Arthropod Population, Phenylalanine Ammonia Lyase Activity, and Fresh Weight of Sweet Basil (*Ocimum basilicum*) as Affected by Plant Age and *Bacillus thuringiensis* Treatment

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More information is needed to evaluate growth dynamics and to understand how arthropod populations affect plants before producers can accurately manage sweet basil for optimum productivity. This investigation evaluated the effects of plant age and the use of the bacterial pathogen *Bacillus thuringiensis* (Bt) on arthropod population, enzyme activity, and yield of sweet basil. Field experiments took place from 11 July through 6 October 1995 during which time half of the plants were subjected to Bt treatment, while the other half received no treatment. Plants were harvested by hand at 11, 18, 25, 30, 44, 55, 61, 75, and 88 d after being transplanted to the field and were assessed for arthropod abundance and diversity, pest damage to plants, phenylalanine ammonia lyase (PAL) activity, and fresh weight (FW) yield. Arthropods were collected and identified to order at 18, 30, 61, and 75 d. Abundance and diversity of arthropods were generally the same in both treatments, although slightly more arthropod pest damage was observed in control plants. Activity of PAL peaked at 44 d in control plants followed by a similar peak at 55 d for Bt-treated plants. Maximum FW yields were observed after peak PAL activity in both treatments between 55 and 75 d. Growth curves for total FW weight were virtually the same for both treatments, although individual measurements indicated occasional differences in biomass production during the last 21 d of growth. Hence, control and Bt-treated plants exhibited similar PAL activity and growth dynamics throughout the study. These results provide evidence for the innate ability of sweet basil to deter herbivory when arthropod populations are limited. ***© 2005 Oklahoma Academy of Science.***

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) belongs to the mint (Lamiaceae) family and is often esteemed for its aromatic and pharmaceutical properties (Werker et al 1993). Sweet basil may be used as a fresh or dry herb, or harvested for its essential oils (Putievsky 1993). In 1990, the United States imported 1,831 metric tons of sweet basil at a value of $2,657,400. By 1994, these figures increased to 3,213 metric tons valued at $4,574,500 (Buzzanell et al 1995). This is not surprising because the United States is the world's largest spice importer, with both imports and consumption on an upward trend (Buzzanell et al 1995). Sweet basil, considered a "spice" by the American Spice Trade Association, is among a half-dozen leading herbs that contribute to the domestic market in the United States (Buzzanell et al 1995), which probably offsets some of the import demands. Better understanding of sweet basil production practices may help to improve the yield and the quality of this crop to keep up with the world's increasing spice demands, particularly in the United States.

Most commercial herb growers are concerned about obtaining maximum yields of high quality herbs. Although the increased yield that comes with maturity of crops is desirable, quality may diminish with age. Aromatic characteristics of sweet basil, attributable to secondary metabolism, provide evidence for natural defenses against herbivory (Dube et al 1988). The enzyme,

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phenylalanine ammonia lyase (PAL), is necessary for the production of many of these defense compounds, increases of which also parallel natural growth and development (Bidlack et al 1995). Metabolites resulting from PAL activity can be generally classified as phenolic derivatives and include coumarins, essential oils, flavanoids, lignin, and tannins (Creasy 1987). These compounds aid in disease and pest resistance, enable plant pigmentation, enhance mechanical support, and possibly assist in seed dispersal as well as pollination efforts (Rhodes 1985). Many factors increase the synthesis of PAL and the resulting plant phenolics. These factors include age (Bidlack et al 1995), concentration of growth regulators (Bidlack and Buxton 1995), herbivory (Olson and Bidlack 1997), and tissue wounding (Vance et al 1976). Highest PAL activity is usually encountered in young developing plants and often levels off or decreases with maturity (McCallum and Walker 1990, Bidlack et al 1995, Olson and Bidlack 1997).

