6. PROJECT DESCRIPTION

(a) Problem, Background and Justification

Pollination services are required for yield in many fruit and vegetable crops. However, conventional management practices generally focus on pest control. The extent to which yield is reduced by pests compared to insufficient pollination is unknown, but a recent study suggests that sufficient pollination can completely reverse yield losses due to pest damage (Strauss and Murch 2004). However, recent declines in managed and native bees due to pesticide use, disease and parasites indicate that management of pollinator as well as pest populations may be essential to maintain crop yield.

Our proposal combines research and extension to evaluate several perimeter trap crops for reducing pesticide use in cucurbit crops and increasing yield via herbivore resistance and pollinator attraction. Perimeter trap crops (PTC), or the use of an attractive crop to surround and protect main crops from herbivores, have proven effective in cucurbits and peppers for reducing pesticide use by >90% while maintaining or improving yield (Boucher et al. 2003a, Boucher and Durgy 2003, 2004, R. Hazzard, unpublished data). Here we propose to (1) screen 20 other cucurbit cultivars for potential as effective PTCs via interactions with pests and pollinators, (2) test 5 PTC cultivars in experimental plots for potential to increase yield through reduced damage and increased pollination, and (3) compare 3 PTC cultivars with conventional management practices on growers' farms in the context of reducing damage, increasing pollination, and increasing yield. Results of these studies will be communicated to growers through electronic and printed publications and educational programs. This research will further the goals of IPM by providing growers with several PTC options to reduce pesticide use and increase pollination services.

Economic value of cucurbits and need for pest control

The value of vegetable crops sold in the United States was $12.7 billion in 2002 (USDA 2002). 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 cucurbit crops (USDA 2002). Yields of winter squash, one of the major cucurbit crops, exceed 20,000 lb/acre with a wholesale value estimated at $3400/acre, or greater than $5 million for the state. Butternut squash is a key winter crop, in part because of strong market demand and excellent storage capability. Forty percent of winter growers store butternut through fall and winter months (Hollingsworth et al. 1998). Butternut can also be processed, which adds value and enhances fall and winter sales; processing accounts for 15% of butternut squash sales (N. Clifton, UMass extension, in preparation).

Cucumber beetles (*Diabrotica* spp, *Acalymma vittatum*) constitute some of most serious pests of cucurbit crops in the world (Metcalf and Metcalf 1992). These beetles are ranked as the most important insect pest in cucurbit crops in the Northeast, and are the primary target of insecticide applications used by growers (Hoffmann et al. 1996a, Hollingsworth et al. 1998, Stivers 1999). The Northeast Vegetable IPM Commodity Working Group, comprised of growers, crop consultants, processors, departments of agriculture, university researchers, extension specialists, and environmentalists from nine northeastern states, ranked the cucumber beetle and bacterial wilt complex as a region-wide problem that causes significant reduction in yield and results in high pesticide use (IPM Priorities for Vegetables in the Northeast: http://northeastipm.org/work_vegepriority.cfm). Conventional pest management for many cucurbit crops requires 2- 8 application of pesticides such as carbaryl (Brust and Foster 1995, 1999) or other carbamates or synthetic pyrethroids (Howell et al. 2004), with an estimated cost of $40-160 per crop (Christensen et al. 2000). Because beetle colonization of fields can occur within a day, proper timing of foliar sprays according to thresholds can be difficult for growers to achieve. Recently, growers in the Northeast have adopted use of systemic insecticides (e.g., imidcloprid) in the furrow at planting (Hazzard 2003) to target early feeding damage; however, the per-acre cost is higher than foliar applications, and adoption in New England remains at <10% (Clifton, in preparation).

Cucumber beetles can reduce yield in cucurbits through direct damage. Cucumber beetles overwinter as adults, colonize cucurbit fields in early to mid June, and often completely destroy newly germinated plants (Ferguson et al. 1983). Active damage by adults to leaves and early flowers takes place over a 4-5 week time period in New England, followed by a period of inactivity aboveground when the first generation of adults have laid eggs in the soil and died. Although the underground larvae are less visible, they can extensively damage cucurbit root systems. Second-generation adults emerge and continue to feed, damaging leaves, flowers, and fruit, before leaving the fields for overwintering sites.

Although yield losses to direct cucumber beetle damage can be extensive, the most damaging effect of cucumber beetle infestation may be through transmission of the bacteria *Erwinia tracheiphila*. Even small amounts of cucumber beetle damage can allow the introduction of bacterial wilt (Brust and Foster 1995, Brust 1997). It is generally thought that cucumber beetles reduce yield to a greater extent through the introduction of bacterial wilt than through direct tissue damage (Brust and Foster 1995), and economic thresholds in wilt-susceptible crops have been established primarily to avoid losses from bacterial wilt (Brust and Foster 1999). Yield losses from simulated feeding damage to young plants occurred only when damage exceeded 20% of leaf area in butternut, and not until it exceeded 80% of leaf area in pumpkin (Hoffmann et al. 2000), suggesting that bacterial wilt is responsible for the greater yield losses due to damage in the field. Yield losses from bacterial wilt in winter squash and pumpkins have increased in the past decade (Hazzard et al. 1999, Zitter 1999, Hazzard et al. 2000, Zitter 2000, Hazzard et al. 2001, Zitter 2001), increasing the necessity for developing control methods that prevent transmission of wilt.

Importance of pollination for squash yield

Pollinators are essential for production of many vegetables, fruits, nuts, cotton and canola. 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); the value of honey bees alone to U.S. crop production has been estimated between $5-14 billion per year (Southwick and Southwick 1992). Nearly all cucurbit crops require pollination to produce fruit (Kemp and Bosch 2001), but the role of pollination on yield is poorly understood. A recent survey revealed that there is a strong perception among Massachusetts growers and agricultural specialists that managed honeybee colonies are declining in quality, and farmers have expressed concerns about pollination and the need for information that affects their production (Hollingsworth 2002). Declines in beekeeping due to pesticide poisoning, disease, parasites, and the spread of Africanized honey bees are all reducing the availability of honey bee pollination services. The development of management strategies that reduce pesticide use and foster pollination are essential to insure future crop yield in the face of declining bee populations.

