

Morphogenic Light Reflected to Developing Cotton Leaves Affects Insect-Attracting Terpene Concentrations

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) leaves accumulate volatile terpenes that have been implicated in the attraction of both insect pests and the arthropods which prey on them. Our objective was to determine if altering the light environment of developing cotton leaves could affect the accumulation of these attractants. Plants were grown in drip-irrigated plots over colored polyethylene soil covers that reflected various combinations and intensities of red (R), far-red (FR), blue (BL), and photosynthetic photon flux (PPF). Individual terpenes were quantified by gas chromatography and identified by mass spectroscopy. Leaves exposed to a low amount of reflected BL and a FR/R ratio higher than that of incoming sunlight were thinner than leaves exposed to high amounts of reflected BL and PPF during development. Increasing the FR/R ratio while decreasing the amount of BL reflected to developing cotton leaves increased the leaf content of insect-attracting terpenes such as α -pinene and β -pinene on both leaf area and fresh weight bases. We conclude that altering the color of light reflected to developing cotton leaves can affect leaf content of insect attractants.

COTTON LEAVES ACCUMULATE VOLATILE TERPENES in mesophyllar glands or subdermal trichomes that attract both insect pests and their natural enemies, arthropods that prey on them. Because of the extensive hectareage of cotton worldwide, and the wide range of insect pests associated with it, the volatile constituents of these glands have been the subject of numerous studies. For example, blends of cotton terpenes containing compounds such as α -pinene, β -pinene, and caryophyllene have been shown to be attractive to pests such as boll weevils (*Anthonomus grandis grandis* Boheman) (Thompson et al., 1971; Hedin, 1976; Chang et al., 1986), tobacco budworm (*Heliothis virescens* Fabricus) (Chang et al., 1988; Rostelien et al., 2000), and cotton bollworm (*Helicoverpa armigera* Hübner) (Jallow et al., 1999), as well as insect parasites and predators such as *Campoletis sonorensis* (Cameron) (Elzen et al., 1983) and *Cotesia marginiventris* (Cresson) (Turlings et al., 1990). The odor profile emitted by growing cotton plants is highly characteristic and serves as an efficient means of location for insects (Chang et al., 1986).

The emission of terpenes by plants is affected by environmental factors such as temperature (Staudt et al., 1997), season (Hedin, 1976), and time of day (Loughrin et al., 1994, 1995, 1997). Light quantity and its spectral distribution are both affected by factors such as

time of day and season. Closeness of neighboring plants, plant residues, and variously colored mulches on the soil surface also affect PPF and morphogenic light that is received by developing plants (Kasperbauer 1971, 1987; Ballaré et al., 1990). For example, nearby growing plants and some colors of mulch can affect the amounts of FR reflected to developing plants. In nature, a high FR/R ratio acts through the natural growth regulating system within a plant to influence allocation and use of photosynthate to favor survival among competing plants. The regulatory system also influences the chemical composition of the developing plant.

Color of light, as a direct visual cue for insects, has been the subject of many studies (Antignus, 2000). Some colors attract certain insects and others repel. Mulch color, for example, has been shown to affect insect populations in crops such as pepper (*Capsicum annuum* L.), tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), and watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] (Lobenstein et al., 1975; Cohen, 1982; Csizinski et al., 1995; Farias-Larios and Orozco-Santos, 1997a, 1997b). The effect of color of light on mesophyllar glands and the synthesis and emission of volatile insect attractants from leaves, however, is not understood.

Recently we found that fragrant compounds including volatile terpenoids emitted by basil (*Ocimum basilicum* L.) leaves could be influenced by altering the color of light reflected from mulches to the developing plants (Loughrin and Kasperbauer, 2000). Like cotton leaves, basil leaves accumulate volatile terpenoids in specialized glands. The objective of the present study was to determine whether color of light reflected to developing cotton leaves could alter the concentrations of terpenes that are known to serve as insect attractants.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Cotton plants were grown in drip-irrigated plots of Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandudults) at the Coastal Plains Soil, Water, and Plant Research Center near Florence, SC. A randomized complete block design with four replicate plots was used each year of the 2-yr study. Raised beds were prepared, irrigation tubes were placed, and the 90-cm wide by 15-cm high raised-bed plots were covered with standard black plastic. In 2000, each plastic-covered plot was divided into three 6-m long subplots, two of which were painted with green or white exterior enamel. The other was covered with a red plastic (SRM-Red, Ken-Bar Agricultural Plastics, Reading, MA) that was held in place by

