Effects of Temperature and Relative Humidity on the Vertical Distribution of Stink Bugs (Hemiptera: Pentatomidae) within a Soybean Canopy and Implications for Field Sampling[1]

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Abstract Stink bugs (Hemiptera: Pentatomidae) are suspected to change their intraplant vertical distribution in response to changes in environmental conditions. As a result, this could influence sweep-net sampling efficiency in a soybean canopy. We examined the effects of both ambient and within-soybean canopy temperature and relative humidity on stink bug vertical distribution in 2 0.38m row spaced commercial soybean fields with full canopies in Virginia, one in 2010 and one in 2011. Temperature and relative humidity were monitored in the upper and lower plant canopy. The within-canopy vertical distribution of a minimum of 20 stink bugs was documented at each of 4 different observation times: observations were replicated on different days 14 times in the morning, 14 around noon, 15 during the midafternoon, and 5 observations were replicated in the early evening. *Chinavia hilaris* Say was the primary species observed with 88% of the total in 2010 and 59% in 2011; the remainder was primarily *Euschistus servus* Say. No significant relationship was observed between the environmental parameters measured or time of day on the vertical distribution of stink bugs in the canopy. Regardless of environmental conditions, an average of 15 - 20% of stink bugs was located below the typical 38cm zone of a sweep net sampling. Efficiency of sweep net sampling for stink bugs in soybean did not appear to be significantly affected by changes in temperature, relative humidity, or time of day, and sweep netting the upper canopy accessed approximately 80% of the total population.

Key words: stink bugs, soybean, temperature, relative humidity, vertical distribution

Stink bugs (Hemiptera: Pentatomidae) are some of the most damaging insect pests of soybean, *Glycine max* (L.) Merrill (Fabaceae), in the southern U.S. (Musser et al. 2010). Growers and crop consultants typically use a 38-cm diameter sweep net to estimate the density of stink bugs to aid in management decisions (Turnipseed 1974, Rudd and Jensen 1977). Extension guidelines recommend samplers to pendulum-swing a 38-cm diameter sweep net crossing the tops of rows, and 'burying' the net to just below the top of the canopy. In general, sweep netting will intercept the upper canopy area, but will not be as effective in providing a reliable sample of stink bugs located below the upper 38cm. Sampling efficiency could be especially challenging in irrigated, early or densely planted soybean which often attain greater plant height than nonirrigated or later-planted soybean (Klocke et al. 1989, Chen and Wiatrak 2011).

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Furthermore, plant height can be influenced by variety as indeterminate varieties are generally taller than determinate varieties (Norberg et al. 2010).

Environmental conditions, such as temperature, wind, and relative humidity can also affect an insect population's distribution in a plant canopy, and possibly sweep net efficiency (DeLong 1932). Few studies have determined how stink bugs are vertically distributed in soybean canopies, and how canopy microclimate may affect their distribution. In the southern U.S., stink bug scouting in soybean is conducted during the summer when ambient temperatures often exceed 35°C during the afternoon. It is unknown whether these high temperature periods stimulate stink bugs to move deeper into the canopy. Espino et al. (2008) found that sweep net catches of rice stink bug, *Oebalus pugnax* F., on rice varied by time of day, with higher stink bug abundance in samples taken during the morning, but possible causes of this relationship were not examined. Rashid et al. (2006) also noted significantly fewer catches of *O. pugnax* on rice during the heat of the day on several sampling dates in July. They suggested avoiding sampling in the afternoon on hot days, as stink bugs moved lower on the rice stems. However, in flooded rice fields, Cherry and Deren (2000) did not see any significant influence of time of day or air temperature on sweep-net catch of *Oebalus* spp.

The objective of this study was to determine the influence of time of day and both ambient and within-canopy microclimate temperature and relative humidity on the diurnal vertical distribution of stink bugs in soybean. If stink bugs move in the soybean canopy throughout the day in response to changing environmental conditions, both the efficiency of sweep-net sampling and insecticide treatment may be affected. If this movement could be predicted, procedures could be adjusted to improve both sampling and control.