Furthermore, yield losses thought to be due to herbivory may actually be due to pollinator preference (Strauss and Murch 2004). Experimentally damaged cantaloupe plants had lower yield compared to undamaged plants when pollinators were not managed. However, *when plants were hand-pollinated, damage did not reduce cantaloupe yield.* This result demonstrates that reduced yield thought to be due to herbivores, is in fact due to herbivores making plants less attractive to pollinators. Thus, managing pollinator preference as well as herbivore resistance is a critical component for increasing yield in cucurbit and many other crops.

Pollinator service may also be improved by developing more attractive crop varieties. Traits that can improve pollinator services include flower morphology (Campbell et al. 1991, Conner et al. 1995, Galen and Cuba 2001), number (Brody and Mitchell 1997), color (Stanton et al. 1989, Rausher and Fry 1993, Melendez-Ackerman et al. 1997, Schemske and Bradshaw 1999), nectar production (Real and Rathcke 1991, Mitchell and Waser 1992, Irwin and Brody 2000), and floral scent (Dobson 1994, Raguso 2001). However, traits attractive to pollinators can also attract pests (Adler and Bronstein 2004). Cucumber beetles are often found in cucurbit flowers, where they feed on pollen (Fronk and Slater 1956, Bach 1977, Fisher et al. 1984, Andersen and Metcalf 1986, 1987, Metcalf and Metcalf 1992). Floral scent volatiles are attractive to several cucumber beetle species (Andersen and Metcalf 1986, Metcalf et al. 1995, Metcalf et al. 1998), and the floral volatiles TIC (1,2,4-trimethoxybenzene, indole, and *trans*-cinnamaldehyde) are particularly effective in baits (Lampman and Metcalf 1987, Lewis et al. 1990, Hoffmann et al. 1996a). Total volatile emissions across *Cucurbita pepo*, *C. maxima*, and *C. moschata* cultivars predicted which blossoms beetles preferred in the field. Thus, scent traits may make crop varieties attractive to both pollinators and cucumber beetles. The relative importance of beetle damage and pollinator service on yield has not been determined for any cucurbit crop except cantaloupe (Strauss and Murch 2004), so we have no data to predict whether the benefits of attracting pollinators outweigh the costs of attracting cucumber beetles.

Aside from the financial expense, environmental impacts, and human health concerns of pesticide use, pesticides can also *reduce* crop yield by killing pollinating bees. In a low pest year, foliar pesticides reduced pollination and thus cantaloupe yield by 18.5% compared to plots with no pesticides (Brust and Foster 1995). In California, organic watermelon farms near natural areas receive sufficient pollination entirely from native bees, while conventional farms had to rely on hired honey bees for full pollination (Kremen et al. 2002). Developing and implementing pest management strategies that reduce pesticide use will have the additional benefit of increasing pollination.

Value of perimeter trap cropping

Perimeter trap cropping ('PTC' hereafter) is a systems approach to pest control that designs the crop layout to take advantage of pest colonization behavior and host preference. Border defenses are established by planting a more attractive trap crop to completely encircle the main crop, resulting in reduced infestation and reduced need for insecticides in the main crop (Aluja et al. 1997, Mitchell et al. 2000, Boucher et al. 2003a, Boucher et al. 2003b). In cucurbits, Blue Hubbard (*C. maxima*) is highly preferred by striped cucumber beetle (*Acalymma vittatum*) relative to butternut squash (*C. moschata*) (McGrath 2002, R. Hazzard, unpublished data), summer squash, and cucumber (*Cucumis sativus)* (Boucher and Durgy 2003, 2004). When early-season beetles encounter a perimeter of Blue Hubbard, they are likely to remain there rather than moving to the main crop. Pesticides can be used to kill pest populations on the perimeter while the need for pesticides is eliminated or dramatically reduced in the commercial crop. While this method has been effective in controlling pest populations and reducing pesticide applications to the main crop, we have little information on how effective different crops function as effective perimeter traps, and what mechanisms (attraction of beetles vs. pollinators) are most important for yield.

The ideal perimeter trap crop in cucurbits is highly attractive to striped cucumber beetle adults, has fast germination and strong seedling growth, has relatively low susceptibility to *E. tracheiphila* so that it does not serve as a reservoir for infection of the main crop, and produces marketable fruit. Blue Hubbard squash meets these criteria. Market demand is not unlimited, but participating growers have marketed their border crops successfully. However, there are several reasons to research the potential of other PTC cultivars and species: (1) Growers want to be able to choose from several possible trap crops to suit their markets, since there is limited demand for Blue Hubbard, (2) We do not know what effect Blue Hubbard or other PTC have on yield via pollinator attraction, and (3) As a systems approach, we want to identify flexible points and underlying principles in the system that growers can build upon. Identification of traits attractive to both pollinators and cucumber beetles will aid in selecting perimeter crops that are most effective at increasing focal crop yield while reducing pesticide use.

(b) Objectives

We propose to address 3 objectives over 2 years:

Research Objectives

Objective 1. (Year 1) Correlate pollination and beetle damage, including bacterial wilt, with floral and vegetative traits across 20 cucurbit species and cultivars to identify traits involved in attraction and resistance, and to predict likely cultivars for PTC.

Objective 2a. (Year 1) Compare yield of butternut squash (*Cucurbita moschata*) using 5 different perimeter trap crops in an experimental farm setting.

Objective 2b. (Year 1) Determine how these 5 PTC affect yield via attraction or resistance to cucumber beetles and pollinating bees.

