Evaluation of a Water-Soluble Bait for Corn Rootworm (Coleoptera: Chrysomelidae) Control[1,2]

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Abstract Laboratory and field-cage experiments evaluated the efficacy of a water-soluble bait for control of adult diabroticites. The bait was composed of a water-soluble feeding stimulant derived from a bitter mutant of Hawkesbury watermelon (*Citrullus vulgaris* Schrad), a dye toxin (phloxine B: D & C Red Dye No. 28), and a modified food starch (Mira Sperse 626). In the laboratory, southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber adults, were exposed to separate bait components and combinations thereof. Eighty percent of adults exposed to the complete bait formulation died within 24 h. Adult mortality increased with higher concentrations of dye and increasing light intensity. Incorporation of the feeding stimulant with the dye reduced the lethal time. The complete bait was most active in sunlight, killing 90% adults in 1 h. Under fluorescent lights, the LT90 for the adults was 48 h. In field cages, using corn plants treated with the bait formulation, numbers of live western corn rootworm, *D. virgifera virgifera* LeConte, adults were reduced by 80% compared with the untreated control. This new water soluble toxic bait appears suitable for testing on a larger scale in the field as a component of an IPM program for corn rootworm pests.

Key Words *Diabrotica virgifera virgifera, Diabrotica undecimpunctata howardi*, cucurbitacin E glycoside, phloxine B, D & C Red Dye No. 28, Hawkesbury watermelon

Growers have routinely applied soil insecticides to 50 to 60% of the corn acreage (12 to 16 million ha) annually (Metcalf 1986) to control corn rootworm beetles (Coleoptera: Chrysomelidae). Insecticides are generally applied prophylactically and are frequently unnecessary and may impose health risks to growers, livestock, and wildlife. Detection of insecticides in ground and surface water is also of concern (Lance and Sutter 1992). Costs to control corn rootworms, combined with crop losses, can exceed $1 billion per year (Metcalf 1986). The northern corn rootworm, *Diabrotica longicornis barberi* Smith and Lawrence; western corn rootworm, *D. virgifera virgifera* LeConte; southern corn rootworm, *D. undecimpunctata howardi* Barber; and Mexican corn rootworm, *D. virgifera zeae* Krysan & Smith comprise the complex of the most destructive pests of corn in North America. We chose two of these insects, southern and western corn rootworms, for our studies.

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[2]This article reports the results of research only. Mention of a proprietary product does not constitute endorsement or a recommendation for its use by the USDA nor does it imply registration under the FIFRA ACT as mentioned.

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Over the past decade, there has been a major effort to replace the ineffective and environmentally undesirable soil insecticides with baits containing cucurbitacins and toxins (Metcalf et al. 1987, Lance 1988, Weissling et al. 1989, Lance and Sutter 1990, Lance and Sutter 1991, Weissling and Meinke, 1991, Brust and Foster 1995). By lacing the baits with cucurbitacins and micro-doses of conventional synthetic insecticides, the diabroticite beetles compulsively feed on it and die. These baits are pest specific and consist of a point source or broadcasted bait capable of killing 99% of the beetles consuming it (Tallamy and Halaweish 1993).

A semiochemical-based insecticide bait (Slam®, Micro-Flo, Lakeland, FL) is being developed that is specific to corn rootworm and striped cucumber beetle. The pesticide carbaryl used in Slam is <5% of the current rate of carbaryl recommended for adult corn rootworm control (Metcalf et al. 1987, Lance and Sutter 1990). Slam is currently being evaluated in the USDA Area-Wide Suppression Program for Corn Rootworm Control, on limited corn acreage in the Midwest (Chandler et al. 1995).