Materials and Methods

2010. The experiment was conducted in Virginia Beach, VA in a field of indeterminate NKS46-U6 soybean planted on 8 May 2010 using a 0.38 m row spacing. At the time of the stink bug observations, soybean plants were at the beginning seed, R5, development stage (Fehr et al. 1971). The plant canopy was full, with well-developed branching, very little defoliation, and a height of 1.2 m. In both years, fields with a fully-developed canopy were intentionally selected because they are typical of non-drought stressed, full season, and double-crop soybean grown in Virginia. Pods were present on all plant nodes 12 cm above the ground and higher. On 11 August, 3 HOBO® Data Loggers (U10 - 003, Onset Computer Corporation, Bourne, MA) were suspended from a 1.2 m pole that was placed in the field. A single data logger was placed into the upper canopy about 6 cm beneath the uppermost trifoliate, another in the middle of the canopy, and the third was placed just above ground surface. They recorded temperature and relative humidity every 8 min for the duration of each observation period.

At the time of the stink bug observations, the study field had an average density of 4.5 stink bug adults and nymphs per 15 sweeps based on several 15-sweep samples taken at random locations throughout the field. Visual observations were made of any naturally encountered stink bug in the soybean canopy at 4 time periods: 6 observations were made on different days in the morning at 0700h-0830h EST (Universal time coordinate (UTC) -5 h), 4 observations around 1200h, 5 in midafternoon at 1500h-1630h, and 5 in the early evening after 1815h for a total of 20 observations between 11 August and 4 September. During each observation period, a minimum of 20 stink bugs (irrespective of stage or species) were located by slowly walking or kneeling between rows while gently pushing the plants back to expose stink bugs and carefully scanning the ground, stems, pods, and leaf undersides. The vertical position in the canopy was measured in cm above the ground surface. Observations were separated into 2 zones: the top 38 cm (the average depth that a sweep net will most often be passed through the canopy) and everything below. Nymphal aggregates were treated as 1 individual (Linker et al. 1999), so as to not bias the calculated distribution of stink bugs at each observation period.

2011. The experiment was conducted again in Virginia Beach, VA in a field of indeterminate Pioneer 94B73 soybean that was planted on 3 May 2011 using a 0.38-cm row spacing. As in 2010, at the time of the stink bug observations, the canopy was full with very little defoliation, a height of 1.0 m, and pods that were distributed along the entire stem to within 12cm above the ground. Only 2 data loggers were used in 2011 because the temperature and relative humidity readings were nearly identical for the bottom and middle data loggers in 2010. The upper data logger was placed 6cm below the uppermost trifoliate and the bottom logger just above the ground. The loggers recorded temperature and relative humidity data every 5 min instead of every 8, as in 2010, to obtain a greater number of readings per observation period. At the time of the stink bug observations, the study field had an average density of 2.5 stink bug adults and nymphs per 15-sweep sample. Observations were not taken during the evening. There were 8 observations on different days in the morning at 0700h-0820h, 10 at 1200h, and 10 in the midafternoon at 1500h-1645h for a total of 28 observations between 21 July and 11 August. Observations of stink bug vertical location in the canopy were taken in the same manner as described in 2010.