Together, these research objectives will help identify crops and traits most appropriate for perimeter trap crops, evaluate the efficacy of 5 trap crop cultivars for increasing yield, and determine the extent to which perimeter trap crops are effective via the mechanisms of reducing pest damage and increasing pollination.

Extension Objectives

Objective 3a. (Year 2) Select 3 cultivars based on data from Objectives 1 and 2, implement PTC using these crops in commercial fields of butternut squash, and evaluate effects on yield compared to fields with conventional pest management practices.

Objective 3b. (Year 2) Determine how these 3 PTC affect yield via attraction or resistance to cucumber beetles and pollinating bees.

The extension objectives will take results from our research and apply them directly in growers' fields. We will compare the efficacy of 3 different perimeter trap crops for reducing pesticide use and increasing yield compared to conventional crops, and determine how each crop influences beetle damage and pollination. Results of these studies will be communicated to growers through electronic and printed publications and educational programs (see **Implementation and Evaluation Plans**).

(c) Approach and Procedures

Study system

Field sites. Experiments in Year 1 will take place at the South Deerfield Research Farm, operated by the University of Massachusetts. Ruth Hazzard has conducted research there on butternut squash and other vegetable crops for 16 years. The station has Occum fine sandy loam soil, provides field preparation and irrigation services, and has adequate space for the proposed research. In Year 2, evaluation of PTC will take place in 25 fields on commercial vegetable farms in Massachusetts.

Cucurbit cultivars. Butternut squash (*C. moschata*), other winter squash, pumpkins and gourds are generally direct-seeded in late May or early June when soils are sufficiently warm for germination. Fruit set begins in early to mid July, and the last fruit that is expected to reach maturity is pollinated by mid August. Harvest takes place in late August and September. Growers have begun to use transplants based on evidence that yield is higher, and we will transplant seedlings at the cotyledon stage for experiments in Year 1. Seed for experimental plots will be obtained from commercial seed suppliers (for commercial cultivars) or USDA seed labs (for wild or unnamed germplasm).

Flowers of most cucurbit crops are open for one day only, providing a short window for pollination. Most cucurbit crops are monoecious, with separate male and female flowers on the same plant. Male flowers are produced first, and female flowers are produced later in the season. Because pollen and ovaries are not in the same flower, squash plants require the services of a pollinator in order to set fruit.

Cucumber beetles and other pests. Cucumber beetles are common pests of cucurbit crops throughout the United States. Cucurbitacins, tetracyclic triterpenoids produced as defenses in members of the squash family, are stimulants that promote compulsive feeding by these beetles (Chambliss and Jones 1966, Sharma and Hall 1973, Metcalf et al. 1980), and the consumption of these compounds does not appear to reduce beetle fitness (Tallamy and Gorski 1997). The major insect pest of cucurbit crops in Massachusetts is *Acalymma vittatum*, the striped cucumber beetle. In Massachusetts, cucumber beetles typically do not become active in crop fields until after June 6, and peak colonization occurs about a week later. Spotted cucumber beetle (*D. undecimpunctata howardi*) is of lesser importance, colonizing fields after flowering. Squash bug (*Anasa tristis*) and aphids are occasional pests, but rarely reach damaging levels in butternut squash.

Pollinating bees. Squash crops are pollinated by specialist squash bees in the genera *Peponapis* and *Xenoglossa* (Anthophoridae), as well as by bumblebees (*Bombus* spp.; Apidae) and the introduced honey bee *Apis mellifera* (Apidae). Native bees are typically more efficient pollinators than honey bees (Stanghellini et al. 1997, Kremen et al. 2002, Stanghellini et al. 2002), requiring fewer flower visits to set fruit. In northeastern states, *Peponapis pruinosa* ranks above honey bees as the most important pollinator of pumpkins (Riggs 2003) and is likely to be similarly important for related crops.

Effectiveness of Perimeter Trap Crops (PTC)

Perimeter trap cropping to arrest striped cucumber beetles as they enter cucurbit fields has been evaluated in small plots and on commercial farms in CT and MA from 2001-2004, using Blue Hubbard squash in a single or double row surrounding a main crop of cucumber, summer squash, or butternut squash. Regardless of the direction of entry into the field, beetles encounter an attractive host plant. The perimeter is established either by hand planting at the same time as the main crop, or in machine-planted fields, growers drive the planter around the perimeter of the field. The same crop spacing is used as in the main crop. Where beetle invasion is expected to be especially heavy, such as adjacent to woodlands or other overwintering sites, a double row may be planted to ensure that the perimeter is not breached. Borders must be planted in good soil where germination will be as rapid as the main crop. Borders are treated with imidicloprid as a furrow drench at planting or with carbaryl as a foliar spray at first observation of any cucumber beetles and weekly thereafter as needed. Thresholds are used to determine spray decisions for the main crop based on weekly scouting.

In 2004, seven MA and CT growers using PTC with Blue Hubbard (*C. maxima*) around butternut squash reduced pesticide use by 90-95% with no loss in yield (R Hazzard, unpublished data). PTC fields received only perimeter sprays because the main crops never exceeded economic threshold of one beetle per plant; conventional fields were treated with imidcloprid at planting or with carbaryl after the threshold was exceeded. During the peak of beetle colonization, beetles in PTC fields were concentrated in blue hubbard borders (1.2 beetles/plant), with fewer beetles (0.16 beetles/plant) in the main butternut crop. In conventional fields, beetles distributed themselves throughout the field and both the main crop and border had high numbers of beetles (1.56 and 1.40 beetles/plant, respectively). PTC reduced pesticide cost by $7 to $80 per acre while also reducing risks to pollinating bees. In summer squash on-farm trials in CT, PTC reduced pesticide use by 95% while yield was increased as a result of fewer losses from bacterial wilt and beetle feeding damage (Boucher and Durgy 2004). Eighty five percent of the growers who participated in these PTC trials stated that they were satisfied or highly satisfied with the PTC system, believe it saved them time, money and pesticide, is easier to use, produces equivalent or better yields, and they plan to continue using it in the future (Boucher and Durgy 2004, R. Hazzard, unpublished data). Additional growers in CT have adopted a PTC system using cherry pepper borders to control pepper maggot fly in bell peppers (Boucher et al. 2003a).

