Title
Visual and chemical cues attracting rednecked cane borer to primocanes
Final Report
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Research Proposal
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Objectives

1) Identify volatile profiles of primocane and floricane blackberry.
The *working hypothesis* for this research objective is that rednecked cane borer (RNCB) feeds on leaves and oviposits in canes of primocanes due to a difference in volatiles emitted by the leaves and canes, allowing the pest to locate primocanes.
2) Identify color differences between primocanes and floricanes blackberry.
The *working hypothesis* for this research objective is that RNCB are attracted to and land on primocanes due to color differences between primocanes and floricanes.

Justification and Description:

Blackberry production in the eastern US faces an insect pest that threatens nearly all the 7,100 tons of fruit produced annually from 2,500 acres of blackberries in the eastern North America (Strik et al. 2007). The rednecked cane borer, *Agrilus ruficollis* (F.), has been shown to affect nearly 72% of blackberry canes (Hixon 1938) where larval girdling of canes predisposes canes to winter injury and reduced yield (Johnson 1992). From late April into June, this pest restricts its feeding and oviposition to primocanes and rarely lands on 1-yr-old floricanes (fruiting) (Johnson and Mayes 1989). Most growers do not scout for RNCB adults. However, it is recommended they apply a preventative prebloom soil drench of imidacloprid (Admire Pro) to a field that contains galled canes. This practice may lead to missed or overuse of the insecticide if the grower is unsure if enough RNCB adults are present to justify a spray. An economic threshold needs to be determined for RNCB. This will require development of a RNCB trap, a monitoring protocol and relate trap counts to subsequent number of galled canes that reduce yield. Having a well timed management tactic will lead to a decrease in RNCB gall formation and winter injury and increase yields. An attractive trap may have potential to mass trap RNCB, reduce the local RNCB density over time and lead to reduced pesticide usage. Less use of insecticide during bloom (concurrent with RNCB oviposition) would lead to a healthier ecosystem for pollinators or natural enemies, and be a more sustainable fruit production practice.

Visual and/or chemical stimuli are integral in the design of attractant traps used to monitor fruit insect pests (Johnson 2009). However, we lack an adequate understanding of host stimuli that attract RNCB to primocanes. A visual comparison of primocanes and floricanes was made during the RNCB flight period in 2011. A spectrometer analysis identified mean L*a*b values (N=4) with significant differences in green-red coloration between primocane and floricane foliage and canes ($P < 0.0001$). Preliminary odor collections and gas chromatograph runs of odor samples from primocanes and floricanes and different blackberry cultivars revealed peaks with different retention times (RT) and quantity of common peaks (peak heights in μV)

Methods:

This proposal will identify differences in volatile components emitted by blackberry primocanes and floricanes and test attractiveness to RNCB of several colored sticky traps that mimic blackberry leaves and canes.

Volatile profiles: Volatiles were collected from several blackberry cultivars currently available in a field planting of nine cultivars (various *Rubus* spp. parentage) at the University of Arkansas farm in Fayetteville, AR, i.e. 'Natchez', 'Oauchita', 'Apache', 'Chester', 'Austin Mayes' and 'Lucretia', 'Rosborough'; 'Brazos' and 'Wye Berry'. 'Wye Berry' is a raspberry/blackberry hybrid that we observed in a local planting that had 100% of canes galled by RNCB with low yields and many dead fruiting canes. Volatiles were collected from four 'Natchez' primocanes inside a clear Teflon bag (American Durafilm, Holliston, MA) (61 cm x 61 cm). Each bag has been customized with an inlet and outlet port along the sides of the bag. Air was delivered through Teflon tubing and an activated charcoal filter at a flow rate of 1.5 liter/min into the bag while volatile ladened air exits through a collection trap with 30 mg Super Q absorbent powder (Alltech Associates) at a rate of 0.5 liter/min using a rotary vane pump (Gast Manufacturing Inc., Benton Harbor, MI). The collection of volatiles was allowed to run for four daylight hrs and trap eluted with 1 ml of hexane (Omni-Solv). The surface of leaves and canes of 'Wye Berry' and 'Natchez' were rinsed with 25 ml dichloromethane and extract dehydrated by passing it through filter containing 25 gm Na_2SO_4 to prepare extract for injection into the GC. All solvent extracts were kept at -80°C before GC-MS analyses (modified from Crook et al. 2008).

For each plant volatile sample, a Varian 3900 GC in our laboratory was used to detect volatiles and quantify peaks following a method described by Crook et al. (2008). A 1µl sample of each Super Q sample of plant volatiles will be injected into the GC in splitless mode with the temperature for the injector at 220°C and FID at 250°C. The carrier gas of Helium will flow through the capillary column (VF-3ms; Agilent Inc.) at 40cm/sec. The GC oven was programmed to increase in temperature at a rate of 8°C/min from 50°C to 230°C with an initial and final hold time of 5 and 10 min, respectively. Volatile collections from primocane and floricane along with a control (empty Teflon bag) were analyzed on GC. Samples that show peaks were injected into a Varian 450 GC coupled with Varian 320 Triple Quadruple EI/CI GC/MS (University of Arkansas State Wide Mass Spectrometry Facility, Fayetteville, AR) to identify and quantify peaks. The GC output into the MS operated in full-scan (M/Z 50-350) and selected ion monitoring modes (TIC at m/z 68, 120, and 93) with electron-impact ionization. To identify each volatile peak, we compared retention times (RT) and mass spectra with those of available authentic synthetic compounds obtained from Sigma-Aldrich Co. (St. Louis, MO) and a computerized mass spectral data library (National Institute of Standards and Technology - Version 2008, Gaithersburg, Maryland).

