ARTICLES

Yield and Enzymatic Activity of Sweet Basil (*Ocimum basilicum*) Subjected to Alternative Pest Control

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ABSTRACT. Selected pest control treatments, consisting of hand removal, horticultural oil, pyrethrum, and *Bacillus thuringiensis* var. *kurstaki* (Bt), were evaluated for sweet basil, *Ocimum basilicum* L. Treatments began 38 days after planting and continued weekly from July through October in Piedmont, Oklahoma. Herbage yield, enzyme activity, trichome number, and relative insect damage were measured at 57 (pre-anthesis) and 93 (post-anthesis) days after establishment of field plots. Yields did not differ significantly among treatments during the first harvest, but phenylalanine ammonium lyase (PAL) activity was lower in untreated control plants and syrin-

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galdazine oxidase (SAO) activity was significantly lower in both control and Bt-treated plants, as compared with other treatments. At the second harvest, both control and Bt-treated plants had significantly higher herbage yield than the other treatments. PAL and SAO activities were influenced by harvest date and pest control treatment. Control and Bt-treated plants exhibited high trichome density and low insect damage compared with other treatments at the first harvest. Although Bt appeared to be the best treatment for controlling lepidopteran pests, the innate ability of sweet basil to discourage herbivory may be sufficient when insects are not abundant. *[Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworth.com]*

KEYWORDS. Herbs, pest control, phenylalanine ammonium lyase, syringaldazine oxidase, yield

INTRODUCTION

Sweet basil, *Ocimum basilicum* L., is an aromatic and pharmaceutical plant cultivated in warm climates for essential oils (31). Between 1990 and 1992, over 6,000 metric tons of sweet basil valued at nearly 9 million dollars were imported to the United States (33), suggesting increased market opportunities for U.S. basil growers. Persistent insect pests observed on commercially-grown sweet basil, however, may reduce the yield and quality of sweet basil (24), suggesting the need for pest control to increase productivity. Currently, herbivorous insects are controlled by hand removal or treatment of plants with horticultural oil, pyrethrum, or *Bacillus thuringiensis* var. *kurstaki* (Berliner; Bt). *Bacillus thuringiensis* is particularly effective in controlling larval lepidopterans, while horticultural oil and pyrethrum are non-selective and act against both pest and beneficial insects (20). The efficacy of these treatments in increasing sweet basil yield is, however, unknown.

Basil, as with other plants, has undoubtedly evolved some defensive strategies for survival against competitors and herbivores (26). Indeed, those secondary metabolites which provide basil with desirable aromatic and flavor qualities may be part of a defensive strategy (19). Most secondary metabolites in plants are synthesized in response to environmental stresses, including attack by herbivores (30). Phenylalanine ammonium lyase (PAL) and syringaldazine oxidase (SAO) are precursory enzymes that lead to formation of secondary metabolites in higher plants, and, in turn, may enhance the innate ability of the plant to deter pests (2,10,27).

This investigation evaluated the effectiveness of alternative pest control measures and the influence of harvest date on sweet basil production. Specific objectives included: determining the influence of harvest date and pest control treatment on yield and metabolism, determining the relationship between enzyme activity and yield as a function of harvest date and method of pest control, and identifying the pest control measure and time of harvest that provides the highest yield of sweet basil.

MATERIALS AND METHODS

Seeds of sweet basil, *Ocimum basilicum* L., cv. Genovese, were obtained from Johnny's Selected Seed Company (Albion, Maine, USA) and planted at a depth of 0.2 cm on July 1 in Farfard growing mix No. 2 (Farfard Soil Company, Quebec, Canada) contained in germination trays. The trays with seeds were placed on a heated table (24°C) in a greenhouse for seed germination. After 7 days, the new seedlings were transplanted into 5.0 cm diameter peat pots containing Farfard Growing Mix No. 2 and moved to an unheated bench in the greenhouse. The seedlings remained in the greenhouse with daily watering until the second set of true leaves had formed (20 days after planting). The peat pots containing sweet basil were then placed under a large shade tree for 7 days to acclimate the plants to field conditions.

