TITLE: *Isolation and identification of host-plant attractants for cranberry weevil and cranberry fruitworm*

SUMMARY

The proposed research will investigate the role of volatiles from flower buds, flowers, and fruit as attractants for the cranberry weevil (CBW) and cranberry fruitworm (CBFW), two major pests in cranberries and blueberries in the Northeast, with the goal to develop traps for monitoring CBW and CBFW populations. In the past, pheromone traps used for monitoring adults of the CBFW have failed to predict fruit damage, and no traps are available to monitor CBW populations. New monitoring tools will prevent unnecessary insecticide applications, reduce management costs, and decrease the development of resistant populations. This proposal will investigate the behavioral and antennal electrophysiological responses of CBW and CBFW to host-plant volatiles, in order to isolate and identify attractants for the development of new traps. Olfactometer assays will be conducted to determine the response of male and female CBW and CBFW to flower buds, flower, and fruit volatiles. Headspace volatile emissions from flowers and fruit will be collected and analyzed via gas chromatography (GC-FID). Active volatile compounds will be identified by coupled gas chromatography and electroantennographic detection (GC-EAD). These studies will provide evidence of host-plant attractants for the development of traps to monitor CBW and CBFW.

PROBLEM, BACKGROUND, AND JUSTIFICATION:

*Economic Importance of Crops*. Highbush blueberries (*Vaccinium corymbosum* L.) and cranberries (*Vaccinium macrocarpon* Aiton) are both native to North America, and have been under commercial cultivation for many years. These are two important crops in the northeast United States: blueberries are grown on 48,310 acres, primarily in Michigan, New Jersey, and Georgia (USDA-NASS 2006a). New Jersey account for more than 15% of the total US blueberry acreage and for approximately 20% of the total utilized production, valued at $55.5 million. Cranberries in the United States are grown in over 39,000 acres, primarily in Wisconsin, Massachusetts, and New Jersey (USDA-NASS 2006b). New Jersey cranberries account for 8% of the total US acreage and total US production, valued at $17.9 million.

*Importance of Pests*. The two insect pests in this study, the cranberry fruitworm (CBFW), *Acrobasis vaccinii* Riley, and cranberry weevil (CBW), *Anthonomus musculus* Say, can cause major economic losses in the northeast US (Marucci 1966; Long and Averill 2003). Both insects feed on the plant's reproductive organs (flower buds, flowers, and fruit), which makes them major direct pests in blueberries and cranberries in the growing areas where they occur; thus, even few individuals may cause a substantial loss to growers. Current limitations on adequate monitoring techniques limit the management of CBFW and CBW populations. For example, pheromone trapping in blueberry monitors only male moth CBFW populations; this makes it difficult to accurately time control measure because females are responsible for laying the eggs. In cranberry, pheromone traps cannot even be used because there is no relationship between male flight and onset of oviposition (Ginnetty and Edgar 1996).

*Cranberry fruitworm*. Because of its direct pest status and lack of effective monitoring techniques, studies on CBFW are a top research priority for berries in the northeastern US (http://northeastipm.org/work_fruipriority.cfm). Cranberry fruitworm is a fruit-eating pyralid infesting cranberries and blueberries and is common throughout the eastern US and Canada. It overwinters as a pre-pupa in a sand-covered hibernaculum on the soil. Because its univoltine life cycle focuses on *Vaccinium* fruit, females are expected to utilize volatile cues from host plants, especially fruit, to find oviposition sites, much like the attraction of female codling moth to volatiles from apple fruit (Bengtsson et al. 2001; Backman et al. 2001; Ansebo et al. 2004; Hern and Dorn 2004). In a pilot choice test comparing CBFW acceptance of *Vaccinium* hosts (blueberry and cranberry) (fruit ripeness and size were standardized), it was found that females from Massachusetts were 6-7 and 27 times more likely to accept cranberry than wild lowbush blueberry (*V. angustifolium* Aiton) or cultivated highbush blueberry (*V. corymbosum*), respectively (Averill, unpublished data). These results support the logical assumption that chemical or physical cues, or both, are important in host acceptance for CBFW.