Recent field studies with sweet basil have shown that arthropod populations accompanying plants include representatives of several insect orders including Coleoptera, Hemiptera, Homoptera, Lepidoptera, and Orthoptera (Olson and Bidlack 1997). Larval lepidopterans were reported as major pests, particularly members of the Families Arctiidae and Noctuidae (Olson and Bidlack, 1997). Several pest control treatments were implemented in these studies, including use of horticultural oil spray, pyrethrum, *Bacillus thuriengiensis* (Bt), and hand removal. The Bt treatment was reported as the best strategy for controlling lepidopteran pests, but it was noted that the innate ability of sweet basil to discourage herbivory may be sufficient when pests are not abundant. These previous studies instigated additional research and served as the basis for more thorough experiments.

Sweet basil was investigated in this experiment to (1) to assess changes in arthropod populations, relative pest damage to plants, enzyme activity, and fresh weight (FW) yield as a function of harvest date and (2) to determine whether or not foliar application of *Bacillus thuriengiensis* affects these measurements of field-grown plants.

MATERIALS AND METHODS

Plants were established, maintained, and treated at Hoggard's Organic Herb Farm in Piedmont, Oklahoma from May through October 1995. Organic farming practices were enforced and involved the use of non-synthetic soil enhancers and a biological pest control. Field plots were fertilized with 14-year-old cattle manure (49 metric tons ha^{-1}). Wet-dry cycling was achieved by watering to capacity by drip tape irrigation approximately every 3 d. Analyses of plants was performed at the Department of Biology, University of Central Oklahoma, Edmond, Oklahoma.

"Genovese" sweet basil seeds were planted on 21 May in germination trays and transplanted after 1 wk to 5-cm diameter peat pots. The seedlings were watered daily and maintained in the greenhouse for 6 wk, when a second set of true leaves formed. Sweet basil seedlings were then placed outside the greenhouse for 10 d before being transplanted to the field. Each peat pot contained a uniform basil plant that was used for this experiment. Two rows of soil were established 60 cm apart by using a Holland® (Holland Transplanter Co., Holland, Michigan, USA) bed-shaper plow. Plants in peat pots were transplanted and spaced 30 cm apart within rows into the sandy-loam (fine, mixed, thermic Udertic Paleustolls) soil and fertilized with 50 mL of fish oil emulsion fertilizer (five parts nitrogen, one part phosphoric acid, one part potash). Black plastic mulch (0.8 mm) was centered on rows to cover approximately 15 cm of soil on either side of each plant. Previous studies have shown that black plastic mulch is effective in reducing erosion, controlling weeds, and maintaining soil moisture and temperature (Ricotta and Masiunas 1991, Palada et al 1999).

Four replications of 40 plants (160 total plants) were arranged in the field to allow for a split plot design with harvest as the main effect and pest control treatment tested against residual error. Border plants surrounded each plant designated for analysis and only one of two plants within each replication was selected for each harvest in order to minimize border effects. Ten harvests were implemented for each of the two pest control treatments, requiring 20 plants per replication and hence 80 total plants for analysis.

After 8 d in the field, the first experimental pest treatment was applied to the plants by using American Brand Thuricide concentrate which contained Bt. A hand-pump sprayer was used to apply the treatment. Plants were sprayed from the top of the plant down to the bottom, with both the abaxial and adaxial portions of the leaves being covered with chemical. Approximately 3.8 g L^{-1} of Bt was applied to the treatment area, providing 0.38 kg active ingredient ha^{-1}. Treatments were applied at 8, 22, 36, 50, 64, and 78 d after transplanting the sweet basil to the field (approximately every 2 wk) for the duration of the experiment.

Prior to removal from the field, plants were evaluated for relative pest damage based on overall visible above-ground damage. Damage included holes in the leaves and irregular or reduced leaf margins resulting from herbivory or other environmental stresses. A score of 1 to 5 was used for each plant with "1" designated for no damage; "2" for 1 to 5% damage; "3" for 6 to 10% damage; "4" for 11 to 15% damage; and "5" for 16 to 20% damage. Percentages were estimated by observation and comparison to other plants in the field that did not show any visual damage. There were no plants that demonstrated more than 20% damage. Scores were recorded for each plant and subjected to the same statistical analysis as other measurements for this experiment.

