Comparison of Perimeter Trap Crop Varieties: Effects on Herbivory, Pollination, and Yield in Butternut Squash

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ABSTRACT Perimeter trap cropping (PTC) is a method of integrated pest management (IPM) in which the main crop is surrounded with a perimeter trap crop that is more attractive to pests. Blue Hubbard (*Cucurbita maxima* Duch.) is a highly effective trap crop for butternut squash (*C. moschata* Duch. ex Poir) attacked by striped cucumber beetles (*Acalymma vittatum* Fabricius), but its limited marketability may reduce adoption of PTC by growers. Research comparing border crop varieties is necessary to provide options for growers. Furthermore, pollinators are critical for cucurbit yield, and the effect of PTC on pollination to main crops is unknown. We examined the effect of five border treatments on herbivory, pollination, and yield in butternut squash and manipulated herbivory and pollination to compare their importance for main crop yield. Blue Hubbard, buttercup squash (*C. maxima* Duch.), and zucchini (*C. pepo* L.) were equally attractive to cucumber beetles. Border treatments did not affect butternut leaf damage, but butternut flowers had the fewest beetles when surrounded by Blue Hubbard or buttercup squash. Yield was highest in the Blue Hubbard and buttercup treatments, but this effect was not statistically significant. Native bees accounted for 87% of pollinator visits, and pollination did not limit yield. There was no evidence that border crops competed with the main crop for pollinators. Our results suggest that both buttercup squash and zucchini may be viable alternatives to Blue Hubbard as borders for the main crop of butternut squash. Thus, growers may have multiple border options that reduce pesticide use, effectively manage pests, and do not disturb mutualist interactions with pollinators.

KEY WORDS *Apis mellifera*, *Bombus* spp., multispecies interactions, *Peponapis pruinosa*, winter squash

Conventional agricultural practices often rely on pesticides that are costly to growers and can have detrimental effects on human health, the environment, and nontarget organisms such as pollinators. Perimeter trap cropping (PTC) is a method of integrated pest management (IPM) that can reduce reliance on pesticides through crop layout design. A trap crop that is attractive to colonizing pests is planted to encircle the main crop, often limiting pesticide use to the border where insects are concentrated as they enter the field. In a wide range of systems, PTC reduced damage or disease to main crops, greatly reduced insecticide use, and increased main crop yield (Aluja et al. 1997; Mitchell et al. 2000; Boucher et al. 2003a, b). However, little research has compared different border crops to provide options for growers and maximize effectiveness. Furthermore, pollinators and pests often have critical impacts on yield. Although the effectiveness of PTC for controlling pests has been shown in many systems, to date there are no studies that examine the impacts of PTC on beneficial insects such as pollinators to provide a more comprehensive view of how PTC affects yield by multiple interactions.

Cucumber beetles (Chrysomelidae: *Acalymma vittatum* Fabricius) are ranked as the most important insect pest in cucurbit crops in the northeastern United States and are the primary target of insecticide applications used by growers (Hoffmann et al. 1996, Hollingsworth et al. 1998, Stivers 1999). Conventional pest management for many cucurbit crops requires multiple applications of foliar pesticides such as carbaryl, carbamates, or synthetic pyrethroids (Brust and Foster 1995, 1999; Howell et al. 2004). Because beetle colonization of fields can occur within a day, proper timing of foliar sprays can be difficult. Systemic insecticides have recently been adopted in the northeast to target early feeding damage (Hazzard 2003) but are used by <10% of growers because of higher costs (Clifton and Duphily 2006).

Northeastern states have a high proportion of their vegetable crop industry invested in cucurbit crops including squash, melons, cucumbers, and pumpkins; in Massachusetts alone, 40% of the vegetable crop acreage is devoted to cucurbits (USDA 2002). Butternut squash is a key crop for fall and winter sales,

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comprising the majority of winter squash grown in New England (Clifton and Duphily 2006). Previous on-farm research found that Blue Hubbard (*Cucurbita maxima* Duch.) is an effective border for butternut (*C. moschata* Duch. ex Poir) and summer squash (*C. pepo* L.), controlling pest populations while reducing pesticide use by >90% (Boucher and Durgy 2004; Cavanagh et al. 2009). However, Blue Hubbard has limited market demand, accounting for only 5% of winter squash grown in New England (Clifton and Duphily 2006). Other cucurbit crops could provide more marketable border options. Alternatively, wild cucurbits would be less profitable than agricultural varieties but could be very effective because of their high levels of cucurbitacins, chemical defenses that attract cucumber beetles (Metcalf et al. 1982).

