Journal of Chemical Ecology, Vol. 22, No. 2, 1996

CORRELATION OF 1-OCTEN-3-ONE WITH ANTIXENOTIC RESISTANCE IN SUBTERRANEAN CLOVER COTYLEDONS TO RED-LEGGED EARTH MITE, *Halotydeus destructor* (ACARINA: PENTHALEIDAE)

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(Received May 26, 1995; accepted October 26, 1995)

Abstract—Artificially damaged cotyledons of subterranean clover (*Trifolium subterraneum* L.) released several volatile metabolites, including 1-octen-3-one, arising from lipid peroxidation. The amount of 1-octen-3-one produced was negatively correlated with feeding damage caused by the red-legged earth mite (*Halotydeus destructor*) in nine out of 10 resistant and susceptible *T. subterraneum* varieties tested. The EC_{50} of this compound in deterring mites from feeding in a membrane bioassay was 50 ppm. Cotyledon toughness was also involved in resistance. The resistant variety, S3615D, which has the lowest toughness value among the resistant varieties, produced the highest amount of 1-octen-3-one recovered from the headspace in 1 hr. Artificially damaged cotyledons of both susceptible Dalkeith and resistant DGI007, growing in shade, showed lower toughness, but had enhanced production of C_8 volatile compounds and were avoided by mites during a 3-hr feeding test. When both 1-octen-3-one content and cotyledon toughness value were taken as cofactors in resistance, the resultant multiplication value yielded a more significantly negative correlation with mite feeding damage scores within the 10 varieties than either factor alone. We conclude that 1-octen-3-one has a role in resistance of subclover cotyledon to the mite.

Key Words—Subterranean clover, *Trifolium subterraneum*, cotyledons, red-legged earth mite, *Halotydeus destructor*, deterrence, 1-octen-3-one, volatiles, host resistance, antixenosis.

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0098-0331/96/0200-0369$09.50/0 © 1996 Plenum Publishing Corporation

INTRODUCTION

The red-legged earth mite, *Halotydeus destructor* (Tucker) (Acarina: Penthaleidae) causes an estimated annual loss of $200 million in production from pastures in southern Australia. Subterranean clover (subclover), *Trifolium subterraneum* L., suffers serious damage from feeding by the mites, especially at the seedling stage. As a consequence, cotyledons and even whole seedlings can be destroyed by mites. Screening for resistant varieties at the seedling stage has therefore become a priority in the control of this pest and a number of resistant varieties have been selected (Gillespie, 1994).

Cotyledons of the resistant varieties show antixenotic properties, with less mites feeding on them and being less damaged than susceptible varieties (Jiang and Ridsdill-Smith, unpublished data). Physical toughness of the cotyledons, regarded as a possible defense against the mite, is negatively correlated with mite feeding damage in at least 16 varieties of subclover (Jiang and Ridsdill-Smith, unpublished data). An exception is the resistant variety S3615D, in which the toughness value is as low as susceptible varieties, suggesting the likelihood that other resistance mechanisms are involved.

Cotyledons of the resistant varieties are not completely immune to mite feeding (Gillespie, 1993; Ridsdill-Smith and Gillespie, 1993). Behavioral observations reveal that mites visit cotyledons of resistant varieties initially in choice tests and some feeding occurs on such cotyledons in nonchoice tests. Silver patches, caused by feeding damage, are visible on cotyledons of resistant as well as susceptible varieties within several minutes (unpublished), although they are limited to small areas for the former varieties. Electron microscopy studies have shown that feeding damage is largely a result of a mechanical process, with mite chelicerae making penetration holes in epidermal cells (Ridsdill-Smith et al., 1996). These observations led us to hypothesize that "antifeedant" properties are not detected until the mite penetrates and damages the cotyledons. Since a mite sucks the content from single cells and penetrates cells quickly, any plant response to cell damage would need to be produced rapidly to have an effect on a feeding mite. The release of volatile compounds can occur within seconds following damage to plant tissue, and such a process could play a significant role in determining resistance. It is possible to investigate the volatile compounds with artificially damaged cotyledons, since Dicke and Sabelis (1992) have demonstrated that artificially damaged Lima bean leaves produce virtually all the same volatile compounds induced by spider mite feeding damage.