Plants were harvested at 11, 18, 25, 30, 39, 44, 55, 61, 75, and 86 d after the plants were transplanted to the field. All harvests were performed between 9:00 and 11:00 AM to ensure consistency in post-harvest analysis. Plants were randomly selected to ensure no bias. The shoot portion of each plant was harvested by cutting the stem at ground level. Arthropods were collected with plants at 18, 30, 61, and 75 d in the field by placing a plastic bag over the entire plant and tying it at the base of the stem. Plants were gently shaken to dislodge arthropods, allowing them to collect in the bag. Additional arthropods were also collected, by grasping them with thumb and index finger or holding them with an enclosed hand, and added to the collection. The bags were then placed on ice to reduce damage to plants, retard degradation of enzymes, and preserve arthropods. The number and type of arthropods were assessed the same day, upon return to the laboratory.

Harvested shoot material was first weighed to obtain total fresh weight (FW) for each plant. Shoot material was then divided by manually removing leaves with petioles and flowers (if present) from the stem. Components were weighed to obtain leaf and stem FW. Five grams of leaf material were kept on ice for enzyme and protein analysis at 25, 30, 39, 44, 55, 61, 75, and 86 d in the field.

The 5 g of fresh leaf material were cut into pieces of approximately 1 cm^2 and homogenized at 4°C in 50 mL of grinding buffer. The grinding solution contained 0.1 M sucrose, 1% polyvinylpyrrolidone, 4 mM cysteine, and 1 mM dithiothreitol buffered to a pH of 7.0 with 50 mM N-tris(hydroxym ethyl)methyl-2-aminoethane-sulfonic acid. Temperature of this buffer was maintained at 0°C thereafter. Homogenized material was decanted through cheesecloth, and the resulting extract was centrifuged at 2,000*g* for 10 min. The pellet was discarded and the supernatant was centrifuged at 3,500*g* for 10 min. Exactly 5.0 mL of the resulting supernatant was placed on ice to prevent enzyme degradation and was used for protein and enzyme analysis.

Protein content was quantified for each

extract using a standard colorimetric procedure (Bradford 1976). A mixture of 0.1 mL of enzyme extract and 5.0 mL of Bradford reagent (Bio-Rad Laboratories, Hercules, California, USA) was allowed to stabilize for 20 min. The mixture was analyzed spectrophotometrically at 595 nm to determine protein concentration in each sample.

Phenylalanine ammonia lyase (PAL) was assayed spectrophotometrically from a mixture of 1.5 mL of enzyme extract and 4.5 mL of 6.68 mM L-phenylalanine in a borate buffer (pH = 8.7) over a period of 1 h at 30°C (Bidlack et al 1995). Enzyme activity was calculated by measuring the production of cinnamic acid at 290 nm and using an extinction coefficient of 9,000 M^{-1} cm^{-1} (Saunders and McClure 1974). The amount of cinnamic acid produced in micromoles h^{-1} was expressed as one unit of activity.

Local weather data were provided by an Oklahoma Climatolotical Station located within 35 km of the experiment. Minimum and maximum daily temperatures, as well as precipitation, were obtained for the period from 11 July to 6 October, 1995 and are provided in Fig. 1.

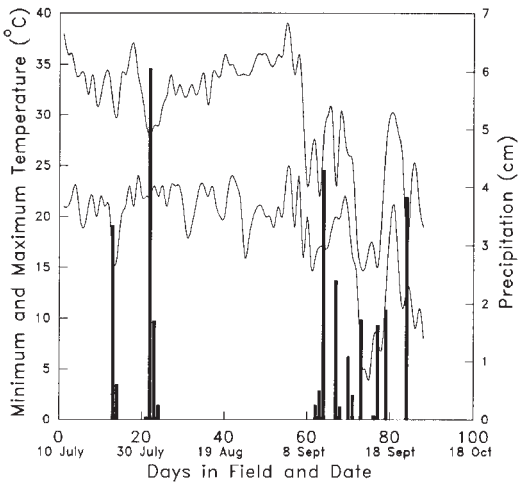

Figure 1. Local climatological data from 11 July to 6 October 1995. Minimum and maximum daily temperatures are shown as lines and daily precipitation is represented by bars.

The general linear model procedure (PROC GLM) of SAS (SAS Institute, 1985) was used for all data except relative pest damage. Because relative pest damage measurements were ranked scores, a nonparametric analysis of variance (ANOVA) that provides linear ranked statistics (SAS PROC NRAR1WAY) was used. Statistical evaluations using ANOVAs, accompanied by graphical representations with standard errors, were used to present results.