Experiments conducted in 2003 using small (20' x 20', separated by 50') butternut plots also demonstrated that PTC is effective at keeping beetles out of main crops. Plots with Blue Hubbard borders had, on average, eight times more beetles per plant in the border than in the main crop. Plots with unsprayed Hubbard borders had 50% fewer beetles than control plots, and plots with sprayed Hubbard borders had 60% fewer beetles than control plots.

Although Blue Hubbard is effective in reducing pests, growers want to be able to choose from several possible trap crops to suit their markets, since there is limited demand for Blue Hubbard. Variety trials suggest that other cultivars may also be effective. Certain varieties of zucchini and summer squash are also highly attractive to beetles (Hoffmann et al. 1996b, McGrath 2002) and may have potential for PTC; however, their susceptibility to wilt is generally high. Wild *Cucurbita* species have much higher cucurbitacin levels than cultivated species, and are many times more attractive to beetles in field trials (Metcalf et al. 1982). Thus, wild or crop-wild hybrids have potential as perimeter trap crops despite producing no marketable fruit, if the yield gain from reduced beetles exceeds the loss in yield from borders.

Experimental Methods

Objective 1. (Year 1) Correlate pollination and beetle damage, including bacterial wilt, with floral and vegetative traits across 20 cucurbit species and cultivars to identify traits involved in attraction and resistance, and to predict likely cultivars for PTC.

There are hundreds of cultivars of cucurbit crops commonly grown in the United States, from at least 8 different species (*Cucurbita pepo, moschata, maxima, argyrosperma, ficifolia, Cucumis melo, sativus,* and *Citrullus lanatus*). Other species are being introduced as crops from Asian, Latino or African cultures and have rapidly increasing markets in Massachusetts, such as *Benincasa hispada* (Chinese winter melon), *Legenaria siceraria* (calabash gourd), and *Momordica charantia* (bitter melon). We will use a subset of 20 cultivars from 14 species, representing a range of commonly grown local crops, cultivars preferred by Latino and Asian markets, and wild species. Identifying traits correlated with beetle and pollinator attraction will provide a valuable tool for assessing the potential of cucurbit cultivars as main crops and PTC.

Design: We will compare 20 cultivars from 14 different species to get the broadest range of variation across floral, leaf and root traits. We will include the commonly grown crops *Cucurbita pepo* (standard pumpkin, zucchini, and sugar pumpkin), *C. moschata* (butternut and calabaza), *C. maxima* (blue hubbard, buttercup, giant pumpkin, and cinderella), *Cucumis melo* (cantaloupe), *Cucumis sativus* (cucumber) and *Citrillus lanatus* (watermelon), the cultivated gourd *Cucurbita argyrosperma,* the recently expanding crops *Benincasa hispada* (Chinese winter melon), *Legenaria siceraria* (calabash gourd), and *Momordica charantia* (bitter melon), and the wild species *Cucurbita texana, C. foetidoessima*, *C. ecuadoriensis*, and *C. andreana*. The four wild species were chosen to represent the range of cucurbitacin types found in roots, leaves and fruits; *C. texana* and *foetidoessima* both contain predominantly cucurbitacins B and D, while *C. ecuadoriensis* and *C. andreana* contain predominantly E and I (Metcalf et al. 1982). Because cucumber beetles are highly attracted to species with high cucurbitacin concentrations (Metcalf et al. 1982), wild species or crop-wild hybrids may provide an unexplored avenue for effective trap cropping (Rhodes et al. 1980).

Plants will be germinated from seed in greenhouse flats, and transplanted to the experimental farm at the one leaf stage, while cotyledons are still healthy and attractive for cucumber beetle feeding. To maintain consistency of crop stage at planting, we will germinate seeds in flats in the greenhouse, and delay faster-growing varieties with cooler temperatures.

We will use 3 replicates of each cultivar, for a total of 60 plants. Cultivars will be planted in a completely randomized design, with 3 m between plants to prevent intertwining. We will initially plant 2 individuals per replicate to insure survival of at least one representative per replicate. If both plants survive, they will be thinned to one plant after they reach the 5-leaf stage. Vines will be trained so that individuals can be discerned easily. Fertility and weeds will be managed according to standard practices for commercial production (Howell et al. 2004) and overhead irrigation will be provided as needed. Beetle numbers and damage, bacterial wilt, and pollinator preference and efficiency will be assessed on these plants at the farm. Five additional replicates of each species will be grown in the greenhouse and used to measure plant traits in the absence of insects, to avoid spurious correlations between traits and damage due to induced defenses.

Pollinator attraction and efficiency. Pollinator visits will be observed at least 3 days/week between 0700 and 1000, when pollinators are most active. The order of observations on individual plants or subplots will be re-randomized daily to prevent bias; every plant will be observed during each day of observations. For every insect visitor, the plant species/cultivar, number of flowers visited, flower sex, time spent per flower, insect behavior (nectar feeding, pollen feeding, floral damage, mating, a mix of behaviors, or no behavior) will be recorded using hand-held tape recorders. Vouchers of all insect visitors will be collected, identified to species, and placed in the University of Massachusetts at Amherst insect collection.

