Diagnosis and Variation in Appearance of Brown Stink Bug (Hemiptera: Pentatomidae) Injury on Apple[1]

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Abstract Adult brown stink bugs, *Euschistus servus* (Say), were caged individually on limbs with apple fruit of 6 cultivars in research orchards in West Virginia. Studies were performed to describe specific characteristics of damage that could be used for field and/or laboratory diagnosis of stink bug injury to apple fruit at harvest. These characteristics were separated into surface and subsurface features. On the apple surface, 3 prevailing types of stink bug injury were observed in the field: (1) a discolored dot, i.e., stink bug feeding puncture; (2) a discolored dot with a depression in the fruit; and (3) a discolored dot with a discolored depression in the fruit. Subsurface characters were related to the extent of damage observed on the fruit skin. Common subsurface damage ranged from a stylet sheath to corky tissue of variable color, shape, and size that sometimes was not contiguous with the skin. Laboratory evaluations under a dissecting microscope revealed that the size of the stink bug feeding puncture was ~0.17 mm diam. This character was the only consistent, definitive symptom of stink bug injury present among all observed damage. Due to variability in other surface and subsurface characters, and potential problems with visual apparency of injury in the field, evaluations of suspected stink bug damage should be performed with 40X magnification in the laboratory to confirm the presence of stink bug feeding punctures.

Key Words stink bug, monitoring, apple, cork spot, IPM

Several stink bug species, including brown stink bug, *Euschistus servus* (Say), are serious pests of stone fruit (Hogmire 1995) and are becoming increasingly important pests of apple in the mid-Atlantic (Brown 2003a, Brown et al. 2006). Economic damage caused by stink bugs in apple orchards appears to have become more prevalent as narrow-spectrum insecticides have replaced broad-spectrum materials such as organophosphates for control of key insect pests. To effectively manage stink bugs in a narrow-spectrum, reduced-spray environment, it is imperative that treatments be triggered by a monitoring system, such as a trap-based system designed to detect increases in stink bug abundance or activity (Leskey and Hogmire 2005, Hogmire and Leskey 2006). The relationship between stink bug trap captures and fruit injury has yet to be established, however. This information is necessary to develop a reliable method for determining the need for and timing of insecticide applications against stink bugs in fruit orchards.

Thus, it must be possible to accurately identify stink bug feeding injury. In the case of peaches, stink bug injury has been described extensively with early-season feeding

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resulting in catfacing, scarring, and dimpling to the developing fruit, whereas injury occurring later in the season creates only water-soaked and gummosis-type injuries (Rings 1957). For apple, however, stink bug injury was recognized only relatively recently (Krupke et al. 2001, Brown 2003a, Brown et al. 2006). Stink bug injury was often confused with symptoms of calcium deficiency in apple, including cork spot and bitter pit (Brown 2003b). Stink bug injury and cork spot have been differentiated from one another based on the presence of a stink bug feeding puncture and a deeper, more circular depression lacking a distinct edge with corky flesh immediately beneath the fruit skin in the case of stink bug injury; corky flesh associated with cork spot is often not found contiguous with the skin. Stink bug injury has been differentiated from bitter pit based on the fact that bitter pit symptoms appear during storage and consist of very small (2 - 3 mm diam.) corky areas of flesh beneath the skin (Brown 2003a).

Unfortunately, the characters for separation of cork spot from stink bug injury have not proven particularly decisive, especially under field conditions. We suspected that a greater level of variation in the overall appearance and apparency of stink bug injury on apple, particularly of the corky tissue beneath the skin, likely exists. Therefore, specific factors were examined that could contribute to variation in the appearance of stink bug injury on apple, including the time interval between stink bug feeding and subsequent injury evaluation, sex of brown stink bug, cultivar phenology, and cultivar blush or color.