Statistical analyses. The percentage of stink bugs found within or below the upper 38 cm of the canopy was calculated. Temperature and relative humidity readings were averaged for each observation time period when stink bugs were observed. The average conditions from the bottom logger were then subtracted from the upper logger to obtain the range of temperature and relative humidity for each time period. Separate linear regressions fit to the 2nd order polynomial of the ambient temperature (approximated by the upper data logger), ambient relative humidity (approximated by the upper data logger), within canopy range of temperature, and within canopy relative humidity versus the percentage of stink bugs below the upper 38cm were performed using the SAS JMP v. 8.0 software (SAS Institute 2009). Species and life stages were pooled due to insufficient sample sizes of each species and life stage and resulting nonnormality and nonlinearity. Temperature and relative humidity values were segregated into 4 groups each, and analysis of variance (ANOVA) was performed to determine if there were a significant temperature or relative humidity grouping effect on vertical distribution of stink bugs. The percentage of stink bugs below the upper 38 cm was arcsine square root transformed. ANOVA was performed to determine if time of day influenced the percentage of stink bugs below the upper 38 cm using SAS JMP v. 8.0 (SAS Institute 2009). A *t*-test was performed on the 2010 and 2011 mean percentage of stink bugs below the upper 38 cm using SAS v 9.2 (SAS Institute 2008) to determine if the two years' results were different due to slight differences in soybean canopy architecture. Due to unequal variance, the Satterthwaite method was used to calculate the *t*-value and probability that the yearly mean was different (SAS Annotated

Output Proc TTest). All reported means were reported as untransformed values ± SE of the means.

Results

Stink bug species composition and yearly mean distribution. Of the 551 stink bugs observed in 2010, the green stink bug, *Chinavia hilaris*, comprised an overall average of 88% of the total, with the remainder identified as brown stink bugs, *Euschistus servus* Say. In 2011, 727 stink bugs were observed; 59% were *C. hilaris* and 39% were *E. servus*. Nymphs comprised 46% of the total observed in 2010 and 25% in 2011. Other species identified in 2011 included *Podisus* spp., *Euschistus quadrator* Rolston, and *Thyanta* spp. The mean percentage of stink bugs below the top 38 cm from all observations was 20.5 ± 7.6% and 15.0 ± 3.9% for 2010 and 2011, respectively, but the yearly means were not significantly different (t = 1.35, df = 29.21, P = 0.19).

Time of day. ANOVA analyses of 2010 and 2011 data indicated no significant difference between the times of day that observations were made and the percentage of stink bugs below the top 38 cm of the soybean canopy (F = 1.00; df = 3, 15; P = 0.42 and F = 0.27; df = 2, 25; P = 0.76, respectively). In 2010, the percentage below the top 38 cm was 12.8 ± 7.4 in the afternoon, 16.8 ± 7.4 in the evening, 25.5 ± 6.7 in the morning, and 27.3 ± 8.3 at noon. In 2011, the percentage below the top 38 cm was lower and more uniform, with 12.9 ± 3.6 low in the morning, 15.2 ± 3.2 in the afternoon, and 16.5 ± 3.2 at noon.

Effects of temperature. Ambient temperatures during the experiment ranged from 22.5 - 40.4°C and from 22.3 - 44.9°C in 2010 and 2011, respectively (Tables 1, 2). There was no significant relationship between temperature range and the percentage of stink bugs below the top 38cm of the canopy in 2010 [*Percentage below* = 10.88548 - 0.2212434 (*Temperature range*) + 4.9844886 (*Temperature range* – 1.78)^2; R^2 = 0.26, F = 3.03, df = 19, P = 0.07] or 2011 [*Percentage below* = 0.138158 + 0.0055897 (*Temperature range*) – 0.0011573 (*Temperature range* – 4.4798)^2; R^2 = 0.03, F = 0.35, df = 27, P = 0.71]. Ambient temperature, as measured by the uppermost logger, also did not have a significant influence on stink bug vertical distribution in either 2010 [*Percentage below* = 21.336372 - 0.2848786 (*Ambient temperature*) + 0.2618396 (*Ambient temperature* – 30.096)^2; R^2 = 0.19, F = 1.99, df = 19, P = 0.17], or 2011 [*Percentage below* = 16.806858 + 0.0218194 (*Ambient temperature*) – 0.078254 (*Ambient temperature* – 33.8128)^2 ; R^2 = 0.09 F = 1.19, df = 27, P = 0.32]. The temperature groupings of 20 - 25, 25 - 30, 30 - 35, and >35°C also did not significantly affect vertical distribution of stink bugs (F = 0.13, df = 3, 44, P = 0.94).

