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Research

Sampling

Stability of Spatial Distributions of Stink Bugs, Boll Injury, and NDVI in Cotton

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Abstract

A 3-yr study was conducted to determine the degree of aggregation of stink bugs and boll injury in cotton, *Gossypium hirsutum* L., and their spatial association with a multispectral vegetation index (normalized difference vegetation index [NDVI]). Using the spatial analysis by distance indices analyses, stink bugs were less frequently aggregated (17% for adults and 4% for nymphs) than boll injury (36%). NDVI values were also significantly aggregated within fields in 19 of 48 analyses (40%), with the majority of significant indices occurring in July and August. Paired NDVI datasets from different sampling dates were frequently associated (86.5% for weekly intervals among datasets). Spatial distributions of both stink bugs and boll injury were less stable than for NDVI, with positive associations varying from 12.5 to 25% for adult stink bugs for weekly intervals, depending on species. Spatial distributions of boll injury from stink bug feeding were more stable than stink bugs, with 46% positive associations among paired datasets with weekly intervals. NDVI values were positively associated with boll injury from stink bug feeding in 11 out of 22 analyses, with no significant negative associations. This indicates that NDVI has potential as a component of site-specific management. Future work should continue to examine the value of remote sensing for insect management in cotton, with an aim to develop tools such as risk assessment maps that will help growers to reduce insecticide inputs.

Key words: spatial analysis by distance indices, spatial aggregation, *Chinavia hilaris*, *Nezara viridula*, *Euschistus servus*

Management decisions for control of insect pests of crops are typically applied to entire fields, yet insects often have highly aggregated distributions (e.g., Taylor et al. 1978, Wilson and Room 1983). Optimized insecticide spray programs that use spatially targeted applications have been described for several insect pests (Weisz et al. 1996, Blom et al. 2002). Formerly known as precision integrated pest management (Fleischer et al. 1999), site-specific management of insects is defined as management of insect pests based on localized densities rather than uniform management across an area using average densities (Park and Krell 2005). This approach is not currently as developed as precision soil fertility and weed management, in part because of the cost of obtaining sufficient data to spatially characterize insect populations (Krell et al. 2003). However, benefits include slowing the development of resistance and preserving natural enemies by maintaining unsprayed refuges in fields (Midgarden et al. 1997, Karimzadeh et al. 2010).

Site-specific insect management requires the mapping of insect distributions and aims to generate prescription maps with management zones (Park and Krell 2005). A limitation is the high cost of sampling required to generate maps (Krell et al. 2003). In addition to reducing the number of samples needed in a given field, identifying portions of fields associated with higher insect densities and, therefore, more prone to crop injury, could reduce costs (Ellsbury and Krell 1999). The relative cost of such sampling can, however, be offset by savings in control costs, particularly if spatial variability in insect pressure is high (Merrill et al. 2014). Models of spatiotemporal dynamics using remotely sensed data can further reduce the costs of site-specific insect management (Merrill et al. 2014). Knowledge needs to be developed on insect pest distributions, related crop injury, and yield loss and their spatial association with remotely sensed data.

As the model crop in our study, cotton, *Gossypium hirsutum* L., is subject to herbivory from a range of insect pests, and cotton producers do not currently use site-specific methods for managing insects in the southeastern United States. The widespread adoption of transgenic cotton containing transgenes from *Bacillus thuringiensis* Berliner (*Bt*) has reduced the pest status of the heliothine complex [tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie)]; the subsequent reduction in insecticide usage has been a key reason for stink bugs becoming major pests of southeastern cotton production (Greene et al. 2001). The predominant species of phytophagous stink bugs in the region are the green stink bug, *Chinavia hilaris* (Say), the southern green stink bug, *Nezara viridula* (L.), and the brown stink bug, *Euschistus servus* (Say) (Reay-Jones et al. 2009). Injury by stink bugs results in reduced yield through abortion of young bolls and diminishing lint turnout (Wene and Sheets 1964, Roach 1988). Furthermore, microorganisms gain entry into cotton bolls through feeding wounds caused by stink bugs, resulting in boll rot. Specifically, *N. viridula* is a confirmed vector of bacterial pathogens affecting boll development (Medrano et al. 2007).

The management of stink bug infestations in cotton currently relies on scouting of bolls injured by stink bug feeding and the use of pyrethroid and organophosphate insecticides applied to entire fields when thresholds are met (Reay-Jones et al. 2010a). Stink bugs are mobile insects, and dispersal across farmscapes is highly dependent on availability of food (Tillman et al. 2009). While stink bugs have aggregated distributions in cotton fields (Reay-Jones et al. 2010b), it is unknown if crop characteristics within fields have a major influence on spatial distributions of densities and boll injury. Remote sensing, which involves collecting information about a subject from a distance, can be used to predict such variability by using in particular in crop protection multispectral vegetation indices (Riley 1989, Hatfield and Pinter 1993). Maps of cotton field habitats can be created using remote sensing, and pest abundance can be estimated within each habitat without large numbers of samples (Willers et al. 2005). While a number of studies have been conducted in cotton associating remote sensing data with crop growth and yield, a very limited number of studies have quantified the spatial association between insect abundance and injury and remote sensing data. A recent study examined the aggregation of stink bugs, boll injury, and normalized difference vegetation index (NDVI) in cotton in North Carolina, with inconsistent spatial associations between boll injury and NDVI (Reisig et al. 2015); however, NDVI was measured on only one date in each year, and the stability of the spatial distributions was not examined. This 3-yr study aimed to 1) determine the aggregation of stink bug densities, boll injury, and NDVI in cotton, 2) determine the spatial association among sampling dates to quantify the stability of the spatial distributions, and 3) determine the spatial association between stink bugs, boll injury, and NDVI. The ultimate goal is to use remote sensing to predict the risk of injury from stink bugs in cotton.

Materials and Methods

Field Trials

Trials were conducted at the Clemson University Pee Dee Research and Education Center (REC) in Florence, SC, and at the Clemson University Edisto REC in Blackville, SC, in 2013, 2014, and 2015. In Florence, the same 5.5-ha field was used in 2013 and 2014 and a 6.5-ha field was used in 2015. In Blackville, a 4.8-ha field was used in 2013 and the same 8.7-ha field was used in 2014 and 2015. Fields were planted on 22 May 2013, 20 May 2014, and 20 May 2015 in Florence, and on 11 May 2013, 23 May 2014, and 1 June 2015 in Blackville. Second-generation *Bt* cotton cultivars (Bollgard II [Deltapine, Memphis, TN]) producing *Cry1Ac* and *Cry2Ab2* proteins were planted: DP 1212 at the Florence location in all years, DP 1137 at the Blackville location in all years. No soil-incorporated insecticide was used at planting other than insecticidal seed treatments of thiamethoxam (0.375 mg A.I. per seed). No foliar insecticides were applied.

Sampling

In each field, a sampling grid consisted of one sampling location (marked with a 1.8-m fiber glass flag) for every 0.12 ha beginning at the field periphery, with each flag separated by 40 m. Fields were spatially mapped, using GIS mapping software (ArcView 10.0, Environmental Systems Research Institute [ESRI] 2010), and then overlaid with a grid of sampling points starting from the margins. The GPS coordinates of all sampling points were recorded using a Trimble GeoXM (Trimble, Sunnyvale, CA). Frequency of sampling varied among years and locations for stink bug sampling with sweep nets (11, 10, and nine sampling dates at the Florence location and zero, two, and three sampling dates at the Blackville location in 2013, 2014, and 2015, respectively) and boll injury (four, five, and five sampling dates at the Florence location and three, two, and three sampling dates at the Blackville location in 2013, 2014, and 2015, respectively).