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taping it over the black plastic. In 2001, each plot was divided into four 6-m long subplots, three of which were painted with green, white, or yellow exterior enamel. The fourth plot was covered with the red plastic as described above. In 2000, seeds of the cultivar McNair 235 were sown, while in 2001, seeds of McNair 235 and 'SC-1' were sown. In both years, 5-cm-diam. holes were cut in the plastic at within-row distances of 0.6 m. Four seeds were sown in each hole. When the seedlings were in the cotyledon stage, all but one per hole were removed by cutting below the cotyledons. This allowed us to avoid disrupting the roots of the remaining seedling in each hole.

Reflected Light Measurements

Light measurements were taken at solar noon \pm 30 min on a cloudless day. The spectral distribution and quantity of upwardly reflected light was measured about 15 cm above the colored surfaces with a LI-COR LI-1800 spectroradiometer (LI-COR Inc., Lincoln, NE) equipped with a remote hemispherical light collector on a 1.5-m fiber optic probe. The spectroradiometer recorded measurements at 5-nm intervals between 400 and 800 nm. A reference spectrum was obtained by measuring incoming sunlight at the same wavelengths. The reflected light values were then calculated as percentages of incoming sunlight at each measured wavelength. We expressed values for R at 645 ± 5 nm because that is the approximate action peak for the red absorbing form of phytochrome (P_R) in green plants due to competitive absorption by chlorophyll at 660 nm (Kasperbauer et al., 1964), which is the absorption peak for P_R in vitro (Butler et al., 1964). Our values for FR were expressed at 735 ± 5 nm, which is the absorption peak for the far-red absorbing form of phytochrome (P_{FR}) in vitro, and FR' was expressed at 755 ± 5 nm, which is the beginning of the far-red reflection plateau (percentagewise) from green leaves (Kasperbauer, 1987). The FR' measurement is important morphologically because prolonged exposure (as occurs in the field) to FR at 735 nm results in a R response due to the overlapping of P_R absorption into that wave band (Kasperbauer et al., 1963). At 755 nm, the P_R absorption is extremely low, resulting in a FR response due to prolonged reflection of the 755- to 770-nm waveband from nearby plants as observed in nature (Kasperbauer, 1987). The FR/R and FR'/R ratios in upwardly reflected light were expressed relative to the ratios in incoming sunlight.

The values for light reflected from the red, green, yellow, and white surfaces were similar to those previously measured from the same colors (Loughrin and Kasperbauer, 2000). The white surface reflected approximately 40% of the BL and PPF that impinged on it, and FR/R and FR'/R ratios were similar to those of incoming sunlight. Yellow also reflected much PPF, but about half as much BL as was reflected by white. Red and green surfaces reflected only about 5% of the BL, relatively low PPF, and higher FR/R and FR'/R ratios than were present in sunlight at the same time and place.

Terpene Analysis

Since volatile emission from leaves is known to vary diurnally (Loughrin et al., 1994, 1997), all leaf samples were collected at 0900 h \pm 20 min to eliminate potential variation from this factor. All leaves were collected within 25 to 30 cm of the soil surface. In 2000, leaves of the cultivar McNair 235 were collected from plants before flowering, on sunny vs. overcast days occurring within 1 wk of each other. In 2001, leaves were collected from McNair 235 and SC-1. Fully expanded leaves were collected from plants of each cultivar at preflowering and again at early boll set. In both 2000 and

2001, leaves were kept cool and in darkness in insulated boxes until sampled.

Each sample consisted of 10 leaf disks that were punched out of two leaves with a No. 10 cork borer (total area per sample was 20 cm²). Care was taken to avoid major leaf veins. The disks were weighed and placed in 8-mL glass vials with Teflon-lined caps. Five milliliters of high purity pentane (amyl hydride) (J.T. Baker, Phillipsburg, NJ) was added to each vial and the samples were stored in darkness at -65°C until analyzed.

Samples were allowed to reach room temperature before analysis. The samples were vortexed and passed through 3-mL solid phase extraction cartridges containing 1000 mg of silica gel absorbent (Supelco Inc., Bellefonte, PA) to remove oxygenated compounds from the extracts. The nonretained portions of the extracts were used for gas chromatographic analyses as described below.