Materials and Methods

Studies were performed in 3 research orchard plots at the USDA-ARS Appalachian Fruit Research Station in Kearneysville, WV, in 2005. The orchards received no insecticide treatments and only minimal fungicide sprays. However, no fungicides were applied while the studies were in progress. Six apple cultivars were studied: 'Ginger Gold', 'Honeycrisp', 'Delicious', 'Golden Delicious', 'Braeburn', and 'Granny Smith'. These cultivars were chosen based on their known susceptibility to stink bug injury (Brown et al. 2006), time of harvest [early- ('Ginger Gold' and 'Honeycrisp'), mid('Golden Delicious' and 'Delicious'), and late-season ('Braeburn' and 'Granny Smith')], and fruit blush [red ripening cultivars ('Honeycrisp', 'Delicious', and 'Braeburn') and green/yellow ripening cultivars ('Ginger Gold', 'Golden Delicious', and 'Granny Smith')]. One orchard contained all 6 cultivars (4.3 × 5.5 m on M9), the second plot contained 'Braeburn', 'Ginger Gold', and 'Golden Delicious' (2.5 × 4.3 m on M9), whereas the third consisted exclusively of 'Granny Smith' (3.7 × 4.9 m on M26).

Beginning on 16 July and continuing until 11 October, adult field-collected brown stink bugs, the most frequently encountered stink bug species in mid-Atlantic apple orchards (Leskey and Hogmire 2005, Hogmire and Leskey 2006), were individually caged on apple limbs with fruit. Cages were 0.30 × 0.20 m and constructed of 0.75 mm flexible polycarbonate frames for side support with a 1.0 mm polycarbonate ring on each end. Each cage was covered with a "no-see-um" polyester mesh bag (Quest Outfitters, Sarasota, FL) and closed around the limb with a twist tie. A wooden dowel rod was fixed in the center of each cage and then attached to the apple limb for stability. Limbs were selected that contained 3 injury-free apples spanning no more than 0.3 m. Larger leaves were removed to increase visibility of insect activity on the limb and fruit and to permit recovery and removal of introduced stink bugs. All stink bugs were sexed prior to placement in the cages to maintain a roughly 1:1 sex ratio for later analysis. After their introduction, stink bugs were permitted to feed inside the cages for 72 h and then were removed.

Limbs containing a caged stink bug were randomly assigned to 1 of 3 treatments: 72 h, 1 wk, or harvest. Each treatment refers to the time interval between stink bug insertion into a cage and subsequent evaluation of the fruit for stink bug injury. After removal of the stink bugs from the cages at 72 h, protective fruit bags, commonly referred to as 'Japanese fruit bags'(Wilson Orchard and Vineyard Supply, Yakima, WA) were placed on individual fruit and stapled closed to protect each apple from further insect injury. This study was repeated biweekly with newly collected stink bugs until the harvest date for each cultivar [fruit were harvested when the majority of apples of each cultivar were rated a '4' on the starch index for fruit maturity (Rudisill 2008)]. Harvest dates for each cultivar were as follows: 'Ginger Gold' on 11 August, 'Honeycrisp' on 29 August, 'Golden Delicious' on 20 September, 'Delicious' on 23 September, 'Braeburn' on 4 October, and 'Granny Smith' on 24 October. For each cultivar, a sample of 6 fruit obtained from 2 cages (each cage containing 3 fruit) were included in each time interval treatment of subsequent injury evaluation, whereas 1 limb with 3 uninjured fruit per cultivar was caged without stink bugs to serve as a control.

Descriptions of any damage seen on the fruit after the treatment period were first made in the field, followed by examinations and descriptions in the laboratory using a Leica GZ4 dissecting scope (Leica Microsystems Inc, Bannockburn, IL) at 10X to 40X magnification. All fruit dissections and subsurface damage assessments occurred in the laboratory. Also measured was the diameter of stink bug punctures (n = 15). A rating system was created to separate the variation observed in stink bug feeding injury. The appearance and severity of stink bug feeding injury to the fruit skin were categorized as, a discolored dot, '1' (Fig. 1A), a discolored dot with a depression, '2' (Fig. 1B), and a discolored dot with a discolored depression, '3' (Fig. 1C). Each injury site observed on every fruit was rated based on this scale, i.e., a single fruit could possess several injury sites of different ratings.

Data were separated into 2 categories, insignificant and significant injury, for statistical comparison of harvest injury. Injury sites rated as a '1' based on our rating scale represent insignificant injury (0) and would not influence fruit grading, whereas injury sites rated as a '2' or '3' represent significant injury (1), which could influence fruit grading. Differences in observed injury based on time interval (72h, 1 wk, and harvest), fruit blush (red- or green/yellow-ripening cultivars), and cultivar phenology (early-, mid, and late-season cultivars) were evaluated using chi-square analyses (PROC FREQ, SAS Institute 2001). Differences in the mean injury rating (1 - 3) for male and female stink bugs was compared using a pooled t-test (PROC TTEST, SAS Institute 2001), with the assumption of a large sample size representing data drawn from a normal population.