We have shown that volatiles from artificially damaged cotyledons are strongly deterrent and/or repellent to the mite at certain concentrations when tested in a membrane sachet bioassay (Jiang et al., unpublished data). The volatile compounds collected from a resistant variety are more deterrent than those from a susceptible variety (Jiang et al., unpublished data). In this paper,

we present evidence that the volatile compound, 1-octen-3-one, was involved in resistance. Bioassay results indicated that the compound was deterrent to the mite in a concentration-dependent manner. Furthermore, in an experiment designed to find effects on mite feeding of cotyledons growing in the shade, it was found, unexpectedly, that although the cotyledons had significantly lower toughness, the mites did not feed on shaded cotyledons and production of 1-octen-3-one was increased in shaded cotyledons.

METHODS AND MATERIALS

Plant Material. Seeds of four susceptible and six resistant subclover varieties were sown in pots (each 12 cm diameter) with standardized sand–loam soil as described by Ridsdill-Smith and Gillespie (1993). For each test, two susceptible and two resistant varieties were grown under the same glasshouse conditions. Seedlings were harvested and processed on the same day for collection of volatile compounds, 12 or 13 days after seed sowing. To study the effects of toughness and volatiles of cotyledons growing in the shade on feeding of mites, seedlings in five pots of each variety (Dalkeith, susceptible; DGI007, resistant) were covered with a cardboard box with four openings of 5 × 5 cm in the middle of the side walls, allowing in dim light to the plants. As controls, seedlings in five other pots were not covered. Cotyledons were used at 12 or 13 days after seed sowing.

Mites. Red-legged earth mites were collected from the field, near Perth, in winter. They were age-graded after collection and only young adult mites were used for bioassays. Before the bioassay, mites were starved for 2 hr in a humid vial.

Chemicals. 1-Octen-3-ol and 2-(*E*)-hexenal were purchased from Aldrich and Sigma Chemical Company, respectively. 1-Octen-3-one was prepared by oxidation of 1-octen-3-ol with pyridinium chlorochromate.

Headspace Collection of Volatile Compounds from Artificially Damaged Cotyledons. A stream of air filtered through granulated charcoal (about 50 g) was drawn into a separating funnel (8 cm diameter) that contained the plant tissues. The air carrying the volatile compounds was passed into a trap made of 50 mg powdered charcoal in a Pasteur pipet. The airflow was controlled by the water pump (about 500 ml/min). Trapping was generally conducted for 1 hr only, unless specified otherwise. For quantitative comparison among 10 varieties, 2.4 g of cotyledon tissues of each variety were used. To determine the 1-octen-3-one content after collection for different times (1 and 6 hr), 7.8 g of cotyledon tissues were used for each variety. To test cotyledons grown in the shade, 3.5 g of tissues were used. Detached cotyledons were frozen in liquid nitrogen and ground (damaged) in a mortar. The powdered tissues were trans-

ferred, with a filter paper, into a round funnel (8 cm diameter), in which another filter paper (Whatman No. 1), cut netlike, was set at the bottom to hold the powder. The tissue was deposited on the walls of the funnel by turning it around. By this time, the tissue had thawed and collection of the volatile compounds was started. To compare different varieties, 5 or 10 μl of hexanol was added as an internal standard. The charcoal traps were stored at $-20°C$.

Gas Chromatography (GC). The volatile compounds were desorbed with 1 ml of dichloromethane. The compounds collected were analyzed by GC, using a Hewlett-Packard 5790A instrument equipped with a fused silica BP 1 column (0.25-μm film thickness, 0.22 mm ID $\times$ 25 m) and an FID detector with hydrogen as the carrier gas (1.5 ml/min). Temperature was programmed from 40°C for 5 min and then to 290°C at 20°C/min. For each sample, at least two chromatograms were recorded.

Gas Chromatography–Mass Spectrometry (GC-MS). Volatile compounds were analyzed using a Hewlett-Packard 5986 GC-MS (EI, 70 eV). The compounds were identified by comparison of their mass spectra and retention time, singly and in admixture, with those of authentic compounds.