In 2000, 10 μg of cumene in pentane was added to each vial as an internal standard. Samples were concentrated to approximately 1 mL under a stream of N before analysis. In 2001, the samples were used directly for analyses after removal of the oxygenated compounds. Compounds were quantified by injections of external standards of α -pinene for monoterpenes and caryophyllene for sesquiterpenes.

Samples were analyzed by injection into a Varian model 3800 gas chromatograph (GC, Varian Associates, Walnut Creek, CA) equipped with a 60-m by 0.32-mm SPB-5 column with a film thickness of 1.0 μm (Supelco, Inc.). Two-microliter injections were made in splitless mode for 1 min with an injector temperature of 220°C , column initial temperature of 50°C for 4 min, and column oven programming at 2°C min^{-1} to 100°C . The column oven was then programmed at 5°C min^{-1} to 230°C . Other GC operating conditions included flame ionization detector at 260°C , column helium linear flow rate of 17 cm s^{-1} , injector split ratio of 75:1, and helium make-up gas flow rate of 25 mL min^{-1} .

Compound Identification

Gas chromatography and mass spectroscopy were performed on a GC equipped with a 30-m by 0.25-mm HP-5 column (Hewlett-Packard, Palo Alto, CA) interfaced to a Hewlett-Packard Model 1800 mass selective detector. Ten-milliliter samples from both cultivars were concentrated to about 1 mL at 50°C with a micro Kudema-Danish concentrator (Supelco Inc.). One-microliter aliquots were injected onto the GC in splitless mode for 1 min and the mass ion detector used a scanning range of 40 to 450 amu. Operating conditions for the GC included injector temperature of 220°C and column oven at 40°C for 1 min then programmed at 3°C min^{-1} to 180°C . Compound identifications were performed by computer database searches and retention time matches of the natural compounds with those of authentic samples of compounds on the SPB-5 column. Authentic samples of compounds were obtained from commercial sources.

Statistical Analysis

In both years, terpene concentrations were expressed relative to leaf weight (ng mg⁻¹ fresh weight) and leaf area (ng cm⁻²) and subjected to analysis of variance with PROC MIXED using the SAS system for Windows (SAS Institute, 1996). Means and standard errors of the mean were calculated by PROC MEANS.

RESULTS AND DISCUSSION

Leaf Morphology

Altering the color of light reflected to developing cotton leaves affected their morphology. Leaf disks were heavier over white mulch than over red or green, and those grown over yellow were intermediate (Fig. 1). Therefore, increasing PPF and BL reflected to leaves resulted in heavier leaves while increasing the ratio of FR to R resulted in lighter leaves. These results are consistent with those of an earlier study of influence of reflected color on leaf morphology and concentration of photosynthetic pigments in cotton seedlings (Bradburne et al., 1989).

Terpene Concentrations

We identified six terpene hydrocarbons from the leaf extracts in 2000, and nine in 2001. The identity of these compounds was confirmed by comparison of fragmentation patterns with samples of authentic compounds and retention time matches on the SPB-5 column. However, an authentic sample of the sesquiterpene δ -guaiene was not available to us and was tentatively identified on the basis of its fragmentation pattern and reports of its occurrence in cotton (Minyard et al., 1966).

2000

The levels of α -pinene, β -pinene, and myrcene from leaves of McNair 235 were affected ($P \leq 0.1$) by color of light received by the leaves during development (Table 1). In contrast, we found no significant effect of sunny vs. overcast weather on the accumulation of volatile terpenes. This is consistent with accumulation in peppermint (*Mentha × piperita* L.) leaves during the vegetative growth stage, in which the synthesis of monoterpenoids in leaves occurred during a relatively short period corresponding with rapid leaf expansion (Gers-

