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Aggregation and Association of NDVI, Boll Injury, and Stink Bugs in North Carolina Cotton

Dominic D. Reisig,^{1,2} F. P. F. Reay-Jones,³ and A. D. Meijer⁴¹Department of Entomology, North Carolina State University, Vernon G. James Research and Extension Center, 207 Research Station Rd., Plymouth, NC 27962²Corresponding author, e-mail: ddreisig@ncsu.edu³School of Agricultural, Forest and Environmental Sciences, Clemson University, 2200 Pocket Rd., Florence, SC 29506⁴Department of Soil Science, North Carolina State University, Vernon G. James Research and Extension Center, 207 Research Station Rd., Plymouth, NC 27962

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ABSTRACT. Sampling of herbivorous stink bugs in southeastern U.S. cotton remains problematic. Remote sensing was explored to improve sampling of these pests and associated boll injury. Two adjacent 14.5-ha cotton fields were grid sampled in 2011 and 2012 by collecting stink bug adults and bolls every week during the third, fourth, and fifth weeks of bloom. Satellite remote sensing data were collected during the third week of bloom during both years, and normalized difference vegetation index (NDVI) values were calculated. Stink bugs were spatially aggregated on the third week of bloom in 2011. Boll injury from stink bugs was spatially aggregated during the fourth week of bloom in 2012. The NDVI values were aggregated during both years. There was a positive association and correlation between stink bug numbers and NDVI values, as well as injured bolls and NDVI values, during the third week of bloom in 2011. During the third week of bloom in 2012, NDVI values were negatively correlated with stink bug numbers. During the fourth week of bloom in 2011, stink bug numbers and boll injury were both positively associated and correlated with NDVI values. During the fourth week of bloom in 2012, stink bugs were negatively correlated with NDVI values, and boll injury was negatively associated and correlated with NDVI values. This study suggests the potential of remote sensing as a tool to assist with sampling stink bugs in cotton, although more research is needed using NDVI and other plant measurements to predict stink bug injury.

Key Words: boll injury, *Euschistus servus* (Say), NDVI, SADIE

Herbivorous stink bug species are one of the most important pest complexes of cotton, *Gossypium hirsutum* (L.), in the southeastern United States. In 2011, an estimated \$34 million was lost due to damage from these insects or due to management costs for these insects (Williams 2012). Stink bugs are difficult to manage in cotton because they are peripatetic, polyphagous, spatially aggregated, and some species are tolerant to commonly used pyrethroid-class insecticides (Todd and Herzog 1980, McPherson and McPherson 2000, Willrich et al. 2003, Reay-Jones 2010). In North Carolina, and throughout the southeast, the most common species in cotton are *Euschistus servus* (Say), *Chinavia hilaris* (Say), and *Nezara viridula* (L.) (Toews et al. 2009).

The recommended sampling method is based on indirect sampling of associated boll injury, rather than direct sampling based on stink bug density. Recommendations include collecting random samples of quarter-sized bolls (~2.5 cm in diameter), with at least one boll collected per 0.4 ha; at least 25 bolls should be sampled in each field at least weekly from the first week of bloom until cutout (Bacheler et al. 2010). This sampling is time- and labor-intensive.

Remote sensing is a precision technology that acquires information about an object without direct contact. Although its greatest applications in agriculture have been in the soil sciences, it has been explored as a detection tool in entomological pest management. For example, another hemipteran insect pest of cotton, *Lygus lineolaris* (Palisot de Beauvois), was shown to be spatially associated with areas of vigorously-growing cotton (Willers et al. 1999). These areas were defined by the Normalized Difference Vegetation Index (NDVI), calculated from aerial remote sensing data. The study presented here was designed to examine the potential of remote sensing as a sampling aid to pinpoint areas of the field where stink bugs may have injured bolls. The ultimate goal will be to improve sampling methods and to reduce the amount of

insecticides applied by combining remote sensing with precision application technologies.