Air Oxidation of 1-Octen-3-ol. 1-Octen-3-ol (1 μl; 98% purity) was treated under the same conditions as described for damaged cotyledon tissues. In another test, 1 ml of 0.05% (v/v) aqueous 1-octen-3-ol solution was added onto the frozen powders of ground cotyledon tissues of a resistant variety (DGI007) and the volatiles were collected as for other treatments.

Membrane Feeding Test. The bioassay of the volatile compounds was conducted by using membrane sachets as described in Jiang et al. (unpublished data). Briefly, compounds were added to pure Tween 80. A solution of glucose in distilled water was added to achieve a final concentration of 5% Tween 80 and 1% glucose. To measure the EC_{50} for deterrence, a range of concentrations of a compound were tested. The aqueous solution used as feeding medium was sandwiched between two layers of parafilm membranes stretched over a plastic ring (2 cm diameter). In the two-choice tests, a sachet with the test compound was a treatment, whereas the other contained only Tween 80 and glucose and served as a control. Two membrane sachets were set into the surface of a sand–loam soil close to each other in a tissue culture cup (5.7 cm diameter). Twenty mites were released onto the soil, and mite numbers were counted every 20 min for the first hour and then every 30 min afterwards for a further 2 hr. There were 10 replicates for each treatment. The averages from seven observations for each replicate were used to calculate the mean and standard errors, which were compared with a t test for statistical significance in a choice test. Mite counts provide a relative estimate of feeding (Jiang and Ridsdill-Smith, unpublished data) based on the formula [(numbers of mites on control $-$ numbers of mites on treatment)/both mite numbers added)] $\times$ 100. Mite selection between the treatment and control membranes was expressed as deterrence of the test

compound (indicated by a positive value) or as preference for the test compound (indicated by a negative value).

Scores of Mite Feeding Damage to Cotyledons over Two Weeks. Seedlings in pots with multivariety choices were exposed to mites for two weeks (Gillespie, 1993). Mite feeding damage to seedlings was scored for each variety from more than two experiments with a 1–10 rating system (1 = 10% feeding damage and 100% = death of seedlings). Damage scores collected by Gillespie from different varieties were used in this study to calculate regression between the resistance and amounts of volatile compounds.

Cotyledon Choice Feeding Test. The choice feeding methods are described in Jiang and Ridsdill-Smith (unpublished data). The cotyledons were cut and two were placed close to each other on the sand–loam in a small Petri dish (5 dm diameter). Cotyledons from the same or a different variety (Dalkeith, susceptible; and DGI007, resistant) were compared for effects of shaded cotyledons on mites. Numbers of mites feeding on cotyledons were counted every 20 min in the first hour and then every 30 min afterwards for 2 hrs. There were 10 replicates for each choice test. Numbers of mites counted from the seven observations during 3 hr were averaged and used to calculate mean, standard error, and statistical significance (t test). Mite preference for a cotyledon was calculated with the above formula.

Toughness Test of Cotyledons. Toughness of cotyledons from varieties (Dalkeith, susceptible; and DGI007, resistant) growing in shade or light was measured with a pipet-balance penetrometer (Jiang and Ridsdill-Smith, unpublished data). Four penetrations were made for each cotyledon, on both sides of the main vein away from the base, with 10 replicate cotyledons for each treatment.

Combined Effects of Toughness and Volatiles. Based on the results obtained in this work for 1-octen-3-one and the results collected by Jiang and Ridsdill-Smith (unpublished data) for toughness, it was assumed that both 1-octen-3-one and toughness contributed to the resistance in cotyledons. It is unknown whether both factors are of equal importance in resistance, but values of both for a variety were multiplied to produce an index. Regressions were made between the index and damage scores for the different varieties.