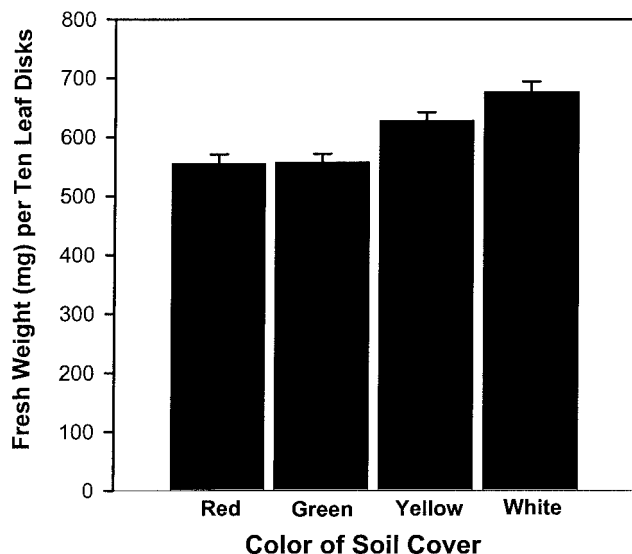


Fig. 1. Mean fresh weight \pm standard error per 10-disk (20 cm²) samples from cotton leaves at early boll stage after development over red, green, yellow, or white soil covers. Means are for 10 such 10-disk samples for each of two cultivars (McNair 235 and SC-1).

Table 1. Analysis of variance comparing the concentrations of volatile terpenes isolated from McNair 235 leaves grown over red, green, or white soil covers and sampled during sunny vs. overcast weather in 2000.

Compound	Color (C)	Weather (W) [†]	C × W
α -Pinene	0.0965 \ddagger	0.8160	0.6366
β -Pinene	0.0619	0.9184	0.4310
Myrcene	0.0696	0.7213	0.9527
Limonene	0.5150	0.5645	0.2104
Caryophyllene	0.6445	0.8302	0.8897
α -Humulene	0.6222	0.7682	0.8534
Total	0.1747	0.9952	0.7529

[†] Sunny vs. overcast.

[‡] Values are probabilities from an analysis of variance with 10 observations. Terpene content was analyzed on a fresh-weight basis.

henson et al., 2000). Apparently no significant losses due to catabolism or volatilization occurred in the accumulated pool thereafter. It appears that terpene accumulation is similarly regulated in cotton; therefore, changes in weather occurring within a relatively short time span would not be expected to affect the level of stored terpenes.

Table 2 presents data on the concentrations of terpenes (ng mg⁻¹) from McNair 235 collected during sunny and overcast days. Although terpene concentrations were variable, greater concentrations of these compounds were found in leaves that had developed over red and green reflectors than those that had developed over white. The same trends were observed when terpene concentrations were expressed in terms of leaf area (ng cm⁻², data not shown).

2001

Differences in the amounts of terpene hydrocarbons accumulated in leaves were found on the basis of cultivar, developmental stage (prebloom vs. early boll set), and color of soil cover. Table 3 presents analysis of variance of the effects of these factors, and their interactions, on the amounts of volatile terpenes per weight of leaf. Color of soil cover was found to be a significant factor ($P \leq 0.05$) affecting the amount of the monoter-

Table 2. Concentrations of terpenes from cotton cultivar McNair 235 grown in 2000 over red, green, or white soil covers and sampled during sunny vs. overcast weather.

Compound	Soil cover		
	Red	Green	White
	ng mg ⁻¹		
	Sunny weather		
α -Pinene	281 \pm 59 [†]	329 \pm 109	211 \pm 50
β -Pinene	61 \pm 9	81 \pm 31	52 \pm 10
Myrcene	125 \pm 31	201 \pm 108	89 \pm 36
Limonene	23 \pm 4	28 \pm 11	27 \pm 5
Caryophyllene	119 \pm 31	142 \pm 40	109 \pm 29
α -Humulene	35 \pm 8	47 \pm 15	33 \pm 8
Total	644 \pm 139	828 \pm 310	522 \pm 133
	Overcast weather		
α -Pinene	410 \pm 96	336 \pm 119	171 \pm 53
β -Pinene	84 \pm 18	74 \pm 19	38 \pm 8
Myrcene	152 \pm 37	167 \pm 59	67 \pm 18
Limonene	30 \pm 7	25 \pm 6	15 \pm 3
Caryophyllene	156 \pm 38	184 \pm 87	118 \pm 35
α -Humulene	48 \pm 11	55 \pm 24	37 \pm 10
Total	879 \pm 196	978 \pm 373	445 \pm 124

[†] Values are the mean of 10 observations \pm standard error of the mean.

Table 3. Analysis of variance comparing the concentrations of volatile terpenes isolated from leaves of two cotton cultivars grown in 2001 over four colors of soil covers and sampled at two developmental stages, expressed on the basis of leaf weight.