All plants were grown at Hoggard's Organic Herb Farm in Piedmont, Oklahoma, USA, during July through October, 1992. Field plots were established in a sandy-loam soil (fine, mixed, thermic Udertic Paleustolls) fertilized with aged cattle manure at a rate of 49 metric tons/ha. The soil was tilled to a depth of 30 cm and raised beds spaced 60 cm apart were formed by a Holland bed-shaper plow (Holland Transplanter Company, Holland, Michigan, USA) to a width of 60 cm and a height of 22 cm. Thick plastic mulch (0.8 mm) was centered on rows to cover approximately 15 cm of soil on either side of each plant to control weeds, reduce erosion, maintain soil moisture and a high soil night temperature, and increase herb yield (28).

The sweet basil plants, including the peat pots, were transplanted to the beds and spaced 30 cm apart within rows. At the time of transplanting, 50 ml of fish emulsion fertilizer containing 5 parts nitrogen, 1 part phosphoric acid, and 1 part potash was added to the transplanting hole for each plant. All plots were subjected to wet-dry cycling for the duration of the experiment by irrigation every 1 or 2 days using a drip tape. Field temperatures ranged from approximately 10° to 40°C.

The experiment consisted of 2 rows of 176 plants, each of which were

divided into 4 random sections of 44 plants for a randomized, complete block, experimental design with 8 replications. In each replicate, sets of 4 plants were designated for each of the 5 treatments and separated by 4 border plants. Sections and treatments were color-coded by spraying paint on the plastic mulch for quick identification.

The experimental treatments, implemented 38 days after planting, included a control in which no pest control techniques were used, hand removal of insect pests, horticultural oil spray (Safer Incorporated Sun Spray "ultra- fine" superior spray oil), pyrethrum (Fairfield American Corporation Pyrenone crop insecticidal spray), and *Bacillus thuringiensis* var. *kurstaki* (American Brand Thuricide concentrate). The horticultural oil, pyrethrum, and *Bacillus thuringiensis* were applied weekly to plants in each treatment area until run-off (approximating 7.6, 3.8, and 3.8 g/liter of horticultural oil, pyrethrum, or Bt, respectively) with a hand-pump sprayer to ensure complete coverage of foliage and resulting in 0.76 kg active ingredient (a.i.)/ha of horticultural oil, 0.38 kg a.i./ha of pyrethrum, and 0.38 kg a.i./ha of Bt. Hand removal of insect pests was done weekly by carefully moving hands through the leaves, starting at the bottom of the plant, to dislodge any insects. Samples of the dislodged insects, captured by hand and forceps, were preserved in vials containing a 70 percent alcohol/glycerin (v/v) solution for later identification. Feeding strategies and beneficial insects were identified using the descriptions of Metcalf and Metcalf (20).

Sampling. Plants were harvested before flowering (57 days after planting) and during full seed production (93 days after planting). A representative plant for each of the treatments within each replication was selected one day before harvesting and evaluated for insect damage and glandular trichome number. A relative score was assigned to the plant based on overall visible damage (Table 1). At harvest, two representative plants per replicate for each of the five treatments were cut at soil level with a knife and the aerial portion was placed in plastic bags (containing holes for ventilation) and packed in a styrofoam cooler with cold packs for transport to the laboratory. Remaining plants in the experimental plots were left in the field and not used for analysis.

Yield and enzyme activities. All plants were analyzed in laboratories at the University of Central Oklahoma, Edmond, Oklahoma, USA. The freshly harvested plant material, after storage at 5°C for 1 to 3 hours to retard tissue degradation, was weighed to determine total shoot fresh weight. The vegetative tissue was subsequently partitioned into smaller components and approximately 7 g of fresh leaf material was removed for determination of enzyme activities. The remaining leaves and stem tissue

TABLE 1. Relative Insect Damage to Plants.

Relative Insect Damage[1]	Visible Damage to Plant[2]
(Relative score)	(%)
1	0
2	1-2
3	3-4
4	5-6
5	7-10
6	11-15
7	16-25
8	26-50
9	51-99
10	Death of plant

[1]The relative insect injury to plants when based on overall damage prior to harvest.
[2]Visual estimate of the total plant.

were placed in paper bags, dried for 96 hours at 45°C, and reweighed to determine dry weight yields.