Stink bugs were sampled from the first week of bloom by taking two 25-sweep samples with a 38.1 cm (dia) net, with each sampling consisting of sweeps covering two rows of cotton. For collection of boll injury data, 10 half-grown ($\sim$2.5 cm in diameter and <600 heat units beyond anthesis [$\sim$24 d], Willrich et al. [2004]) soft bolls were collected at each flag. Internal damage to an individual boll (classified on a binomial scale) consisted of callus growths (warts) or stained lint (Greene et al. 1999, Bundy et al. 2000). The response variable was the sum of bolls in the 10-boll sample with at least one symptom of boll injury.

Crop reflectance was recorded using the NDVI (Rouse et al. 1973) measured at each flag using either tractor-mounted (Florence in 2014) or handheld (all other fields and years) GreenSeeker (NTech Industries, Ukiah, CA) or Crop Circle (Holland Scientific, Lincoln, NE) sensors. Tractor-mounted measurements were recorded with GPS coordinates over two rows immediately adjacent to sampling flags. Handheld sensors recorded NDVI on $\sim$8 m of one row centered at each flag. This commonly used remote sensing index uses normalized red (R) and near-infrared (NIR) spectral bands: $NDVI = (NIR - R)/(NIR + R)$. Values range from -1 to $+1$. NDVI was measured on 12 dates in Florence in 2013 (10 June–31 July), one date in Blackville in 2013 (30 July), 16 dates in Florence in 2014 (30 May–16 September), eight dates in Blackville in 2014 (6 June–28 August), eight dates in Florence in 2015 (18 June–20 August), and three dates in Blackville in 2015 (11 August–26 August).

Data Analysis

Spatial analysis by distance indices (SADIE Version 1.22, Perry et al. 1999) analyses were conducted for stink bug counts, boll injury, and NDVI. Stink bugs summed across the 50 sweeps per flag and boll injury expressed as the number of injured bolls per sample at each flag were analyzed. For NDVI data, values were first averaged over the 8-m row section for the handheld measurements or within a 2.5-m radius circle of each flag using ArcView for data from the tractor-mounted sensors. NDVI values were then expressed as the integer of 100 times the average at each flag.

The SADIE red-blue methodology was used for grid sampling locations expressed as absolute positions. This analysis determines the minimum distance D needed to achieve regularity, which is the distance moved by counts in the observed sample to reach the most uniform distribution possible. A clustering index was assigned to every location, with either a positive cluster index ($\bar{v}_i$) for counts above the mean of each field-date combination or a negative gap index ($\bar{v}_j$) for counts below the mean. A random spatial pattern has indices $\bar{v}_i = -\bar{v}_j = 1$. Nonrandomness is quantified by comparing observed patterns with rearrangements in which the sample counts are randomly redistributed across the sampling locations. The overall index of dispersion (I_a) indicates either an aggregated (>1), random (= 1), or uniform pattern (<1). The one-tailed null hypothesis of spatial randomness is rejected for a probability $P < 0.05$ indicating aggregation. A total of ~6,000 randomizations were used for each test.

The SADIE association tool (Perry and Dixon 2002) was used to determine spatial associations between stink bug counts, boll injury, and NDVI. Associations between NDVI and stink bugs or boll injury were generally restricted to datasets resulting from samples taken within a week or less of each other. An exception was for NDVI in Florence in 2013 where the last measurement of NDVI was taken on 31 July; this dataset was used with the spatial association tool with boll injury recorded on 20 and 26 August. 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. Interpolation maps of local aggregation indices were generated using the inverse distance weighted (IDW) spatial method with the geographical information systems software ArcView 10.0 (ESRI 2010). Rather than using more traditional geostatistical methods such as semiovariograms and kriging, SADIE was chosen because of its ability to describe and illustrate local variability of spatial distribution

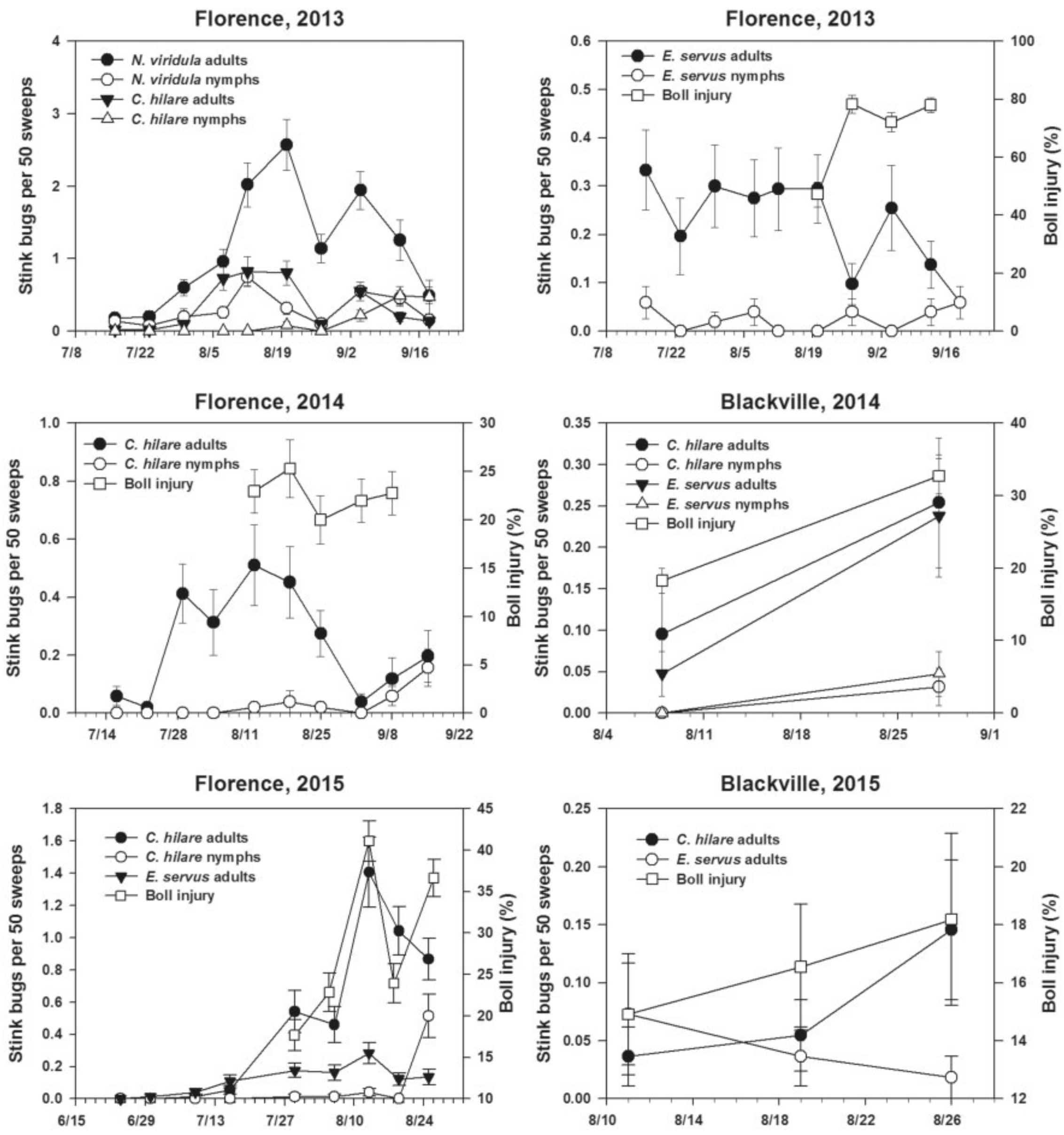

Fig. 1. Densities of stink bugs and boll injury (± SEM) in cotton trials in Florence and Blackville, South Carolina (2013–2015).