Compound	Color (C)	Stage (S) [†]	Cultivar (V)	C × V	C × S
α-Pinene	0.0002‡	0.4928	0.1806	0.1404	0.4369
β-Pinene	0.0001	0.8610	0.2517	0.0779	0.3114
2-Carene	0.1107	0.0001	0.0846	0.3205	0.2332
Myrcene	0.0028	0.9227	0.1261	0.0852	0.8547
Limonene	0.2214	0.2739	0.7814	0.1033	0.9308
γ-Terpinene	0.0421	0.0093	0.0030	0.0824	0.3487
Caryophyllene	0.0013	0.1377	0.0432	0.1699	0.0512
α-Humulene	0.0016	0.3184	0.1263	0.4379	0.4431
δ-Guaiene	0.4150	0.0028	0.0317	0.1399	0.4347
Total terpenes	0.0106	0.0840	0.0315	0.2340	0.2866

[†] Prebloom vs. boll set.

[‡] Values are probabilities from an analysis of variance with eight observations. Terpene content was analyzed on a fresh-weight basis.

penes α-pinene, β-pinene, myrcene, γ-terpinene, and the sesquiterpenes caryophyllene and α-humulene as well as total terpenes. Developmental stage of the plants was a significant factor affecting the amounts of 2-carene, γ-terpinene, and δ-guaiene. Significant differences between cultivars were seen in the amounts of γ-terpinene, caryophyllene, δ-guaiene, and total terpenes. No interactions were found between color of light reflected to the developing leaves and cotton cultivar nor color of light and developmental stage of the plant.

When analyzing each cultivar separately, color of light significantly ($P \leq 0.05$) affected α-pinene, β-pinene, γ-terpinene, caryophyllene, and α-humulene, as well as total terpenes in McNair 235 (Table 4). Developmental stage was a significant factor affecting α-pinene, β-pinene, 2-carene, myrcene, γ-terpinene, caryophyllene, α-humulene, δ-guaiene, and total terpenes. Significant interactions between color of light and developmental stage were seen for α-pinene, β-pinene, caryophyllene, and total terpenes. Color of light reflected to developing

Table 4. Analysis of variance comparing the concentrations of volatile terpenes isolated from leaves of two cotton cultivars grown in 2001 over four colors of soil covers and sampled at two developmental stages, expressed on the basis of leaf weight.

Compound	Color (C)		Stage (S) [†]	C × S
	'McNair 235'			
α-Pinene	0.0008‡	0.0001	0.0001	0.0185
β-Pinene	0.0001	0.0001	0.0001	0.0102
2-Carene	0.1173	0.0001	0.0001	0.4487
Myrcene	0.0773	0.0001	0.0001	0.4179
Limonene	0.1983	0.6295	0.6609	0.6609
γ-Terpinene	0.0344	0.0225	0.1971	0.1971
Caryophyllene	0.0115	0.0009	0.0374	0.0374
α-Humulene	0.0127	0.0023	0.0659	0.0659
δ-Guaiene	0.3286	0.0168	0.5064	0.5064
Total	0.0249	0.0001	0.0476	0.0476
Compound	Color (C)		Stage (S) [†]	C × S
	'SC-1'			
α-Pinene	0.0025	0.0001	0.0001	0.2916
β-Pinene	0.0014	0.0001	0.0001	0.3902
2-Carene	0.2384	0.2691	0.3029	0.3029
Myrcene	0.0002	0.0001	0.1663	0.1663
Limonene	0.1366	0.0540	0.8814	0.8814
γ-Terpinene	0.4303	0.1923	0.6329	0.6329
Caryophyllene	0.1428	0.0217	0.1072	0.1072
α-humulene	0.0139	0.0011	0.2190	0.2190
δ-guaiene	0.1645	0.0728	0.6056	0.6056
Total	0.0084	0.0001	0.4526	0.4526

[†] Prebloom vs. boll set.

[‡] Values are probabilities from an analysis of variance with eight observations. Terpene content was analyzed on a fresh-weight basis.

Table 5. Concentrations of terpenes from cotton cultivar McNair grown over four colors of soil covers and sampled at two developmental stages in 2001.