As expected for a direct pest, there is low threshold for damage, especially for the percent of fruit that is marketed as fresh. In the Northeast, infestation levels may exceed thresholds from fruit set until harvest, requiring a series of sprays. Still, damaged fruit may contain larvae at harvest, which is unacceptable to all handlers. Tolerance among cranberry and blueberry growers for CBFW contamination is extremely low, making CBFW one of the most important pests in New Jersey, especially in blueberries. In New Jersey cranberries, it can become a particular problem when spring temperatures are cool causing the emergence of adults to synchronize with cranberry fruit set.

In cranberry, IPM programs do not rely on monitoring for the initial sprays and instead, recommend two prophylactic sprays based upon crop phenology; these are followed by collection and visual examination of fruit for presence of visual eggs. In blueberry, monitoring techniques for CBFW involve the use of sex-pheromone baited traps for capturing male moths, combined with berry inspections (Mallampalli and Isaacs 2002). Berry inspection is both expensive and labor-intensive. Further, due to the narrow window of opportunity for fruitworm control, insecticide applications need to be made before fruitworm larvae enter the fruit. If larvae escape treatments and enter into the fruit, control is much more difficult. Thus, a low-cost, easy-to-use, and reliable monitoring technique is desperately needed to precisely time applications. Timing based on the first captures of female moths is expected to improve control effectiveness compared to monitoring male moths. Because CBFW populations are variable between years, between and within farms, and in the timing of their damage, a reliable sampling method will provide growers with a better tool for determining when and where intervention is required and reduce unnecessary insecticide applications at sites where populations of CBFW are low.

*Cranberry weevil* (also known as blossom weevil). Cranberry weevil feed as adults on developing anthers, pistils, and leaf buds of cranberries and blueberries. This insect is also regularly found across the northeastern US. Females lay their eggs into the unopened flower and the larvae complete their development within the flower buds in which they are deposited as eggs. Volatiles from host plants may provide information to foraging females on suitable oviposition sites. Pilot choice bioassays in a simple still-air arena where plant parts or a blank port were provided to cranberry-origin CBW (Averill, unpublished data), showed positive and negative responses to host (wild blueberry flowers, cranberry blossom buds) and non-host material, respectively (Table 1). Several congeners of CBW, e.g. *Anthonomis pomorum*, *A. grandis*, and *A. rubi*, have shown electrophysiological responses to host volatiles (Dickens 1990; Kalinova et al. 2000; Bichao et al. 2005) and several species of weevils, such as the pine weevil, banana weevil, and vine weevil (Budenberg et al. 1993; Wibe et al. 1997; van Tol and Visser 2002) responded to host-plant volatiles in behavioral bioassays.

Weevil-infested flower buds become purple in color, fail to open, and eventually fall to the ground. Currently, CBW is a major pest in cranberries in some Northeastern states. For example, outbreak populations were observed throughout the state of Massachusetts in 2002-2004 owing to the appearance of organophosphate-resistant populations and the absence of effective management alternatives. This was rectified through EPA approval of a Crisis Exemption petition for indoxacarb. Cranberry weevil is a secondary pest in blueberries in New Jersey where populations have been kept under economic threshold with applications of broad-spectrum insecticides. However, recent regulatory restrictions on the use of organophosphates and carbamates create a potential for this insect to become a major pest in New Jersey and other growing regions.

Adult CBW are monitored using sweeping (action threshold has been an average of 4.5 weevils per 25 sweeps). This method, however, can be costly and labor-intensive. In addition, populations are exceedingly patchy and CBW adults are very small and will, when disturbed, fall to the ground; taken together these traits make sweep-net sampling variable and often unreliable among scouts. A reliable method for monitoring CBW adults is critical to accurately time insecticide applications to avoid interference with the activity of honey bees. Traps using volatiles from host plants may provide growers with a cost-effective, low-disturbance, and reliable method to monitor CBW populations across blueberry and cranberry growing states.