Cucurbitacins, the tetracyclic triterpenoids that comprise the bitter tasting characteristics of Curcubitaceae (Rehm et al. 1957, Guha and Sen 1975), are powerful feeding stimulants to many of the corn rootworm pests (Metcalf and Metcalf 1992, Metcalf 1994). Studies have been conducted to identify dependable high-yielding cucurbit fruits possessing substantial cucurbitacin content (Rhodes et al. 1980) and to demonstrate their use in a ground dry state, for toxic baits (Metcalf et al. 1987).

Consistent with these emerging bait technologies is the need for environmentally-compatible water soluble baits composed of an attractant or arrestant (such as a sex pheromone or feeding stimulant) with a biocontrol agent or a toxicant with low mammalian toxicity.

In recent years, the development of photoactive molecules as candidate pesticides is gaining renewed interest. The class of compounds shown to be most effective against insects are the halogenated xanthenes. According to Heitz (1995), many insects are susceptible to photodynamic action by dyes, but their effects against the corn rootworm are unknown.

The fruit of a bitter mutant of Hawkesbury watermelon, *Citrullus vulgaris* Schrad, contains cucurbitacin (Rehm et al. 1957, Guha and Sen 1975, Peterson and Schalk 1985) and extracts of the melon elicit a strong visitation and feeding stimulant/arrestant response to the banded cucumber beetle *Diabrotica balteata* Le Conte (Peterson and Schalk 1985). These reports coupled with laboratory observations on the phagostimulancy of bitter mutant Hawkesbury watermelon to corn rootworms (DeMilo et al., unpubl. data) led us to investigate the melon as a reliable source of a corn rootworm feeding stimulant that could be formulated with a candidate safe water-soluble photoactive toxicant, phloxine B (D & C Red Dye No. 28).

In this paper, we report on the results of laboratory and field cage tests of new water soluble bait formulation incorporating phloxine B (hereafter referred to as "dye") as a toxin and the extract of the bitter mutant of Hawkesbury melon as a feeding stimulant against the western and southern corn rootworms.

Tests were conducted to determine the effect of varying bait components or selected combinations varying feeding stimulant and dye concentrations and artificial versus sunlight on corn rootworm mortality because the photodynamic action of the dye is a function of light intensity (Martin et al., unpubl. data). The efficacies of the bait formulations for control of the corn rootworm adults were determined in walk-in cages placed in a sweet corn field.

Materials and Methods

Source of insects. Adult southern corn rootworms were supplied by French Agricultural Research, Inc., Lamberton, MN. Adults were maintained in screen cages at 21°C, 70% RH, a photoperiod of 16:8 (D:L) h, and fed a dry diet (Guss and Krysan 1973) and water. Adult western corn rootworms were supplied by the USDA, ARS, Northern Grain Insects Laboratory, Brookings, SD. These adults were maintained as described for southern corn rootworms.

Preparation of feeding stimulant. The feeding stimulant used for laboratory and field-cage experiments was prepared as follows. One kilogram of the sliced rind from bitter Hawkesbury watermelon was placed in a 3-liter stainless-steel blender along with 1-liter methanol. The rind was homogenized for 1 to 2 min. An additional liter of methanol was added, the homogenate transferred to an Erlenmeyer flask, and stored in the refrigerator. After 2 d at 5° to 7°C, the homogenate was filtered through a medium-porosity sintered-glass funnel. The bottom of the sintered-glass funnel was lined with 25 to 50 mm layer of fine glass wool to minimize plugging of the filter plate by the suspended solids. The filtrate was stripped to dryness (bath temperature, 45° ± 5°C) using a Buchi Model RE 111 rotary evaporator (Brinkmann Instruments, Inc., Westbury, NY) equipped with a dry-ice trap. The residue was further dried under high vacuum (0.1 Torr). The solid residue was diluted with water to form the appropriate concentrations for the tests.