Effects of relative humidity. Relative humidity ranged from 35.6 - 92.6% and from 39.8 - 91.7% in 2010 and 2011, respectively (Tables 1, 2). Relative humidity was highest in the morning and lowest in the afternoon. In both years, the relative humidity measured by the bottom logger was higher than that measured by the top logger, so the calculated range was negative (Tables 1, 2). However, the relative humidity range did not have a significant influence on stink bug distribution in either 2010 [*Percentage below* = 28.153866 - 0.0877282 (*Relative humidity range*) -0.2395068 (*Relative humidity range* – 9.562)^2; R^2 = 0.11, F = 1.01, df = 19, P = 0.38], or 2011 [*Percentage below* = 14.054084 - 0.2967638 (*Relative humidity range*) – 0.0410105 (*Relative humidity range* – 11.465)^2; R^2 = 0.11, F = 1.01, df = 27,

Table 1. Environmental conditions and stink bug position during each observation period, 2010. Averages were taken from data logger readings, and ranges were taken by subtracting the lower sensor from the upper sensor

Date	Time of day*	Number of bugs observed	Temp. of upper canopy (°C)	Temp. range (°C)	Relative humidity of upper canopy	Range of relative humidity	Percentage of stink bugs in lower canopy
11 Aug.	noon	21	39.6	3.1	51.4	−14.1	5.0
12 Aug.	morn	26	26.3	0.1	92.6	0.0	3.8
13 Aug.	morn	24	27.1	1.1	90.0	−4.3	25.0
13 Aug.	aft	30	30.4	2.3	67.6	−15.7	3.3
13 Aug.	even	35	25.2	0.3	82.1	−5.0	20.0
14 Aug.	even	29	24.0	0.8	81.7	−3.2	3.4
17 Aug.	morn	25	28.3	2.3	82.7	−9.3	44.0
17 Aug.	noon	26	40.4	4.5	46.5	−15.6	61.5
17 Aug.	aft	30	37.1	3.3	53.7	−13.2	16.7
17 Aug.	even	28	29.9	0.3	76.6	−3.2	14.3
18 Aug.	morn	30	26.0	0.3	89.9	−3.8	26.7
18 Aug.	morn	22	35.3	3.9	58.9	−18.2	13.6
18 Aug.	noon	35	37.0	3.5	56.1	−16.1	42.9
18 Aug.	aft	22	29.7	0.5	81.0	−5.8	18.2
3 Sept.	aft	29	27.2	1.7	74.1	−14.8	10.3
3 Sept.	even	26	24.4	0.5	84.9	−3.9	26.9
4 Sept.	morn	25	22.5	0.3	77.0	−3.7	40.0
4 Sept.	noon	31	33.3	2.4	40.6	−17.5	0.0
4 Sept.	aft	32	34.5	3.6	35.6	−16.7	15.6
4 Sept.	even	26	23.8	0.8	54.8	−7.1	19.2

* Morn = morning observations (0,700 - 0830h), midmorn = midmorning (1030h), aft = afternoon observations (1500 - 1615h), and even = early evening observations (after 1815h)

P = 0.38]. Likewise, ambient relative humidity, as measured by the uppermost logger, also had no significant effect on stink bug distribution in either 2010 [*Percentage below* = 23.333209 - 0.0222369 (*Ambient relative humidity*) – 0.0042803 (*Ambient relative humidity* – 68.89)^2; R^2 = 0.00, F = 0.04, df = 19, P = 0.96], or 2011 [*Percentage below* = 13.63637 + 0.0571796 (*Ambient relative humidity*) – 0.0089739 (*Ambient relative humidity*-64.1303)^2; R^2 = 0.05, F = 0.60, df = 27, P = 0.56]. The relative humidity groupings of <45, 45 - 60, 60 - 75, and >75% RH did not significantly

Table 2. Environmental conditions and stink bug position during each observation period, 2011. Averages were taken from data logger readings, and ranges were taken by subtracting the lower sensor from the upper sensor