RESULTS

Identification of Compounds Present in Headspace Collection. No qualitative difference in the volatile compounds produced was found among the varieties examined (Figure 1). Of the five major compounds, three, 2-(E)-hexenal, 1-octen-3-one, and 1-octen-3-ol, have been identified. Comparison of their mass spectrometric and chromatographic properties with those of authentic samples

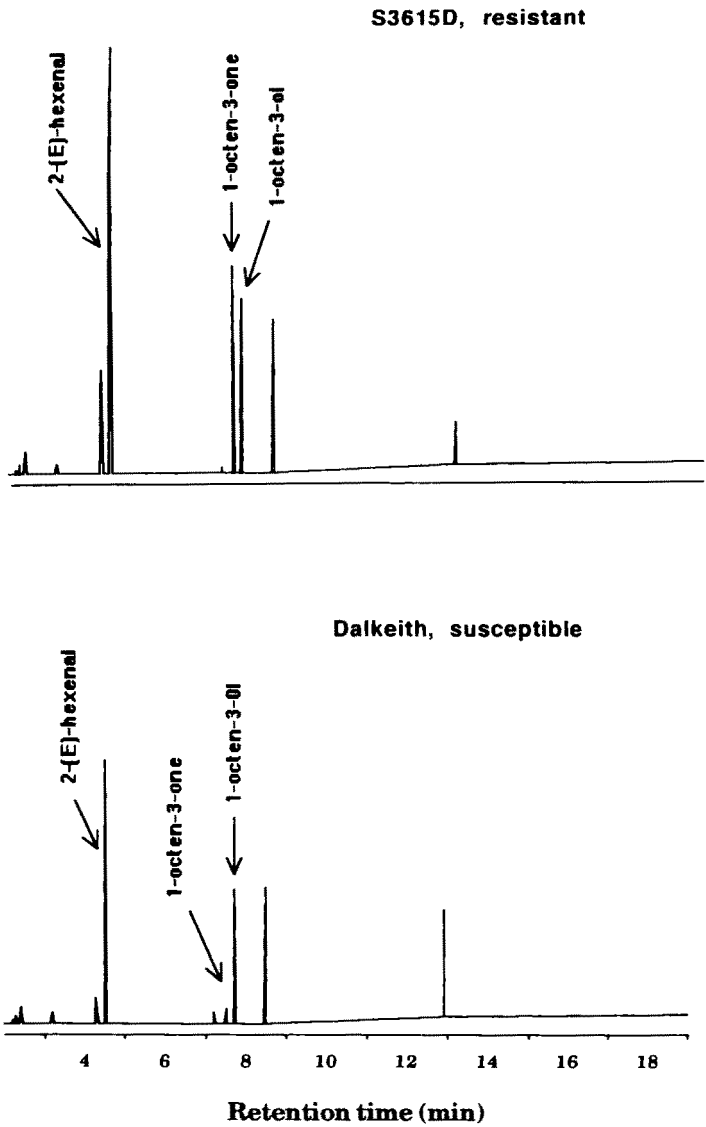

FIG. 1. Gas chromatograms of headspace volatile compounds collected from damaged cotyledons of subclover. Varieties shown here were S3615D (resistant) and Dalkeith (susceptible). Positions of 2-(*E*)-hexenal, 1-octen-3-ol, and 1-octen-3-one are indicated. Some volatile compounds are unidentified.

established their identity. Not more than 0.5% 1-octen-3-one was detected from the headspaces of either 1-octen-3-ol or combined 1-octen-3-ol and ground DGI007 cotyledon tissues, indicating negligible air oxidation of this metabolite.

Quantitation of Volatile Compounds and Correlation with Mite Feeding Damage. The relative amounts of the three compounds collected from 10 varieties over a 1-hr period are presented in Table 1. There was variation of the compounds between varieties, but only 1-octen-3-one was consistently higher in resistant than in susceptible varieties, with one exception (82S47.16.2). There is a significant negative correlation ($R^2 = 0.713$) between the levels of 1-octen-3-one produced and scores of mite feeding damage within a two-week testing period.

Collection of 1-octen-3-one over 6 hr yielded a higher amount than in 1 hr in all three varieties (Figure 2). The estimated amount of 1-octen-3-one collected within 1 hr from the damaged cotyledons was about 5 ppm in the susceptible varieties, whereas in resistant varieties it was about 15 ppm (19 ppm in S3615D). The amount of 1-octen-3-ol collected was generally higher than that of 1-octen-3-one and varied from 2 ppm (variety 82S47.16.2) to 28 ppm (variety S3617D). 2-(*E*)-Hexenal also varied from 11 ppm (variety Dalkeith) to 30 ppm (variety DGI007).