RESULTS

The hottest, driest part of the growing season occurred within the first 60 d. Conditions were cooler and wetter during the last 21 d of the growing season. Climate conditions while the plants were in the field were normal when compared to the century averages.

Six orders of arthropods belonging to the Classes Arachnida and Insecta were collected (Table 1). There were occasional grasshoppers (Orthoptera) present in the field, but their numbers averaged less than one per plant during collections and were not included in data analysis. Arthropods were classified to the family level, and guild was noted for each. The term *guild* was used to refer to the feeding pattern of groups of arthropods (Metcalf and Metcalf 1993). Arthropods were not as abundant during the first 30 d of the experiment as they were at 61 and 75 d in the field. In both Bt and control treatments, representatives of Hemiptera (phloem feeders) were generally more abundant than other members of Insecta at 61 and 75 d in the field (Fig. 2). This was accompanied by relatively high numbers of Araneida, which may have been present as predators of the insect pests. Some lepidopterans were also present at 61 and 75 d, but there were no significant differences between the number of these insects in Bt and control treatments.

Analysis of variance demonstrated significant differences in all measurements, as affected by harvest (Table 2). However,

Table 1. Survey of arthropod populations obtained from sweet basil during collection.

Class	Order	Family	Guild
Arachnida	Araneida		Predator
Insecta	Coleoptera	Cantharidae	Predator
		Chrysomelidae	Foliage feeder
		Coccinellidae	Predator
	Diptera	Culicidae	Predator / Phloem feeder
	Hemiptera	Lygaeidae	Seed feeder
		Miridae	Phloem feeder
		Pentatomidae	Phloem feeder
		Reduviidae	Predator / Phloem feeder
	Homoptera	Aphidae	Phloem feeder
		Cicadellidae	Phloem feeder
	Lepidoptera	Arctiidae	Foliage feeder
		Geometridae	Foliage feeder
		Noctuidae	Foliage feeder

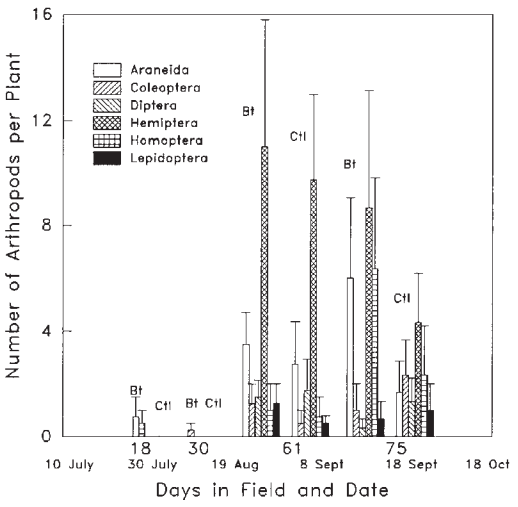

Figure 2. Number of arthropods per plant collected on the day of harvest for control (Ctl) and *Bacillus thuringiensis* (Bt) treatments. Standard errors are indicated by error bars and were calculated for the average of four replications within each treatment.

only relative pest damage (RPD) varied significantly as affected by pest control treatment.

Relative pest damage (RPD) of both treatments was higher during the last four harvests of the experiment (Fig. 3), which coincided with cooler temperatures, more precipitation, and greater arthropod numbers. Control plants demonstrated similar or higher pest damage compared with Bt-treated plants, although any differences were limited to less than one RPD unit (less than 5%). The significant differences in RPD between control and Bt-treated plants were observed at 18, 55, 61, 75, and 86 d in the field.

Phenylalanine ammonia lyase activity initially increased with plant age and then decreased (Fig. 4). In both control and Bt-treated plants, the highest PAL activity values were observed between 39 and 61 d. Enzyme activity achieved a higher value in control plants than it did in Bt-treated plants at 44 d in the field.

Table 2. Significance of mean squares[1] for relative pest damage (RPD), phelyalanine ammomia lyase (PAL) activity, total fresh weight (TFW), leaf fresh weight (LFW), and stem fresh weight (SFW) of sweet basil.

