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SURFACE LIPIDS OF *TRIFOLIUM* SPECIES

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Key Word Index—*Trifolium pratense*; *T. resupinatum*; Leguminosae; clover; surface lipids; hydrocarbons; alcohols; esters.

Abstract—Fatty acids comprised 27.7 and 30.3% of the surface lipids of *Trifolium pratense* and *T. resupinatum*, respectively. The hydrocarbons contained 11 *n*-alkanes with chain lengths ranging from C_{25} to C_{35}. Nonacosane and hentriacontane were the major hydrocarbons in both species. The alcohols comprised 25% of the surface lipids of *T. resupinatum* and 6% in *T. pratense*, whereas esters comprised 14% of the surface lipids in *T. resupinatum* and 26% in *T. pratense*.

INTRODUCTION

Extensive reviews of the role of volatile secondary plant substances in regulating insect behavior [1,2] have shown that these compounds offer major clues to host plant selection by invading insects. The volatile substances may allow host insects to exhibit their preferences within the family or higher taxonomic group in the plant kingdom, although they apparently have little effect on insect–plant response within the genus and species level. Preliminary studies on cotton, clover and other plants [3–8] have implicated the less volatile chemicals as a source of insect resistance within these plant lines.

This study was conducted to investigate a possible chemical basis for the reported preference and non-preference of the clover head weevil, *Hypes meles*, on clovers of the genus *Trifolium* [9]. Persian clover, *T. resupinatum*, which is susceptible to the clover head weevil and a more resistant red clover, *T. pratense*, were selected for study. Surface lipids were examined because weevil feeding and oviposition contact initially involves this class of compounds.

RESULTS AND DISCUSSION

The average concentrations of leaf surface lipids were 6 mg/g fr. plant tissue in red clover and 3 mg/g in the insect-susceptible Persian clover. The percentages of components identified in the surface lipid were 60.3 and 66,5% for red and Persian clover, respectively.

The total fatty acids (Table 1) comprised 27.6% in red clover and 30.3% in Persian clover. The small amount of free fatty acids (>0.2%) in both clover lines indicates that the acids are mainly esterified. TLC and column chromatography on silicic acid showed that mono-, di- and triglycerides were the major classes of non volatile esters. Volatile esters comprised 13.7% of the total surface wax. Emery and Gear [10] found a relative abundance of 18.6% acids in the wax of sweet clover, *Melilotus alba*. Body [11] found a similar wax ester composition for white clover.

Table 1. Composition of total fatty acids in the surface lipid of red and Persian clovers

Fatty acid	% of total surface lipid	
	Red clover	Persian clover
16:0	1.3	7.1
18:0	1.8	1.4
18:1	2.0	1.0
18:2	1.3	0.8
18:3	2.0	2.8
20:0	12.3	6.8
21:0	1.0	1.9
22:0	2.0	3.9
23:0	2.0	2.0
24:0	2.0	2.6

The hydrocarbons, which accounted for 26% of the surface lipids (Table 2), were straight-chain compounds, principally nonacosane and hentriacontane. These hydrocarbons are qualitatively and quantitatively similar to those found in the surface lipid of glabrous cotton, *Gossypium hirsutum* [12, 13]. The hydrocarbon concentration in red clover is also similar to that observed for *Hebe* spp. (Scrophulariaceae), in which the ratio of C_{29} to C_{31} is *ca* 2:1 [14]. The ratio of C_{29} to C_{31} hydrocarbons is *ca* the same in the wax of Persian clover as it is in *Phaseolus aureus* (Leguminosae) [14].

The primary alcohol content is 5 times as great in Persian clover (25.7%) as it is in red clover (5.9%). 1-Triacontanol accounts for 18.4% of the total wax of Persion clover and is present in the highest concentration of any single component in both lines. The major alcohols in the identified esters of both clovers were *n*-C_{18} alcohols and the monoterpene geraniol.

Chibnall *et al.* [15] investigated the surface lipids of white clover (*Trifolium repens*) by mixed melting point and assigned a concentration of 50% to 1-triacontanol. They estimated equimolar concentrations of C_{26}, C_{28} and C_{30} fatty acids from MW determination by titration.