Antifeeding Activity of 1-Octen-3-one with Membrane Feeding Tests. At high concentrations, all three volatile compounds, 1-octen-3-one, 1-octen-3-ol,

TABLE 1. VOLATILE COMPOUNDS RELATIVE TO *n*-HEXANOL IN HEADSPACE OF ARTIFICIALLY DAMAGED COTYLEDONS (2.4 g) OF SUBCLOVER VARIETIES[a]

Varieties	Character	Compound (mean ± SE)		
		1-Octen-3-one	1-Octen-3-ol	2-(*E*)-Hexenal
Dalkeith	Susceptible	0.13 ± 0.001	0.44 ± 0.001	0.47 ± 0.026
Denmark	Susceptible	0.11 ± 0.001	0.21 ± 0.001	2.84 ± 0.010
Junee	Susceptible	0.11 ± 0.001	0.54 ± 0.002	2.53 ± 0.001
Seaton Park	Susceptible	0.12 ± 0.001	0.62 ± 0.001	0.55 ± 0.010
82S47.162	Resistant	0.15 ± 0.001	0.09 ± 0.001	2.07 ± 0.020
S3617D	Resistant	0.27 ± 0.003	1.18 ± 0.001	1.77 ± 0.005
S3623A	Resistant	0.39 ± 0.002	0.80 ± 0.005	1.41 ± 0.005
S3615H	Resistant	0.29 ± 0.001	0.31 ± 0.001	1.39 ± 0.010
DGI007	Resistant	0.39 ± 0.001	0.85 ± 0.003	4.95 ± 0.005
S3615D	Resistant	0.48 ± 0.005	0.65 ± 0.002	2.69 ± 0.005

[a]Collection of compounds was completed at 1 hr. The internal standard was 10 μl *n*-hexanol ($n = 2$).

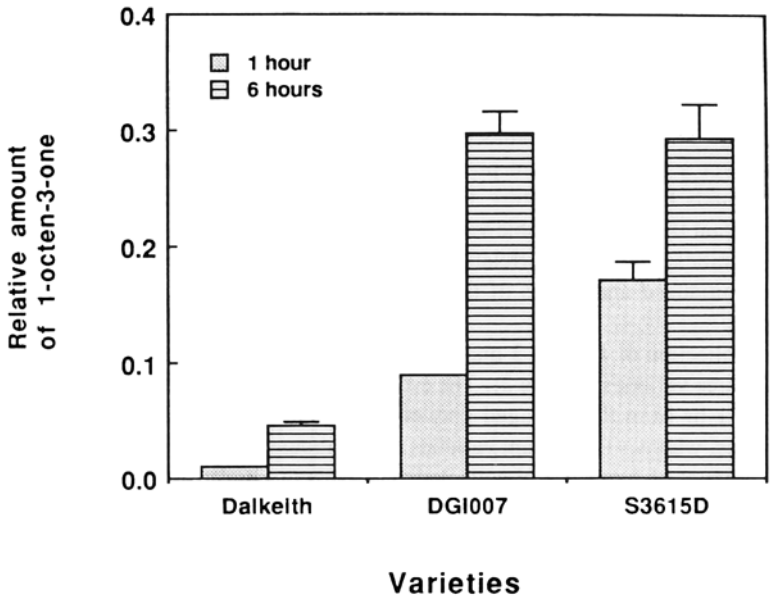

FIG. 2. Relative amounts of 1-octen-3-one collected for 1 and 6 hr from the headspace of damaged cotyledons of three subclover varieties, Dalkeith (susceptible), DGI007 (resistant), and S3615D (resistant).

and 2-(*E*)-hexenal, exhibited antifeeding activity towards mites in the membrane feeding test. This is illustrated for 1-octen-3-one (Figure 3). The EC_{50} for deterrent activity was 50 ppm for 1-octen-3-one, 55 ppm for 2-(*E*)-hexenal, and 285 ppm for 1-octen-3-ol. For 1-octen-3-one and 2-(*E*)-hexenal, all mites were repelled from feeding on both treatment and control membrane sachets with concentrations of 1000 ppm and higher. At low concentration (about 1 ppm), 1-octen-3-one and 2-(*E*)-hexenal were attractive to the mites in the three hour test.