To assess the efficiency of different pollinating bees, we will bag female buds one day before opening to prevent pollinator visitation. We will open bags during observation on each plant, collect the stigma after the first pollinator visit, and record the identity of the pollinator. We will attempt to collect at least 10 stigmas per common pollinator species per cultivar. Stigmas will be collected into clean microcentrifuge vials and stored in a portable cooler while being transported to a laboratory refrigerator. Stigmas will be softened by treating with KOH for 6 hours, briefly stained in 3% fuchsin, squashed onto microscope slides, and scored at 40X magnification (as in Kremen et al. 2002 for watermelon pollination). This will provide a measure of how much pollen is deposited by each of the common floral visitors, including cucumber beetles, and whether pollinator efficiency varies across different cucurbit cultivars or species.

Beetle resistance, damage and bacterial wilt. We will census the number and species of cucumber beetles on each plant weekly from planting to the five-leaf stage, and twice during flowering. Beetles will be counted on cotyledons, leaves, stem, and on soil at the base of the stem. During flowering, wilted flowers from that day will be collected into plastic bags, placed in coolers and brought to the lab to count and identify beetles in blossoms (as in Andersen and Metcalf 1987). Leaf and flower damage will be rated on a 0-5 scale of 20% increments (as in Hoffmann et al. 1996b). Bacterial wilt will be assessed with ratings on a 0-3 scale (0 = no symptoms, 3 = dead); the crown of dead plants will be returned to the laboratory to confirm presence of *Erwinia* in the vascular system. All bacterial wilt analyses will be performed by Dr. Robert Wick, a plant pathologist in charge of the vegetable diagnostics lab at the University of Massachusetts at Amherst (see Letter of Support).

Vegetative traits in greenhouse plants. We will grow 5 replicate plants of each cultivar in the greenhouse to measure traits in the absence of interactions with herbivores. Toughness will be measured using a penetrometer at the tip (next to the midrib) of the 3rd fully expanded leaf of each plant. Trichome density will be assessed by counting all trichomes using a dissecting microscope on the disc cut by the penetrometer. We will measure water content by collecting and immediately weighing the 5th fully expanded leaf on each plant. This leaf will then be dried and re-weighed to calculate water content. The same leaf will be ground and analyzed for total tissue P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, B, Mo, Pb, Cd, Ni, and Cr by ICP spectrometry of dry-ashed sample in 10% HCl. In addition, total nitrogen will be analyzed by catalytic combustion. The University of Massachusetts Soil and Plant Tissue Testing Laboratory provides these analyses for a standard fee (http://www.umass.edu/plsoils/soiltest/services1.htm). Due to budget constraints, nutritional analyses will be performed on a subset of 3 randomly chosen plants per cultivar. Because larval cucumber beetles feed on roots, elemental analyses will also be conducted on 3 samples of root tissue per cultivar, collected by destructive sampling after floral traits are measured.

Cucurbitacins in greenhouse plants. Previous studies have found no cucurbitacins in the leaves, roots or fruits of cultivated *C. maxima, C. moschata,* and *C. pepo* (Metcalf et al. 1982). However, *C. maxima* flowers have curcurbitacins, while *C. pepo* and *C. moschata* do not (Andersen and Metcalf 1987); cucurbitacin levels in flowers of other species are unknown. We will measure cucurbitacin levels on 2 flower samples from each cultivar, and in 3 leaf and root samples from all species except *C. maxima, pepo* and *moschata*.

Floral traits in greenhouse plants. In a previous study, *C. maxima* blossoms were preferred by cucumber beetles over blossoms of *C. pepo* and *C. moschata* cultivars (Andersen and Metcalf 1987). Cucurbitacins were found only in the stamens of *C. maxima* cultivars. Additionally, floral volatile emissions were higher in cultivars of *C. maxima* than *C. moschata* or *C. pepo* (Andersen and Metcalf 1987). Thus, cucurbitacins and/or floral scent are likely candidate traits involved in beetle attraction. By screening these and other floral traits across a range of cultivars from an extended group of species, we hope to find a range of volatile, chemical, reward and color combinations to dissect which traits, singly or in combination, are most responsible for pest resistance or attraction.

We will measure morphology and rewards on the first 3 male and female flowers per plant. Flowers are open for only one morning. We will measure floral length, diameter, and style/anther height, and total mg pollen on male flowers. We will collect nectar with microcapillary tubes to measure volume, and measure sugar concentration with a pocket refractometer. Flowers will then be collected and pooled for cucurbitacin analysis (see above).

Floral volatile measures will be conducted by Dr. Nina Theis of the Arnold Arboretum, who has broad experience analyzing floral scent in a range of systems. Volatiles will be collected from 3 flowers per plant (2 male and one female) using dynamic headspace sampling. Ambient air will be blown over enclosed floral samples. The outgoing air-stream flows over an adsorbent material (Poropak) for collection. The compounds are then eluted from the adsorbent with hexane, concentrated and injected into a gas chromatogram attached to a mass spectrometer (GC-MS) for identification and quantification. Compounds are identified through a combination of retention time (from previously injected standards) and mass spectral libraries [Wiley 1995 and NIST 1998 libraries (with greater than 120,000 mass spectra)]. Quantification is accomplished by relating mass ions of each scent compound to the mass ions of the internal standard, using previously run serial dilutions (Theis and Raguso in review, Theis et al. in review-a, b). Volatile profiles are typically complex, and we will use principal components analysis to reduce the number of variables (Sharma 1996).

Statistical analyses. We will use multiple regression to determine how vegetative and floral traits affect pollinator preference, beetle abundance, beetle damage, and bacterial wilt. While multiple regression analyzes correlational rather than causational relationships, it is a powerful tool to identify likely traits involved in pest resistance and pollinator attraction that can be experimentally tested in future studies. We will test for correlations between predictor variables that could lead to multicollinearity and unstable models by examining eigenvalues and variance inflation factors (Philippi 1993). If serious multicollinearity exists, we will use stepwise regression to select subsets of traits that best predict pest resistance and pollinator preference. By using a wide range of cucurbit species and cultivars, we hope to sample a broad range of trait variation and combinations to effectively dissect traits involved in these interactions.