Influence of components/combinations of bait formulations on southern corn rootworm adult mortality. Ten adults were placed in a plastic Petri dish (100 × 15 mm), and presented with one of the following bait formulations: 10% (wt/vol) starch and distilled water; 10% starch and 10% feeding stimulant; 10% starch and 5% dye; or 10% starch/5% dye/10% feeding stimulant combination. The starch product used in these experiments was Mira Sperse 626® (A.E. Staley Manufact. Co., Decatur, IL). Each treatment was replicated three times. Petri dishes were randomly arranged 10.5 cm under Cool White fluorescent lights (ISCO, 1982, 5200-5900 lux) in a growth chamber. Lux measurements were used as an estimate of light intensity. The chamber was set at a photoperiod of 16:8 (L:D)h and maintained at 25°C. Mortality was recorded at 16, 20 and 24 h.

Influence of varying dye concentrations on southern corn rootworm adult mortality with and without feeding stimulant. In an experiment similar to that described above, adults were exposed to five concentrations of dye (0.05%, 0.1%, 0.5%, 1%, 5%), with and without feeding stimulant (10%). Mortality was recorded at 16, 24, and 40 h.

Influence of reduced dye concentrations on southern corn rootworm adult mortality exposed to artificial light. The same procedure was used, except reduced dye concentrations (0.0001%, 0.001%, 0.01%, 0.1%) were tested in combination with feeding stimulant (10%). Mortality was recorded at 24 and 48 h.

Influence of reduced dye concentrations on southern corn rootworm adult mortality exposed to direct sunlight. In further experiments, reduced dye concentrations (000.1%, 0.001%, 0.01%, 0.1%) were tested in combination with feeding stimulant (10%). In this test, dishes containing 15 adults were exposed to direct sunlight (72,000-95,000 lux) for a total of 2.5 h and then placed in the growth chamber for the duration of the test. Mortality was recorded at 1, 1.5, 17, and 21 h.

Field cage study using feeding stimulant bait formulation. Efficacy of bait formulations against the western corn rootworm adults were tested in walk-in cages

(2 × 2 × 2 m). Cages were constructed of 20-mesh plastic screen with a zipper closure hung on PVC or conduit pipes (Bioquip, Gardenia, CA). Cages were placed over sweet corn, *Zea mays* L. (var. 'Silver Queen') plants (approximately 17 plants/cage) in a 0.5-ha field at the University of Maryland Farm at Beltsville. Corn was approximately 2 m tall, in the silking and tassle stages. One hundred fifty adults (1 week old) were released into each cage and given 24 h to become acclimated. Due to the unavailability of southern corn rootworm adults, western corn roorworm adults were used in these field tests. The bait applied to the corn plants consisted of feeding stimulant (5%), starch (5%), dye (0.06%), and Gelva® (2%), a multipolymer emulsion supplied by Monsanto (St. Louis, MO) in water. Approximately 66 ml of the bait formulation was sprayed on the upper half of the corn plants in each of 3 cages. The bait was applied with a hand sprayer (Consolidated Plastics Co., Inc., Twinsburg, OH). In addition, 66 ml of the above solution was prepared without the dye or feeding stimulant, and similarly applied to corn plants in a fourth cage, which served as the control. Western corn rootworm adults in the cages were sampled 48 h after release. All live and dead beetles were aspirated and counted. This experiment was repeated using 0.25% feeding stimulant. The western corn rootworm exposure period to the bait was increased to 6 d.

Laboratory data analysis. Analysis of variance (ANOVA) was used to test the various components of the bait and concentrations on corn rootworm mortality (converted to percent and log transformed) using MIXED procedure (SAS Institute 1996). The LC50 for the reduced red dye concentration in artificial light was determined using PROBIT analysis (SAS Institute 1996).

Results and Discussion

Influence of feeding components/combinations on corn rootworm mortality. The dye and feeding stimulant were the most significant factors impacting on the increased southern corn rootworm mortality ($P < 0.003$). Exposure time significantly affected the increase in southern corn rootworm mortality, particularly during the 16 to 20 h period ($P = 0.04$). The percent mortality of southern corn rootworm adults exposed to the complete bait formulation (dye, starch and feeding stimulant) was 80% in 24 h (Fig. 1). The 30% mortality of southern corn rootworm exposed to the dye and starch alone may be attributed to a no-choice food situation in which the adults eventually must feed on the dye and starch to survive, thus the exposure to the dye toxin killed them. In a field test situation, southern corn rootworm would have a choice to move and forage elsewhere.