Correlation of 1-Octen-3-one Content and Toughness in Relation to Mite Feeding Damage. Most resistant varieties tested not only produce high amounts of 1-octen-3-one but also have high toughness values (Jiang and Ridsdill-Smith, unpublished data), although 1-octen-3-one is low for variety 82S47.16.2 and toughness for variety S3615D (Figure 4). Multiplication between 1-octen-3-one content and the toughness value for each variety yields an index that was negatively correlated with mite feeding damage scores. The R^2 value (0.873) obtained was higher than that (0.713) in the correlation between 1-octen-3-one

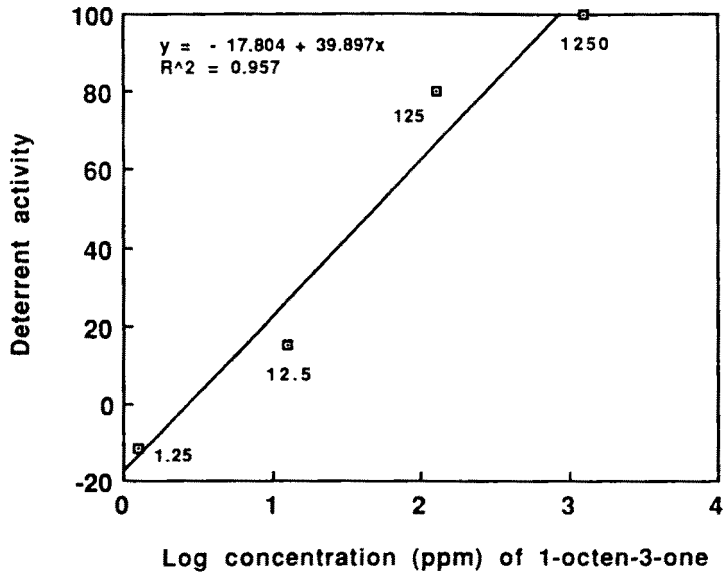

FIG. 3. Responses of the red-legged earth mite to 1-octen-3-one tested at four concentrations (1.25, 12.5, 125, and 1250 ppm) in a membrane bioassay. Note attractiveness of the compound at 1.25 ppm.

alone and damage score and that (0.752) in the correlation between toughness and damage score.

Shading Effect on Mite Feeding and Volatile Content. Cotyledons of both Dalkeith (susceptible) and DGI007 (resistant) grown under shading were smaller than nonshaded cotyledons, but they were green. The shaded cotyledons of both varieties were completely avoided by mites in choice feeding tests during 3 hr (Table 2). Nonshaded cotyledons of DGI007 were more acceptable than shaded Dalkeith cotyledons. Toughness test indicated that shaded cotyledons of both varieties had significantly lower toughness values ($P < 0.01$) than the nonshaded counterparts: with Dalkeith (susceptible), toughness was 59.1 $\pm$ 0.93 for nonshaded and 43.9 $\pm$ 0.46 for shaded cotyledons; with DGI007 (resistant), this was 81.8 $\pm$ 1.04 for nonshaded and 52.4 $\pm$ 1.15 for shaded cotyledons. However, analyses of volatile compounds showed that C_8 volatile compounds, especially 1-octen-3-one, increased by more than 50%, whereas 2-(*E*)-hexenal decreased 9% in both varieties with shading (Table 3).