Objective 2a. (Year 1) Compare yield of butternut squash (*Cucurbita moschata*) using 5 different perimeter trap crops in an experimental farm setting.

The ideal PTC is attractive to beetles, but we do not know whether attractiveness to pollinators is desirable to increase butternut yield. If an attractive PTC brings more pollinators to the whole plot, this could result in greater pollination of the main crop. However, if an attractive PTC competes with butternut squash for pollinators, then the ideal PTC choice would be a cultivar that attracts beetles but not pollinators. We will evaluate the relative effects of different PTC cultivars on butternut squash yield via pest resistance and pollinator attraction in an experimental farm setting.

Cultivars will include *C. maxima*, (Blue Hubbard and Red Kuri, a buttercup type), *C. pepo* (zucchini), *C. foetidissima*, a native wild species highly attractive to diabroticite beetles (Sharma and Hall 1973), and butternut borders (*C. moschata*) as a control. These cultivars were selected by the following criteria: (1) all have been demonstrated to be highly attractive to cucumber beetles in field studies (Sharma and Hall 1973, Metcalf et al. 1982, Andersen and Metcalf 1987), and (2) these species vary 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.

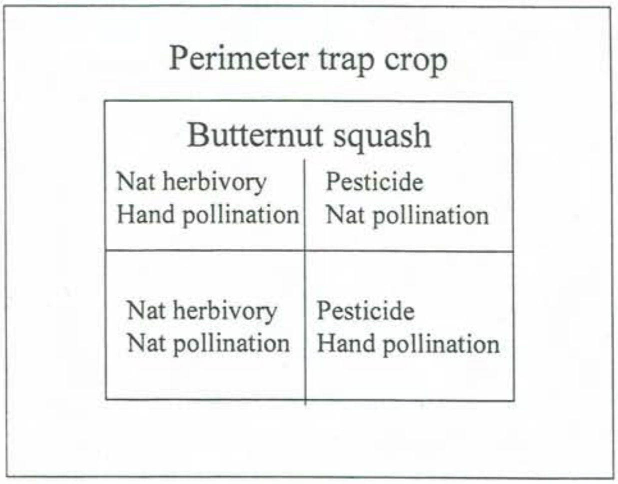
Design: Butternut squash and PTC will be germinated from seed in greenhouse flats and transplanted to the South Deerfield experimental farm at the 1-leaf stage in the first week of June. We will plant 5 replicate plots of each PTC surrounding a focal crop of butternut squash, for a total of 25 plots. Border treatments will be randomly assigned to fields. Each plot will be 50 X 35 ft, with 2 feet in-row and 5 ft between-row spacing, surrounded by a perimeter of one row of PTC plants. Perimeter plants will be treated with imidicloprid (Admire 27 @0.02ml/plant) 24h prior to planting. Plots will be separated by a fallow area fifty feet wide to reduce the influence of borders on adjacent plots. Butternut squash will be planted in 4 quadrats in each plot separated by an empty row of 8 ft for additional treatments described in **Obj 2b**. Plants will be thinned one row inside the border after establishment to prevent competition with the border crop, and vines will be trained to stay within their quadrat. PTC cultivars will be assigned randomly to plots. Fertility (preplant with side dress as needed), weed management (pre-emergence herbicide and cultivation) and irrigation (overhead) will follow standard commercial practices. Butternut squash yield will be assessed by harvesting fruit at maturity, counting and weighing all squash in each plot.

Statistical analyses: The effect of PTC cultivar on butternut yield will be analyzed within a larger MANOVA model (see **Obj 2b**, *Statistical analyses*) to determine how perimeter crop affects the number of squash produced and mean squash weight per plot. This experiment will provide a test case to compare the efficacy of new cultivars and butternut squash controls as effective PTCs for increasing squash yield.

Objective 2b. (Year 1) Determine how these 4 PTC affect yield via resistance to cucumber beetles and attraction of pollinating bees.

This experiment will use the same plots as **Obj 2a**. We will subdivide each plot into four quadrats, each assigned to a different treatment combination. We will use 2x2 factorial combinations of pollination treatments (natural pollination vs. hand pollination) and herbivory

Figure 1. Example of plot layout for Objective 2b.



treatments (natural herbivory vs. conventional pesticide; Fig. 1). Because flowers are open for only one day, all female flowers in the hand pollination treatments will be pollinated every day from the onset of female flowering in mid July through the second week of August. Fruit that sets after that date is unlikely to mature. Pollen will be collected from randomly selected male flowers across plots and mixed in a microcentrifuge vial for use in hand pollination treatments. Pollen will be added with a camelhair paintbrush to cover the entire stigmatic surface of all flowers in the hand-pollination treatment. All plants will also be open to natural pollinator visits. For the conventional pesticide treatment, seedlings will be treated with imidicloprid prior to planting as described above; natural herbivory plants will be treated with water only to control for effects of added water.

Beetle resistance, damage and bacterial wilt. We will census beetle resistance, damage and bacterial wilt as in **Obj. 1** on 3 randomly chosen plants per treatment combination per plot (12 plants/plot; 300 plants total) each week from the time of transplanting until flowering. Values will be averaged within quadrats in each plot for analysis.

Pollinator attraction. Insect visits to flowers in each quadrat per plot will be observed for 5 minutes each day between 0600 and 1000 at least 4 times per week (4 quadrats/plot x 25 plots x 5 minutes = 500 minutes; 2 observers will be used simultaneously) following the methods of **Obj 1**. Because squash plants are vines that readily intertwine, we will not distinguish between individual plants.

Butternut squash yield. Yield will be assessed as described in **Obj 2a**, but will be measured separately for each quadrat in each plot.