Pollinators and herbivores play a key role in the production of many crops. The value of pollination services in agriculture and rangelands has been estimated as $117 billion per year in the United States (Costanza et al. 1997). With recent, dramatic losses of honey bees (*Apis mellifera* L.) because of colony collapse disorder (Cox-Foster et al. 2007), it is imperative to determine whether native bees can provide sufficient pollination for yield. Nearly all cucurbit crops require pollination to produce fruit (Kemp and Bosch 2001). *Cucurbita* species, including butternut squash and Blue Hubbard, are visited by the native specialist squash bee pollinator *Peponapis pruinosa* Say (Apidae), as well as generalist bumble bees (*Bombus* spp., Apidae) (Shuler et al. 2005, Walters and Taylor 2006). However, honey bees are often used ensure adequate pollination. For example, processing pumpkin (*C. moschata*), a conspecific of butternut squash, had 70% heavier fruits with the addition of honey bee colonies (Walters and Taylor 2006), indicating that native bees did not provide adequate pollination for maximum yield. Yield in other cucurbits, such as cucumber, cantaloupe, and watermelon, was also limited by pollinator visitation, and in many cases, honey bees were relied on to maximize yield (Stanghellini et al. 1997, 1998; Kremen et al. 2002; Strauss and Murch 2004; but see Winfree et al. 2007).

Pollinators and insect pests may respond to different border varieties, but predicting such effects will depend on the scale at which pollinators are attracted to cucurbit fields. For example, if border varieties are more attractive than the main crop, this could reduce main crop yield because of competition for the same pollinators. Alternatively, if attractive borders bring more pollinators overall to the field, there may be higher pollination in the main crop because of facilitation. Although the role of competition or facilitation mediating pollination has been examined in wild systems (Brown et al. 2002, Moeller 2004), much less is known about how these interactions influence pollination in agricultural settings (Kremen et al. 2007). Examining the effects of different border crops on yield by pollination and herbivory will provide a more comprehensive assessment of the mechanisms by which PTC could influence yield.

In this study, we examined the effect of border treatments, herbivory, and pollination on yield in butternut squash as a main crop. Specifically, we (1) compared attractiveness of border varieties to herbivores and pollinators, (2) assessed the impacts of different border varieties on herbivory, pollination, and yield in the main crop, and (3) manipulated herbivory and pollination in the main crop in a 2 by 2 factorial design to determine the effects of these interactions on yield.

Materials and Methods

Border Treatments

We compared the effectiveness of five border treatments on herbivory, pollination and yield in butternut squash (*C. moschata* cultivar Waltham): Blue Hubbard (*C. maxima*), buttercup squash (*C. maxima* cultivar Burgess), zucchini (*C. pepo* cultivar Embassy), wild gourd (*C. pepo* ssp. *texana*) mixed with zucchini (*C. pepo* cultivar Elite), and butternut (cultivar Waltham) as a control. The wild gourd treatment was mixed with zucchini in a 2:3 ratio to determine whether a wild gourd could provide additional protection without entirely sacrificing the border row for crop production. Butternut and Blue Hubbard were provided by Johnny's Selected Seeds (Winslow, ME), buttercup and zucchini were provided by Rupp Seeds (Wauseon, OH), and wild gourd was provided by A. G. Stephenson (Penn State University). Border taxa (varieties hereafter) were selected by the following criteria: (1) attractiveness to cucumber beetles in field studies (Andersen and Metcalf 1987, Pair 1997, McGrath 2000, Petzoldt 2001; A. Cavanagh, L.S.A., and R.V.H., unpublished data), (2) potential marketability (buttercup and zucchini), and (3) variation in the level of floral volatile emissions and cucurbitacins (Andersen and Metcalf 1987), both of which are attractive to beetles but whose effects on pollinators are unknown.