Compound	Soil cover			
	Red	Green	Yellow	White
ng mg ⁻¹				
Prebloom stage				
α-Pinene	180 ± 13 [†]	80 ± 26	76 ± 7	76 ± 33
β-Pinene	38 ± 4	19 ± 7	16 ± 2	17 ± 2
2-Carene	43 ± 6	37 ± 10	38 ± 5	39 ± 6
Myrcene	42 ± 4	32 ± 12	22 ± 4	32 ± 9
Limonene	44 ± 5	35 ± 6	38 ± 4	38 ± 4
γ-Terpinene	32 ± 3	39 ± 15	24 ± 3	24 ± 3
Caryophyllene	58 ± 5	35 ± 12	23 ± 3	23 ± 3
α-Humulene	30 ± 4	12 ± 3	8 ± 2	5 ± 1
δ-Guaiene	29 ± 3	45 ± 10	66 ± 13	49 ± 8
Total	734 ± 28	420 ± 112	447 ± 54	381 ± 34
Early boll set stage				
α-Pinene	708 ± 198	335 ± 50	160 ± 18	103 ± 15
β-Pinene	128 ± 31	61 ± 7	25 ± 4	23 ± 3
2-Carene	32 ± 8	18 ± 7	7 ± 5	12 ± 6
Myrcene	111 ± 30	76 ± 16	60 ± 10	30 ± 6
Limonene	54 ± 7	40 ± 6	36 ± 5	38 ± 5
γ-Terpinene	56 ± 15	60 ± 8	23 ± 4	27 ± 3
Caryophyllene	183 ± 65	128 ± 21	37 ± 4	252 ± 85
α-Humulene	48 ± 18	34 ± 6	9 ± 2	61 ± 22
Total	1660 ± 417	967 ± 102	512 ± 29	819 ± 157

[†] Values are the mean of eight observations ± standard error of the mean.

leaves significantly affected α-pinene, β-pinene, myrcene, α-humulene, and total terpenes in SC-1. Also in SC-1, developmental stage significantly affected α-pinene, β-pinene, myrcene, caryophyllene, α-humulene, and total terpenes. However, there were no significant interactions between color of reflected light and developmental stage for SC-1.

While amounts of foliar terpenes increased from prebloom to early boll set for the cultivar McNair 235 and decreased for the cultivar SC-1, some trends were apparent (Tables 5, 6). For McNair 235, the highest levels of total terpenes were obtained from leaves which were

Table 6. Concentrations of terpenes from cotton cultivar SC-1 grown over four colors of soil covers and sampled at two developmental stages in 2001.

Compound	Soil cover			
	Green	Yellow	White	
ng mg ⁻¹				
Prebloom stage				
α-Pinene	383 ± 90 [†]	374 ± 48	276 ± 60	80 ± 26
β-Pinene	74 ± 16	75 ± 9	55 ± 9	21 ± 3
2-Carene	38 ± 7	43 ± 10	32 ± 7	17 ± 6
Myrcene	53 ± 11	131 ± 26	68 ± 15	12 ± 2
Limonene	45 ± 9	54 ± 8	48 ± 5	34 ± 7
γ-Terpinene	25 ± 6	32 ± 4	25 ± 4	15 ± 4
Caryophyllene	89 ± 24	44 ± 16	55 ± 15	14 ± 2
α-Humulene	28 ± 6	26 ± 7	18 ± 5	5 ± 1
δ-Guaiene	50 ± 5	60 ± 8	58 ± 10	41 ± 6
Total terpenes	923 ± 180	1050 ± 104	770 ± 120	337 ± 36
Early boll set stage				
α-Pinene	84 ± 18	130 ± 19	44 ± 7	18 ± 3
β-Pinene	18 ± 4	26 ± 5	7 ± 2	3 ± 1
Myrcene	12 ± 2	36 ± 7	15 ± 9	3 ± 1
Limonene	32 ± 5	41 ± 5	39 ± 6	30 ± 5
γ-Terpinene	25 ± 4	27 ± 4	27 ± 5	22 ± 2
Caryophyllene	20 ± 5	38 ± 8	14 ± 2	10 ± 1
α-Humulene	6 ± 2	9 ± 2	8 ± 3	2 ± 1
δ-Guaiene	62 ± 3	70 ± 11	57 ± 8	36 ± 7
Total terpenes	401 ± 32	523 ± 57	333 ± 34	217 ± 22

[†] Values are the mean of eight observations ± standard error of the mean.

grown over the red reflective surfaces at both developmental stages. For SC-1, leaves which had been grown over green surfaces contained the highest levels of total terpenes, followed by leaves which had been grown over red. The same pattern occurred at both growth stages. This is consistent because both the red and green soil covers reflected a relatively low PPF and a high FR/R ratio as compared with the white and yellow surfaces.