Influence of dye concentrations on southern corn rootworm mortality with and without feeding stimulant. The dye concentrations, feeding stimulant, and exposure time significantly increased the southern corn rootworm mortality compared to the control ($P < 0.0001$). At dye concentrations higher than 0.1%, all insects died at 40 h (Fig. 2a). At 16 h, there was no consistent mortality response to the various dye concentrations. At 24 h, mortality increased with increasing concentrations of dye. There was no mortality in the controls during the observation periods. The feeding stimulant treatment significantly increased the southern corn rootworm mortality compared to the controls without feeding stimulant (compare Fig. 2a and 2b). The increase in mortality over time in the dye and feeding stimulant treatment can be attributed to the compulsive feeding behavior the adults exhibit when exposed to the feeding stimulant. Thus, the beetles consume more dye over time. In the absence of

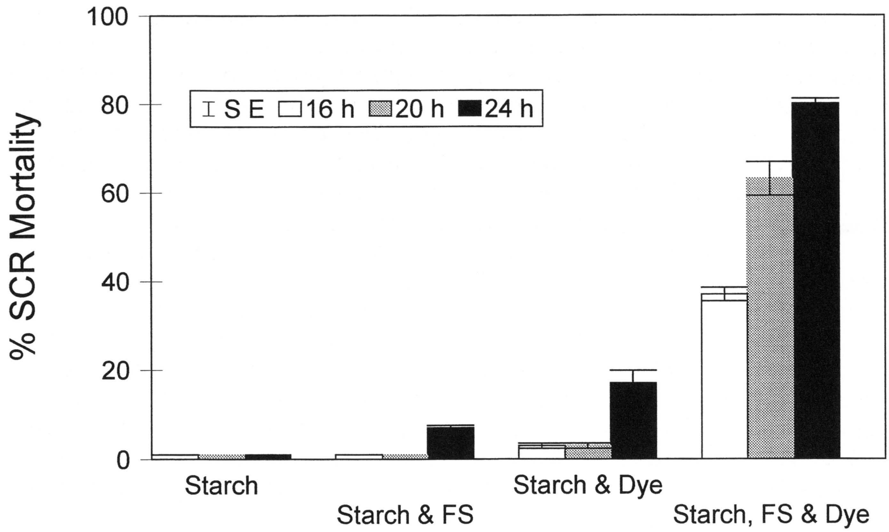

Fig. 1. Effect of various bait components and combinations (starch, feeding stimulant, and dye) on southern corn rootworm mortality over 24 h. Error bars indicate +/– one SEM.

the feeding stimulant, the beetles initially avoid the starch-dye combination (Fig. 2b). During the study, without a feeding choice, they eventually feed on the starch-dye combination and die. Therefore, time is a factor that significantly influences the mortality effect ($P < 0.0001$).

Influence of dye concentrations on mortality of southern corn rootworm exposed to fluorescent light. The increased mortality is primarily due to the 0.1% dye concentration at 24 h. Mortality at 0.1% is significantly higher than the control and all other dye concentrations (Fig. 3). There is also a significant increase in mortality from 24 to 48 h ($P < 0.0001$). This is probably due to increased exposure to the dye with time. The difference here is that the mode of action (i.e., slow-acting toxicant) of the dye is such that it mediates corn rootworm mortality over time. The LC50 calculated for the dye at 24 h was 0.07% (95% CI, 0.056 to 0.090). The high mortality in the 0.0001% concentration at 48 h was due to beetles searching for food and drowning in the starch.