Octadecanyl icosanoate ($C_{38}H_{57}O_2$) was the major

Table 2. Chemical components in the surface lipids of red and Persian clover

Constituent	% of total surface lipid Red clover	Persion clover
Aliphatic hydrocarbons		
Pentacosane	0.3	0.6
Hexacosane	0.1	0.0
Heptacosane	1.1	1.5
Octacosane	0.5	0.3
Nonacosane	8.8	7.9
Triacontane	0.3	0.8
Hentriacontane	4.0	11.6
Dotriacontane	0.0	0.5
Tritriacontane	0.0	1.9
Tetratriacontane	2.2	0.0
Pentatriacontane	0.9	0.7
Alcohols		
1-Pentacosanol	2.7	0.2
1-Hexacosanol	1.1	2.0
1-Octacosanol	0.5	4.0
1-Nonacosanol	0.3	1.1
1-Triacontanol	1.3	18.4
Esters		
Methyl icosanoate	0.1	0.5
Geranyl mexadecanoate	2.5	0.1
Geranyl octadecanoate	4.4	5.8
Gernayl octadecanoate	4.1	4.6
Octadecanyl octadecanoate	4.6	0.9
Octadecanyl icosanoate	11.8	1.8
Sterols		
Cholesterol	0.8	0.6
Stigmasterol	2.0	0.9

ester present in red clover (11.8%). MS gave a strong M^+ at *m/e* 564 with a parent ion at 313 corresponding to icosanoic acid ($C_{20}H_{41}O_2$). Further evidence for the structure was obtained by hydrolysis and GC–MS of the isolated acid and alcohol.

The small amount of sterols in the clovers probably preclude the involvement of these compounds in insect–plant response, though they are required as an exogenous source of sterol for normal insect growth [16]. However, the relative concentrations of sterols of the plant may be more important than concentrations of any individual sterol in causing a plant to be resistant or susceptible to insects and diseases [17]. Identification of the plant constituents and analysis of their concentrations may provide information needed to develop laboratory techniques that will give a true measure of insect responses. When the plant–insect responses depend on a multi-chemical relationship, the difficulty of determining the nature of this relationship from chemical studies increases in proportion to the number of components involved. We are attempting to develop a laboratory bioassay that will measure the insect–plant interaction as observed in the field.

EXPERIMENTAL

Isolation and fractionation. Samples of Persian and red clover in bloom were collected from adjacent fields by clipping plants just above the ground. Great care was taken to prevent breaking or crushing the plant leaves and stems to lessen the extraction of internal chemical components. The samples were weighed and the wax was extracted by dipping them × 5 for 5 sec each in 3 portions of purified CH_2Cl_2. The CH_2Cl_2 extracts were combined, dried, filtered and the solvent was removed *in vacuo* at 30°. The sample was weighed, made to vol. with CH_2Cl_2, and held in the freezer at −10° until fractionated.

The surface lipids were chromatographed on a 2 × 30 cm silicic acid column. Hydrocarbons were eluted with 200 ml pentane, and polar compounds were successively eluted with 200 ml each of 5, 10, 20 and 50% CH_2Cl_2 in pentane and finally with CH_2Cl_2 [18]. Progress of the elution and recombination of all fractions into 5 reconstructed fractions was monitored by Si gel TLC [19]. The solvent was removed from each fraction *in vacuo* and weighed.

GC–MS. Fractions were introduced into a Hewlett-Packard [20] 5930 quadrupole MS from a 2.5 m × 2 mm column packed with Gas-Chrom Q coated with 3% OV-101. The GLC was programmed from 120 to 240° at 2°/min; gas flow was 30 ml/min. Components in sufficient concentration were trapped from a stream splitter and introduced into the MS through the direct proble. MS were obtained at 70 eV. Peak identity was confirmed by comparison with standard spectra and authentic compounds where possible [21]. Total fatty acids were determined as Me esters after transmethylation [22] and GLC on a 2.5 m × 2 mm, 10% DEGA column at 190°.

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