Color differences: Another aspect of RNCB adult preference for primocanes that was investigated was the coloration of leaves and canes. In 2011, a local hardware store mixed paints to mimic color of different blackberry primocane or floricane leaves or canes. A Jazz spectrometer (Ocean Optics) was used to confirm that the visible spectrum and percent reflectance from these paints closely mimicked those from the respective blackberry plant leaves and canes. These flat paints were applied to rectangles of gray, primed sheet metal (3.8 cm X 8.9 cm). We compared the attractiveness of these paint colors mimicking a blackberry plant to traditional trap colors for leaf feeding insects, e.g., John Deere yellow and dark purple, light purple, and green that attracts other Buprestid beetles such as Emerald ash borer (Crook et al.

2008) and an unpainted control. During the RNCB flight period in May and June, traps with each test color were positioned vertically (cane traps) or tilted down at 45º angle (leaf traps). Four painted metal leaf traps were alternately attached onto 2ft long x 1/2 inch diameter dowel rods. These colored leaf traps were positioned 3 m apart in blackberry rows and the rod anchored in a vertical orientation to a piece of rebar exposed 6 inches above soil. To test the color attractiveness of the canes, 2 ft long wooden dowel rods 1/2 inch in diameter were either painted a cane mimic color, yellow, or unpainted (control). Also, three emerald ash borer panel traps (dark purple, light purple, and green) were modified to 2ft long x 1 inch wide prism traps to mimic canes. At similar spacing in the blackberry row, painted rods were anchored vertically to a piece of rebar exposed 6 inch above soil. All traps were coated with Tanglefoot® after deployment into the field. Just before the initiation of flight of RNCB adults in late April, traps were placed in several bramble plantings each in a randomized complete block design with four replicates (Fruit Station in Clarksville, and a grower in Fayetteville, AR). Traps were checked weekly when all captured RNCB adults was counted and removed. Treatment traps were rearranged after each count period to eliminate trap location effect. Clean sticky traps were set out every two weeks. One-way analysis of variance with Waller-Duncan k-ratio t test ($\alpha = 0.05$) was conducted to identify significant differences between mean treatment counts of rednecked caner borer adults for all colored traps: shaped as vertical canes; rectangular leaves; or accumulative means for all traps shaped as vertical canes or as rectangular leaves. Tukey pairwise comparison analysis ($\alpha < 0.05$) was used to identify differences between mean counts of rednecked caner borer adults on cane and leaf traps of the same color.

Results

Volatile profiles:

Samples collected from surface rinses of 'Wye Berry' primocane and 'Natchez' blackberry primocanes were analyzed with GC/MS. A few volatile organic compounds were identified from primocanes from both 'Wye Berry' and 'Natchez' blackberry primocanes (Fig. 1 A), but no compounds could be identified from the volatiles emitted by 'Natchez' primocanes (Fig. 2). 'Wye Berry' and 'Natchez' blackberry primocanes emitted very low quantities of volatiles. As a result, we are attempting to accumulate more primocane volatiles for future injection for GC-MS analysis and peak identification.

Color differences:

A comparison of color analyses of colored traps and blackberry plant parts identified differences in the mean reflected wavelength and percentage reflectance (Table 1). The peak wavelength of light emitted by blackberry plant parts varied from green light at 546 nm for primocane cane, 554 nm for primocane leaf and 550 nm for floricane leaf to a reddish-brown floricane cane at 630 nm. In comparison, the EAB green trap had peak reflection at 544 nm but had 50% reflectance compared to < 25% reflectance from all blackberry plant parts. All the traps painted to mimic a blackberry plant part had used a flat paint that reflected from 17 to 25% of the light compared to 50% light reflectance by the EAB green trap. Overall, significantly more rednecked cane borer adults were captured on traps mimicking a blackberry cane than traps mimicking a blackberry leaf (Table 2) ($F = 11.43$, df= 1, 96, $P = 0.001$). The EAB green traps shaped as a vertical cane captured significantly more beetles than any other colored cane or colored leaf trap ($F = 6.09$, df= 6, 42, $P = 0.0001$). The EAB green trap shaped as a blackberry leaf captured significantly more beetles than any other colored leaf trap ($F = 14.87$, df= 6, 42, $P < 0.0001$).

Similar beetle catches were obtained by cane traps colored yellow, EAB light purple, and mimics of a primocane or floricane.

Conclusion

Volatiles collected from plant samples have demonstrated that there are some differences between surface rinses and aeration collections. Further studies are needed to correctly identify the unknown compounds and also to determine which primocane compounds demonstrate antennal activity.

Traps colored EAB green or painted to mimic a primocane cane or leaf each had a mean reflected light wavelength that was similar to the actual blackberry plant cane or leaf. EAB green trap and traps mimicking a blackberry cane were the most attractive to rednecked cane borer adults. Yellow was similar in trap capture to primocane for the cane mimics, this could be associated with the high reflectance of the yellow trap. Francese et al. (2010) demonstrated that green traps with higher reflectance (23% - 66%) caught significantly more emerald ash borer than other traps. Having a high reflectance could be an explanation of the greater trap capture on EAB green traps than primocane cane traps.

Impact Statement

Although rednecked cane borer may not be considered a major pest, leaving populations uncontrolled can lead to reductions in yield due to an increase in winter injury of primocanes. Knowing the attractive volatiles and color for this pest will allow the development of an effective monitoring or possibly a mass trapping program. An effective trap can allow growers to detect rednecked cane borer presence, note start and end of the flight period, justify need for an insecticide and properly time that insecticide application. Providing a mass trapping option can allow organic and even conventional growers the opportunity to save money by eliminating an insecticide application and conserve pollinators.