Materials and Methods

Two adjacent 14.5 ha fields of cotton were selected for the study near Pantego, NC in 2011 (DP 1028 B2RF) and 2012 (DG 2570 B2RF). The fields were separated by a 0.9-m ditch and surrounded on three sides by cotton and on one side by a road, with more cotton across the road. Sampling grids were marked every 0.4 ha in both fields, so that 36 sampling points were defined in each field. Sampling points on field edges were located within 30 m from the field perimeter. The sampling points were identified using a 2.4-m fiberglass pole with a flag attached. Points were georeferenced using a Trimble GeoXT (Trimble, Sunnyvale, CA) for later geographical information systems post-processing.

Cotton was sampled at each point during the third, fourth, and fifth weeks of bloom (21, 28 July, and 4 August 2011 and 27 July, 3 and 10 August 2012) by taking 25-sweep samples with a 41-cm-diameter sweep net and, two rows over from the area that was swept, 10 quarter-sized boll samples. Stink bugs were identified to species at each location, and bolls were assessed for internal boll injury following North Carolina State University extension guidelines (Bacheler et al. 2010). After the fourth week of bloom in 2011, cotton exceeded the recommended treatment threshold guidelines and was sprayed with dicrotophos and *beta*-cyfluthrin (Bidrin 8 at 560 g ai/ha and Baythroid XL at 23.5 g ai/ha). Stink bug injury was not detected in the field during the fifth week of bloom in 2011, and data from this week were dropped from the study. After the third week of bloom in 2012, cotton exceeded the recommended treatment threshold guidelines and was sprayed with dicrotophos and *beta*-cyfluthrin using the same rates as in 2011. Because stink bug injury was still present, the field was sprayed again

after the fourth week of bloom in 2012 using the same chemicals and rates as before. The 2012 field was sprayed using strips, because it was part of a separate study analyzing the effectiveness of strip sprays for stink bug management; hence only the 36 sample points from 2012 with the spatially uniform spray were used for analysis from the fourth and fifth week of bloom in 2012. In contrast, the 2011 field was uniformly sprayed following common grower practices, so all 72 sampling points could be used.

Remote sensing data were collected during the third week of bloom on 22 July 2011 using the RapidEye satellite (RapidEye AG, Berlin, Germany) and on 26 July 2012 using a DigitalGlobe satellite, QuickBird (DigitalGlobe Inc., Longmont, CO), both of which were equipped with a pushbroom multispectral satellite. Satellite providers calibrate to reflectance from the earth's surface using objects with known units of reflectance and a radiative transfer model with sensitivities for atmospheric scattering and absorption. The frequency of the calibration is proprietary and truly calibrated reflectance values were not available. Satellite data were imported into ArcMap 10.0 (ESRI 2011, Redlands, CA), and regions of interest were drawn around the sampling area at each 0.4 ha. This encompassed an area that was 36–39 pixels in the RapidEye image (pixel ~ 16.5 m) and 204–207 pixels (pixel ~ 2.4 m) in the QuickBird image. Not all the sampling points were included in the RapidEye image, so only 66 sampling points could be analyzed. Values from these regions of interest were extracted by mask in ArcMap and imported into an electronic spreadsheet. An NDVI value for each location was calculated using the following equation (Rouse et al. 1974):

$$\frac{(R_{\text{NIR}} - R_{\text{RED}})}{(R_{\text{NIR}} + R_{\text{RED}})}$$

where R_{NIR} = the value encompassed in the wavelengths from the near infrared region and R_{RED} = the value encompassed in the wavelengths from the red region.

The Spatial Analysis by Distance IndicEs (SADIE version 1.22, Perry et al. 1999) tool was used to determine aggregation of stink bug densities, boll injury, and NDVI. The NDVI values were multiplied by 100 and expressed as integers for analysis. The overall index of dispersion (I_a) indicates either an aggregated (>1), random ($=1$), or uniform pattern (<1). The probability (P) is derived after a large number of randomizations as a formal test of randomness: the null hypothesis of spatial randomness is rejected for $P < 0.025$ (aggregation) or $P > 0.975$ (uniformity) with a 5% error rate. In total, $\approx 6,000$ randomizations were used for each test. There were insufficient adult stink bug numbers and boll injury on 4 August 2011 and 3 August 2012 for SADIE aggregation analysis.