Results

Field observations. A total of 992 fruit were assessed for stink bug damage, and 15.12% (n = 150) possessed at least one injury site. The most common injury type observed was a discolored dot that primarily appeared green or gray, although black and red dots were also observed. Dots were always present on damaged fruit and became easier to discern with repeated experience. Discolored depressions appeared primarily green, but also were occasionally observed with varying amounts of brown

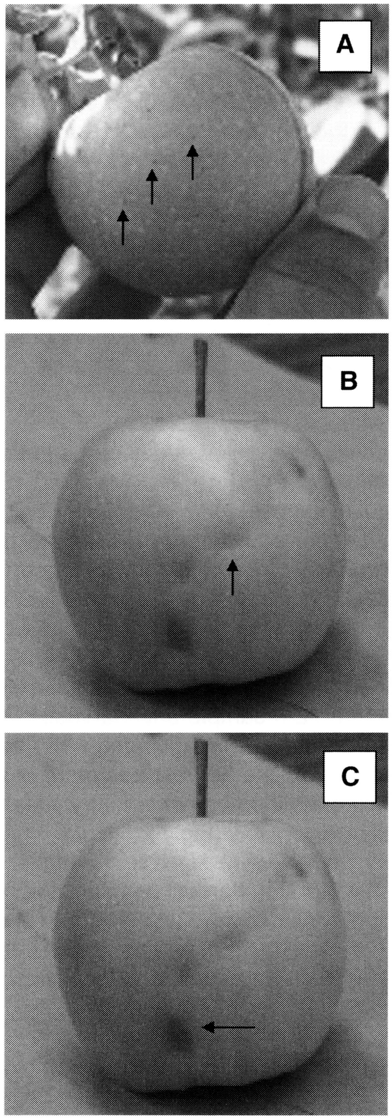

Fig. 1. (A) Gray discolored dots on surface, (B) depression on surface, and (C) discolored depression on surface of 'Granny Smith' apple as seen in the field.

or red. 'Delicious' fruit tended to be the exception, where discolored depressions were mostly red and green with some injury appearing almost black. 'Braeburn', 'Delicious', 'Ginger Gold', and 'Granny Smith' exhibited a combination of the different types of damage.

Laboratory observations. Without exception, stink bug feeding resulted in the presence of a uniformly circular hole (mean ± SE) diameter: n = 15; 0.174 ± 0.007 mm) (Fig. 2) in the skin of the fruit that had been recorded as a discolored dot in field assessments. No variation in the size, shape, or color of the hole itself was observed among cultivars. Fruit cut in the laboratory revealed that variation existed in the tissue beneath observed surface injury. Beneath a discolored dot or stink bug feeding hole, existed a stylet sheath (Fig. 3); the stylet sheaths ranged in color from white to light brown, with an occasional green tube. When the fruit skin was depressed, corking typically increased around the stylet sheath, but did not usually obscure it from the view. Significant corking, ranging from yellow to dark brown, was visible beneath the skin of fruit possessing a discolored depression on the surface. Typically, the corky area beneath the surface was contiguous with the fruit skin and uniform in shape (Fig. 4A), although this was not always the case, as the area of corking was sometimes separated from the fruit skin and was nonuniform in shape (Fig. 4B). No injury was observed on fruit caged without stink bugs.

Injury ratings. There was no significant difference in the mean injury rating between male and female stink bugs (t=1.49; df=686; P = 0.1364), so these data were pooled for further analysis. Across all treatments, 56% of all injured fruit did not have a depression or discolored depression; but possessed only the discolored dot, i.e. the site of the feeding puncture. All 3 types of injury were observed throughout the study. The percentage of surface damage rated '1', '2', and '3' was 88% (n = 63), 1% (n = 1), and 11% (n = 8), respectively, from the 72 h sample, 63% (n = 43), 21% (n = 14), and 16% (n = 11), respectively, from the 1 wk sample, and 47% (n = 150), 19% (n = 59), and 34% (n = 107), respectively, from the harvest sample. Not only did the number of injury sites increase from the 72 h sample until harvest, but the percentage of sites rated a '3' increased, whereas the percentage rated a '1' decreased. The mean injury rating increased from the 72 h to the harvest treatment of all cultivars except 'Delicious' and 'Honeycrisp', although the number of injury sites for these cultivars for the 72 h and 1 wk treatments was very low (Fig. 5).