*Reason for the study*. The proposed research will address a top research priority stated by the Fruit IPM Working Group, which is to develop and implement *"Effective monitoring strategies for key pests in which technologies currently do not exist"* http://northeastipm.org/work_fruipriority.cfm. This study, if successful, will develop cost-effective and reliable monitoring techniques for CBFW and CBW based on host-plant attractants, which can be implemented into IPM programs in cranberries and blueberries across the northeast and other US regions. The new monitoring technologies will replace the current practices that are costly and ineffective for monitoring CBFW and CBW populations and, thus, may help improve the timing of insecticide applications.

Volatiles from plants are important cues for many phytophagous insects (Bernays and Chapman 1994), which aid foraging insects in finding hosts, mates, and oviposition sites (Miller and Strickler 1984; Visser 1986). Host-plant volatiles may provide a natural source for the development of attractants for monitoring insect pests that are safe to the environment. The use of attractant traps has been well established for other systems. One of the best known success cases is the use of attractant traps to monitor and control fruit flies (e.g., Prokopy et al. 1973; Reissig 1974; Carle et al. 1986; Prokopy and Vargas 1996; Prokopy et al. 1996; Linn et al. 2003). Attractants (baits) for fruit flies are now commercially available (e.g., Nu Lure, GF-120, Naturalure). There are, however, no previous studies conducted to determine the behavioral and antennal electrophysiological responses of CBFW and CBW to host-plant volatiles. Cranberry fruitworm attacks cranberry and blueberry fruit, which have specific volatiles (Hall et al. 1970; Hall et al. 1971; Hirvi and Honkanen 1983a,b; Horvat and Senter 1985; http://www.sisweb.com/referenc/applnote/app-35-a.htm). Cranberry weevil adults deposit eggs in flower buds and feeds on both buds and leaves, all of which are expected to produce specific volatiles.

By improving methods to monitor CBFW and CBW populations, and better timing application of insecticides, I expect to see a reduction in insecticide use in cranberry and blueberry fields. Unnecessary insecticide applications will reduce pesticide residues in fruit and maximize farm worker protection. Furthermore, use of traps will reduce the need for scout visits that are costly and labor-intensive, and thus minimize exposure of scouts to pesticide residues.

OBJECTIVES AND ANTICIPATED IMPACTS:

**Objectives:**

1. Assess the behavioral responses of adult cranberry fruitworm and cranberry weevil to host-plant volatiles.

2. Isolate and identify volatiles important in attraction of the cranberry fruitworm and cranberry weevil to host plants.

**Anticipated Impacts:**

*Implementation of IPM*. This study's ultimate goal is to provide cranberry and blueberry growers with new tools for monitoring CBFW and CBW populations. This work will have a strong likelihood to contribute towards our ongoing efforts to implement better monitoring techniques into pest management programs in cranberries and blueberries.

*Economic and environmental benefits*. This study will develop and implement novel traps that are economically feasible, easy-to-use, and reliable for cranberry and blueberry growers.

This will result in several benefits to growers and the environment that include:
a) More effective pest monitoring techniques;
b) Reduced monitoring costs;
c) Reduced insecticide use;
e) Lower likelihood for the development of resistant populations;
g) Greater preservation of beneficial insects; and
h) Better protection to workers.

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## Appendix

**Table 1.** Response of *A. musculus* from Massachusetts to host or non-hosts in choice tests comprised of plant material paired with a blank port

| | ***Mean # arrivals/replicate*** |
|---|---|
| ***Host plant; plant part*** | ***Treatment/Blank***[1] |
| Cultivated highbush blueberry, *V. corymbosum* | |
| Blossom buds | 6.4/5.4 |
| Flowers | 5.7/5.3 |
| Leaves | 4.2/5.0 |
| Wild lowbush blueberry, *V. angustifolium* | |
| Flowers | 13.3/4.0* |
| Cranberry, *V. macrocarpon* | |
| Blossom buds | 13.5/5.5* |
| Non host, *Lonicera* | |
| Blossom buds | 2.2/3.8 |
| Flowers | 2.0/6.7* |
| Leaves | 1.5/8.5* |

[1] Treatment/blank pairs accompanied by an asterisk (*) were significantly different (P <0.05, Chi square test)