Table 1. Characterization of spatial distribution of stink bugs and associated boll injury counts in cotton fields in Florence, SC, with SADIE index of dispersion (I_a)

Year	Variable	Date									
		16 July	23 July	30 July	7 Aug.	12 Aug.	20 Aug.	27 Aug.	4 Sept.	12 Sept.	8 Sept.
2013	*N. viridula* (A)	1.296	0.766	1.094	1.058	1.034	1.681*	1.414	1.066	1.368	1.044
	N. viridula (N)	0.791	0.754	0.883	1.182	1.795**	1.035	1.037	1.100	0.808	0.921
	C. hilaris (A)	–	–	1.403	1.826**	2.038**	1.897**	0.834	1.468	0.876	1.080
	C. hilaris (N)	–	–	–	–	–	0.799	–	1.173	1.320	1.320
	E. servus (A)	1.507	0.904	1.198	1.333	1.055	0.965	0.751	1.282	1.382	1.073
	E. servus (N)	1.364	–	–	0.823	–	–	0.866	–	0.702	1.125
	Boll injury						1.479	1.178	0.991	1.544	
2014		16 July	22 July	29 July	4 Aug.	12 Aug.	19 Aug.	25 Aug.	2 Sept.	8 Sept.	15 Sept.
	C. hilaris (A)	1.080	–	1.286	2.038***	1.129	1.450	1.650*	1.877**	1.084	0.830
	C. hilaris (N)	–	–	–	–	–	–	–	–	0.908	–
	E. servus (A)	0.861	1.029	1.325	–	1.333	0.701	–	0.865	–	–
	E. servus (N)	–	–	–	–	–	–	–	–	–	–
	Boll injury					2.044**	1.917**	1.985**	1.254	1.15	
2015		9 July	16 July	29 July	6 Aug.	13 Aug.	19 Aug.	25 Aug.			
	C. hilaris (A)	–	0.915	0.979	0.991	1.364*	1.101	1.212			
	C. hilaris (N)	–	–	–	–	1.117	–	1.103			
	E. servus (A)	0.782	0.856	1.365*	0.836	1.207	1.061	1.843***			
	Boll injury			1.461*	1.641**	1.039	1.220	0.990			

Overall index of dispersion indicates aggregated pattern for $I_a > 1$: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.
Missing data represented by "–" indicate that insect counts were insufficient to generate aggregation indices.
A, adults; N, nymphs.

Table 2. Characterization of spatial distribution of stink bugs and boll injury in cotton fields in Blackville, SC, with SADIE index of dispersion (I_a)

Year	Variable	Date		
		24 July	30 July	7 Aug.
2013	Boll injury	1.807*	0.917	0.744
2014		8 Aug.	28 Aug.	–
	C. hilaris (A)	1.035	1.557*	–
	C. hilaris (N)	–	0.889	–
	E. servus (A)	0.966	0.766	–
	E. servus (N)	–	0.820	–
	Boll injury	1.082	1.173	–
2015		11 Aug.	19 Aug.	26 Aug.
	C. hilaris (A)	0.828	0.484	0.993
	E. servus (A)	1.253	0.809	–
	Boll injury	2.089**	1.276	1.885**

Overall index of dispersion indicates aggregated pattern for $I_a > 1$: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.
Missing data represented by "–" indicate that insect counts were insufficient to generate aggregation indices.
A, adults; N, nymphs.

Table 3. Indices of spatial association (X) from SADIE analyses between adult stink bugs and boll injury datasets in cotton in South Carolina

Year	Location	Date	Association 1		Association 2	
			Species	X	Species	X
2013	Florence	20 Aug.	*C. hilaris*	0.431**	*N. viridula*	0.280
		26 Aug.	*C. hilaris*	0.114	*N. viridula*	0.173
		4 Sept.	*C. hilaris*	0.165	*N. viridula*	0.115
		10 Sept.	*C. hilaris*	0.223	*N. viridula*	−0.101
2014	Florence	12 Aug.	*C. hilaris*	−0.039	*E. servus*	−0.430**
		19 Aug.	*C. hilaris*	0.256	*E. servus*	0.189
		25 Aug.	*C. hilaris*	0.434**	–	–
		2 Sept.	*C. hilaris*	0.226	*E. servus*	0.214
		8 Sept.	*C. hilaris*	0.132	–	–
	Blackville	14 Aug.	*C. hilaris*	−0.356*	*E. servus*	0.091
		24 Aug.	*C. hilaris*	0.026	*E. servus*	0.041
2015	Florence	29 July	*C. hilaris*	0.026	*E. servus*	0.0036
		6 Aug.	*C. hilaris*	0.141	*E. servus*	0.223
		13 Aug.	*C. hilaris*	0.236	*E. servus*	0.093
		20 Aug.	*C. hilaris*	0.196	*E. servus*	−0.137
		26 Aug.	*C. hilaris*	0.175	*E. servus*	0.169
	Blackville	11 Aug.	*C. hilaris*	0.083	*E. servus*	0.239
		19 Aug.	*C. hilaris*	−0.271	*E. servus*	−0.258
		26 Aug.	*C. hilaris*	0.275	–	–

Overall index of association between each of the paired data sets, with a positive association for $X > 0$ ($P < 0.025$) and a negative association for $X < 0$ ($P > 0.975$). ***$P < 0.001$; **$P < 0.01$; *$P < 0.025$.

and association among datasets sharing the same locations (Perry et al. 2002).