Experimental Design

Seed were sown in greenhouse flats on 16 May 2005 and transplanted to the South Deerfield experimental farm, UMass-Amherst, at the one-leaf stage from 6 to 10 June. This site has Occum fine sandy loam soil; fields were previously used for vegetable crop experiments including sweet corn, peppers, field corn, *Brassica*, and vine crops, with a winter cover crop of rye. We planted five replicate plots of each border treatment surrounding a main crop of butternut squash, for a total of 25 plots arranged in five blocks, with one replicate per border treatment per block. Each plot was 15.24 by 15.24 m, with 0.61-m in-row and 1.52-m between-row spacing, surrounded by one row of border plants. On-farm studies with much larger fields have found one to two border rows to be effective at controlling beetle damage with greatly reduced pesticide use (Cavanagh et al. 2009). Border treatments were randomly assigned to plots within blocks. Border plants were

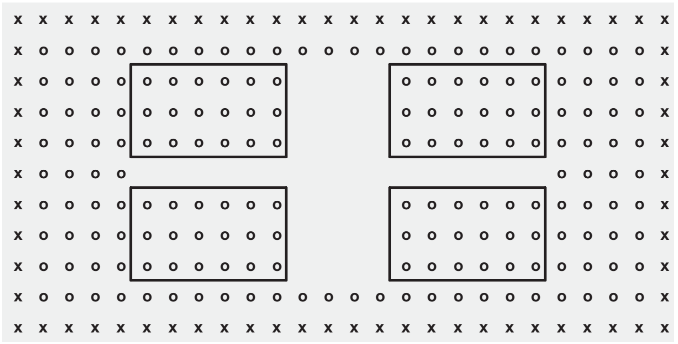

Fig. 1. Example layout for one plot, where x represents a border plant and o represents a main crop plant (butternut squash). The four central quadrats (18 plants each) were randomly assigned to one of four factorial combinations of herbivory (natural herbivory versus pesticide) and pollination (natural pollination versus hand pollination) treatments. Each experimental block consisted of five such plots, each with a different perimeter trap crop treatment.

treated with imidacloprid, a systemic pesticide (Admire 2 F @0.01 ml, or 0.0021 [AI], dissolved in 3.5 ml water per plant) on 7 June (24 h before planting), as a drench followed by an application of 1 ml water per plant to rinse off the leaves and soak the chemical into the soil. Previous work found that treating border plants with systemic rather than foliar pesticides made PTC more effective by protecting borders against rapid decimation by beetles (Cavanagh et al. 2009). Border plants that died were replaced as needed through 15 June. Plots were separated by a fallow area 15.24 m wide to reduce the influence of borders on adjacent plots. Butternut squash was planted in four quadrats in each plot with 18 plants per quadrat, separated by an empty row of 2.44 m. At the six-leaf stage, butternut plants were removed from one row inside the border to prevent competition with the border crop, and vines were trained to stay within their quadrat. At this point, beetles are past their initial colonization period, and the crops are less subject to mortality from direct beetle damage. Based on soil tests and recommendations from the New England Vegetable Management Guide (Howell 2008–2009), 560.4 kg/ha (500 lb/acre) of 19–19-19 (Crop Production Services, South Deerfield, MA) was used on two of the blocks, and on the other three, we used 896.6 kg/ha (800 lb/acre) of 5–10-10 (Crop Production Services). Fertilizers were granular, broadcast, and incorporated into the soil 3 d before planting. Weeds were managed with mechanical cultivation before vining and by hand hoeing after vining. Overhead irrigation was provided as needed.

To determine whether border varieties alter the relative importance of damage and pollination for main crop yield, we used a 2 by 2 factorial combination of pollination (natural pollination versus hand pollination) and pesticides (natural herbivory versus conventional pesticide) treatments within plots (Fig. 1). Each plot was divided into four quadrats that were randomly assigned to pollination–pesticide treatment combinations. Because flowers are open for only 1 d, all female flowers in the hand pollination treatments were pollinated 5 d/wk from the onset of female flowering in mid-July through the second week of August. Fruits that set after that date are unlikely to mature. For the hand pollination treatment, pollen was collected from randomly selected male flowers across plots, mixed in petri dishes, and added with a camelhair paintbrush to cover the entire stigmatic surface of all flowers. All plants were also open to natural pollinator visits. For the conventional pesticide treatment, seedlings were treated with imidacloprid before planting as described above; natural herbivory plants were treated with water only. Although imidacloprid may be toxic to bees at certain concentrations (Suchail et al. 2000), studies that have examined the effect of imidacloprid on honey bee or bumble bee pollination in agricultural systems found no effect (Gels et al. 2002, Elzen et al. 2004).