The SADIE association tool was one of two methods used to investigate the association between boll injury from stink bugs and the NDVI value from each location. Spatial associations were measured between NDVI values of each year and adult stink bug numbers at each week of bloom, as well as NDVI values and boll injury at each week of bloom. An overall index of association (X) was determined between each of the paired datasets, with a positive association for $X > 0$ ($P < 0.025$) and a negative association for $X < 0$ ($P < 0.975$). Mean X is calculated from the local spatial associations (X_k) at each sampling location k . At the local scale, a positive association between two variables indicates the presence of either a patch or a gap for both variables; a negative association indicates the presence of a patch for one variable and gap for the other variable at the same location. Local association and aggregation indices were interpolated using the inverse distance weighting spatial method in ArcMap 10.0 (Environmental Systems Research Institute 2011).

Correlation analysis (PROC CORR, SAS Institute 2008) was conducted in 2011 for NDVI values and the numbers of stink bug adult and injured bolls for the third (21 July) and fourth week of bloom (28 July).

Not enough stink bugs were captured in the fifth week of bloom in 2011 for correlation analysis. The 2011 remote sensing image did not include all the sampling points, and six were dropped from the analysis. Separate correlation analyses for 2012 data were conducted for NDVI values and the numbers of stink bug adult and injured bolls in the third (27 July), fourth (3 August), and the fifth week of bloom (10 August). Similar correlation analyses were performed for total stink bug adult numbers at each week of bloom and NDVI values. Only adult numbers were used, since few nymphs were captured. Values were transformed ($\sqrt{x+1}$), when needed, to satisfy the assumptions of the correlation (Zar 1999). A visual representation of the association between boll injury and NDVI values was generated by interpolating the percent injured boll values and both the local aggregation and association indices using inverse distance weighting in ArcGIS.

Results

The primary stink bug species captured in sweep samples was *E. servus* during 2011. Over the course of the 2011 sampling period, there were 11, 7, and 3 *E. servus* adults captured on the third, fourth, and fifth weeks of bloom, respectively, and 4, 1, and 0 *E. servus* nymphs captured on the third, fourth, and fifth weeks of bloom, respectively; there were also five *C. hilare* Say adults and one *C. hilare* nymph captured on the fourth week of bloom in 2011. Only *E. servus* was captured in 2012, 17, 0, and 5 *E. servus* adults captured on the third, fourth, and fifth weeks of bloom, respectively, and three *E. servus* nymphs captured on the third weeks of bloom. Adult stink bugs were more abundant than nymphs in both years. Stink bug injury was numerically lower in 2011 than 2012. For example, 6.2% injured bolls were found during the third week of bloom in 2011 compared with 28.2% injured bolls in 2012 during the third week of bloom. Even after insecticide treatment after the third week of bloom in 2012, 10.8% of the bolls were injured in the fourth week of bloom. After the second insecticide treatment in 2012, injured bolls were reduced to 2.5%, below the treatment threshold of 10% injured bolls. In contrast, boll injury was essentially undetectable after the insecticide treatment following the fourth week of bloom in 2011.

Aggregation. In 2011, adult stink bugs showed a trend to being spatially aggregated on the third week of bloom ($I = 1.510$, $P = 0.0402$; Fig. 1A) but not on the fourth week of bloom ($I = 1.081$, $P = 0.2693$; Fig. 1A). Insufficient numbers of stink bugs were captured for analysis during the fifth week of bloom. In 2012, adult stink bugs were not spatially aggregated during the third ($I = 1.086$, $P = 0.2618$) and fifth week of bloom ($I = 0.692$, $P = 0.8579$) and not enough were captured for analysis during the fourth week of bloom. In 2011, boll injury due to stink bugs was not spatially aggregated on the third ($I = 1.039$, $P = 0.5934$) or fourth ($I = 0.881$, $P = 0.6404$) week of bloom (Fig. 1A). In contrast, the 2011 NDVI values were spatially aggregated ($I = 2.658$, $P = 0.0002$; Fig. 1A). In 2012, boll injury due to stink bugs was not spatially aggregated on the third ($I = 0.903$, $P = 0.5934$) or fifth ($I = 0.789$, $P = 0.7161$) weeks of bloom, but a trend for aggregated injury was observed on the fourth week of bloom ($I = 1.705$, $P = 0.0409$; Fig. 2A). The NDVI values were aggregated across all 72 locations used for associations with stink bug densities and boll injury on the third week of bloom ($I = 3.949$, $P = 0.0002$; Fig. 2A), and across half (36) of the sampling locations used for associations with stink bug densities and boll injury on the fourth and fifth weeks of bloom ($I = 3.643$, $P = 0.0002$; Fig. 2A).