Results

Across the three years of study, the main adult phytophagous stink bugs were *C. hilaris* (total of 677; 0.33 ± 0.02 [SEM] per 50 sweeps), *N. viridula* (total of 630; 0.31 ± 0.02), and *E. servus* (total of 246; 0.12 ± 0.01). The main nymphal phytophagous stink bugs were *N. viridula* (total of 166; 0.08 ± 0.01), *C. hilaris* (total of 134; 0.07 ± 0.01), and *E. servus* (total of 22; 0.01 ± 0.002). A total of 12,620 cotton bolls were collected, with an average of 32.5 ± 0.75% injured bolls. Densities of insects and boll injury fluctuated between sampling dates and fields (Fig. 1). *Nezara viridula* was the most abundant species in 2013 in Florence, with a distinct population peak of adults followed by a decrease as the crop matured (Fig. 1). *Chinavia hilaris* adults had a similar trend in the

Table 4. Indices of spatial association (X) from SADIE analyses among datasets of boll injury caused by stink bugs in cotton in South Carolina

Year	Location	Date 1	Date 2	X	P
2013	Florence	20 Aug.	26 Aug.	0.562	0.0001
			4 Sept.	0.027	0.4370
			10 Sept.	0.128	0.258
		26 Aug.	4 Sept.	0.083	0.297
			10 Sept.	0.108	0.236
		4 Sept.	10 Sept.	0.187	0.103
2014	Florence	12 Aug.	19 Aug.	0.304	0.0193
			25 Aug.	0.381	0.0045
			2 Sept.	−0.025	0.560
			8 Sept.	−0.010	0.533
		19 Aug.	25 Aug.	0.602	0.0002
			2 Sept.	0.195	0.092
			8 Sept.	−0.009	0.526
		25 Aug.	2 Sept.	0.447	0.0008
			8 Sept.	0.144	0.192
		2 Sept.	8 Sept.	0.342	0.0173
	Blackville	14 Aug.	24 Aug.	0.255	0.0377
2015	Florence	29 July	6 Aug.	0.0678	0.297
			13 Aug.	0.309	0.0043
			20 Aug.	0.137	0.124
			26 Aug.	0.0517	0.333
		6 Aug.	13 Aug.	0.106	0.223
			20 Aug.	0.294	0.022
			26 Aug.	0.114	0.218
		13 Aug.	20 Aug.	0.315	0.0128
			26 Aug.	0.280	0.021
		20 Aug.	26 Aug.	0.201	0.087
	Blackville	11 Aug.	19 Aug.	−0.181	0.899
			26 Aug.	0.564	0.0001
		19 Aug.	26 Aug.	0.015	0.472

Overall index of association between each of the paired data sets, with a positive association for $X > 0$ ($P < 0.025$) and a negative association for $X < 0$ ($P > 0.975$).

same field in both 2013 and 2014. Trends for *E. servus* adults were less apparent in Florence in 2014 and 2015, with only a limited number of nymphs. Levels of boll injury in Blackville in 2013 were 24.6 ± 2.6% on 24 July, 29.8 ± 2.7% on 30 July, and 52.4 ± 3.3% on 7 August (data not shown in figure). Average boll injury was greatest in Florence in 2013 at 67.5%, with 35.6% in Blackville in 2013, 22.6% in Florence in 2014, 25.5% in Blackville in 2014, 28.3% in Florence in 2015, and 16.5% in Blackville in 2015.

According to SADIE aggregation indices (Table 1), stink bug counts were significantly ($P < 0.025$) aggregated in Florence in 2013 in four out of 28 analyses for adults (14%), one out of 19 analyses for nymphs (5%), and zero out of four analyses for boll injury. In Blackville during 2013, boll injury was significantly aggregated in one out of three analyses (33%; Table 2). In Florence during 2014, stink bug counts were significantly aggregated in three out of 15 analyses for adults (20%), zero out of one analyses for nymphs, and three out of five analyses for boll injury (60%; Table 1). In Blackville in 2014, stink bug counts were significantly aggregated in one out of four analyses for adults (25%), zero out of two analyses for nymphs, and zero out of two analyses for boll injury (Table 2). In Florence during 2015, stink bug counts were significantly aggregated in three out of 13 analyses for adults (23%), zero out of two analyses for nymphs, and two out of five analyses for boll injury (40%; Table 1). In Blackville in 2015, stink bug counts were significantly aggregated in zero out of four analyses for adults (25%), and two out of three analyses for boll injury (Table 2). SADIE association indices between stink bug densities and boll injury collected on the same sampling date showed only two positive associations and two negative associations out of 35 paired datasets (Table 3). Both positive associations were for *C. hilaris* in Florence (once in 2013 and once in 2014). The negative associations were for *E. servus* in Florence in 2014 and *C. hilaris* in Blackville in 2014.

Spatial stability in time as quantified by SADIE association indices between boll injury datasets at different sampling dates showed overall that 11 out 30 pairs of datasets (36.6%) were positively associated across both locations and all years (Table 4). The range and variability of the spatial associations among datasets is illustrated by box charts in Fig. 2. None of the paired datasets had significant negative associations, and only four of the 34 indices had negative values. When considering only weekly intervals between datasets, six out of 13 paired datasets (46.2%) were positively associated across all years and locations. Stink bug datasets were less often spatially associated among sampling dates than boll injury (Fig. 2); adults of *C. hilaris* were positively associated among sampling dates on 19 of 83 paired datasets (22.9%), with no negative associations; for weekly intervals only, associations for *C. hilaris* were significantly positive in only five out of 20 paired datasets (25%). Adults of *E. servus* were positively associated among sampling dates on four of 38 paired datasets (10.5%) and negatively associated among sampling dates on three of 38 paired datasets (7.9%); for weekly intervals only, associations for *E. servus* were significantly positive in only one out of eight paired datasets (12.5%) and significantly negative in only one out of eight paired datasets (12.5%). Adults of *N. viridula* were positively associated among sampling dates on eight of 45 paired datasets (17.8%) and negatively associated among sampling dates on three of 45 paired datasets (6.7%); for weekly intervals only, no associations were significant. Nymphs of *N. viridula* were positively associated among sampling dates on three of 45 paired datasets (6.7%) and negatively associated among sampling dates on three of 45 paired datasets (6.7%); for weekly intervals only, associations for *N. viridula* nymphs were significantly positive in only one paired dataset (11.1%) and significantly negative in only one (11.1%) out of nine paired datasets.

NDVI increased in each field up to a plateau that occurred approximately on 14 July 2013, 24 July 2014, and 16 July 2015 in Florence, and on 16 July 2014 and on 21 July 2015 in Blackville (Fig. 3). NDVI was recorded only once in Blackville in 2013 on 31 July (0.825 ± 0.013). SADIE indicated significant aggregation for NDVI values in four out of 12 sampling dates in 2013 (33.3%), 11 out of 16 sampling dates in 2014 (68.7%), and zero out of eight sampling dates in 2015 in Florence (Fig. 3). NDVI values were significantly aggregated in zero out of one sampling date in 2013 ($I_a = 1.0005$; $P = 0.3895$), two out of eight sampling dates in 2014 (25%), and two out of three sampling dates in 2015 (66.7%) in Blackville.

Spatial stability in time as quantified by SADIE association indices between NDVI datasets at different sampling dates showed, out of 277 pairs of datasets, significant positive associations in 192 pairs of datasets (69.3%), significant negative associations in 59 pairs of datasets (21.3%), and no association in 26 pairs of datasets (9.4%; Fig. 2). When considering only weekly intervals among pairs of datasets, significant positive associations were noted for 32 out of 37 paired datasets (86.5%) with no significant negative associations, indicating a greater stability than either boll injury or stink bugs.