Source	RPD	PAL	TFW	LFW	SFW
Harvest	**	**	**	**	**
Replication	NS	NS	NS	NS	NS
Error a					
Treatment	*	NS	NS	NS	NS
Harvest X Treatment	NS	NS	NS	NS	NS
Error b					

[1]Because RPD included measurements of ranked scores, a nonparametric analysis of variance (ANOVA) was used. Measurements of PAL were subjected to ANOVA for eight of the nine harvests because not enough tissue was obtained during the first harvest. Measurements of TFW, LFW, and SFW were subjected to ANOVA for all nine harvests. **,*Significant at the 0.01 and 0.05 probability levels, respectively; NS indicates not significant.

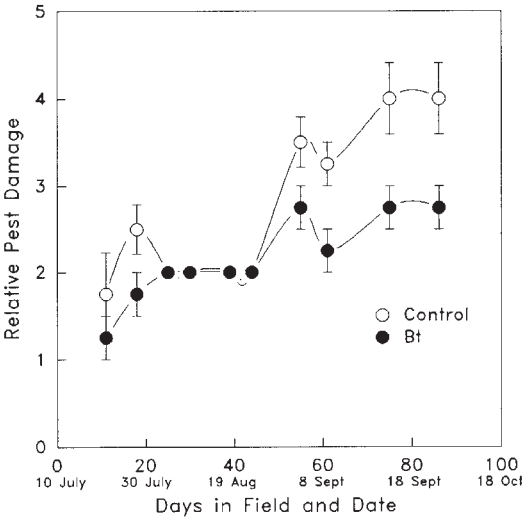

Figure 3. Relative pest damage per plant (means) recorded on the day of harvest. Values range from 1 (no damage) to 5 (20% damage). Standard errors are indicated by error bars and were calculated for the average of four replications within each treatment.

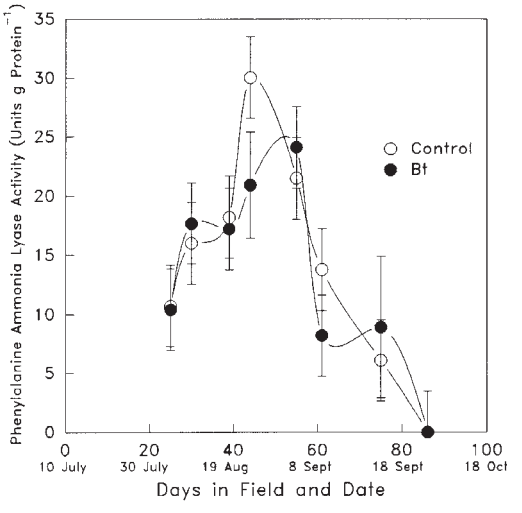

Figure 4. Phenylalanine ammonia lyase (mean values) activity per plant measured and recorded on the day of harvest. Standard errors are indicated by error bars and were calculated for the average of four replications within each treatment.

In general, total plant FW increased with age to a maximum, and then leveled off or decreased (Fig. 5). Plant growth rate was fastest during the first 7 wk of the field-growing season, particularly between 30 and 55 d. Growth curves for leaf and stem FW (data not shown) paralleled growth curves for total FW, although leaf FW in both treatments demonstrated a more pronounced decrease during the last 2 wk than did other FW measurements. Fluctuations in total FW during the last 3 wk of the experiment may be partially attributable to leaf loss and senescence as well as pest damage accompanying increased arthropod numbers during the same growth period.

DISCUSSION

Mean monthly minimum and maximum daily temperatures were within four degrees of the century average according to the USA Today Weather Almanac (Williams 1994). Precipitation during July, August, and October was within 3 cm of the average. Precipitation in September was almost twice the average. Because drip line irrigation was implemented, plants received adequate amounts of water even during dryer periods. The direct effect of precipitation on plant growth was probably minimal, although increases in arthropod populations during wetter periods may have indirectly affected enzyme activity and FW yield of sweet basil. Herbivory and competition have been identified as important factors affecting the general health and well-being of a plant (Herms and Mattson 1992). In this investigation, the experimental design was developed, in part, to assess arthropod populations in relation to plant growth and to evaluate the effect of herbivory on plant health. All members of the order Araneida, two of the three families of coleopterans, and others listed in Table 1 were predators of accompanying arthropods. Perhaps these predators were responsible for controlling herbivore populations and, by virtue of their presence, sustained healthy plants.