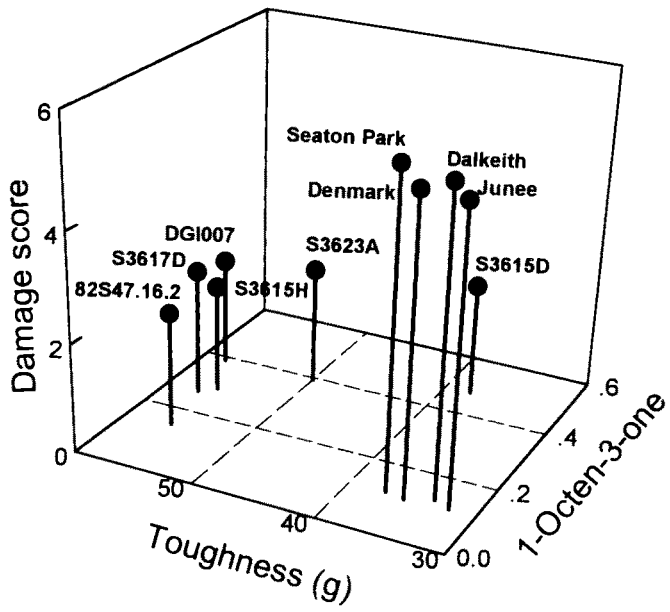

FIG. 4. Three-dimensional relationships between mite feeding damage scores, relative amount of 1-octen-3-one, and toughness value of the same 10 varieties of subclover. Note the four susceptible varieties are grouped as having both the lowest content of 1-octen-3-one and toughness values, whereas the resistant varieties are scattered around with either high 1-octen-3-one content or toughness values or both.

DISCUSSION

There is no visible difference in surface structure between subclover cotyledons of resistant and susceptible varieties. The cotyledons do not possess trichomes (Ridsdill-Smith et al., 1995), which are important chemical and physical barriers against spider mites in many other plant species. Furthermore, no antifeedant activity was found in nonvolatile extracts from resistant varieties, in comparison to susceptible varieties.

Higher amounts of 1-octen-3-one are produced in most resistant than in susceptible varieties, in particular the S-lines, which originate from Sicily, Italy. The significant correlation between 1-octen-3-one produced and resistance and its concentration-dependent deterrent activity strongly suggest that it could be responsible, at least in part, for the deterrent activity of the variety S3615D, which has low cotyledon toughness, as well as in other varieties with high cotyledon toughness such as DGI007. The concentration of 1-octen-3-one occur-

TABLE 2. MITE NUMBERS ON DETACHED COTYLEDONS GROWING UNDER SHADE (−) AND LIGHT (+) FROM TWO VARIETIES (DALKEITH, SUSCEPTIBLE; DGI007, RESISTANT) IN PAIRWISE CHOICE TESTS[a]

Choices	Mites ($N \pm$ SE)	Preference for the 1st choice	P level from t test
(+) Dalkeith-(− Dalkeith)	11.2 ± 0.38 − 1.4 ± 0.25	77.8	<0.001
(+) DGI007-(−) DGI007	7.5 ± 0.22 − 0.6 ± 0.27	85.2	<0.001
(−) Dalkeith-(−) DGI007	4.5 ± 0.19 − 1.7 ± 0.09	45.2	<0.001
(+) Dalkeith-(−) DGI007	12.4 ± 0.39 − 0.2 ± 0.05	96.8	<0.001
(−) Dalkeith-(+) DGI007	1.3 ± 0.26 − 8.2 ± 0.38	−72.6	<0.001

[a]There are highly significant differences between each choice ($P < 0.001$).

TABLE 3. SHADING EFFECTS ON RELATIVE AMOUNT OF VOLATILE COMPOUNDS IN HEADSPACE OF ARTIFICIALLY DAMAGED COTYLEDONS (3.5 g) OF SUBCLOVER VARIETIES DALKEITH (SUSCEPTIBLE) AND DGI007 (RESISTANT)[a]

Treatments	Compound (mean ± SE)		
	1-Octen-3-one	1-Octen-3-ol	2-(*E*)-Hexenal
Dalkeith (susceptible)			
Non-shaded	0.14 ± 0.001	0.70 ± 0.003	2.04 ± 0.001
Shaded	0.21 ± 0.002	1.16 ± 0.002	1.85 ± 0.005
DGI007 (resistant)			
Non-shaded	0.26 ± 0.005	1.11 ± 0.006	3.36 ± 0.010
Shaded	0.49 ± 0.005	1.17 ± 0.001	3.05 ± 0.010

[a]The difference between shaded and nonshaded cotyledons is significant within the same variety using the t test ($P < 0.05$). An internal standard of 5 μl n-hexanol was used in quantification ($N = 2$).

ring in the resistant variety S3615D (19 ppm, collected within 1 hr) is within the range of concentrations for which mite deterrence is observed. With the susceptible variety Dalkeith, we were able to demonstrate production of 2-(*E*)-hexenal, 1-octen-3-ol, and 1-octen-3-one from cotyledons damaged by red-legged earth mites feeding activity (unpublished). Thus, the volatile compounds pro-

duced by artificially damaged cotyledons are representative of those produced by cotyledons after mite feeding.