Across cultivars, injury ratings were significantly higher at 1 wk compared with 72 h (χ^2=11.1979; df=1; P = 0.0008), harvest compared with 72 h (χ^2=37.9525; df=1; P < 0.0001), and harvest compared with 1 wk (χ^2=5.5646; df=1; P = 0.0183). Mean injury rating at 72 h (n = 72), 1 wk (n = 68), and harvest (n = 316) were 0.13 ± 0.04 SE, 0.37 ± 0.06 SE, and 0.53 ± 0.03 SE, respectively. There was a significant difference in

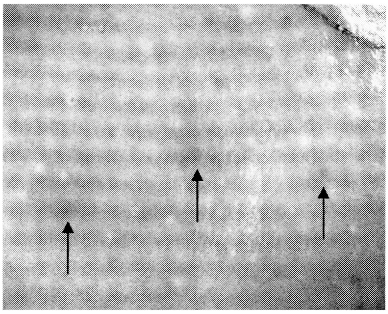

Fig. 2. Puncture sites (10x) in the fruit skin attributed to stink bug feeding.

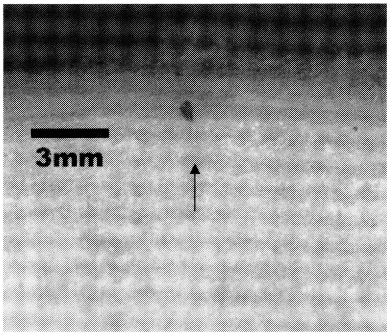

Fig. 3. Transverse section of apple showing a stink bug stylet sheath (40x).

injury associated with fruit blush (χ^2=4.2608; df=1; P = 0.0390); the mean injury rating was higher for red cultivars (n = 173; 0.57 ± 0.04 SE) than nonred cultivars (n = 143; 0.46 ± 0.04 SE) at harvest.

The injury rating for mid and late-season harvested cultivars was not significantly different (χ^2=1.9468; df=1; P = 0.1629). However, the early-season cultivars had a lower injury rating and were significantly different than the mid (χ^2=6.5421; df=1; P =

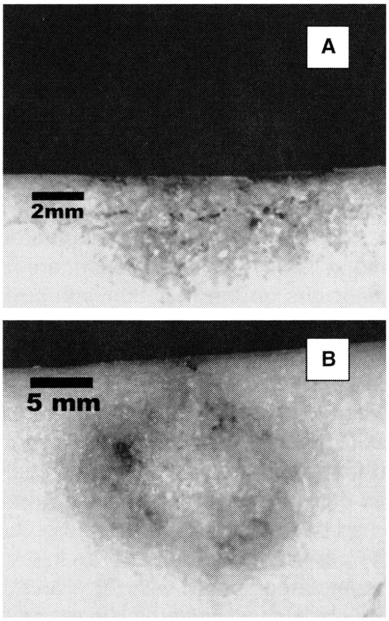

Fig. 4. (A) Transverse section of stink bug injury to apple with typical uniform, discolored corky tissue contiguous with the fruit skin and (B) with non-uniform, discolored corky tissue separated from the fruit skin (40x).

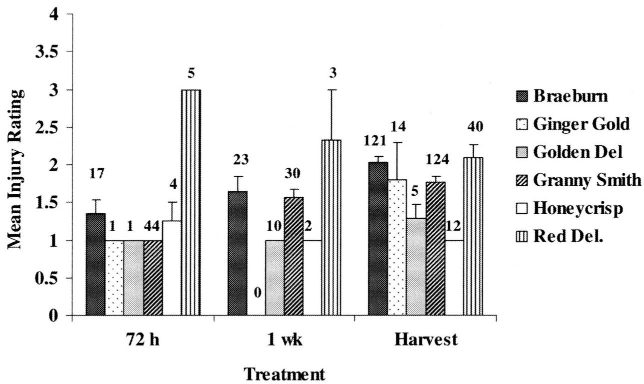

Fig. 5. Mean injury rating ± SE and total number of injury sites for 'Braeburn', 'Ginger Gold', 'Golden Delicious', 'Granny Smith', 'Honeycrisp', and 'Delicious' from 72 h, 1 wk, and harvest sample interval treatments.

