Purdue University
Department of Entomology
Undergraduate Capstone
Project Summary

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Project Title:

A Gut Content ELISA as a Potential Tracking Method for Variant Western Corn Rootworm

Project Summary:

Introduction and Background

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is a serious pest of corn throughout the corn belt of the Midwestern United States. The larval stage causes damage by feeding on corn, *Zea mays* L., roots during the early growing season, leading to lodging and reduced nutrient uptake. Larvae hatch in late May and pupate from late June to early July. Adult beetles emerge throughout July and are present until late September. Females lay eggs (the overwintering stage) throughout this period (Mabry & Spencer 2003).

Crop rotation between corn and soybean, *Glycine max* L., was widely used in the past to manage western corn rootworm; since rootworm larvae cannot live on soybean roots, they quickly starve when hatching in soybean, thus disrupting the rootworm life cycle, and decreasing populations over a wide area (Levine and Oloumi-Sadeghi 1996). Furthermore, wild-type western corn rootworm adult females do not lay eggs in crops other than corn in significant numbers. Unfortunately, during the late 1980's rootworm damage to first year corn grown in rotation with soybeans began to occur in east-central Illinois in a significant number of fields (Levine and Oloumi-Sadeghi 1996). The prevalence of this damage in first-year corn has increased over time, and was soon found to include parts of Illinois, Indiana, Iowa, Michigan, and Ohio (O'Neal et al 2002).

Extended diapause is a mechanism by which some insects adapt to unpredictable environments. The northern corn rootworm, *Diabrotica barberi* Smith and Lawrence, can overcome crop rotation by extended diapause

of the egg stage (Krysan et al 1984). Northern corn rootworm eggs that are in a state of extended diapause remain dormant for two winters, hatching a year and a half after oviposition. In some areas, over 50% of northern corn rootworm eggs go through an extended diapause period (Levine et al 1992). In a corn-soybean rotation system, an egg laid in a cornfield remains dormant the following year when the field is planted with soybean, then hatches a year later when corn is present again, allowing a significant segment of the population to circumvent the rotation.

Unlike the northern corn rootworm, however, extended diapause of eggs was ruled out as a mechanism of crop rotation resistance for western corn rootworm; instead, rootworm oviposition in soybean fields the previous year was found to be the culprit (Levine and Oloumi-Sadeghi 1996). More recent data, obtained through soil sampling at different depths, have shown that rootworm eggs laid in soybean are found at a similar range of soil depths in corn and soybean, meaning egg overwintering survival is likely similar in the two crops (Pierce and Gray 2006a). In areas where crop rotation is heavily practiced, such as most of the state of Indiana, eggs laid in soybean will hatch in a cornfield the following spring, resulting in the potential for rootworm damage.

It was originally hypothesized that a variant population of western corn rootworm had developed an attraction to soybean as an oviposition site (Sammons et al 1997). Subsequent studies, however, have refuted this notion. A 1999 study conducted using a wind tunnel showed no evidence of variant rootworm attraction to soybean (Spencer et al 1999). A later study showed that variant populations lay eggs in crops other than corn and soybean at significant levels (Rondon and Gray 2003). Finally, electroantennogram tests showed that insects from variant populations were no more sensitive to olfactory stimuli from soybean than those from wild-type populations (Hibbard et al 2002). Studies have shown that a primary difference between wild-type and variant females is that variants are generally more active (Knolhoff et al 2006). Based on these and other studies, the current consensus is that rootworm variant oviposition in soybean and other non-corn crops is due to a lack of fidelity to corn as an oviposition site for adult females, rather than an increased attraction to soybean or an extended diapause.

Dispersal of gravid females from corn into soybean is influenced by the developmental stage of the corn; thus, oviposition outside of cornfields occurs later than oviposition within cornfields (Rondon and Gray 2004). One explanation for this phenomenon is that rootworm adults react to corn phenology, seeking new food sources as corn silks, foliage, and tassels mature (O'Neal et al 2002). Although feeding on non-host plant foliage reduces longevity, rootworm females that exhibit this behavior have been demonstrated to survive and produce viable offspring (Siegfried and Mullin 1990).