Phenylalanine ammonium lyase and syringaldazine oxidase activities, were obtained using the procedures of Bidlack et al. (1) to prepare acetone powders (25 g) from the fresh leaf samples while avoiding problems that may be encountered with the enoltautomer-borate complexes associated with direct aqueous extracts (8). The acetone powder samples were subsequently stored at −5°C until analyzed. To determine enzyme activity, the frozen acetone powder was mixed with 25 ml of 100 mM borate buffer, pH 8.7 (for PAL), or 25 ml of 100 mM phosphate buffer, pH 7.5 (for SAO), at 0° to 2°C. The resulting suspensions were kept on ice, shaken at least once every 5 min for 1 h, and then filtered through Whatman No. 54 filter paper to give a clear, crude enzyme extract containing PAL associated with the endoplasmic reticulum (34) and SAO associated with the cell wall (11). Protein concentrations of the extracts were determined according to the procedure of Bradford (3).

The PAL activity was assayed spectrophotometrically (Hewlett-Packard HP8452A diode array spectrophotometer) by monitoring changes at 290 nm in a mixture of 1.5 ml of extract and 4.5 ml of 6.68 mM phenylalanine in borate buffer (pH 8.7) over a period of 1 h at 30°C (1). Activity was expressed as the μmoles of cinnamic acid produced per hour per gram of protein.

Syringaldazine oxidase was assayed using a modification of the spectrophotometric procedures reported by Imberty et al. (14). All reagents were made the day of the enzyme assay. Within 5 sec of beginning the

assay, 0.10 ml of 2 mM syringaldazine (in dimethylsulfoxide) was mixed with 7.4 ml of 3.0 percent hydrogen peroxide (in 100 mM phosphate buffer pH 7.5). This mixture was added to 1.5 ml of enzyme extract and observed at 525 nm for synthesis of tetramethoxy-azo-bis-methylenquinone using an extinction coefficient of 65,000 mole/liter · cm^2 for 3 min. Activity was expressed as the μmoles of quinone produced per minute per gram of protein.

Statistical analyses. All experiments were analyzed as a split-pot, randomized complete block design described by Freund and Littel (9). Analyses of variance (ANOVA) and least significant differences tests were performed using the general linear model procedure (SAS PROC GLM). Simple correlations were calculated by the correlation procedure (SAS PROC CORR) in SAS (29). The ANOVA was designed with harvest as the main effect and the treatment by harvest interaction as the effect tested against residual error.

RESULTS

Insects observed on sweet basil foliage included representatives of the orders Coleoptera, Diptera, Hemiptera, Homoptera, Lepidoptera, and Orthoptera, some of which, such as the larval lepidopterans, were major insect pests (Table 2). Visitations to plants by most insects were for a relatively short time period and the observable per plant insect density was usually confined to less than 10 insects per plant at any given time. Damage to foliage by herbivorous insects was usually observed as holes in the leaves and cutting around the edges of leaves. No general infestations of any insect order occurred during the study.

Leaf, stem, and total fresh weight were significantly different between harvests and a significantly different harvest by treatment interaction was noted for stem and total fresh weight (Table 3). Fresh weight yields were not significantly different within the first harvest, but in the second harvest the total fresh weight of plants treated with Bt was significantly higher than the total fresh weight of hand, oil, and pyrethrum treatments. The Bt treatment significantly increased stem fresh weight, but not total yield, as compared with the non-treated control in the second harvest. None of the treatments produced yields of leaf fresh weight that differed significantly from the non-treated control, although plants treated with Bt had the highest yield and significantly higher leaf fresh weight as compared with pyrethrum and hand treatments.

The dry weight yields differed significantly between harvests and a significant harvest by treatment interaction was observed for stem and

TABLE 2. Identification of General Pests on Basil.