SADIE spatial association between NDVI and boll injury showed significant positive associations in seven paired datasets (32%) at $P < 0.025$ and four additional paired datasets (18%) at

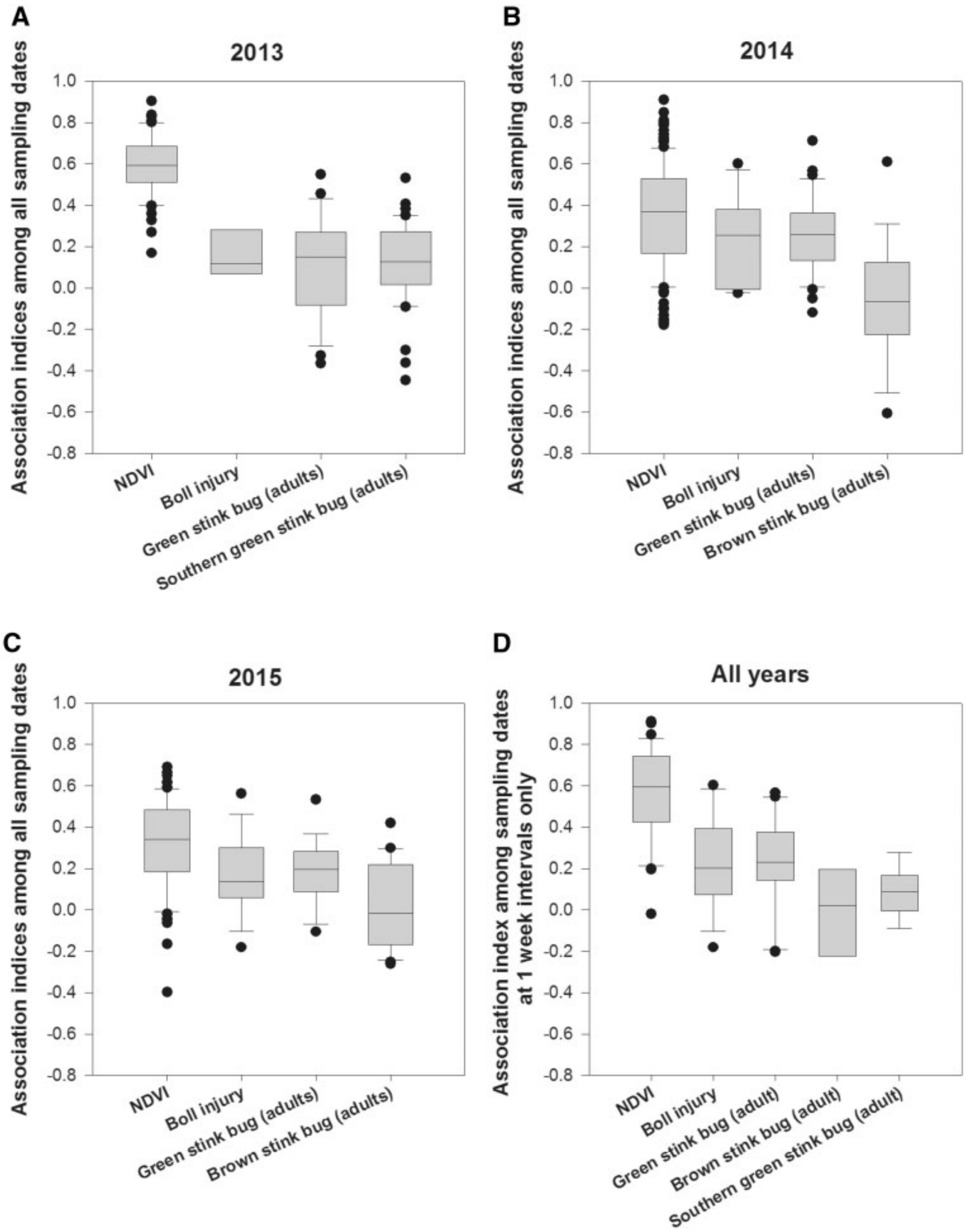

Fig. 2. Box plots of SADIE association indices among sampling dates for NDVI, boll injury, and stink bug densities in Florence and Blackville, South Carolina (2013–2015). All combinations of sampling date associations were used for (**A–C**), and only datasets at weekly intervals were used for (**D**).

$0.025 < P < 0.05$, for a total of 11 paired datasets (50%) at $P < 0.05$ out of a total of 22 paired datasets (Fig. 4). Only two SADIE association indices had negative values, and both were not significant ($0.025 < P < 0.975$). Selected interpolation maps are shown with significant clusters and gaps for different insect variables which frequently shared similar locations in fields in Florence (Fig. 5) and in Blackville (Fig. 6). In Florence where cotton was planted in the same field in 2013 and 2014, clusters of elevated levels of boll injury were present in the north eastern side of the field (Fig. 5A, C, G, I, K, M, and O). Five of the nine paired NDVI and boll injury datasets showed significant associations, indicating that gaps and clusters of high values of boll injury and NDVI occurred in the same areas of the field. This is apparent in Fig. 5B, D, H, L, and N where significant positive association indices were generally located in the south western corner of the field (where cotton plants were smaller and NDVI values were smaller) and in the north eastern corner of the field (where cotton grew more vigorously and NDVI values were higher). In Florence in 2015, associations among boll injury and NDVI spatial patterns were weaker, with stronger associations along some of the field edges (Fig. 5T and X). In Blackville, local association indices within fields were more frequently positive than negative, indicating positive associations between clusters of boll injury and NDVI values (Fig. 6J and N).

Discussion

Since its creation (Perry 1995), SADIE analyses have been used to quantify the spatial structure of count data in a number of ecological studies (e.g., Thomas et al. 2001, Cocu et al. 2005, Blackshaw and Vernon 2006, Reay-Jones et al. 2010b). Here, SADIE was used for the analysis of insect densities as well as boll injury and NDVI