Soybean is not an ideal diet for rootworm adults, including the variant. (Mabry and Spencer 2003) It contains a cysteine proteinase inhibitor (soyacystatin N) that inhibits rootworm growth and causes dietary stress (Koiwa et al 2000). Despite this, beetles from variant populations have been observed to have soybean foliage in their gut contents at higher frequencies than wild-type adults (Spencer et al 1999). This apparently maladaptive behavior can be explained by the observation that variant adults have been shown to be less sensitive to switches in diet than wild-type adults, meaning they likely remain in soybean fields longer after feeding on soybean (Mabry et al 2004). Dietary stress has been demonstrated to induce rootworm females to oviposit in multiple studies (Mabry and Spencer 2003; Mabry et al 2004; Pierce and Gray 2006b; Siegfried and Mullin 1990). Variant beetles' relative insensitivity to switches in diet may lead them to stay in soybean long enough after feeding on soybean foliage to be induced to oviposit by dietary stress (Mabry et al 2004). The mechanism for this lack of fidelity to corn as an oviposition site may be similar to that of the Hessian fly, *Mayetiola destructor* Say. This insect will normally lay eggs only in wheat, but will use oats as an oviposition site if deprived of wheat for several hours. It is hypothesized that the Hessian fly's acceptance threshold widens as the threat of mortality due to starvation increases (Harris and Rose 1989).

Due to the substantial threat of injury to first-year corn in Indiana as a result of the rootworm variant, and the variability of the threat throughout different areas of the state, methods to identify the presence of the variant in an area are needed by producers and agricultural consultants. Throughout much of the state, the presence of the variant is sporadic, leaving producers unsure of whether or not to treat first-year corn at planting time. The decision of whether or not to treat has direct financial implications for producers; unnecessary treatments are a needless expense, while failure to apply a necessary treatment can lead to substantial losses.

The current methods most commonly used to sample for the variant, field sweeps and Pherocon AM sticky card traps, both have their strengths and weaknesses. Field sweeps, which have been conducted by Purdue University throughout the state since 1993, are used to determine the presence and relative abundance of beetles in soybean fields. Sweeps can be conducted by untrained personnel, and are widely used as a measure of abundance for a variety of other insect pests. The major problem with this method is that sweeps taken at different times cannot be compared, as beetle activity varies widely throughout the day and throughout the season (Isard et al 2000). The other method, use of Pherocon AM traps, involves the weekly placement and monitoring of traps throughout a soybean field, which attract and trap adult beetles. After they are collected from the field, these traps can be

analyzed immediately, or frozen to be checked at a later date. The major drawback of this method is the difficulty of handling and replacing the traps on a weekly basis; also, due to the attractiveness that these traps have to adult beetles, traps placed too close to the edge of the field can result in inaccurately high beetle counts, as beetles are attracted from adjacent fields.

Since adult beetles from variant populations are more likely to contain soybean foliage in their gut, the presence of this foliage may be a suitable marker to identify the presence of variant beetles in a given area (Spencer et al 1999). An enzyme-linked immunosorbent assay (ELISA) is a procedure that can be used to detect a wide variety of protein markers, including two that are specific to soybean. We plan to use ELISA technology to test the gut contents of adult beetles captured throughout the state of Indiana for the presence of soybean foliage. A similar concept, using protein expression tests to identify the presence of transgenic corn tissue in corn rootworm gut contents, has been proposed as a way to monitor the movement of beetles to and from transgenic cornfields (Spencer et al 2003).

The beetles that we will test will be captured using Trece WCR lure traps. These traps retain and kill beetles that encounter them without the use of sticky material, which will allow us to easily extract individual beetles from the traps for ELISA testing in the laboratory. We hope to combine data on the rate of soybean herbivory by beetles captured in these traps with data on the amount of root damage to first year corn in the fields of origin of the tested beetles. If the rate of soybean herbivory of beetles captured in vial traps proves to be a positive indicator of rootworm larval damage to corn planted the following year, this procedure could be used to confirm the presence of variant rootworms in a given area. This would allow the implementation of a system similar to the present system of soil analysis, in which producers could collect beetles from their fields using vial traps, send the beetles to a laboratory to be tested for the presence of soybean foliage in their gut contents, and make a decision on rootworm treatment based on the results.