Influence of dye concentrations on mortality of southern corn rootworm exposed to direct sunlight. The dye treatments, exposure time, and the dye-exposure time interaction significantly increased southern corn rootworm mortality ($P < 0.0089$). At the high dye rate (0.1%), 93% of the southern corn rootworm adults were dead in 1 h (Fig. 4). The southern corn rootworm mortality rate for the 0.01% dye concentration gradually increased from less than 1% at 1 h to 93% in 21 h. There were no other significant increases in mortality of southern corn rootworm treated at the lower rates.

Under fluorescent versus sunlight conditions there was a great disparity in exposure time required (48 h vs 1 h, respectively) to achieve greater than 90% mortality of southern corn rootworm adults.

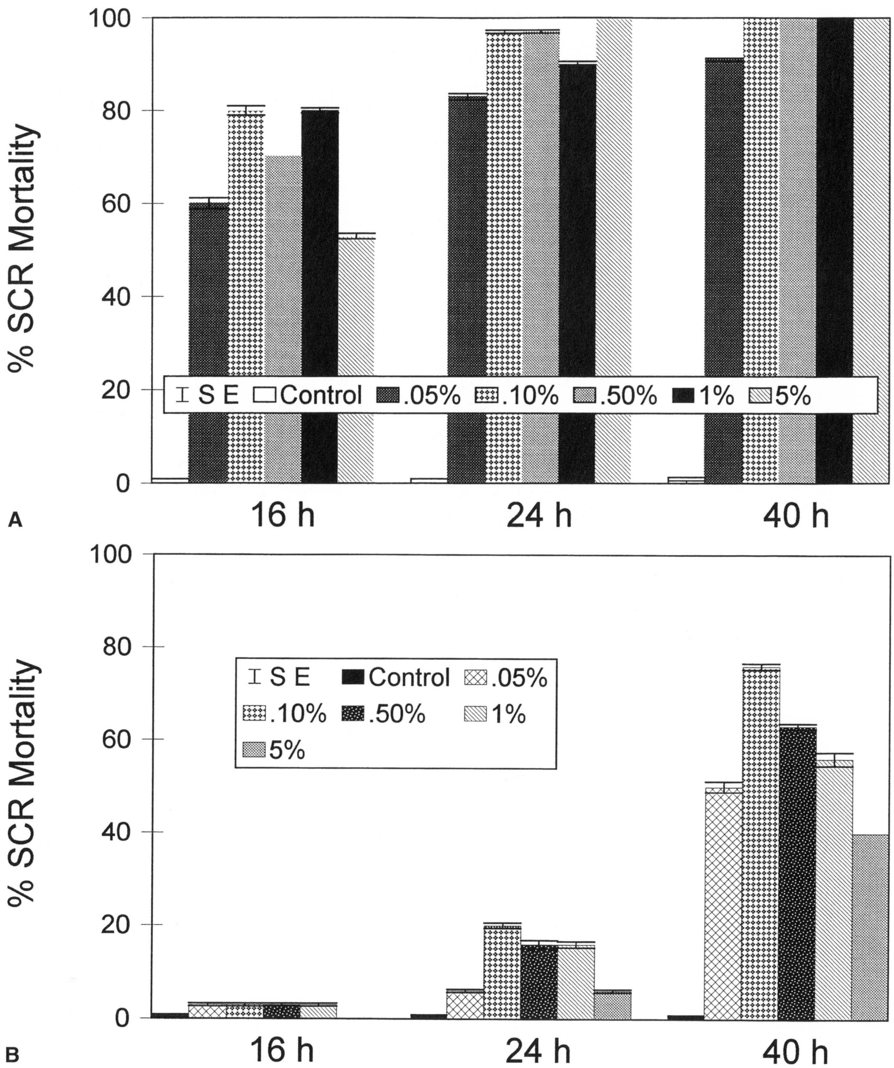

Fig. 2. Effect of increasing concentrations of dye with (a) and without (b) 10% feeding stimulant on southern corn rootworm mortality over 40 h. Error bars indicate +/– one SEM.