Association. Total number of adult stink bugs during the third week of bloom were positively correlated ($r = 0.28$, $df = 66$, $P = 0.0220$) and positively associated ($X = 0.5288$, $P < 0.0001$; Fig. 1B) with NDVI values in 2011 and negatively correlated ($r = -0.25$, $df = 72$, $P = 0.0356$) and unassociated ($X = -0.3838$, $P = 0.9996$) with NDVI values in 2012. In the fourth week of bloom, total number of adult stink bugs were positively associated ($X = 0.5918$, $P < 0.0001$; Fig. 1B) with NDVI values in 2011, and no stink bugs were found in the fourth week

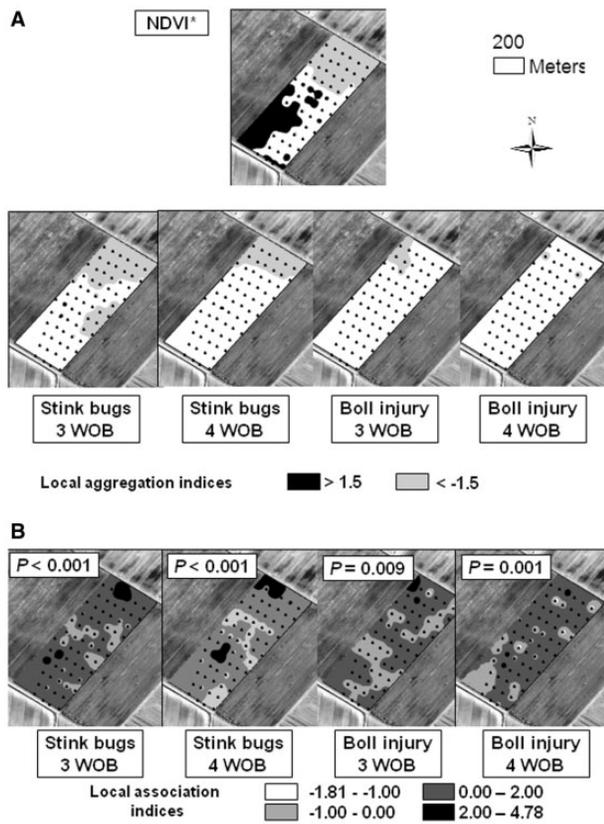


Fig. 1. 2011 inverse distance weighted spatial interpolations of (A) local SADIE aggregation indices of NDVI values, stink bug adult numbers, and boll injury from the third week and fourth week of bloom; (B) local SADIE association indices between NDVI values from the third week of bloom and stink bug adult numbers and boll injury from the third and fourth weeks of bloom; WOB, week of bloom.

of bloom in 2012. Finally, total number of adult stink bugs were uncorrelated ($r = 0.05$, $df = 36$, $P = 0.7548$) and unassociated ($X = 0.1064$, $P = 0.2735$) with NDVI values in 2012 during the fifth week of bloom.