Methods

For this Capstone project, an ELISA designed to detect soybean protein was conducted on samples consisting of a high and low concentration solution of soybean seed and water and a high and low concentration solution of soybean foliage and water. The concentrations used were relative to each other for each assay and not standardized,

as the goal of this project was only to test and perfect the ELISA protocol's effectiveness in detecting soybean protein.

For each assay, a 96-well microplate was used. Samples were added to each microplate well and suspended with varying concentrations of Tris-borate-(Ethylenedinitrilo) Tetraacetic acid, a suspension buffer. Each microplate contained 80 uL total of sample solution and buffer. Control wells consisted entirely of buffer. After samples were added, each plate was incubated at 37 C° for 120 minutes. After incubation, the plates were washed five times with a Phosphate buffered saline-Triton X- Polyethylene glycol (PBST-PEG) mixture using a microplate washer. The plates were then aspirated to remove all fluid, and 80 uL of PBS-bovine serum (PBS-BS) were added as a blocking buffer. After a 60 minute incubation period at 37 C°, two washes with PBST-PEG, and aspiration, the primary antibody, rabbit antiserum to soy protein, was added in 80 uL aliquots. The antibody solution was diluted into PBS-BS by a factor of 2 uL: 8 mL. An incubation period of 60 minutes at C° was used, followed by 7 washes with PBST-PEG and aspiration. The secondary antibody, donkey antiserum to rabbit protein, was diluted into PBS-BS by a factor of 1 uL: 8 mL and added to the microplate in 80 uL aliquots. An incubation period of 120 minutes at C° was used here, followed by three washes with PBS-sodium dodecyl sulfate (a more stringent wash solution), three washes with PBST-PEG, and aspiration. The substrate, TMB-ELISA, was added to yield a color change in wells that were positive for soybean. After ten minutes at room temperature, the reaction was stopped with sulfuric acid. Since technical difficulties prevented spectrophotometric analysis of the plates, visual analysis was used to determine whether or not color change occurred.

Results

Unfortunately, no quantitative results could be obtained due to technical difficulties with the connection between the microplate reader and the printer, the sole source of output for the reader. By simple visual analysis, it was concluded that the assay yielded positive results for both the high and low concentration solutions of soybean seed. This was possible because the seed solutions yielded an obvious color change almost every time. For the high and low concentration solutions of soybean foliage, no conclusion could be drawn by visual analysis; although spotty color change could be seen, these results were not obvious enough to be confirmed or denied as positive without quantitative data from the microplate reader. It should be noted that contamination due to operator error was a

factor in this experiment, as several control wells yielded positive results despite not being purposefully inundated with any soybean sample throughout the course of the ELISA test. This was likely due to faulty pipette techniques.

Discussion

The visual confirmation of positive results for soybean protein in the seed samples indicates that the ELISA protocol used in this project was effective to some degree in detecting the presence of soybeans. The failure to visually confirm positive results in soybean foliage could mean one of two things: the protocol may need to be altered to make it more sensitive, or the color change may have been too subtle to pick up with visual analysis. Quantitative analysis with a microplate reader will be necessary before any conclusions can be drawn as to the functionality of this particular protocol for the analysis of soybean feeding in adult western corn rootworm beetles described at the end of the introduction of this paper. Once a protocol is established that can detect soy protein effectively, it will have to be tested against various corn samples to ensure that it yields negative results for proteins found in corn. This is extremely important for the usefulness of the protocol, as its purpose will be to separate beetles that have fed on soybean from those that have fed on corn. The next step will be to test the protocol on beetles known to have fed on either soybean or corn. Once a protocol is established that yields positive results for beetles that have fed on soybean and negative results for those that have fed on corn, it will be ready for use on field-collected beetles.

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