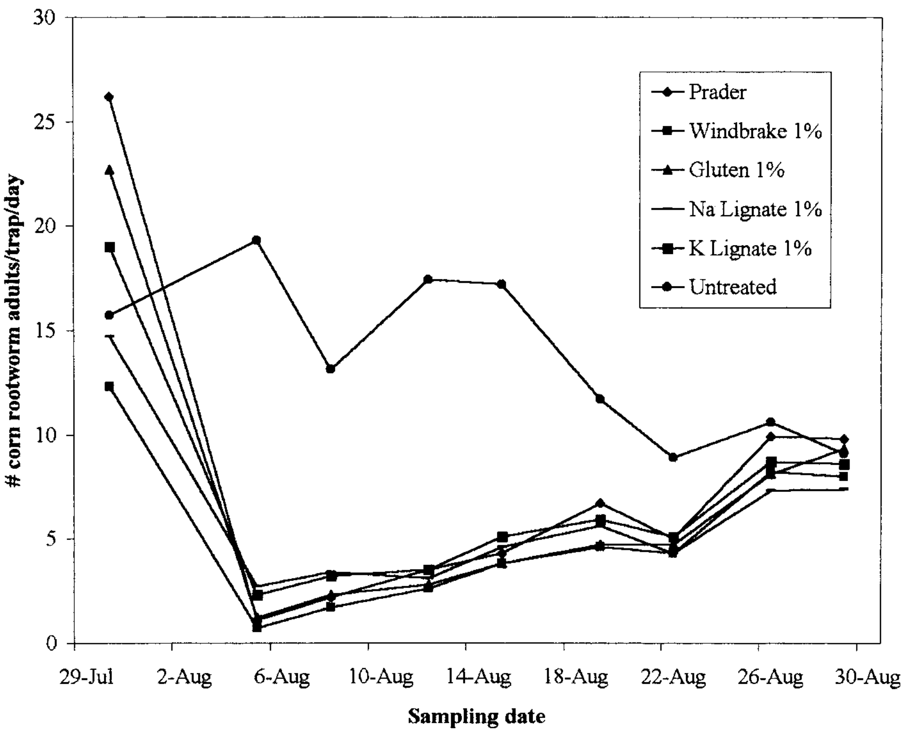

Fig. 1. Mean number of corn rootworm adults (*D. v. virgifera* and *D. barberi*) captured per trap per day in 1997 before and after application of Slam treatments on 31 July.

6 Aug when the adult rootworm density (averaged ≈8 rootworm adults/trap/day) was above the economic threshold. On 7 Aug, trap counts of adults averaged <1 per trap per day within all insecticidal treatment plots with the exception of Slam SD with sodium lignate at 3%, which averaged 1.06 rootworm adults/trap/day (Fig. 2). There was an ≈87% reduction in the relative numbers of corn rootworms within the insecticidal treatment plots when compared with the control plots. Trap counts in insecticide treated plots remained below 1 adult rootworm/trap/day for the 3-wk sampling period following application. Over the entire post-application period, significantly fewer corn rootworms were trapped in plots treated with the insecticidal bait formulations when each was compared with the control plots.

Total rainfall during the 1997-postapplication period averaged 6.25 cm for the 4 fields. The measurable precipitation occurred during the sampling periods ending on 12 Aug (3 cm), 15 Aug (0.5 cm), and 19 Aug (2.75 cm). In 1998, the total average rainfall for the post-application period was 7.35 cm with precipitation occurring during the sampling periods ending on 11 Aug (0.65 cm), 18 Aug (0.9 cm), 25 Aug (2.2 cm), and 28 Aug (3.6 cm).

In 1997, the average number of natural enemies increased throughout the sampling period in all treatment plots from 0.12 natural enemies/trap/day on 30 July to

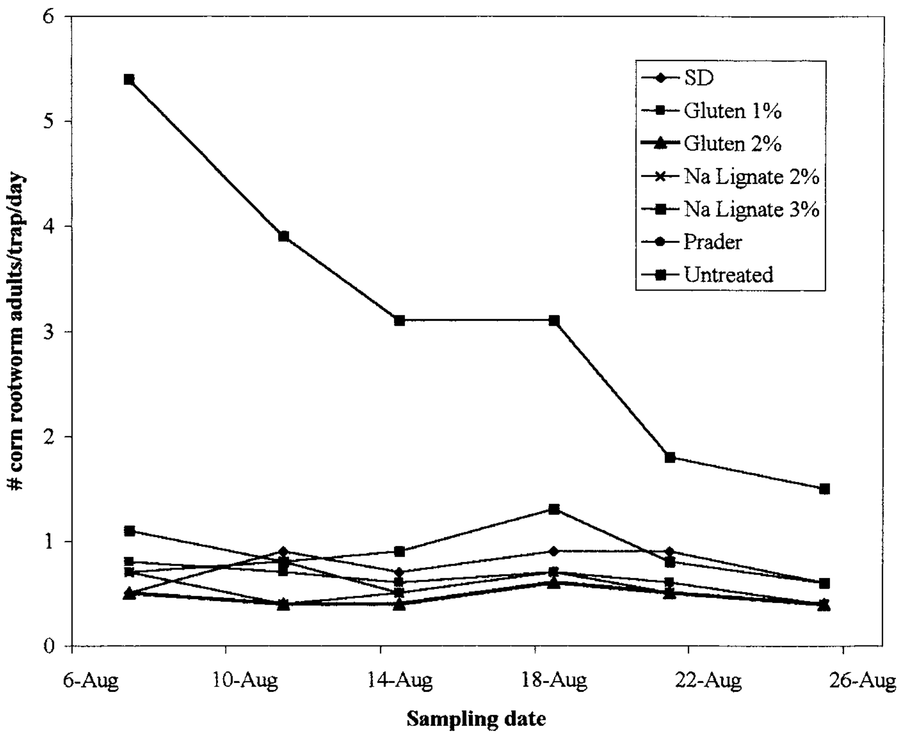

Fig. 2. Mean number of corn rootworm adults (*D. v. virgifera* and *D. barberi*) captured per trap per day in 1998 after application of Slam treatments on 6 Aug.