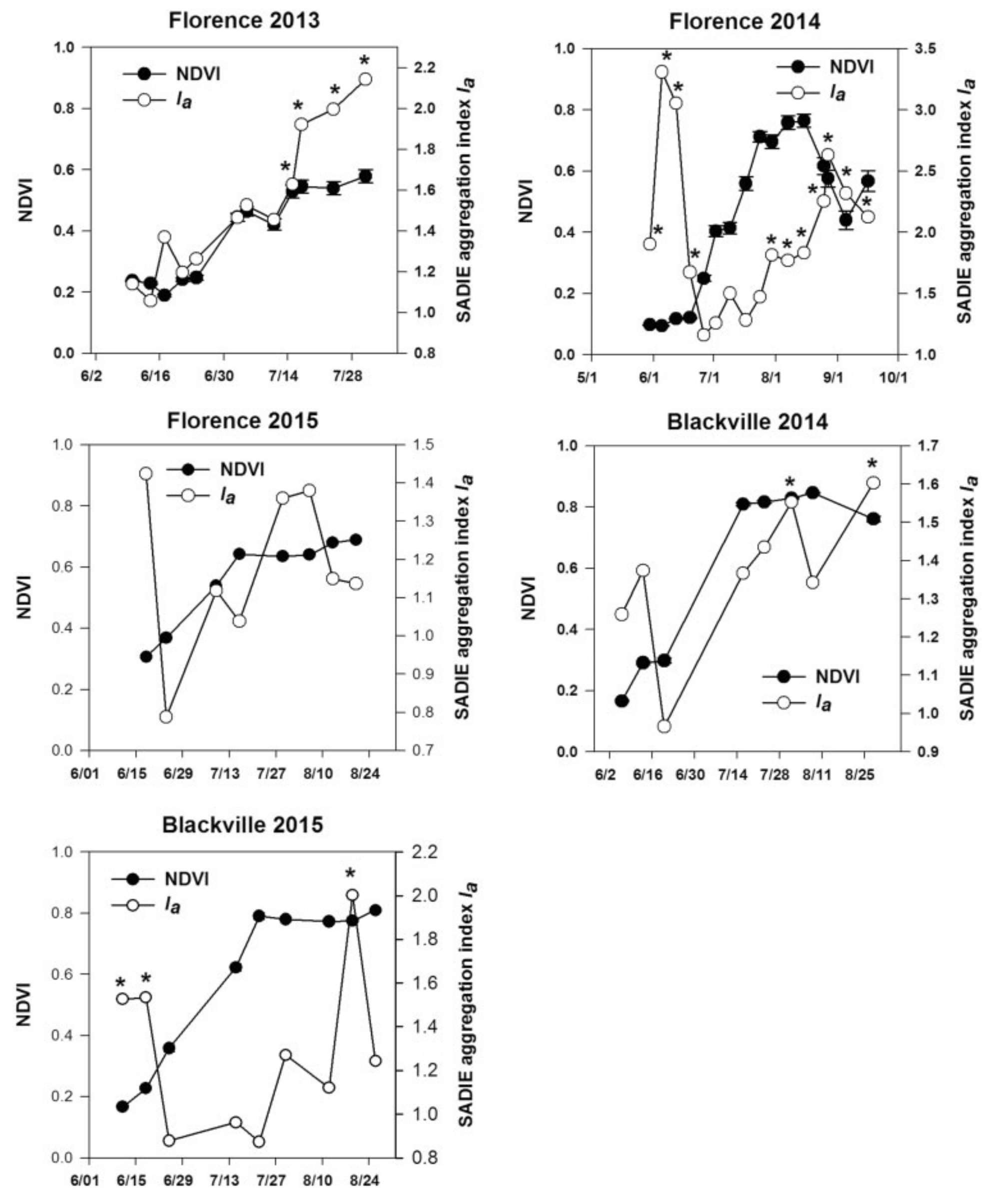

Fig. 3. Average NDVI and SADIE aggregation indices in cotton fields in Florence (2013–2015) and Blackville (2014–2015), South Carolina. Significant ($P < 0.05$) aggregation indices are indicated by asterisks.

expressed as integers. SADIE has previously been used to analyze spatial patterns of environmental variables including temperature (Cocu et al. 2005), boll injury from stink bug feeding (Reay-Jones et al. 2010b, Reisig et al. 2015), and NDVI in cotton (Reisig et al. 2015). Perry et al. (1999) indicated that SADIE can be used for binary data and continuous data that have been categorized prior to analysis. Results from this study showed variation in levels of both insect densities and crop injury within fields at different sampling locations. Most species of arthropods are spatially aggregated (Taylor et al. 1978, Wilson and Room 1983). The degree of spatial aggregation can vary considerably between species and often between life stages. Stink bugs were less frequently aggregated (17% for adults and 4% for nymphs) than boll injury (36%). However, a previous study in cotton showed that only 3.7% of SADIE indices of aggregation for average densities of stink bugs were significant at the $\alpha = 0.05$ level, in part due to low densities of some of the species and life stages (Reay-Jones et al. 2010b).

Studies have shown that SADIE is not a sensitive method when using small sample sizes (Thomas et al. 2001), and significance of SADIE analyses can be affected by the number of sampling locations. The distance to regularity D for the I_a index is impacted by the relative and absolute position of the clusters (Perry and Klukowski 1997). The I_a index is known to be influenced more by the number and position of the clusters rather than cluster size (Xu and Madden 2004). The number of possible clusters within a field increases with the number of sampling locations. SADIE aggregation indices were significant in 35% of analyses for stink bugs in wheat, *Triticum aestivum* L., in a study that used an average of 87 sampling locations per field (Reay-Jones 2014). An average of 57 sampling locations was used in our study, which was substantially more than a previous study in cotton (21; Reay-Jones et al. 2010b), which may explain the increase in the significance of spatial structures detected by SADIE for stink bugs compared with previous studies.

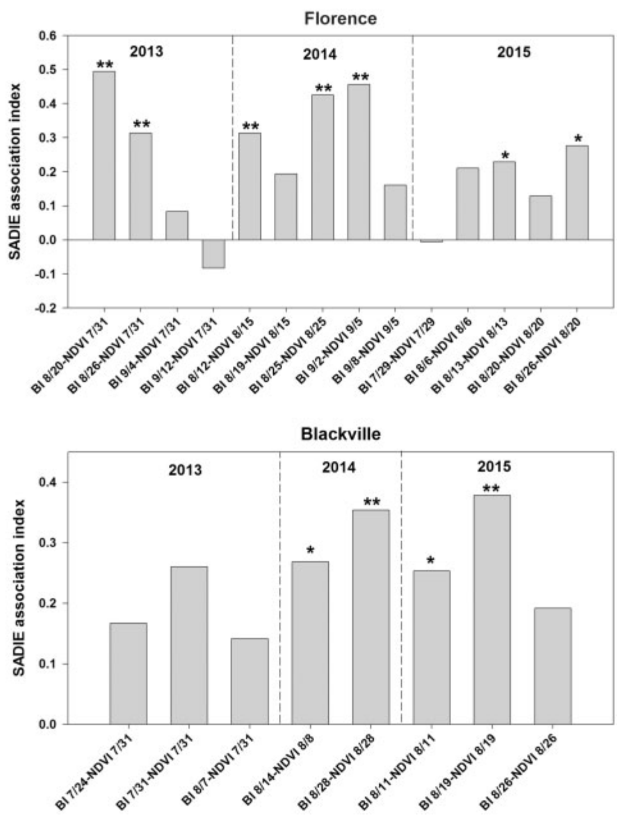

Fig. 4. Indices of spatial association (X) from SADIE analyses among boll injury (BI) caused by stink bugs and NDVI datasets in cotton in South Carolina. Significant association indices are indicated by asterisks (*$P < 0.05$; **$P < 0.025$).

While NDVI can increase with weed intensity in cotton fields (Sui et al. 2008) and can therefore be confounded with cotton growth, weeds were generally sparse across the cotton fields used in our study. Average NDVI values generally increased with time (Fig. 3) as plant ground cover increased. Other studies have shown a similar increase in NDVI values until ground cover is complete, followed by a decrease as defoliation and opening of cotton bolls occurs (Li et al. 2001). This decrease was observed only in Florence in 2014 when NDVI measurements continued until September. NDVI values expressed as integers were also significantly aggregated within fields in 19 of 48 analyses (40%), with the majority of significant indices occurring in July and August. Paired NDVI datasets from different sampling dates were frequently associated (86.5% for weekly intervals among datasets). This clearly indicated that locations of patches and gaps of NDVI were fairly stable within fields from week to week. The southeastern coastal plains in the United States are characterized by highly variable soils (Duffera et al. 2007), and NDVI values in cotton have been shown to be considerably lower for cotton grown on sandy soils compared with loamy soils (Plant et al. 2000). The consistency in spatial distributions of NDVI values in our study likely reflects seasonal variability in plant growth caused by variability in soil texture.