Class	Order	Family	Guild	Disposition
Arachnida	Araneida		Predator	Beneficial
Insecta	Coleoptera	Cantharidae	Predator	Beneficial
		Chrysomelidae[1]	Foliage feeder	Pest
		Coccinellidae	Predator	Beneficial
		Lampyridae	Predator	Beneficial
	Diptera	Culicidae	Phloem feeder	Pest
	Herniptera	Corimelaenidae	Phloem feeder	Pest
		Berytidae	Phloem feeder	Pest
		Lygaeidae	Seed feeder	Pest
		Miridae[1]	Phloem feeder	Pest
		Pentatomidae	Phloem feeder	Pest
		Rhopalidae	Phloem feeder	Pest
	Homoptera	Cicadellidae[1]	Phloem feeder	Pest
		Aphidae[1]	Phloem feeder	Pest
	Lepidoptera	Arctiidae[1]	Foliage feeder	Pest
		Geometridae	Foliage feeder	Pest
		Noctuidae[1]	Foliage feeder	Pest
		Pterophoridae	Foliage feeder	Pest
		Pyralididae	Foliage feeder	Pest
		Thyatiridae	Foliage feeder	Pest
	Orthoptera	Acrididae[1]	Foliage feeder	Pest
		Gryllidae	Foliage feeder	Pest

[1]Major pest during study as measured by general density and feeding behavior; all insects were captured by hand at 57 and/or 93 days after planting.

total dry weight yields (data not shown). No differences in dry weight yield were noted among treatments in the first harvest, but the use of Bt significantly increased total dry weight yield as compared with hand, oil, and pyrethrum treatments in the second harvest. Similar differences between fresh weight and dry weight yields for all treatments indicated that sweet basil plants had similar moisture content and dried uniformly under the conditions of the experiment.

Phenylalanine ammonium lyase and syringaldazine oxidase activities differed between harvests (Figures 1 and 2). The activities of these enzymes were highest for all treatments during early growth and lowest at maturity.

Trichome density was significantly affected by the treatment and the harvest by treatment interaction, whereas relative insect damage was sig-

TABLE 3. Sweet Basil Yields.

Insect control[3]	57 days after planting[1]			93 days after planting[2]		
	Leaf	Stem	Total	Leaf	Stem	Total
	(g F.Wt./plant)					
Control	170.2	95.0	265.2	737.0	382.6	1119.6
Hand	192.4	93.0	285.3	685.1	305.4	990.5
Oil	187.3	87.4	274.7	702.7	386.6	1089.2
Pyrethrum	190.3	101.4	291.7	663.2	369.9	1033.1
Bacillus	192.6	96.3	288.9	815.3	433.3	1248.6
$LSD_{0.05}$	31.3	19.7	41.6	121.7	48.0	145.6

[1]First harvest date.

[2]Second harvest date.

[3]Control = no insect control, Hand = removed of insects by running hands through foliage, Oil = spraying with horticultural oil, Pyrethrum = spraying with pyrethrum, *Bacillus* = spraying with *Bacillus thuringiensis*.

FIGURE 1. Phenylalanine Ammonium Lyase (PAL) Activity of Sweet Basil Leaf Tissue.