Statistical analyses. The design of this experiment is a split-plot, with PTC cultivar as the main plot and herbivory and pollination treatments as subplot effects. We will analyze the effect of herbivory, pollination, PTC cultivar and their interactions on components of yield to determine whether (1) pollination increases yield (pollination treatment effect), (2) herbivory reduces yield (herbivory treatment effect); (3) PTC cultivar affects yield (cultivar effect, incorporating Tukey's pairwise comparison of means to determine which cultivars are statistically different); (4) whether the effect of pollination on yield changes with different PTC crops (pollination x cultivar interaction), which could occur if some PTC cultivars are more beneficial for pollinators than others, (5) whether the effect of pests on yield changes with different PTC crops (herbivory x cultivar interaction), which could occur if some PTC cultivars confer greater resistance to pests than others, and (6) whether supplemental pollination reduces the effect of pests on yield (herbivory x pollination interaction), as was found in Strauss et. al. (2004) using cantaloupes. **Our design will allow us to determine which PTC have the greatest effects on yield, and how much of this benefit is due to attraction of pollinators *versus* resistance to beetles.**

Objective 3a. (Year 2) Select 3 cultivars based on data from Objectives 1 and 2, implement PTC using these crops in commercial fields of butternut squash, and evaluate effects on yield compared to fields with conventional pest management practices.

Selection of PTC cultivars. We will use the most suitable cultivars tested in **Obj. 1** and **Obj. 2**, based on several criteria: high attraction of beetles, low seed cost, potential marketability, and low susceptibility to bacterial wilt. Pollinator criteria will depend on the outcome of PTC plot studies in Objective 2, in which we will determine whether pollinator attractiveness in PTC increases or decreases yield in the main crop.

Design. We will use 5 fields for each cultivar and 5 additional fields with conventional butternut without a border crop, for a total of 20 fields. Growers will plant whole fields (1-5 acres per field). No fields will have been planted to butternut the previous year. Fields will be separated by at least 250 feet of non-agricultural habitat (woods, roads, fallow fields) or other non-cucurbit crops. This will ensure that colonization of each field by cucumber beetles is not influenced by presence or absence of borders in other fields. Crops will be planted during a 3-week period between May 20 and June 10 following grower's normal schedules. The trap crop will be seeded at the same time and spacing as the main crop, in good soil, along the entire crop perimeter. For machine planted fields, growers will drive their planter across the rows to seed the trap crop at the ends of the fields. For hand planted fields, growers will seed two plants at the end of every row of the main crop. Crops will be grown according to standard grower practices, including fertilizer application according to soil tests, and irrigated as needed.

Perimeters will be treated with imidicloprid at planting (furrow drench, 1.1oz Admire/1000 feet of row) or with carbaryl (Sevin XLR Plus @1qt/acre) as a banded foliar spray at first observation of beetles in the border, and weekly thereafter if beetle activity remains high. Insecticide (carbaryl) will be applied to the main crop only if beetle numbers exceed 2 beetles per plant.

Yield. Growers will keep records of management practices and total yield for the field. Yield samples will be also taken from six 6' X 12' quadrats per field; fruit will be counted, weighed, and graded for marketable and cull fruit.

Statistical analyses. Because fields managed by individual farmers are scattered throughout the growing region, fields will be treated as independent. Thus, field is the unit of replication ($n = 5$). We will analyze the effect of PTC cultivars and conventional agricultural (i.e., 4 treatments) on components of yield using one-way ANOVA.

Objective 3b. (Year 2) Determine how these 3 PTC affect yield via attraction or resistance to cucumber beetles and pollinating bees.

Assessing beetle damage and bacterial wilt. This experiment will use the same fields as **Obj 3a**. Fields will be scouted twice weekly from emergence to the 4-leaf stage, and weekly thereafter. We will sample 5 sets of 5 plants each in borders and in the main field along a transect midway from the border to the center of the field. Live and dead beetles on leaves or soil within 12" of the plant will be counted; cotyledons and leaves will be rated for defoliation on a scale from 0 to 5 in 20% increments. Border and main field plants will be rated for bacterial wilt twice in July at flower initiation and two weeks later, when symptoms typically develop. We will walk transects along the border and through the center of the main crop and rate plants at every 10 ft on a 0-3 scale (0=no symptoms, 3= dead) for bacterial wilt damage.

Pollinator abundance and pollen limitation. Commercial hives are scattered throughout the local area, so it is anticipated that honeybees will be present in every field. We will observe pollinators, recording the identity of all bee species, at randomly selected points in main and border crops at least twice per field during the flowering season. We will use these estimates of pollinator abundance combined with pollinator efficiency of each bee species (measured in **Obj. 1**) to estimate the extent to which honey bees and native bees pollinate butternut squash (as in Kremen et al. 2002).

To determine whether squash receive sufficient pollination for full fruit set, we will perform hand-pollination treatments. Because squash flowers are only open for one day, we can compare fruit set and the probability of abortion due to lack of pollination on comparable flowers open on the same day. We will visit each field two times during the season in a random order; each field will be visited once before any is visited a second time. At each visit we will define 3 5'x5' plots within the field, hand-pollinate every female flower, and mark flowers with a piece of colored twine. All female flowers in 3 additional 5'x5' plots will be marked with a different color twine but not pollinated. After 2 weeks, each flower will be scored as having set fruit or aborted, and twine will be removed. We will calculate the average percent fruit set in hand-pollinated and control plots in each field.

Statistical analyses. The extent of pollen limitation will be determined with a t-test comparing the percent fruit set in hand-pollinated and naturally pollinated plants, using field as the unit of replication. The effect of cultivar on pollinator diversity and abundance, and on beetle abundance, damage and bacterial wilt, will be analyzed with one-way MANOVAs. This experiment will provide a comprehensive assessment of the effect of several PTC cultivars on pest management, pollinator activity and crop yield in local farms, and allow growers to compare yield from conventional farming practices to those of PTC.