The two- to threefold increase in 1-octen-3-one collected with time suggests that the compound is produced in a time-dependent manner and/or that collection within 1 hr underestimates the amount of compounds. Because the mites readily distinguish between cotyledons of resistant and susceptible varieties within 1 hr, it is reasonable to correlate mite resistance with the compounds collected within 1 hr. Terpenoids do not appear to be present in detectable amounts. We have shown that this is not due to the trapping system employed, since terpenes from damaged *Eucalyptus* leaves were trapped effectively using the same technique (unpublished). Dicke and Sabelis (1992) also found no production of terpenes in artificially damaged Lima bean leaves.

The striking negative effect of shade-grown cotyledons on mite feeding behavior and on the relative proportions of the volatile compounds are consistent with the results above. Shade-grown cotyledons have much lower toughness than nonshaded ones, but mites apparently do not like gathering and feeding on them. Although other changes, such as morphology and content of sugar, in these cotyledons could also affect mite feeding behavior, production of C_8 compounds (1-octen-3-one and 1-octen-3-ol) in both shade-grown susceptible and resistant varieties increased considerably. This could explain, at least in part, the reduced acceptance of the cotyledons by mites. The DGI007 variety is highly resistant to the red-legged earth mite (Gillespie, personal communication), and it appears that both high toughness value and high 1-octen-3-one production contribute to this resistance. However, the experiments with shade-grown cotyledons tend to suggest that the role of 1-octen-3-one in resistance is more important than toughness.

2-(*E*)-Hexenal has been shown to arise from the unsaturated fatty acid, linolenic acid (Hatanaka, 1993; Vick and Zimmerman, 1987). This is present in greater amounts in green leaves, particularly in the membranes of chloroplasts, than in nongreen and dark-grown leaf tissues (Harwood, 1980). On the other hand, the C_8 volatile compounds originate from linoleic acid (Mau et al., 1994), which is produced in greater amounts in dark-grown leaves than in green leaves and more in white than in the green parts in *Chlorophytum* (Harwood, 1980).

The above observations further support the correlation between 1-octen-3-one and mite resistance and help to rationalize the lack of correlation between levels of 2-(*E*)-hexenal and mite resistance. We presume that 2-(*E*)-hexenal collected from artificially damaged cotyledons is produced by the green palisade and spongy mesophyll cells that are not the major feeding sites of the mite. However, C_8 volatile compounds are probably produced, for the most part, in the nongreen epidermal cells. If this is true, the C_8 compounds should have a

much higher local concentration than is revealed from headspace trapping experiments.

Hildebrand et al. (1986) have observed enhanced lipid peroxidation and lipoxygenase activity in soybean leaves infested with spider mites. The authors suggest that the metabolite hexenal played a role in the induced resistance of soybean to spider mite (Kasu et al., 1994). We are not in a position to judge whether or not hexenal could also be involved in resistance because our measurements were for the whole cotyledon tissue, rather than for those specific tissues subjected to feeding mites.

In conclusion, the results strongly suggest that wound-induced C_8 compound, 1-octen-3-one, plays a significant role in the deterrence of cotyledons of some resistant subclover varieties to the red-legged earth mites. The combined effects of the compound and physical toughness confer more resistance to a variety than either factor alone. In the future, we need to establish whether or not other chemical compounds, e.g., those specifically induced by mite feeding, are also involved in the antixenotic resistance of subclover cotyledons to the redlegged earth mite.

Acknowledgments—Bioassay and toughness measurement of cotyledons were performed by J. D'Arcy-Evans. T. Picen helped in preparation of mites in some experiments. We thank D.J. Gillespie for providing seeds of subclover varieties and unpublished data on screening.

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