Responses Measured

Herbivory. The striped cucumber beetle (*A. vittatum*) is the most common cucurbit insect pest in the northeastern United States generally (Hoffmann et al. 1996, Hollingsworth et al. 1998, Stivers 1999, Boucher and Durgy 2003, Clifton and Duphily 2006) and was the primary aboveground herbivore in our study. We found very occasional spotted cucumber beetles (*Diabrotica undecimpunctata;* <1% of beetles) and did not observe squash bugs (*Anasa tristis*) or lepidopteran pests. We counted striped cucumber beetles and recorded damage to crop and border plants weekly from 13 June through 12 July. We counted live and dead beetles within a 10-cm radius of each plant and scored damage on the four youngest leaves of a randomly chosen vine from each plant using the following scale: 0 = no damage, 1 = 1–25% damage, 2 = 26–50% damage, 3 = 51–75% damage, and 4 = 76–100% damage. At the first census we also recorded cotyledon damage (presence/absence), and after the second census, we introduced an additional measure of 0.5 for leaves with 1–12% damage to increase the precision of our measurements. Each quadrat in the main crop consisted of 18 plants (three rows of six); we censused the six plants in the middle third of the quadrat. In the borders, we censused four plants per side starting from a randomly selected plant (16 total). We censused the same number of border plants in the mixed treatment, but noted whether each plant was zucchini or wild gourd. During pollinator observations, we counted beetles in open flowers (see *Pollinator Attraction*).

Floral Traits. We measured floral traits because reduced herbivory, caused by either border or pesticide treatments, could influence pollinator attraction by affecting the number, size, or shape of flowers. We counted male and female flowers in each quadrat 5 d/wk from 13 July through 14 August, the main flowering period. We measured morphological traits on up to two male and two female flowers per quadrat. For each flower, we measured flower diameter, petal length, petal width, and nectary diameter. For female flowers, we also measured ovary length and width and estimated the area of one stigma lobe as (length) $\times$ [(width at top + width at bottom)/2]. For male flowers, we estimated the size of one anther as anther length $\times$ anther width. All measurements were to the nearest 0.01 mm. Measurements were conducted twice: 3–5 and 16 August.

Pollinator Attraction. We observed insect visits to flowers in the border and main crop on 14 separate dates between 14 July and 18 August. We conducted 5-min observations on individual male and female flowers, for a total of 98.7 h in the main crop and 56.3 h in the border crops. Whenever possible, we controlled for temporal variation by using five simultaneous observers, one on each plot of the same block. All observations took place between 0530 and 1130 hours, when flowers are open and pollinators are active. We recorded the number of visits, time per visit, and insect species for all visits, and we counted beetles in each observed flower. Because squash plants are vines that readily intertwine, we did not distinguish between individual plants.

Crop Yield. Butternut squash were harvested from 2 to 7 September. All squash were counted and weighed from each quadrat in each plot. We also estimated fruit set as the number of squash per total female flowers for each quadrat. For four randomly selected squash per quadrat, we recorded viable seeds, nonviable seeds, and total seed weight as additional measures of pollinator effectiveness.

Statistical Analyses

General Points. In several cases, multiple responses were analyzed with MANOVA as indicators of an underlying response of interest (e.g., herbivory, pollination, yield). Significant MANOVAs were followed by univariate ANOVAs to examine which individual responses were most affected by treatments (Scheiner 1993). Tukey's tests were used to distinguish significantly different treatments at $P < 0.05$. All analyses were conducted with SAS v. 9.1 (SAS Institute 2004), and we examined all responses for normality using the Shapiro-Wilk test in the UNIVARIATE procedure. Data were normal without transformation except as indicated.