1.02 natural enemies/trap/day on 29 Aug. Per sampling date, there were no consistent significant differences for the number of natural enemies/trap/day among treatments after their application. For the entire post-application period, fewer natural enemies were found in the control plots (0.36 natural enemies/trap/day) versus the insecticide treated plots (0.47 natural enemies/trap/day). In 1998, a similar increase in the number of natural enemies was found in all plots from an average of 0.25 per trap per day on 7 Aug to 1.18 per trap per day on 18 Aug, after which there was a decline in numbers on the last two sampling dates. Over the entire post-application period, the average number of natural enemies trapped in the insecticide treated plots was not significantly different from that in the control plots. The majority of natural enemies captured on the Pherocon traps during the sampling period were Coccinellidae; the highest relative number of lady beetles (1.05/trap/day) in the treatment plots occurred on 18 Aug (Fig. 3). There were significantly fewer Coccinellidae found in all insecticide treated plots when compared with the untreated control only on the first sampling date after treatment application on 7 Aug. The average maximum number of lacewings (0.22/trap/day) occurred on 21 Aug, and significantly fewer were trapped in insecticidal plots when compared with control plots on 21 and 25 Aug ($F = 9.58$; df = 1; $P = 0.0022$ and $F = 9.20$; df = 1; $P = 0.0027$, respectively).

Large-scale field experiments did not demonstrate dramatic differences in efficacy

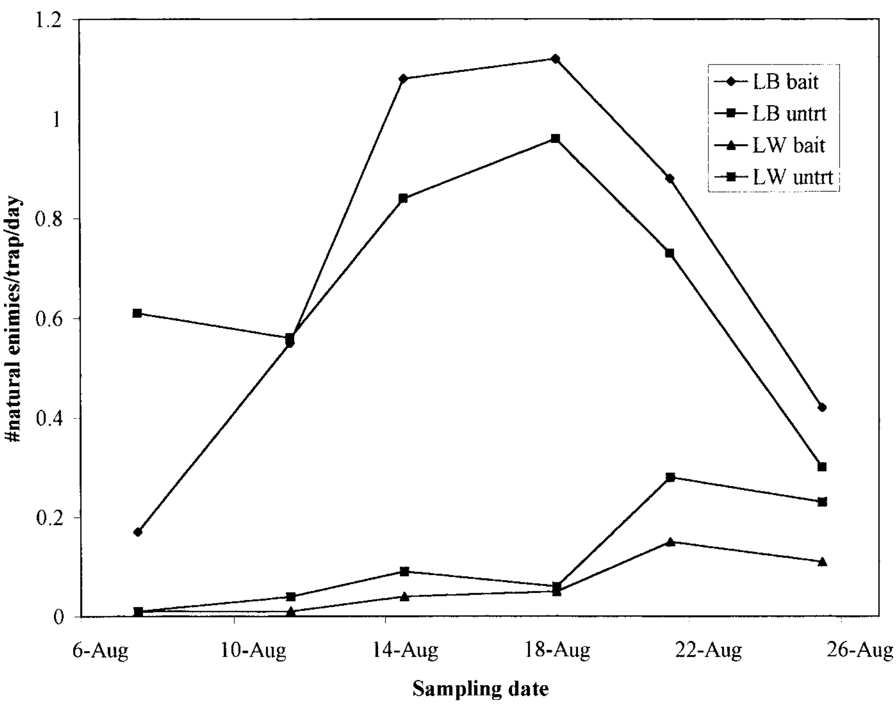

Fig. 3. Mean number of adult Coccinellidae (LB) and Chrysopidae/Hemerobiidae (LW) captured in Slam (bait) treated plots vs control (untrt) per trap per day in 1998 after application of treatments on 6 Aug.

of these materials as adjuvants or as part of the formulations. In 1997, the lack of difference among treatments could be attributed to the lack of rain shortly after application, which did not allow for a rigorous test of rainfastness. In 1998, the population levels were considerably lower than that of 1997. In 1998, fewer corn rootworms were captured in plots where 2% gluten and lignin formulations were applied; however, all bait treatments kept the adult corn rootworm population well below the economic threshold throughout the sampling period.

Despite the limited difference among bait treatments during 2 yrs of experiment, a positive aspect is that the Slam Prader formulation does have a reasonably long residual life, i.e., ≈3 wk or more. In small-plot and large-plot experiments, this formulation retained at least 50% of original activity and was able to maintain corn rootworm populations below damaging levels, respectively. An additional benefit of any insecticide would be minimal impact on natural enemy complex of the corn ecosystem. In both years, there was little evidence that the Slam insecticide applications had detrimental effects on the two groups of natural enemies monitored.

Acknowledgments

We thank Doug Freeze, Monica Wetzel, Erica Bailey, Holly Goebel, Jeff Bradshaw, and Paul Rohlwing for their technical support. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendations or endorsement by the U.S. Department of Agriculture.

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