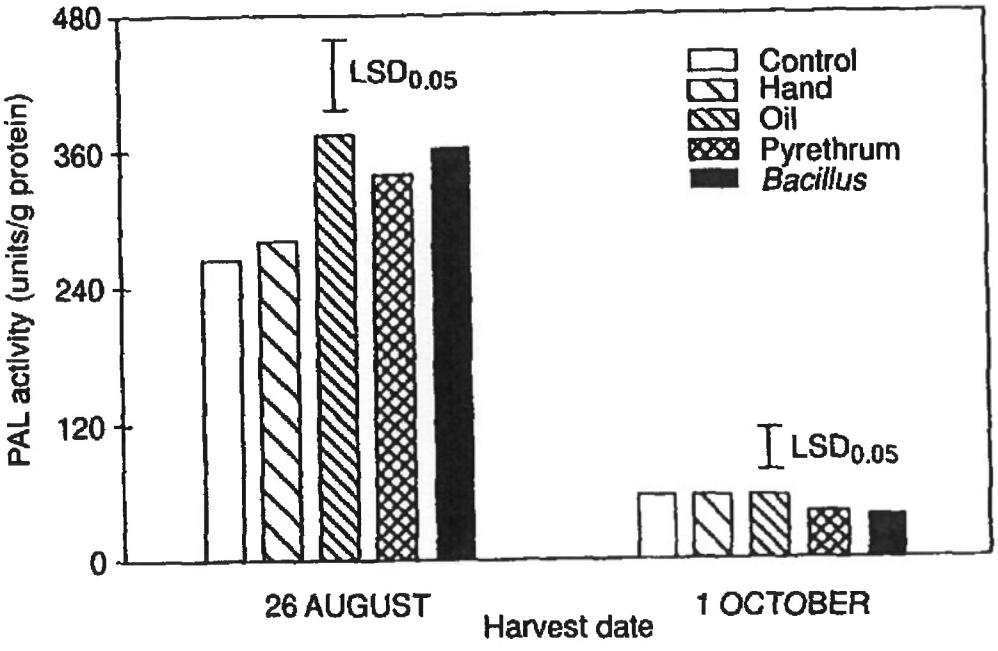
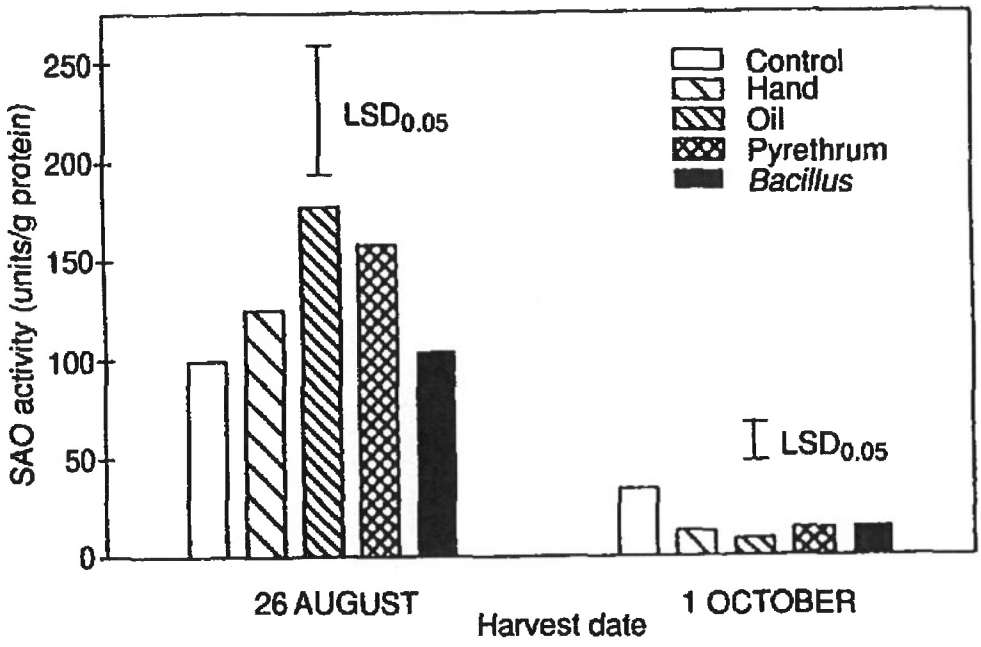


FIGURE 2. Syringaldazine Oxidase (SAO) Activity of Sweet Basil Leaf Tissue.



nificantly affected by both harvest and treatment (Figures 3 and 4). Leaf trichome number was significantly higher in control, hand, and Bt treatments as compared with horticultural oil and pyrethrum treatments at the first harvest, and control plants had significantly higher trichome number than hand treatments at the second harvest. Sweet basil treated with horticultural oil received the most relative insect damage whereas pyrethrum and Bt treated plants demonstrated the least relative insect damage during the first harvest.

Total dry weight was positively correlated with stem and leaf dry weight, and negatively correlated with PAL and SAO activities (Table 4). The negative correlation of total dry weight with PAL and SAO activities indicated that plants continued to grow even after maximum PAL and SAO activities were achieved and those activities subsequently decreased with plant senescence.