Comparisons in Border Varieties. We used MANOVA to ask how border variety and block affected the number of beetles and amount of herbivory in the borders. Herbivory measures were averaged across censuses. To compare the attractiveness of border varieties to pollinators, we analyzed the number of pollinator visits by bumble bees, honey bees, and squash bees with MANOVA. Time spent per flower by squash bees was analyzed with separate analysis of variance (ANOVA) because not all borders were visited; honey and bumble bees did not visit enough replicate plots to analyze time per flower. Squash bee time per flower was $\log(x)$ transformed. Because this analysis compares border plants, pesticide and pollination treatments (which were conducted on main crop plants) were not relevant here. Because one border treatment (mixed) included two species that could have different effects on insects, we consider zucchini-mixed and wild gourd-mixed as separate categories for these analyses only. Although this slightly inflates our error degrees of freedom, treating the two species separately is the most biologically relevant approach to compare herbivory and pollination on different taxa.

Comparisons in the Main Crop. We compared the effect of border variety, pesticide, and pollination treatments on herbivory, pollination, and yield in the main crop. The experiment was a split-plot design with border treatment as the main plot treatment and pesticide and pollination treatments as the subplot treatments. We considered block a random factor so that treatment effects were tested over the treatment by block interaction terms (Potvin 2001). Plot or subplot was the unit of replication for all analyses of main plot and subplot effects, respectively, and data were averaged within subplot unless otherwise stated. Two plots in one block (buttercup and Blue Hubbard borders) were removed from the yield analysis only because of high levels of bacterial wilt, leaving 23 plots in this analysis. It is possible that removing these two plots could mask biologically important effects of our treatments because wilt is transmitted by cucumber beetle damage. However, we feel that including these plots is not appropriate for several reasons. We noted the occurrence of wilt during our final herbivory census on 12 July. We found high incidences of wilt in the main crop plants of those two plots (33 and 50% of plants affected), but no wilt in any of the other 23 plots. These data suggest that wilt was not common at our site and had a very patchy incidence that could not be attributed to particular treatments. Furthermore, wilt was lower and yield was higher in pesticide-treated treated subplots for one of the wilted plots, but the reverse pattern was found in the other plot. This suggests that reduced herbivory caused by pesticides did not protect these plants from wilt. Finally, yield in those two plots was one half that of most other plots and was 2 SD below the mean (total squash weights of 52.0 and 51.3 kg compared with overall mean $\pm$ SD of 98.4 $\pm$ 21.3 kg). Including these two plots changes our results, but we would not feel it appropriate to report results that are dependent on the inclusion of two

Table 1. ANOVAs reporting effect of border variety and block on measures of herbivory in border plants

Response	Source	df	Sums of Squares	*F*
Dead beetles	Border variety	5	0.0017	0.95 NS
	Block	4	0.0056	3.88[a]
	Error	19	0.0068	
Live beetles	Border variety	5	6.77	18.03[b]
	Block	4	0.45	1.51 NS
	Error	19	1.43	
Cotyledon damage	Border variety	5	1.25	8.89[c]
	Block	4	0.37	3.27[a]
	Error	19	0.53	
Leaf damage	Border variety	5	0.48	19.45[b]
	Block	4	0.095	4.83[d]
	Error	19	0.093	

[a] $P < 0.05$.
[b] $P < 0.0001$.
[c] $P < 0.001$.
[d] $P < 0.01$.
NS, not significant.

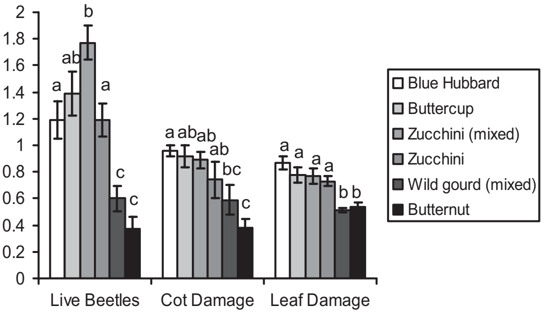

Fig. 2. Comparison of herbivory in border varieties. The *y*-axis indicates mean beetles/plant for live beetles and a damage scale (described in methods) for cotyledon and leaf damage. Error bars represent SE, and letters denote significantly different means by Tukey's test within response.

outlier data points. For all of these reasons, we do not feel it is appropriate to include these two plots in our yield analysis.