(d) Cooperation and Institutional Units Involved

This research involves a collaboration between two members of the Division of Entomology in the Department of Plant, Soil and Insect of the University of Massachusetts at Amherst: Ruth Hazzard, a vegetable crops extension specialist with 16 years of experience studying squash and other vegetable crops, and Dr. Lynn Adler, a new faculty member who studies the ecology of plant-insect interactions. We are both excited about the synergy of basic and applied interests to address questions that are of fundamental interest to vegetable growers in the Northeast, and the applications of this research to reduce pesticide use and increase crop yield in Massachusetts. Because cucurbit crops are extensively grown throughout the Northeast, we anticipate expanding our efforts to include research and extension with researchers and growers in other states after establishing this research program.

This proposal also involves the cooperation of Dr. Nina Theis of the Arnold Arboretum, who will conduct floral scent analyses and contribute to the analysis and publication of **Objective 1**. Dr. Theis has broad experience analyzing floral scent in a range of systems from her PhD and postdoctoral research. In addition, Dr. Rob Wick, a plant pathologist who heads the vegetable diagnostics lab at the University of Massachusetts at Amherst, will cooperate in this project by providing diagnostic confirmation of bacterial wilt in squash samples.

The University of Massachusetts Agronomy Farm will provide fields, plot preparation, and farm equipment for establishing and maintaining experimental plots. Vegetable farmers in Massachusetts will collaborate with the project by establishing and maintaining perimeter trap crops in commercial butternut fields, in accordance with the experimental plan (see letters of support).

(e) Implementation and Evaluation Plans

Objectives 1 and 2: The development of perimeter trap cropping systems in cucurbit crops builds upon development and implementation of IPM in winter squash and pumpkin that started in 1998. This program has emphasized management of cucumber beetle and bacterial wilt as well as key diseases such as *Phytophthora*, blackrot and *Plectosporium*. Grower response to PTC trials conducted from 2003-2004 in MA and CT of the blue hubbard system in cucumber, summer squash, pumpkin and butternut has been very positive; we believe that the time and cost savings combined with equal or improved control and yield will encourage wider adoption of PTC in all vine crops. Better understanding of pollinators and whether lack of effective pollinators may be limiting yield will add an important element to reduced-risk IPM/ICM and integrates easily with PTC, especially since PTC reduces pesticide risk to pollinators.

Objective 3: We will maintain a close working relationship with cooperating growers, building upon past connections from 16 years of IPM implementation in Massachusetts. We will meet with growers as a group both before and after the growing season to plan and to evaluate; in particular, we will solicit input on their practical concerns for implementing PTC. In addition, each participant will be interviewed after the growing season, using a survey instrument developed for our current NE/SARE project LNE-03-177 "Popularizing a novel whole-farm systems approach to pest management on vegetable farms," in which Ruth Hazzard is co-PD with Dr. Jude Boucher of University of Connecticut. This survey will be modified to measure growers' assessment of pollinators in addition to pest management. These surveys include detailed questions about PTC benefits and implementation costs, including pre- and post-adoption pesticide use history and estimates for pest damage/crop quality/yield losses, estimates of all implementation costs and income, and ratings for impacts on environment/landwater, personal liability, and personal exposure for applicators and workers. It will also use ratings for simplicity of PTC use and overall user satisfaction, and likelihood of continued adoption of PTC in butternut and other crops. Growers will also provide pesticide and yield records, and we will take yield samples in each field. This information will allow us to construct partial enterprise budgets for PTC vs. conventional practices and to assess quantitative and qualitative impacts (i.e. pesticide reductions/user satisfaction).

Extension activities will include reports, fact sheets and newsletter articles disseminated through newsletters (*UMass Vegetable Notes*, e.g. Boucher et al 2003b) and websites (www.umassvegetable.org and regional websites) as well as presentations at educational programs including twilight meetings at cooperating farms and regional conferences (e. g., New England Vegetable and Berry Conference). R. Hazzard serves on program planning committees of the New England Vegetable and Berry Extension Conference and the NEVBGA, and as team leader coordinates programs for the UMass Vegetable Program.

We will also develop more active relationships with beekeeper associations in Massachusetts and with commercial beekeepers in order to foster wider knowledge about and use of pollinators in vine crops, and to disseminate information about pollination services. Surveys conducted annually in conjunction with the New England Vegetable and Berry Growers Association on pesticide use in vegetable crops will include questions regarding use of PTC and pollinators in cucurbit crops. These surveys are funded for winters of 2006 and 2007 by an EPA Region I Grant under the leadership of Dr. A. Richard Bonanno, UMass Extension weed specialist, and are administered through the NEVBGA.

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(9) Key Personnel

(a) This proposal involves the PD, Lynn S. Adler, the co-PD, Ruth Hazzard, and two consultants, Dr. Rob Wick and Dr. Nina Theis.

Roles and responsibilities

Lynn S. Adler of the Division of Entomology, University of Massachusetts at Amherst, will be responsible for overseeing and implementing all research goals of this proposal (Year 1), including publication of results. Ruth Hazzard will provide support in Year 1 for planting and management of all experiments based on her 16 years of research experience in vegetable crops.

Ruth Hazzard of the Division of Entomology, University of Massachusetts at Amherst, will be responsible for overseeing and implementing all extension goals of this proposal (Year 2), including communication of results to growers. Lynn Adler will assist in pollinator and herbivore censuses, data management, and analysis in Year 2 based on her experience in insect ecology and statistical analysis.

Nina Theis of the Arnold Arboretum will serve as a collaborator for the analysis of floral volatiles outlined in Objective 1. Dr. Theis, through her PhD and postdoctoral research, has extensive experience in floral volatile chemistry, as well as access to all necessary equipment for this work.

Rob Wick of the Division of Plant and Soil Sciences, University of Massachusetts at Amherst, will provide diagnostic confirmation of bacterial wilt in all affected samples from both years of the project. Dr. Wick is a plant pathologist and the head of the vegetable crops diagnostics lab at UMass Amherst.