Plant Physiol. (1992) 100, 1403–1405
0032-0889/92/100/1403/03/$01.00/0

Received for publication April 10, 1992
Accepted July 24, 1992

Temperature and Photoperiod Influence Trichome Density and Sesquiterpene Content of *Lycopersicon hirsutum* f. *hirsutum*[1]

Thomas J. Gianfagna*, Catherine D. Carter, and John N. Sacalis

Horticulture Department, Rutgers University, New Brunswick, New Jersey 08903 (T.J.G., J.N.S.); and Plant Science Department, South Dakota State University, Brookings, South Dakota 57007 (C.D.C.)

ABSTRACT

Resistance to Colorado potato beetle in a clone of *Lycopersicon hirsutum* f. *hirsutum* L. is attributed to the presence of the sesquiterpene zingiberene in the type VI leaf trichomes; however, both day/night temperature regimen and photoperiod affect zingiberene content and trichome density. In short days (SD), zingiberene content per trichome is more than 3-fold greater than in long days. In SD, trichome density per unit leaf surface is 2-fold greater at 25/20°C (day/night) than at either 30/25°C or 20/15°C, thus indirectly influencing zingiberene content per cm^2. In long days, temperature regimen had little effect on either trichome density or zingiberene content, although trichome density was greater than or equal to that in SD.

Glandular trichomes that accumulate large quantities of terpenes and other essential oils have been found to be associated with insect resistance in a number of species of tomato (6, 11, 13). *Lycopersicon hirsutum* f. *hirsutum* Humb. and Bonpl. is resistant to several arthropod pests, including the Colorado potato beetle (*Leptinotarsa decemlineata* Say), although the degree of resistance may vary in different environments and with different genetic backgrounds. Resistance to this pest has been associated with the presence of type VI trichomes, capitate glandular hairs, 0.2 to 0.5 mm long, which contain a four-celled head. The secretory head cells of the type VI trichomes from resistant genotypes contain high amounts of the sesquiterpene zingiberene (2, 9). Because *L. esculentum* contains type VI trichomes and can be successfully crossed with *L. hirsutum*, it may be possible to transfer this trait. To better understand the effect of environment on trichome density, zingiberene content, and insect resistance, the following research was undertaken to determine whether temperature and photoperiod modify the expression of the genes for type VI trichomes and zingiberene production.

MATERIALS AND METHODS

Lycopersicon hirsutum f. *hirsutum* PI 126445 was propagated by cuttings and grown for 12 weeks in chambers at 30/25°C, 25/20°C, or 20/15°C (day/night) in either SD with a 10-h photoperiod (450 μmole m^{-2} s^{-1}) or LD, an 18-h photoperiod provided by extending the light period with 8 h of incandescent light. Sesquiterpenes were extracted from leaf tissue obtained from the third or fourth node below the apical bud (4–6 cm in length, about 50% expanded) by immersing the leaf in hexanes overnight at 4°C according the methods described by Carter et al. (2). Sesquiterpenes previously identified by GC-MS (1) were quantified by GC using a wide-bore capillary column (1.5-μm DB-1 film, 30 m in length; J & W Scientific, Inc., Rancho Cordova, CA) that was at 110°C for 5 min and then temperature programmed at 5°C/min to 135°C. Farnesol (Sigma, St. Louis, MO) was used as an internal standard. Percentage recovery ranged from 65 to 90% (n = 12).

Leaf area was determined by using either an empirically derived conversion factor relating leaf weight to area, for each of the environmental conditions described above, or a Li-Cor LI-300 portable leaf area meter. Trichome density was determined by counting trichomes with the aid of a binocular microscope, within five 19.6-mm^2 circles from the interveinal region on the abaxial and adaxial surfaces of a lateral leaflet, obtained from the third or fourth leaf below the apical bud. The average value per unit surface area was obtained for 12 leaves. The entire experiment was repeated twice.