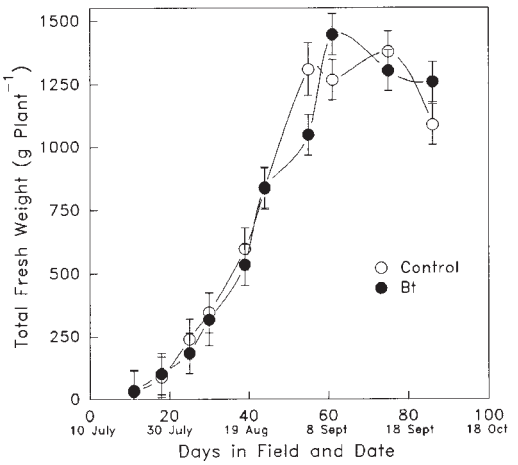

Figure 5. Total fresh weight per plant (mean values) recorded on the day of harvest. Standard errors are indicated by error bars and were calculated for the average of four replications within each treatment.

Although previous studies with sweet basil (Olson and Bidlack 1997) demonstrated that Bt provided potent insecticidal activity against lepidopterans, use of this natural agent did not appear to significantly reduce pests in this investigation. An average of less than three lepidopterans per plant were collected from both treatments during the course of this experiment. There were no significant differences in the number of lepidopterans per plant between treatments. However, slightly more pest damage was observed in control plants compared with those treated with Bt, suggesting that the persistence of lepidopteran pests may be a significant factor affecting RPD. The amount of damage may have been limited to 20% as a consequence of PAL enzyme products that contribute to pest resistance.

Because PAL plays an important role in plant defense reactions (Yoshioka and Doke 1994), both control and Bt-treated plants may have responded to pest attacks by increasing PAL activity. In this investigation, PAL peaked between 44 and 55 d in the field, about a week prior to maximum FW yields that occurred between 55 and 75 d in

the field. Interestingly, PAL activity peaked in control plants prior to Bt-treated plants, which may partially explain why arthropod numbers did not significantly differ during the collection periods.

Previous studies have indicated that herbivory can be an important factor in altering the yield and metabolism of sweet basil (Olson 1993) when pests are abundant. This is important because the FW yield and aroma of sweet basil contribute towards its value. In this study, PAL activity peaked prior to maturity and about the same time when arthropods became more abundant, implying that both developmental and environmental factors may have influenced activity of the enzyme. Moderate pest populations can stimulate secondary metabolism and increase the aromatic value of plants used for herbs, but excessive herbivory can reduce the FW yield and essential oil production of these plants. Metabolites produced by PAL that provide sweet basil with innate resistance may play a pivotal role in the balance between yield and quality of this crop.

Decreased growth rates can result from the shuttling of resources from leaf production to secondary metabolic pathways and expansion of structural defenses (Herms and Mattson 1992). Growth rates may decrease soon after pest populations and herbivory become abundant. Perhaps plants begin to invest more energy into defense rather than growth during pest attack. In this experiment, plants demonstrated fluctuations in growth late in the growing season and when arthropods were more abundant.

Fresh weight increased rapidly for 55 d in the field and fluctuated thereafter. Fluctuation of FW yield components after 55 d and during the month of August suggests that sweet basil growth is susceptible to influence by temperature and pest damage, as well as maturation of the plant. Recent studies have shown that higher temperatures are more favorable to growth in French basil (*Ocimum basilicum*) and that herb yields increase with each subsequent developmental stage (Randhawa and Gill 1995). Fresh weight yield of sweet basil in this experiment increased with maturity, and then decreased. The decrease was probably due to leaf drop and dehydration, a happenstance of senescence.

Regardless of slight variations in trends, both control and Bt-treated plants demonstrated similar PAL activity and growth dynamics throughout this experiment. These results suggest that nature's way may be the best way to deter herbivory when arthropod populations are limited.

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