Boll injury during the third week of bloom was positively correlated ($r = 0.29$, $df = 66$, $P = 0.0195$) and positively associated ($X = 0.3000$, $P = 0.0094$; Fig. 1B) with NDVI values in 2011 and were uncorrelated ($r = -0.02$, $df = 72$, $P = 0.8570$) and unassociated with NDVI values in 2012 ($X = -0.0655$, $P = 0.7075$). Boll injury during the fourth week of bloom was positively correlated ($r = 0.45$, $df = 66$, $P < 0.0001$) and positively associated ($X = 0.3853$, $P = 0.0010$) with NDVI values in 2011 but were negatively correlated ($r = -0.49$, $df = 36$, $P = 0.0023$) and negatively associated ($X = -0.4495$, $P = 0.9967$, Fig. 1B) in 2012. Finally, boll injury was uncorrelated ($r = -0.03$, $df = 36$, $P = 0.8824$) and unassociated ($X = -0.1693$, $P = 0.8341$) with NDVI values in 2012 during the fifth week of bloom.

Discussion

Both stink bug adult number and stink bug boll injury were associated with NDVI values. Associations with stink bug numbers and NDVI were strongest in 2011 in the third and fourth week of bloom, within a week that the remote sensing image was captured. Associations with NDVI and boll injury were strongest in 2011 with boll injury in the fourth week of bloom. Because the procedure for determining injured bolls is an indirect method—based on the plant response to stink bug feeding—a time-lag between the phenological stage of the plant and stink bug injury should be expected. However, it is unknown why stink bug numbers and stink bug injury were more prevalent in areas with high NDVI values in 2011 but less prevalent in the same areas during 2012. Many factors could have contributed to the differences between the 2 yr. One caveat is that,

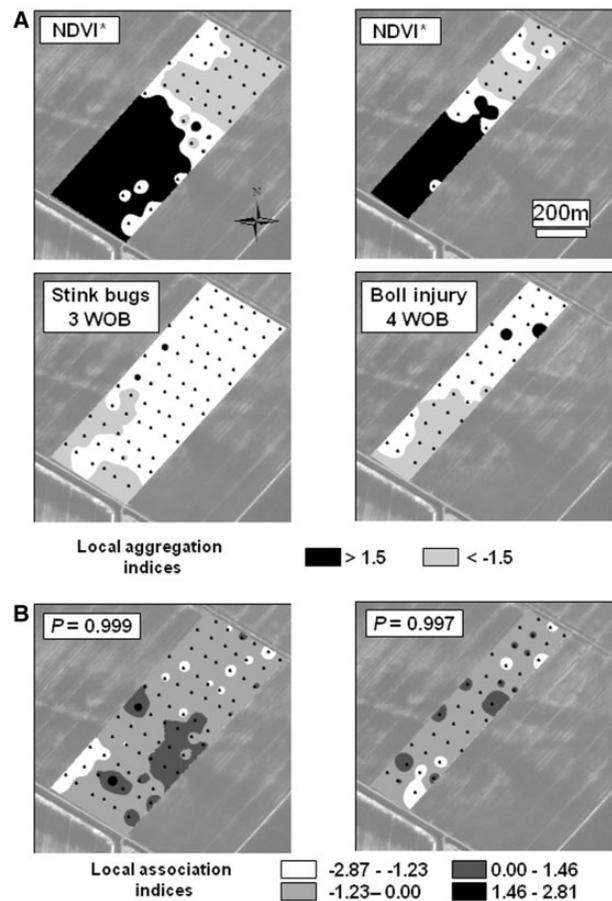


Fig. 2. 2012 inverse distance weighted spatial interpolations of (A) local SADIE aggregation indices of NDVI values and stink bug adult numbers from the third week of bloom and boll injury from the fourth week of bloom; (B) associations between NDVI values and stink bug adult numbers from the third week of bloom and boll injury from the fourth week of bloom. WOB, week of bloom.

relatively low numbers if stink bugs were captured in the study. Hence, the sample size might have been small to explain meaningful phenomena, even though the numbers we captured paralleled similar studies using the sweep net (Reay-Jones et al. 2009, Tillman and Cottrell 2015). Furthermore, an early-maturing variety was used in 2011, while an early to mid-maturing variety was used in 2012. Finally, responses in plant growth likely differed in both years due to the interaction of the variety, weather (e.g., from 15 May to 15 August 2011, 35 more centimeters of precipitation and 167 more heat units, calculated with 16°C as a base temperature (DD60), were recorded compared with the same dates in 2012), planting date and other unrecorded or unknown factors. NDVI values between both years tended to be consistently lower or higher relative to the same parts of the field. Therefore, although the plant growth patterns were likely different between the 2 yr, there was a range of growth within the field during each year. However, our hypothesis that stink bugs would be associated with highly vigorous cotton, as expressed by high-relative NDVI values, regardless of the conditions was only true in 1 yr.