RESULTS AND DISCUSSION

Three sesquiterpenes were previously identified in foliar extracts: zingiberene, curcumene, and bisabolene (1). By collecting the glandular tips from all type VI trichomes on both surfaces of the leaflets and comparing the sesquiterpene content of these cells with that of the rest of the leaf, we determined that zingiberene and the related sesquiterpenes occur almost exclusively in the glandular tips of the type VI trichome (1). Each of these compounds was found in the six temperature-photoperiod regimens in similar proportions of approximately 85% zingiberene, 10% bisabolene, and 5% curcumene. The levels of zingiberene and the other sesquiterpenes (not shown) were significantly greater in SD than LD regardless of the temperature regimen (Table I). In SD, however, the optimum conditions for zingiberene production were 25/20°C. Zingiberene levels decreased by about 50% when the temperature was either raised or lowered by 5°C. In addition to the lowest levels of zingiberene, the 20/15°C

[1] Supported by U.S. Department of Agriculture competitive research grant No. 89–37153–4943, state, and Hatch funds. New Jersey Agricultural Experiment Station publication No. 12145–6–92.

Table I. *Effect of Photoperiod and Temperature Regimen on Type VI Trichome Density and Zingiberene Content of the Leaves of L. hirsutum f. hirsutum*

Photoperiod[a]	Temperature Regimen (day/night)	Trichome Density	Zingiberene	Zingiberene
	°C/°C	No./cm²	μg/cm²	ng/trichome[b]
SD	20/15	541 ± 40	32 ± 2	59
	25/20	1204 ± 58	67 ± 5	56
	30/25	514 ± 39	32 ± 2	63
LD	20/15	829 ± 41	11 ± 1	13
	25/20	1122 ± 78	19 ± 2	17
	30/25	1000 ± 57	17 ± 2	17

[a] SD, 10 h of light, 14 h of dark. LD, 18 h of light, 6 h of dark. [b] Calculated from measurements of trichome density and zingiberene content per unit surface area.

temperature regimen resulted in the development of a purple color to the leaves, reminiscent of a phosphorus deficiency. Temperature had little effect on zingiberene levels in leaves of plants held in LD. Final leaf size was also affected by both photoperiod and temperature. Leaves growing in LD or at the 30/25°C temperature regimen tended to be somewhat smaller and more narrow at maturity than in SD (not shown).

Trichome density was affected by both photoperiod and temperature (Table I). Except for plants in the 25/20°C temperature regimen, LD resulted in generally greater trichome densities than SD. Density of trichomes was high in the 25/20°C temperature regimen regardless of the photoperiod. Within LD, there was little affect of temperature on trichome density. In SD, however, temperature had a significant effect, with higher quantities in the 25/20°C temperature regimen and less than 50% of that amount when the temperature was either raised or lowered by 5°C. Lower night than day temperature regimens were chosen because there is some evidence that vegetative growth in tomato is superior under these conditions (15).

Working with the subspecies *L. hirsutum* f. *glabratum*, Kennedy et al. (10) found that type VI trichome density was greater in LD than in SD, as were 2-tridecanone content and resistance to tobacco hornworm. Snyder and Hyatt (14) found higher trichome densities in LD in both *L. hirsutum* f. *hirsutum* and *L. hirsutum* f. *glabratum*. They identified two carbon-15 compounds from *L. hirsutum* f. *hirsutum* trichomes that were likely to be sesquiterpenes, although they did not find any differences in their levels on a per unit surface area as a result of photoperiod. In a later study, Good and Snyder (9) did find higher trichome densities in LD and higher quantities of zingiberene. Our results contrast with those of Good and Snyder (9). This could be the result of the *L. hirsutum* f. *hirsutum* clone that we are using, which produces zingiberene in amounts 3- to 10-fold greater than the clones they surveyed. In addition, their SD and LD conditions differed considerably from ours in that their experiments were conducted in the greenhouse in either winter (SD) or summer conditions (LD).

Our results indicate that photoperiod affects the amount of zingiberene per unit surface area by controlling zingiberene content per trichome. Although the factors controlling zingiberene synthesis may be different from those controlling trichome density, in SD at least, temperature affects the amount of zingiberene per unit surface area by controlling trichome formation.