Spatial distributions of both stink bugs and boll injury were less stable than for NDVI, with positive associations among sampling dates varying from 12.5 to 25% for adult stink bugs for weekly intervals, depending on species. Locations of their patches and gaps within fields, therefore, varied more frequently from week to week than for NDVI. A study in wheat showed significant spatial associations among sampling dates ranging from 11–43% for *E. servus* and 7–50% for *Oebalus pugnax* (F.) across four fields (Reay-Jones 2014). Spatial stability of stink bug distributions was therefore sometimes greater in wheat, possibly due to the increased size of plant canopy in cotton that may hinder the use of sweep nets compared to wheat where stink bugs generally remain on the head of the plant. Spatial distributions of boll injury from stink bug feeding were more stable than stink bugs, with 46% positive associations among paired datasets with weekly intervals. A possible explanation is that highly mobile insects such as stink bugs can be difficult to sample with sweep nets. The permanent nature of boll injury relative to the temporal presence of stink bugs on a cotton plant, combined with the limited portion of a cotton plant being sampled with a sweep net, emphasizes the added value of using injury as a measure of stink bug presence for making management decisions (Reay-Jones et al. 2010a).

The sweep net sampling method used in our study has been shown to be more cost-reliable than using the beat cloth sampling method in cotton for stink bugs at low densities (Reay-Jones et al. 2009), which was generally the case in our study, except for *N. viridula* adults in Florence in 2013 (Fig. 1). Stink bug nymphs are known to be generally more aggregated than adults in cotton (Reay-Jones et al. 2009), because eggs are laid in masses and nymphs are unable to fly (Todd 1989). A greater sample size is therefore required to estimate densities of stink bug nymphs relative to adults with a given level of reliability (Reay-Jones et al. 2009). This may explain why only limited numbers of nymphs were sampled with sweep nets in our study, in addition to cotton being a poor reproductive host for stink bugs when compared to a crop such as soybean (Herbert and Toews 2012). The proportion of each stink bug species varied in each field, though there was generally one dominant species (*E. servus*, *N. viridula*, or *C. hilaris*) in each field. Willrich et al. (2004) state that *E. servus* adults can cause significant injury to cotton bolls similar to other stink bug species and life stages. As such, stink bugs are generally considered as a complex in cotton and in other crops such as soybean (McPherson et al. 1979).

NDVI values were positively associated with boll injury from stink bug feeding in 11 out of 22 analyses ($P < 0.05$), with no significant negative associations. Gaps and patches of high and low values of boll injury and NDVI therefore often occurred in similar areas of cotton fields. Because the proportion of injured bolls increased with NDVI values, we can assume that either stink bugs were attracted to vigorously growing cotton with high NDVI values, or that stink bugs were more prone to cause injury to cotton bolls in areas of the field with more vigorously growing cotton. However, associations between NDVI and stink bug densities were not as consistent as those with boll injury. A likely explanation could again be the permanent nature of boll injury relative to the temporal presence of stink bugs on a cotton plant. A previous study in cotton in North Carolina showed an inconsistent relationship between boll injury in cotton and NDVI, with a positive spatial association in one year and a negative association in another (Reisig et al. 2015). Differences in weather patterns and varieties between years were suggested as reasons explaining the differing results in each year. Another reason could be the lower densities of stink bugs and lower levels of boll injury observed in Reisig et al. (2015) compared to our study. The lack of any significant negative association between NDVI and boll injury across multiple sampling dates over three years of data indicates that NDVI has potential as a component of site-specific management due to the widespread use of boll injury as a sampling tool for stink bug control. Because populations of susceptible bolls increase as the flowering period progresses and then decrease as the

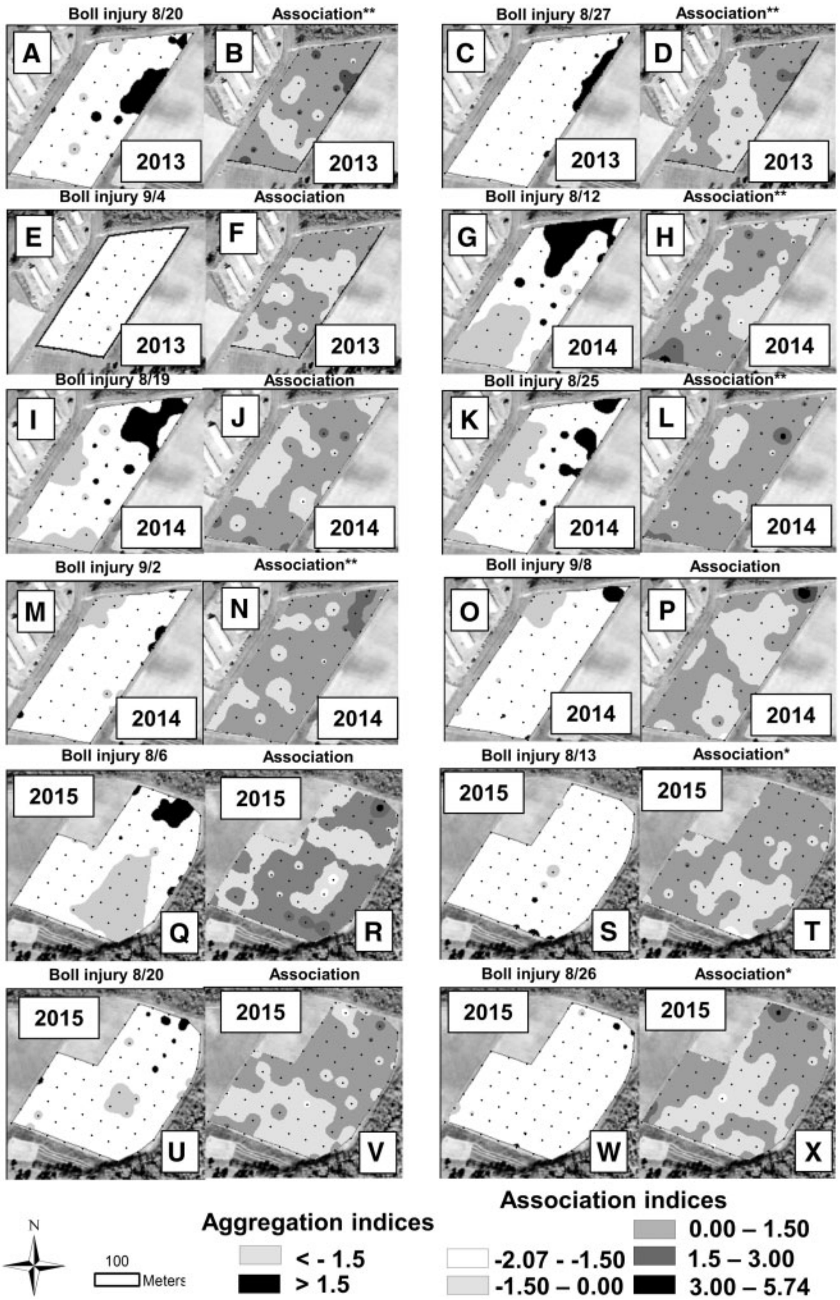

Fig. 5. (A–**X**) Spatial interpolation of SADIE local aggregation indices for boll injury caused by stink bugs and local association indices for paired boll injury and NDVI datasets in Florence, South Carolina (2013–2015). Clusters are indicated by excluding aggregation indices between −1.5 and 1.5, with patches indicated in black ($\bar{v}_i > 1.5$) and gaps indicated in grey ($\bar{v}_j < -1.5$). Significant association indices are indicated by asterisks (*$P < 0.05$; **$P < 0.025$).

crop matures, a dynamic threshold is recommended for stink bug control in the southeastern United States based on week of bloom (50, 30, 10, 10, 10, 30, 30, and 50% injured bolls for each week respectively; Bacheler et al. 2007, Greene et al. 2009).