Date	Time of day*	Number of bugs observed	Temperature of upper canopy (°C)	Temp. range (°C)	Relative humidity of upper canopy	Range of relative humidity	Percentage of stink bugs in lower canopy
21 July	aft	21	42.5	6.3	51.8	–24.7	4.8
22 July	morn	21	31.1	3.6	74.8	–16.7	19.0
22 July	noon	21	40.5	2.5	54.6	–13.0	0.0
22 July	aft	21	44.9	7.3	41.6	–27.6	19.0
25 July	noon	27	38.5	5.5	56.0	–25.4	29.6
25 July	aft	24	39.2	5.4	56.3	–24.3	0.0
27 July	morn	20	25.0	0.5	92.0	–4.5	15.0
27 July	noon	21	35.8	3.4	46.3	–18.4	14.3
27 July	aft	22	33.9	2.2	55.5	–15.3	13.6
28 July	morn	21	26.8	1.3	91.7	–1.7	4.8
28 July	noon	24	36.8	3.2	60.7	–15.5	20.8
28 July	aft	21	34.4	1.0	71.3	–6.1	42.9
29 July	morn	24	26.7	0.4	85.7	–3.8	20.8
29 July	noon	22	39.2	3.9	59.0	–14.9	31.8
29 July	aft	23	37.9	3.7	60.2	–16.0	13.0
3 Aug.	morn	22	26.3	0.9	85.5	–5.0	13.6
3 Aug.	noon	21	35.3	2.9	64.5	–12.7	14.3
3 Aug.	aft	22	37.7	1.7	55.0	–7.2	18.2
4 Aug.	morn	23	25.8	1.1	86.3	–6.7	26.1
4 Aug.	noon	26	32.7	1.6	74.9	–8.5	15.4
4 Aug.	aft	28	33.7	3.2	66.8	–17.4	17.9
5 Aug.	morn	25	25.9	1.0	84.3	–6.9	4.0
5 Aug.	noon	25	31.8	1.8	62.3	–10.0	16.0
10 Aug.	noon	24	38.2	0.6	43.6	0.1	4.2
10 Aug.	aft	20	33.3	0.5	52.2	–2.7	10.0
11 Aug.	morn	24	22.3	0.0	82.5	–2.1	0.0
11 Aug.	noon	27	35.5	1.8	40.5	–8.8	18.5
11 Aug.	aft	23	35.0	2.1	39.8	–5.4	13.0

* Morn = morning observations (0,700 - 0830h), aft = afternoon observations (1500 - 1615 h)

affect the percentage of stink bugs below the upper 38cm ($F = 0.40$, df = 3, 44, $P = 0.75$).

Discussion

In this study, time of day, ambient and within-soybean canopy temperature, and relative humidity did not influence the vertical distribution of stink bugs in a predictable manner. Regardless of climatic conditions, 15 - 20% of stink bugs were located below the recommended sweep net sampling zone (~38 cm from the top of the canopy). Ambient temperatures during the observation periods reached levels that were several degrees above average July (30.5°C) and August (29.3°C) high temperatures (National Climatic Data Center 2008). Although temperatures during the study were unusually hot for Virginia, they are not uncommon in many other soybean-growing states south of Virginia. Waite (1980) suggested that if the upper canopy were too warm for stink bugs, they might seek shelter lower in the canopy. This was not the case in our studies, even though cooler temperatures were available in the lower canopy.