The trend observed in 2001 was consistent with that observed in 2000 (see Table 2). That is, cotton leaves that developed over soil covers that reflected a FR/R ratio higher than that of incoming sunlight contained the highest levels of terpenes. It appears, therefore, that increasing the FR/R ratio reflected to developing cotton leaves can increase the amount of volatile terpenoids accumulated in leaves despite their having less weight per unit area. This, in turn, could increase the emission of volatile insect attractants from cotton. It should be noted, however, that in the present study the red and green surfaces reflected a FR/R ratio higher than was present in sunlight without decreasing incoming PPF. Whether a high FR/R ratio at reduced PPF, as occurs when the FR/R ratio is altered by nearness of other plants, would result in increased accumulation of terpenes is unclear and beyond the scope of the present study.

Conversely, increased PPF as was reflected from the yellow and white soil covers was less effective in inducing the accumulation of leaf terpenes than was a high FR/R ratio. Thus, within the range of natural outdoor light intensities used in the present study, altering R and FR light reflected to developing leaves was more effective in inducing terpene accumulation than was altering PPF reflected to the leaves.

In summary, phytophagous insects locate hosts by a combination of visual and olfactory cues. Many studies have shown that colored soil covers and traps can affect insect populations in crops, presumably by acting as a direct visual cue (Antignus, 2000). Results from the present work show that reflected color may also affect insect orientation to plants by affecting plant metabolism and boosting available olfactory cues. Our work clearly shows that altering morphogenic light reflected to developing cotton leaves can modify the concentration of volatile insect attractants. This finding has potential application in any cultural practice or integrated pest management program that can influence morphogenic light received by growing cotton leaves.

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REFERENCES

Antignus, Y. 2000. Manipulation of wavelength-dependent behaviour of insects: An IPM tool to impede insects and restrict epidemics of insect-borne viruses. *Virus Res.* 71:213–220.