Each analysis included border treatment (main plot effect), pesticide treatment (subplot effect), pollination treatment (subplot effect), block, and all two- and three-way interaction terms. This model was used in separate MANOVAs to determine how these factors affected the following responses in the main crop: (1) leaf herbivory, estimated as number of live beetles, number of dead beetles, cotyledon damage, and leaf damage with data averaged across censuses; (2) floral herbivory, estimated as the number of beetles in flowers; (3) pollination, measured as mean bumble bee, squash bee, and honey bee visits over a 5-min period; (4) floral morphology for male and female flowers in separate MANOVAs for each sex; (5) agricultural yield, measured as total weight of squash, number of squash, and fruit set per female flower; and (6) ecological reproduction, measured as viable seeds, nonviable seeds, and seed weight. Number of beetles in flowers was $\log(x + 1)$ transformed and honey bee visits were square root(x) transformed to best satisfy assumptions of normality.

Results

Attractiveness of Border Varieties to Herbivores and Pollinators

We compared herbivory and pollination in borders to determine attractiveness of border varieties. Both border variety and block significantly affected herbivory (MANOVA, border: Wilks lambda = 0.03, $F_{20,54.02} = 4.98$, $P < 0.0001$; block: Wilks lambda = 0.18, $F_{16,49.52} = 2.35$, $P = 0.012$). Examination of univariate analyses showed that border varieties had different numbers of live but not dead beetles and different amounts of cotyledon and leaf damage (Table 1), with beetles and damage typically highest in Blue Hubbard, buttercup, and zucchini, and lowest in butternut squash and wild gourd (Fig. 2). There was no significant difference in pollinator visits to border varieties or blocks (Wilks lambda > 0.38, $F < 1.4$, $P > 0.2$ for both). However, squash bees spent significantly longer in Blue Hubbard compared with butternut flowers, with other varieties intermediate ($F_{5,18} = 3.80$, $P < 0.02$; Fig. 3). The number of beetles per flower did not differ in the border varieties ($P > 0.1$), but did vary with block ($F_{4,20} = 4.18$, $P < 0.015$).

Herbivory in Main Crop

As expected, the pesticide treatment reduced herbivory in the main crop (MANOVA: Wilks lambda = 0.02, $F_{4,4} = 42.13$, $P = 0.0016$). Subplots with pesticides had more dead ($F_{1,7.53} = 87.41$, $P = 0.0001$) but not live beetles ($P > 0.15$), marginally less cotyledon damage ($F_{1,5.23} = 6.19$, $P = 0.053$), and less leaf damage ($F_{1,4.26} = 11.21$, $P = 0.026$; Fig. 4). No other treatment or interaction affected herbivory (MANOVA: Wilks lambda > 0.08, $F < 1.0$, $P > 0.5$ for all). The number of beetles per open flower was marginally affected by border variety ($F_{4,16} = 2.65$, $P = 0.073$), block ($F_{4,6.15} = 4.18$, $P = 0.057$), and the border by pollination interaction ($F_{4,16} = 3.57$, $P = 0.03$) in male flowers but not in female flowers ($F < 2.5$, $P > 0.08$ for all). There were over eight times as many beetles in main crop male flowers with a control (butternut) border compared with buttercup borders,

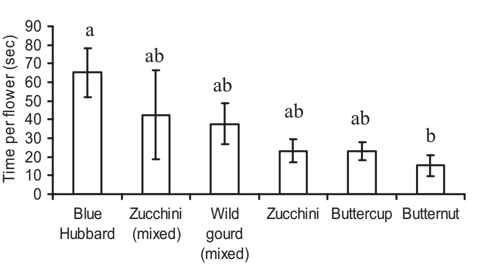

Fig. 3. Comparison of border varieties for squash bee mean time per flower. Error bars represent SE, and letters denote significantly different means by Tukey's test.

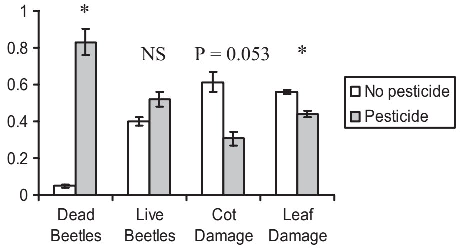

Fig. 4. Effect of pesticides on herbivory, measured as the number of dead beetles per plant, live beetles per plant, cotyledon damage (0 or 1 for absence/presence), and leaf damage (scale described in Materials and Methods). Error bars represent SE. *Significant effects at $P < 0.05$ from univariate ANOVAs for each response after significant MANOVA.

with other border treatments intermediate (mean ± SE beetles/flower: butternut, 0.26 ± 0.06; mixed, 0.22 ± 0.06; Blue Hubbard, 0.20 ± 0.06; zucchini, 0.17 ± 0.06; buttercup, 0.03 ± 0.02). There were no other treatment effects on beetle abundance in male or female flowers.