Environmental influences on the behavior of other hemipterans have been reported and, in general, differ from our results in that most samples for hemipterans vary with diurnal or environmental changes. Romney (1945) found that sweep-net catches of beet leafhoppers, *Eutettix tenellus* Baker (Hemiptera: Cicadellidae) in pepperwort, *Lepidium alyssoides* L. (Brassicaceae), varied widely with temperature, wind speed, and time of day. In soybean, population density estimates of *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) taken by sweep net varied among time of day, temperature, and cloud cover influences with more insects caught in the evening and on clear, cool days (Dumas et al. 1964). Other predatory true bug counts vary with both soil temperature and cloud cover (Dumas et al. 1962, 1964). *Geocoris spp*. (Hemiptera: Lygaeidae) moved low enough in soybean canopies to affect sweep-net catches as temperatures rose throughout the day (Shepard et al. 1974). A study of the behavior of the southern green stink bug, *Nezara viridula* (L.), in Australia found that basking behavior was greatest between 0700h and 0900h (UTC + 10 h). After that time, bugs began to move under leaves or lower in the canopy (Waite 1980). Musser et al. (2007) found that, during the heat of the day, fewer *Lygus* spp. (Hemiptera: Miridae) were visually observed in cotton, *Gossypium hirsutum* L. (Malvaceae) terminals and flowers. However, in that study time of day did not have a significant influence on sweep catches, which suggested that bugs moved to the interior of the canopy as the day warmed rather than lower and so were still accessible by sweep net.

Different canopy heights might influence stink bug distribution. It would be logical to assume that vertical distribution of stink bugs would be more uniform in shorter plant canopies than were used in our studies and that sweep-net efficiency would also be greater as the net would intercept a large portion of the total canopy. We did see slight differences in plant canopy height between years (1.2 m in 2010 versus 1.0 m in 2011) and a slightly smaller proportion of stink bugs were below the sweep net intercept zone in the shorter 2011 canopy. Research across a greater range of plant heights would help clarify this relationship.

The authors also recognize that this study was confined to only one row spacing, 0.38 m, which was selected because it is the most common for our region, and that additional studies in other row spacings need to be done. Other row spacings might influence the microclimate, for example, wide row spacings where the canopy does not close could result in more uniform temperature and humidity levels. Conversely, narrower row spacings, such as 17 cm grain drilled fields, could have slightly greater temperature and humidity gradients, depending on plant height. It is also possible that the vertical distribution of stink bugs would be different in indeterminate compared with determinate soybean varieties due to differences in pod location and development (Russin et al. 1987). In determinate varieties, pods are at the same development stage throughout the canopy, but an indeterminate variety might have developed pods at the bottom of the plant while still producing flowers near the top. This offering of food sources with different levels of attractiveness could influence where stink bugs feed on the plant with concentrations where more preferred pods are located. While stink bugs might go lower into a canopy of indeterminate soybeans to feed on the most mature pods in the early reproductive stages, our study was initiated in a field of R5-R6 growth stages (pods with seed/full seed on the upper nodes) so the pod maturity gradient would have been slight.

It is also possible that stink bug density could influence distribution in a canopy. Russin et al. (1987) noted that stink bug feeding injury in a determinate soybean canopy was confined to the upper halves of soybean plants until average populations reached high levels of 3.8 or more per row m. As populations increased to 9.5 per row m, apparently due to crowding, bugs moved lower in the canopy to feed on less damaged seeds. Our work was limited to the natural infestation levels encountered, a field average of 4.5 and 2.5 stink bugs per row m in the 2 years, but these levels are typical for this area, and at or above current economic thresholds. It is possible that our results may have been different if stink bug densities had been higher.

Using current recommendations for stink bug management in soybean from across the southeastern U.S., converting a density per row m to a per sweep-net sample, the mean density required to reach a threshold level corresponds to between 4.1 - 6.2 stink bugs per 15 sweep sample (Lorenz et al. 2006, Roberts and McPherson 2010, Baldwin et al. 2010, Reisig and Roberson 2011). Because of this wide range, there is a need to more sharply define sweep-net thresholds for stink bugs. The study presented here did not address this question, but provides evidence that time of day and changes in temperature and relative humidity should not influence efficiency of sweep-net captures in fields with tall, full-canopy structure for the stink bug species we observed. Also, given the full-plant canopy used in this study, it appears that approximately 80% of the stink bug population can be accessed by sweeping the upper 38 cm of the canopy.

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