Field cage tests using 5% and 0.25% feeding stimulant bait formulations. The number of field cages (3 treatment cages and 1 control cage) would not allow the data collected on surviving adults to be statistically validated. However, actual counts of surviving western corn rootworm adults exposed to the 5% feeding stimulant bait showed an 85% reduction in numbers at 48 h compared with a 7% reduction in numbers in the control (Fig. 5). Counts of surviving western corn rootworm adults

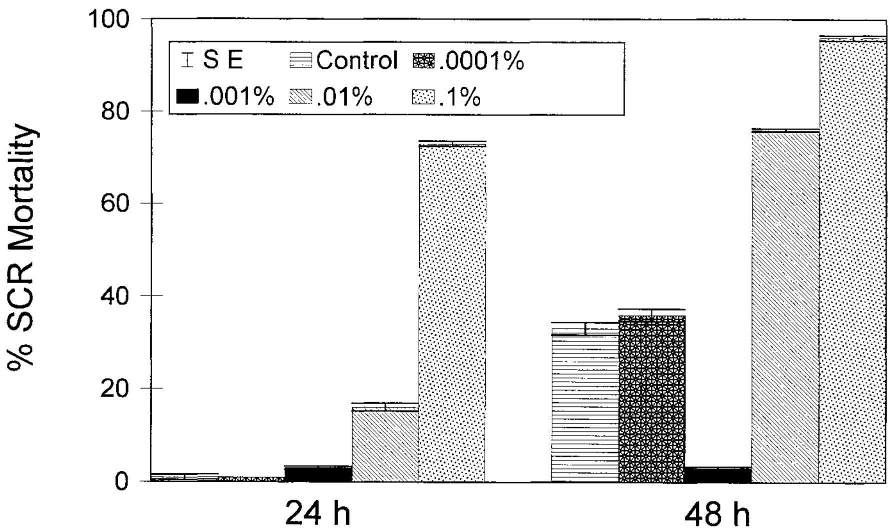

Fig. 3. Effect of reduced concentrations of dye with 10% feeding stimulant on southern corn rootworm mortality when exposed to fluorescent light (approximately 5600 lux) over 48 h. Error bars indicate +/– one SEM.

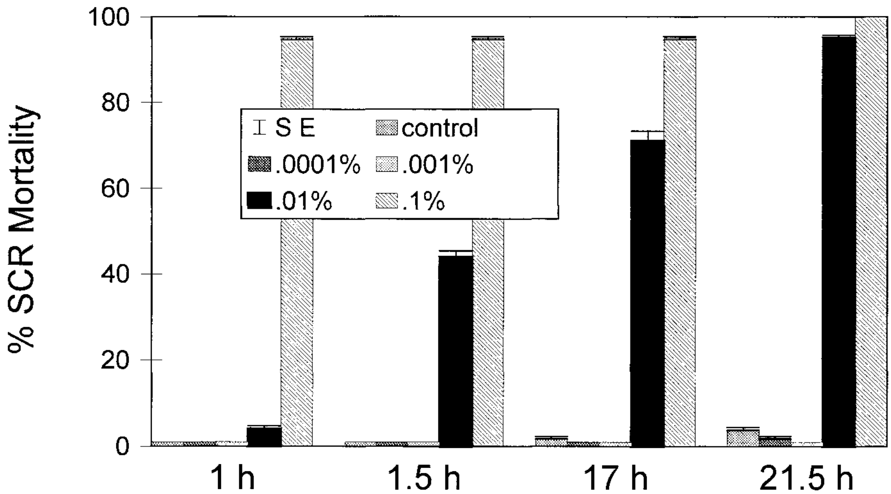

Fig. 4. Effect of reduced concentrations of dye with 10% feeding stimulant on southern corn rootworm mortality exposed to sunlight (2.5 h, approximately 82,000 lux) over 21.5 h. Error bars indicate +/– one SEM.