Pollination in Main Crop

Squash bees were the most common visitors to butternut flowers (54% of 861 total flower visits), followed by bumble bees (33%) and honey bees (13%). There was no effect of border variety, pesticides, hand pollination, block, or their interactions on any measure of pollinator visitation to the main crop (MANOVA: Wilks lambda > 0.55, $F < 3.3$, $P > 0.05$ for all).

Main Crop Floral Traits

There was no effect of border variety, herbivory, pollination, or their interactions on any measure of main crop female or male floral traits (MANOVA: Wilks lambda < 0.7, $F < 1.1$, $P > 0.4$ for all). Male but not female traits varied significantly with block (Wilks lambda = 0.06, $F_{20,40.75} = 2.7$, $P = 0.004$).

Main Crop Yield and Reproduction

We found no effect of border variety, pesticides, pollination, or their interactions on any measure of yield (MANOVA: Wilks lambda < 0.25, $F < 2.0$, $P > 0.1$ for all). Although the effect of border variety was not statistically significant ($P = 0.11$), we note that plots with Blue Hubbard and buttercup borders produced ≈12.5% more squash by weight and 8% by number than plots with mixed and butternut borders, with zucchini borders intermediate (means ± SE total weight: Blue Hubbard, 108.8 ± 2.1 kg; buttercup, 108.8 ± 2.6 kg; zucchini, 102.7 ± 3.2; mixed, 98.3 ± 4.8; control, 96.1 ± 2.7; number of squash: Blue Hubbard, 81.7 ± 1.4; buttercup, 80.0 ± 2.0; zucchini, 80.6 ± 2.2; mixed, 76.5 ± 2.7; control, 75.5 ± 2.1).

Discussion

One of the main goals of this study was to test the effectiveness of several cucurbits as borders around butternut squash. Previous research has shown that Blue Hubbard is an effective border for butternut squash, reducing pesticide use by >90% while controlling herbivore damage and maintaining yield (Cavanagh et al. 2009). However, the limited marketability of Blue Hubbard may prevent widespread adoption by growers. Field trials suggested that more marketable crops such as buttercup squash might be as attractive to beetles as Blue Hubbard (A. Cavanagh, L.S.A., and R.V.H., unpublished data). In this study, we found that buttercup and zucchini were as attractive to beetles as Blue Hubbard (Fig. 2), suggesting that they may be effective borders. Wild gourd was surprisingly unattractive to beetles despite relatively high cucurbitacin levels compared with cultivated taxa (Metcalf et al. 1982). Because wild gourd also does not provide any marketable crop, our data suggest it has little potential for use as a border.

Although pollination is critical for yield in >90 major U.S. crops including many cucurbits (Delaplane and Mayer 2000, Kemp and Bosch 2001), variation in pollinator attraction between crop cultivars is rarely studied. We compared pollinator attraction to border varieties and found no difference in the number of bee visits. However, specialist squash bees spent over five times as long in Blue Hubbard flowers compared with butternut flowers. Squash bee preference for borders rather than the main crop has the potential to result in competition for pollination. However, we found no effect of hand pollination on yield, suggesting that butternut squash yield is not limited by lack of pollination. Furthermore, pollinator visitation to the main crop was unaffected by border treatment. Both these results suggest that competition for pollination between the main crop and borders should not reduce yield even when the border variety is highly attractive. In larger commercial fields, the ratio of border to main crop plants would be lower than in our experimental plots, further reducing the potential for competition between border and main crop plants. Yield could potentially be reduced if heterospecific pollen is transferred from borders to the main crop and prevents fruit set by clogging stigmas. Previous research indicates that Blue Hubbard pollen does not inhibit fruit set in butternut squash as long as butternut pollen is also deposited (Hladun and Adler 2008), suggesting that Blue Hubbard borders should not reduce butternut yield by pollen transfer. Similar studies have not been conducted for buttercup or zucchini squash, but these varieties were not more attractive to squash bees than butternut squash (Fig. 3).