0.0105) and late-season (χ^2=12.9347; df=1; P = 0.0003) cultivars. The mean injury ratings for early- (n = 17), mid (n = 54), and late-season (n = 245) cultivars were 0.12 ± 0.08 SE, 0.46 ± 0.07 SE, and 0.57 ± 0.03 SE, respectively.

Discussion

The presence of the stink bug feeding puncture was the only reliable and consistent characteristic for determination of stink bug injury. Corky tissue associated with stink bug injury may not be contiguous with the fruit skin (Fig. 4B) and, therefore, this characteristic may not be used to separate it from the physiological disorder cork spot. Field-based evaluations of purported stink bug injury can be performed based on puncture site recognition, i.e., discolored dots, but laboratory assessments under a dissecting microscope, with at least 10X magnification, are required for absolute confirmation. Discolored depressions on the fruit skin with corky tissue below may enhance observer recognition of potential injury, but cannot stand alone in confirming stink bug injury identification.

The most common injury encountered was rated a '1', a discolored dot present on fruit, even at harvest. Interestingly, the total number of injury sites recorded as a '1' in the 72 h and 1 wk treatment samples was 63 and 43, respectively. However, 150 sites rated a '1' were identified from the harvest treatment samples, potentially indicating that 60 - 70% of discolored dots present on fruit were not visually apparent within the first week after the injury had been inflicted. Furthermore, the mean injury rating was significantly higher and the percentage of fruit with an injury rating of '3' was highest in the harvest treatment sample compared with 72 h and 1 wk treatment samples. Also, early-season cultivars had significantly lower injury ratings than mid or late-season cultivars. These data suggest a possible progression in the visual apparency and severity of injury over the course of the growing season, which will initially appear as a discolored dot, but over time may develop into an injury site that includes a discolored dot with a discolored depression on the fruit surface. Brown stink bug injury to other crops such as cotton varies in severity depending on the age of the plant as well (Willrich et al. 2004).

We hypothesized that the source of variation in the appearance of stink bug injury was related to the nature of stink bug feeding, i.e. probing would result in discolored dots whereas sustained feeding would more likely produce depressions in the fruit skin. We simulated stink bug probing injury by mechanically injuring 'Braeburn' and 'Granny Smith' apples with an acupuncture needle. Fruit were injured by the needle with a quick, in-and-out puncture to reflect breaking of the fruit skin, without any insertion of enzymes or removal of fluids that would be expected from sustained stink bug feeding. 'Braeburn' and 'Granny Smith' are considered to be preferred cultivars for stink bug feeding, i.e., both of these cultivars had high levels of stink bug injury based on the presence of depressed or discolored areas on fruit skin with underlying flesh having a corky appearance (Brown et al. 2006). Interestingly, in our trials all 'Granny Smith' injury sites developed into depressions, and 'Braeburn' injury sites developed into discolored depressions (unpubl. data) demonstrating that the nature of stink bug feeding did not necessarily translate into the type of injury observed. Simply breaking the fruit skin was enough to create a discolored depression on the fruit surface. However, the variation between the type of injury observed on the fruit skin of 'Braeburn' and 'Granny Smith' also suggested that there may be individual cultivar differences related to the injury development and appearance. Similarly, injury to red blush fruit was significantly higher than nonred fruit suggesting inherent differences may exist between green and red fruit as has been observed from wound-sites on tomato (Parsons and Mattoo 1991).

In conclusion, as stink bug injury continues to be a threat to many apple cultivars (Brown et al. 2006), it is important to be able to accurately detect and identify stink bug injury. In our studies, variation in the ability to detect damage seems to be most strongly linked to the duration of time between actual stink bug injury and subsequent fruit evaluation. We suggest that other factors that may contribute to this variation include fruit maturity at the time of injury and potential fruit oxidation and phytochemical processes, which are currently under investigation.

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