Larger cotton plants can have more bolls than small cotton plants. Stink bugs may be attracted to such plants which represent a greater concentration of resources. Plants growing more vigorously have been shown to sometimes be more attractive to herbivores (Solomon 1981), and variability in plant growth has been shown to be spatially associated with insect pests in crop systems (Ferguson et al. 2003). In cotton, populations of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), can be more abundant in areas of fields with higher NDVI values (Willers et al. 1999); attraction to vigorously growing plants as well as greater protection from insecticides by the larger canopy were suggested as reasons explaining this association. Remote sensing using NDVI measurements can be used to optimize sampling plans for *L. lineolaris* (Willers et al. 1999, 2005). Because foliar insecticide applications were not made in any of the fields in our study, focusing on areas of fields that are vigorously growing as measured by remote sensing may help to improve sampling and management of stink bugs in cotton.

All fields in our study were planted with transgenic cotton expressing Bt toxins. The reduction in insecticide usage in Bt cotton has been a key reason secondary pests such as stink bugs in the southeastern United States (Greene et al. 2001) and mirid bugs in China (Lu et al. 2010) have now become major pests in cotton.

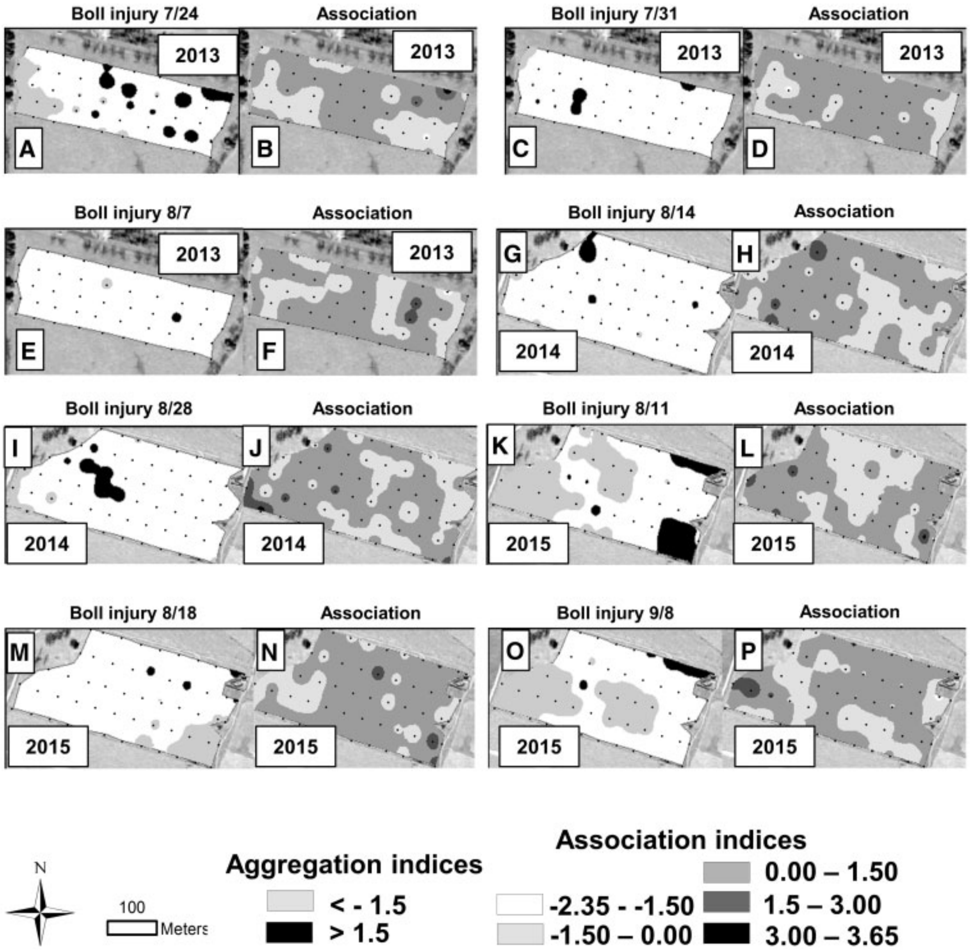

Fig. 6. (A–**P**) Spatial interpolation of SADIE local aggregation indices for boll injury caused by stink bugs and local association indices for paired boll injury and NDVI datasets in Blackville, South Carolina (2013–2015). Clusters are indicated by excluding aggregation indices between −1.5 and 1.5, with patches indicated in black ($\bar{v}_i > 1.5$) and gaps indicated in grey ($\bar{v}_j < -1.5$). Significant association indices are indicated by asterisks (*$P < 0.05$; **$P < 0.025$).

However, studies have shown that, in the absence of insecticide applications on both Bt and non-Bt cotton, abundance of non-lepidopteran herbivores can increase on Bt cotton due to reduced leaf feeding by heliothine pests (Wilson et al. 1992). In addition to insecticide release, outbreaks of secondary pests in Bt cotton may also be caused by the release from direct resource competition from heliothine pests susceptible to Bt toxins (Whitehouse et al. 2007, Zeilinger et al. 2011), or from indirect competition by herbivore-induced secondary metabolites on Bt cotton (Hagenbucher et al. 2013). Although no studies have suggested that differences in the pest status of stink bugs between Bt and non-Bt cotton fields may impact their spatial distributions, >90% of the cotton planted in the southeastern United States is Bt cotton (USDA ERS 2016); our study on Bt cotton is therefore representative of the majority of cotton fields in the region.

This study showed significant variability in stink bug densities, boll injury, and NDVI within cotton fields. Site-specific pest management is considered to be more effective for insects that are spatially aggregated and have limited dispersal ability (Park et al. 2007), such as corn rootworms (Park and Tollefson 2006). The degree of aggregation of boll injury in our study and the association with NDVI, combined with the highly variable soils in the southeastern coastal plains in the United States (Duffera et al. 2007), suggests that site-specific management has potential and deserves further study. Site-specific management in potato, *Solanum tuberosum* L., was more successful with the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), than with the potato leafhopper, *Empoasca fabae* (Harris), in part due to the greater spatial stability of *L. decemlineata* (Weisz et al. 1996, Fleischer et al. 1999). The greater spatial stability of boll injury relative to stink bugs also underlines the greater potential of developing a successful site-specific management program in cotton with boll injury than with stink bugs. A study in South Carolina showed that cotton yield and quality are highly sensitive to variability in the properties of soils (Johnson et al. 2002). Because insect herbivores are often sensitive to plant growth (Solomon 1981), site-specific management may be particularly suited for the region. Developing our understanding of the interactions between the environment and the dynamics of insect pests in crop systems is needed to optimize management practices with reduced use of insecticides. If spatial heterogeneity in insect densities can be correlated with variables measured by remote sensing, models can be developed to generate risk assessment maps (Merrill et al. 2009, 2014). Because only 50% of the spatial associations between NDVI and boll injury were significant, future work should examine other soil and plant measurements that may correlate with stink bug injury. The value of remote sensing for insect management in cotton needs further study, with an aim to develop tools such as risk assessment maps that will help growers to reduce insecticide inputs.

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