In general, environmental or developmental factors that promote photosynthesis or availability of photosynthate concomitantly increase terpene accumulation (4, 7). Higher light intensity and duration usually promote terpene accumulation (8, 16), and cool nights have been found to increase monoterpene concentration in peppermint (*Mentha piperita* L.) before flowering (12). Monoterpene concentrations are generally higher in young leaves and peak at or before flowering (3, 12), with increased rates of catabolism at the time of flowering (4, 5).

LITERATURE CITED

1. **Carter CD, Gianfagna TJ, Sacalis JN** (1989) Sesquiterpenes in glandular trichomes of a wild tomato species are toxic to Colorado potato beetle. J Agric Food Chem **37:** 1425–1428
2. **Carter CD, Sacalis JN, Gianfagna TJ** (1989) Zingiberene and resistance to Colorado potato beetle in *Lycopersicon hirsutum* f. *hirsutum*. J Agric Food Chem **37:** 206–210
3. **Croteau R, Felton M, Karp F, Kjonaas R** (1981) Relationship of camphor biosynthesis to leaf development in sage (*Salvia officinalis*). Plant Physiol **67:** 820–824
4. **Croteau R, Johnson MA** (1984) Biosynthesis of terpenoids in glandular trichomes. *In* E Rodriguez, PL Healey, I Mehta, eds, Biology and Chemistry of Plant Trichomes. Plenum Press, New York, pp 133–185
5. **Croteau R, Martinkus C** (1979) Metabolism of monoterpenes. Demonstration of (+)-neomenthyl-β-D-glucoside as a major metabolite of (−)-menthone in peppermint (*Mentha piperita* L.) Plant Physiol **64:** 169–175
6. **Fery RL, Kennedy GG** (1987) Genetic analysis of 2-tridecanone concentration, leaf trichome characteristics, and tobacco hornworm resistance in tomato. J Am Soc Hort Sci **112:** 886–891
7. **Gershenzon J, Croteau R** (1990) Regulation of monoterpene biosynthesis in higher plants. Recent Adv Phytochem **24:** 99–160
8. **Gleizes M, Pauly G, Bernard-Dagen C, Jacques R** (1980) Effects of light on terpene hydrocarbon synthesis in *Pinus pinaster*. Plant Physiol **50:** 16–20
9. **Good DE Jr, Snyder JC** (1988) Seasonal variation of leaves and mite resistance of *Lycopersicon* interspecific hybrids. HortScience **23:** 891–894
10. **Kennedy GG, Yamamoto RT, Dimock MB, Williams WG, Bordner J** (1981) Effect of day length and light intensity on 2-tridecanone levels and resistance in *Lycopersicon hirsutum* f. *glabratum* to *Manduca sexta*. J Chem Ecol **7:** 707–716
11. **Lin SYH, Trumble JT, Kumamoto J** (1987) Activity of volatile compounds in glandular trichomes of *Lycopersicon* species against two insect herbivores. J Chem Ecol **13:** 837–850

12. **Loomis WD, Croteau R** (1973) Biochemistry and physiology of lower order terpenoids. Recent Adv Phytochem **6:** 147–185
13. **Snyder JC, Carter CD** (1984) Leaf trichomes and resistance of *Lycopersicon hirsutum* and *L. esculentum* to spider mites. J Am Soc Hort Sci **109:** 837–843
14. **Snyder JC, Hyatt JP** (1984) Influence of daylength on trichome densities and leaf volatiles of *Lycopersicon* species. Plant Sci Lett **37:** 177–181
15. **Went FW** (1944) Plant growth under controlled conditions. II. Thermoperiodicity in growth and fruiting of the tomato. Am J Bot **31:** 135–150
16. **Yamaura T, Tanaka S, Tabata M** (1989) Light-dependent formation of glandular trichomes and monoterpenes in thyme seedlings. Phytochemistry **28:** 741–744