Recent declines in both managed and native bees (Allen-Wardell et al. 1998, Kearns et al. 1998, Cox-Foster et al. 2007, Winfree et al. 2007) have prompted concerns about agricultural losses caused by lack of pollination services. We found that butternut squash yield was not limited by lack of pollination at our site; plots that were hand-pollinated had nearly identical yield compared with plots with natural pollination only. Furthermore, native bees made up the vast majority (87%) of visits to main crop flowers. Few other studies have tested whether crop cucurbits receive sufficient pollination for full yield. Hand pollination and the addition of honey bee colonies increased canteloupe yield (*Cucumis melo*) in California (Strauss and Murch 2004), indicating that naturally occurring bees were not providing sufficient pollination for maximum yield. Agricultural intensification may reduce native bee pollination services in California; only organic farms near natural habitat had sufficient pollination from native bees for full yield in watermelon (*Citrullus lanatus*), whereas all other farms relied on managed honey bees (Kremen et al. 2002). However, native bees provided sufficient pollen to most watermelon farms in New Jersey (Winfree et al. 2007). The authors suggest that increased heterogeneity of natural and managed patches in the eastern United States may contribute to higher native bee diversity and abundance in agricultural lands. Our results are consistent with the finding of Winfree et al. (2007) that native bee populations provide sufficient pollination for cucurbit crops in the eastern United States.

Trap cropping systems can be beneficial for growers by reducing damage and pesticide use, increasing yield, or both (Hokkanen 1991). Although we found no significant effects of border treatments on butternut yield, it is interesting to note that yield was 12.5% higher in plots surrounded by Blue Hubbard or buttercup squash compared with control plots and that control plots had the lowest yield of any treatment. The mechanism behind potential differences in yield is unclear. Although we surprisingly found no effect of border variety on butternut leaf herbivory, beetles in male butternut flowers were highest in control plots and lowest in plots surrounded by buttercup squash. Although florivory is not generally studied as a source of concern in cucurbits, *Diabotrica* adults can be abundant in cucurbit flowers (Andersen and Metcalf 1987), and floral herbivores can reduce reproduction in other systems by damaging reproductive structures or deterring pollinators (McCall and Irwin 2006). In addition, greater floral volatiles and greater numbers of beetles in flowers of the congener *C. pepo* subsp. *texana* are associated with higher incidence of bacterial wilt (Ferrari et al. 2006, 2007). Although the relationship between floral beetles and yield is speculative, floral herbivory merits examination as a potential mechanism for border effects on main crop yield.

Pesticides reduced herbivory in this study, but replicates with and without pesticides had nearly identical yield (pesticide: 103.5 ± 2.1 kg/subplot; natural herbivory: 101.5 ± 2.3 kg/subplot). This result was initially surprising, because cucumber beetles are ranked as the most important insect pest in cucurbit crops in the northeast and are the primary target of insecticide applications by growers (Hoffmann et al. 1996, Hollingsworth et al. 1998, Stivers 1999). *A. vittatum* may have particular potential for reducing yield because larvae are specialists on cucurbit roots. In contrast, *Diabrotica* larvae are generalists that often prefer grass species, so that these species incur damage aboveground only (Eben et al. 1997). However, damage levels were relatively low at our site, averaging ~15% in the natural herbivory treatments (Fig. 4). Other work has shown butternut squash yield is unaffected when <20% of the leaf area is removed (Hoffmann et al. 2000); our results are consistent with this finding. The exception is that even small amounts of cucumber beetle damage can allow the introduction of bacterial wilt (Brust and Foster 1995, Brust 1997), but wilt was relatively rare in our field. Because both herbivory and wilt were low, our study provided a conservative test of the benefits of both pesticide use and border treatments for yield. However, other studies have found that yield in cucumber was substantially increased with the use of Blue Hubbard as a border crop (Boucher et al. 2003b).

In conclusion, our results suggest that both buttercup squash and zucchini may be viable alternatives to Blue Hubbard as borders, because all these varieties were equally attractive to cucumber beetles. The inclusion of multiple options may increase growers' adoption of the PTC system by addressing the need to maximize marketability. Pollination did not limit yield at our site, and border varieties did not seem to attract pollinators away from the main crop. Thus, growers may have multiple border options that reduce pesticide use, effectively manage pests, and do not disturb mutualist interactions with pollinators.

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