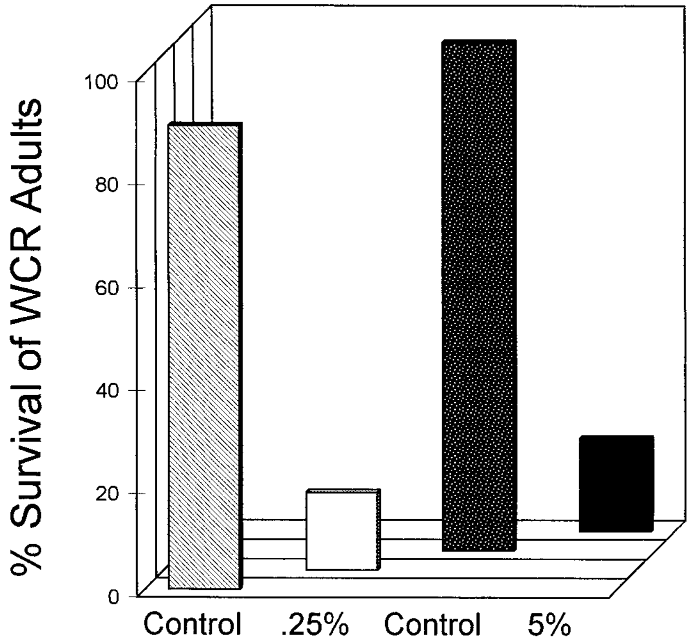

Fig. 5. Survival of western corn rootworm adults exposed to 0.25% and 5% feeding stimulant and 0.06% dye applied to corn in field cages. Light intensity in cages averaged ca. 56,500 lux.

exposed to 0.25% feeding stimulant showed an 81% reduction in 6 d compared with a 1% reduction in the control. Lux readings showed a 57% reduction in light intensity inside the cages from ambient sunlight (reduced from 99,000 lux to 56,500 lux).

The feeding stimulant prepared as a crude extract offers considerable promise for use in a bait(s) to control the southern corn rootworm, western corn rootworm, and other corn rootworm pests because the extract exhibits high stimulant activity. The extract is easy and economical to prepare from the melon, and readily lends itself to commercial production. Seed source of the melon is available through USDA.

Another advantage of the new feeding stimulant is its high water solubility, a property that offers considerable flexibility in the preparation of aqueous bait formulations.

Due to the water solubility of the dye, it is readily washed from application equipment. Considering the projected low volume application rate of 28g red dye per gallon per hectare, the off-target drift is minimized.

Because the dyes exhibit insecticidal activity against many insects and some are approved by FDA for human consumption (Lipman 1995), new dye application technologies are expected to emerge in the near future. This research suggests that these photoactive dyes will be more effective in the field because of increased activity in the more intense natural sunlight. EPA has already approved experimental use of the dyes against the Mexican fruit fly, *Anastrepha ludens* (Loew) (Moreno and Mangan 1995).

For a variety of reasons, the management of corn rootworm will become more complicated and challenging in the future. Levine and Oloumi-Sadeghi (1991) state the factors such as microbial degradation of soil-applied insecticides, insecticide resistance, extended diapause, pesticide laws and regulations, and concern about use of pesticides in general, affect strategies for corn rootworm management. Due to the numerous cases of control failures of corn rootworm, the focus of current strategies is on the integrated approach including baits, crop rotation, scouting to determine the need to curtail silk clipping and root damage the following year, crop management, use of insecticides based on need, and the consideration of biological, chemical, and physical factors that impact on corn rootworm control.

We are hopeful that the new water-soluble toxic bait developed from our research will provide a key tool as an alternative control of corn rootworm pests. The low mammalian toxicity of the toxicant coupled with the high specificity of the feeding stimulant are advantages that offer the prospect of a more environmentally compatible corn rootworm control agent.

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