DISCUSSION

Greater variation in PAL and SAO activity at the first harvest as compared with less variation at the second harvest indicated that sweet basil was more susceptible to insect herbivory and selected pest control treat-

FIGURE 3. Foliar Density of Sunken Glandular Capitate Trichomes of Sweet Basil Leaves.

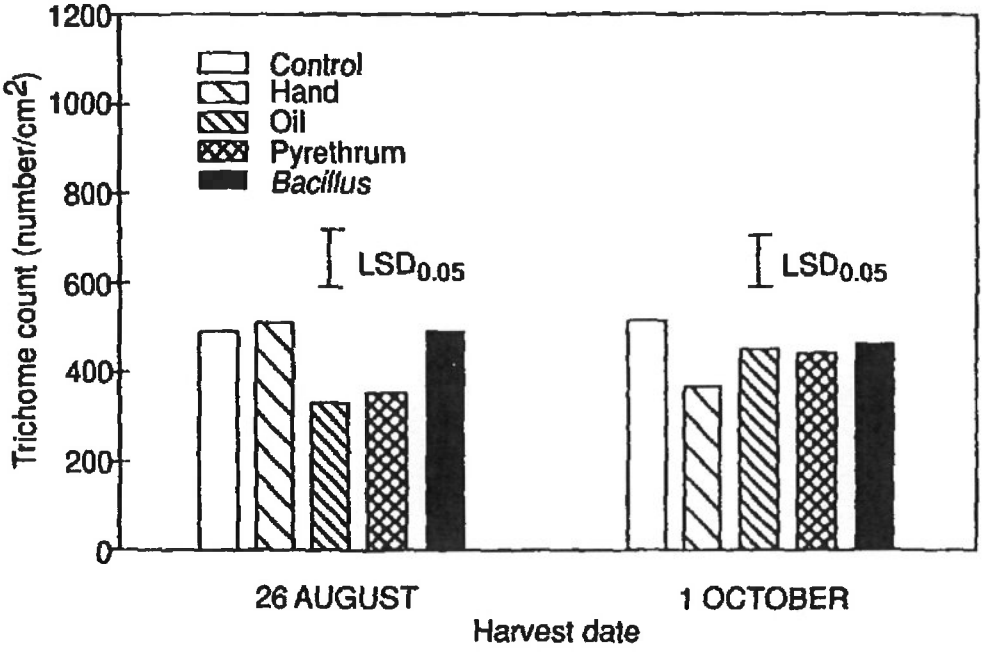


FIGURE 4. Relative Insect Damage of Sweet Basil at 57 and 93 Days After Planting.