One striking difference between the 2 yr was the prevalence of stink bugs in 2012 compared with 2011. Stink bugs may have been attracted to vigorously growing cotton in 2011, similar to *L. lineolaris* (Willers et al. 1999), but the effect of conspecific stink bug density was a stronger driver of spatial distribution than the effect of plant vigor in 2012. Stink bug injury was much higher in 2012 than 2011; therefore, one would expect the injury to be more spatially widespread given the fact that stink bugs are thought to feed on individual locules from multiple bolls rather than remaining on individual bolls and feeding on multiple

locules (Willrich et al. 2004, Huang and Toews 2012). In our dataset, there was no association between injured bolls during the third week of bloom and the NDVI values in 2012, but adult stink bug numbers were negatively associated with NDVI values in the third week of bloom. In contrast, there was a positive association in 2011 between injured bolls during the third week of bloom and NDVI values, in addition to a positive association with stink bug adult numbers and NDVI values. As a result, the third week of bloom in 2012 could have been a transition period, where stink bugs had injured most of the bolls of a preferred size in vigorously growing cotton and were moving to less vigorous growing areas to feed on previously unfed upon bolls of the appropriate size. Without multiple points of remote sensing data and more data from the crop, this is not possible to determine, especially given the indeterminate growth pattern of cotton. Future studies should focus on the association among other crop characteristics and stink bug injury for remote sensing to be useful.

The above hypothesis is contingent on the association of vigorously growing cotton and the presence of medium-sized bolls that *E. servus* prefers (Siebert et al. 2005, Huang and Toews 2012). Cotton growth patterns and boll size can vary from year to year (Siebert et al. 2005), among varieties, and within the field, as seen in this experiment. The ability of *E. servus* to distinguish among boll sizes combined with its mobility may render this insect one of the more difficult to predict using remote sensing.

In both years, abundances were likely affected by insecticides applied to reduce infestation levels. However, stink bug captures at the rate of 0.010 adults and 0.002 nymphs per sweep in 2011, as well as 0.013 adults and 0.001 nymphs in 2012 were in-line with a previous published paper from South Carolina (Reay-Jones et al. 2009) finding 0.006 adults and 0.003 nymphs per sweep in cotton. Similarly, although total stink bug numbers were not reported, based on extrapolation from interpolation maps, similar numbers of *C. hilaris* were also captured with a sweep net in cotton in a Georgia study (Tillman and Cottrell 2015). However, relatively low stink bug captures in this study were one reason that boll injury was also assessed and are another reason that boll injury, rather than direct sampling of stink bugs, is the basis of the threshold for stink bug treatment in cotton (Bacheler et al. 2009). Boll injury in this study also showed only a trend to being spatially aggregated once in 2012 but not in 2011. Our results mirror previous findings where stink bug densities and boll injury were shown to be aggregated using SADIE only 25% of the time in southeastern US cotton (Reay-Jones et al. 2010).

The SADIE analysis determined associations and aggregations, particularly in 2011 for boll injury, when stink bug injury was relatively low. During the third week of bloom, areas of the field with local association indices between 1.66 and 3.98 could be visually overlaid with areas of the field with higher relative boll injury levels to a high degree of accuracy. In 2012, when stink bug injury was higher, association indexes were not significant and could not be visually matched with aggregation indexes to the same degree. Correlation analyses only provided a “rough” estimate of association. In general, associations between areas of boll injury and NDVI values were difficult to visually match on an intrafield basis, although on some dates they were significantly associated on a whole field level. This study suggests the potential of remote sensing as a tool to assist with sampling stink bugs in cotton, although more research is needed using NDVI and other plant measurements to better predict the incidence of stink bug injury.

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