- Ballaré, C.L., A.L. Scopel, and R.A. Sánchez. 1990. Far-red radiation reflected from adjacent leaves: An early signal of competition in plant canopies. *Science* (Washington, DC) 247:329–332.
- Bradburne, J.A., M.J. Kasperbauer, and J.N. Mathis. 1989. Reflected far-red light effects on chlorophyll and light-harvesting chlorophyll protein (LHC-II) contents under field conditions. *Plant Physiol.* 91:800–803.
- Butler, W.L., S.B. Hendricks, and H.W. Siegelman. 1964. Action spectra of phytochrome in-vitro. *Photochem. Photobiol.* 3:521–528.
- Chang, J.F., J.H. Benedict, T.L. Payne, and B.J. Camp. 1986. Methods for collection and identification of volatile terpenes from cotton, and evaluation of their attractiveness to boll weevils. *Southwest Entomol.* 11:233–241.
- Chang, J.F., J.H. Benedict, T.L. Payne, and B.J. Camp. 1988. Volatile monoterpenes collected from the air surrounding flower buds of seven cotton genotypes. *Crop Sci.* 28:685–688.
- Cohen, S. 1982. Control of whitefly vectors of viruses by colour mulches. p. 45–46. In K.F. Harris and K. Maramorosch (ed.) *Pathogen, vectors, and plant diseases: Approaches to control.* Academic Press, New York.
- Csizinski, A.A., D.J. Schuster, and J.B. Kring. 1995. Colour mulches influence yield and insect pest populations in tomatoes. *J. Am. Soc. Hortic. Sci.* 120:778–784.
- Elzen, G.W., H.J. Williams, and S.B. Vinson. 1983. Response by the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) to synomones in plants: Implications for host habitat location. *Environ. Entomol.* 12:1873–1877.
- Farias-Larios, J., and M. Orozco-Santos. 1997a. Effect of polyethylene mulch color on aphid populations, soil temperature, fruit quality and yield of watermelon under tropical conditions. *N. Z. J. Crop Hortic. Sci.* 25:369–374.
- Farias-Larios, J., and M. Orozco-Santos. 1997b. Color polyethylene mulches increase fruit quality and yield in watermelon and reduce insect pest populations in dry tropics. *Gartenbauwissenschaft* 62:255–260.
- Gershenson, J., M.E. McConkey, and R.B. Crouteau. 2000. Regulation of monoterpene accumulation in leaves of peppermint. *Plant Physiol.* 122:205–213.
- Hedin, P.A. 1976. Seasonal variations in the emissions of volatiles by cotton plants growing in the field. *Environ. Entomol.* 5:1234–1238.
- Jallow, M.F.A., M.P. Zalucki, and G.P. Fitt. 1999. Role of chemical cues from cotton in mediating host selection and oviposition behaviour in *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Austr. J. Entomol.* 38:359–366.
- Kasperbauer, M.J. 1971. Spectral distribution of light in a tobacco canopy and effects of end-of-day light quality on growth and development. *Plant Physiol.* 47:775–778.
- Kasperbauer, M.J. 1987. Far-red light reflection from green leaves and effects on phytochrome-mediated assimilate partitioning under field conditions. *Plant Physiol.* 85:350–354.
- Kasperbauer, M.J., H.A. Borthwick, and S.B. Hendricks. 1963. Inhibition of flowering of *Chenopodium rubrum* by prolonged far-red radiation. *Bot. Gaz. (Chicago)* 124:444–451.
- Kasperbauer, M.J., H.A. Borthwick, and S.B. Hendricks. 1964. Reversion of phytochrome 730 (Pfr) to P660 (Pr) in *Chenopodium rubrum*. *Bot. Gaz. (Chicago)* 125:85–90.
- Lobenstein, G., M. Alper, D. Levy, D. Palevitch, and E. Menager. 1975. Protecting peppers from aphid-borne viruses with aluminum foil and plastic mulch. *Phytoparasitica* 3:43–53.
- Loughrin, J.H., and M.J. Kasperbauer. 2000. Light reflected from colored mulches affects aroma and phenol content of sweet basil (*Ocimum basilicum* L.) leaves. *J. Agric. Food Chem.* 49:1331–1335.
- Loughrin, J.H., A. Manukian, R.R. Heath, and J.H. Tumlinson. 1995. Volatiles emitted by different cotton varieties damaged by feeding beet armyworm larvae. *J. Chem. Ecol.* 21:1217–1227.
- Loughrin, J.H., D.A. Potter, T.R. Hamilton-Kemp, and M.E. Byers. 1997. Diurnal emission of volatile compounds by Japanese beetle-damaged grape leaves. *Phytochemistry* 45:919–923.
- Loughrin, J.H., T.C.J. Turlings, A. Manukian, R.R. Heath, and J.H. Tumlinson. 1994. Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton. *Proc. Natl. Acad. Sci. USA* 92:11836–11840.
- Minyard, J.P., J.H. Tumlinson, A.C. Thompson, and P.A. Hedin. 1966.

- Constituents of the cotton bud. Sesquiterpene hydrocarbons. *J. Agric. Food Chem.* 14:332–335.
- Rostelien, T., A.K. Borg-Karlson, and H. Mustaparta. 2000. Selective receptor neurone responses to E-beta-ocimene, beta-myrcene, E,E-alpha-farnesene and homo-farnesene in the moth *Heliothis virescens*, identified by gas chromatography linked to electrophysiology. *J. Comp. Physiol. [A]* 186:833–847.
- SAS Institute. 1996. SAS system for Windows. Version 6.12. SAS Inst., Cary, NC.
- Staudt, M., N. Bertin, U. Hansen, G. Seufert, P. Ciccioli, P. Foster, B. Frenzel, and J.L. Fugit. 1997. Seasonal and diurnal patterns of monoterpene emissions from *Pinus pinea* (L.) under field conditions. *Atmos. Environ.* 31(Suppl. 1):145–156.
- Thompson, A.C. 1971. Identification and quantitative analysis of the volatile substances emitted by maturing cotton in the field. *Plant Physiol.* 48:50–52.
- Turlings, T.C.J., J.W.A. Scheepmaker, L.E.M. Vet, J.H. Tumlinson, and W.J. Lewis. 1990. How contact foraging experiences affect preferences for host-related odors in the larval parasitoid *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae). *J. Chem. Ecol.* 16:577–589.