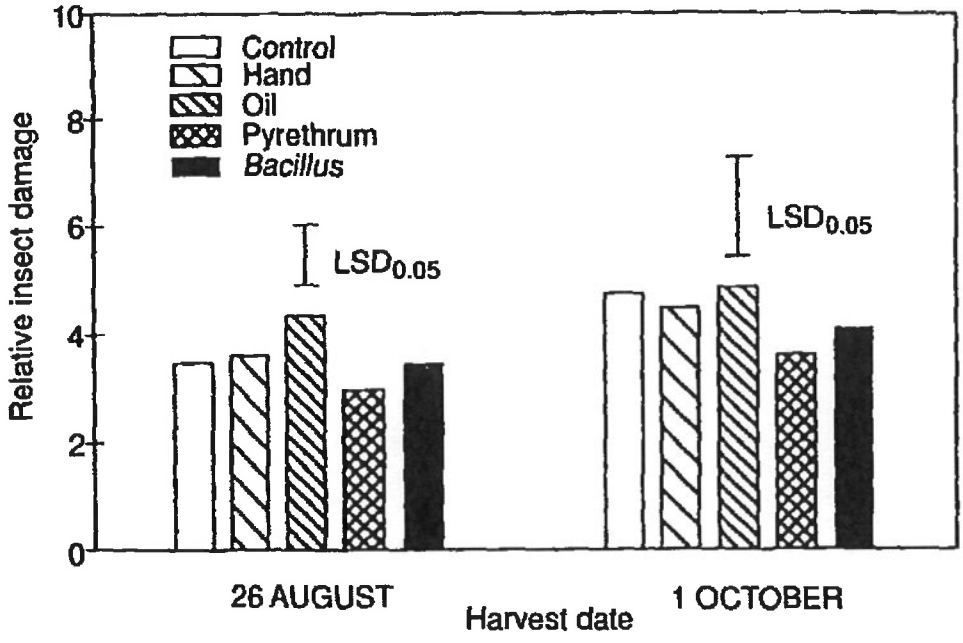


TABLE 4. Correlations Among Dry Weight Yield and Enzyme Activities on Sweet Basil for All Harvests and Treatments Combined.

	SDW[1]	LDW	PAL	SAO
TDW	0.99*	0.99*	−0.96*	−0.92*
SDW		0.99*	−0.96*	−0.91*
LDW			−0.96*	−0.92*
PAL				0.95*

[1]SDW = stem dry weight; LDW = leaf dry weight; PAL = phenylalanine ammonium lyase; SAO = syringaldazine oxidase; TDW = total dry weight.

*For all correlations shown, Pearson's rank coefficient differs significantly from zero, based on the 0.001 level of probability.

ments when young, actively growing tissues were present. Regression analysis suggested that 91 percent of the variability in SAO activity could be explained by a linear model (data not shown), indicating that SAO is attributable to precursory action of PAL and that synthesis of lignin monomers may be required prior to the polymerization and incorporation of these monomers into the cell wall matrix (2).

Bacillus thuringiensis var. *kurstaki* is known to have potent insecticidal activity against lepidopterans with no detectable phytotoxic effects, suggesting that this organism may deter certain pest insects without disrupting the general metabolism and growth of sweet basil (17). In our study, *Bacillus thuringiensis* var. *kurstaki* deterred Lepidopteran pests, without significantly altering plant growth, and development. Since high fresh-weight yield is generally desirable to herb growers, Bt provided the best pest control in this study.

Insect feeding, which can increase lateral branching, leaf production, and oil yield in spearmint (*Mentha spicata* L.) and peppermint (*Mentha piperita* L.)(25), may have induced higher yields in sweet basil in plots not receiving any pest control. Untreated control plants may also have demonstrated high yields because essential oils produced by glandular trichomes discouraged insect feeding, while lower trichome numbers in the horticultural oil and pyrethrum treatments, as compared with other treatments, may have left these plants susceptible to insect damage that decreased yield. Alternatively, plant tissue injury from the penetrating action of horticultural oils (21,32) and pyrethrum (7) and some physical tissue damage during hand removal of insects, may have lowered yields in these treatments as compared with control plants and those treated with Bt. Treatment differences in fresh weight and dry weight yield components for the second harvest revealed that insect control treatments were de-

tected only after the first harvest. Significant differences encountered in the second (post-anthesis) harvest may have resulted from treatments applied during the vegetative or pre-anthesis period (4,6,13,17,22,23).

While plant yield provides evidence of the efficacy of pest control treatments, enzyme activities may help explain metabolic events which lead to altered sweet basil production. Phenylalanine ammonium lyase is a precursory enzyme of lignification (1,5) and related phenylpropanoid pathways (2). Activity of PAL in plants has been shown to increase initially, and then "level-off" or decrease as a plant ages (1,16,18). Syringaldazine oxidase is more closely related to lignificaton (11) and would be expected to peak, as observed, prior to maximum lignification in sweet basil. Although PAL is required for lignin biosynthesis, activation of this enzyme by numerous environmental stimuli (12) could result in phenylpropanoid products other than lignin (15) and subsequently improve innate pest resistance (12).

In summary, this study demonstrates that, under the general level of insect population observed in the field (no serious infestations), untreated sweet basil plants yield as well as plants subjected to alternative pest control measures. If insects are abundant, Bt would appear to be the best treatment for targeted insect control. While horticultural oil and pyrethrum control a broad spectrum of insects in sweet basil, this study indicates these substances are phytotoxic and reduce the numbers of trichomes on a leaf, especially when applications are excessive.

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