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(*Z*)-OXACYCLOTRIDEC-10-EN-2-ONE, AN ALFALFA WEEVIL FEEDING DETERRENT FROM *MEDICAGO RUGOSA**

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Key Word Index—*Medicago rugosa*; Leguminosae; *Hypera postica*; Curculionidae; alfalfa weevil; feeding deterrent; repellent; (*Z*)-oxacyclotridec-10-en-2-one; *cis*-9-dodecen-12-olide; (*Z*)-12-hydroxydodec-9-enoic acid lactone.

Abstract—(*Z*)-Oxacyclotridec-10-en-2-one, a constituent of a steam volatile fraction from *Medicago rugosa* leaves and stems, inhibits adult alfalfa weevil feeding on an inert substrate, and may be responsible for the strong resistance to weevil feeding exhibited by this annual *Medicago* species.

INTRODUCTION

The alfalfa weevil, *Hypera postica* Gyllenhal is an important pest of alfalfa, *Medicago sativa* L. [1]. The insect feeds only on plants within the genus *Medicago* and on a few other legumes [1]. Some *Medicago* species are resistant either to larval weevil feeding, to adult weevil feeding, or to both [2–5]. Larval resistance may relate to the presence of erect glandular trichomes, which secrete materials that immobilize the insects [6, 7]. With adult weevils immobilization does not occur, and resistance to feeding is attributed to antixenosis [4].

Medicago rugosa Desr., a glandular-haired annual is particularly resistant to adult alfalfa weevil feeding [2, 4, 5, 8]. Observation of weevils in free-choice tests suggests that olfactory cues may be responsible for conferring resistance on this species [8]. The work discussed below was undertaken to determine whether *M. rugosa* possesses volatile materials that could act as alfalfa weevil feeding deterrents.

RESULTS AND DISCUSSION

The resistance of *M. rugosa* relative to *M. sativa* cv Ranger was demonstrated in a number of choice tests that were carried out during the period the study discussed below was conducted. In a typical test with trifoliolate leaves, alfalfa weevil feeding (area basis) on cultivated alfalfa was nearly 10 times that on *M. rugosa* [8.0 $\pm$3.5 mm^2 per leaf on *M. rugosa versus* 78.5$\pm$17.3 mm^2 on 'Ranger' ($\bar{X}\pm$s.e. for 10 arenas with 8 weevils per arena, and a 24-hour bioassay period)]. Hence, our work confirms earlier reports that *M. rugosa* is resistant to feeding by adult alfalfa weevils [2, 4, 5, 8].

Steam distillate from *M. rugosa* inhibited weevil feeding on 13 mm diameter membrane filters treated with a phagostimulatory fraction from *M. sativa* 'Ranger' [9]. The average ($\pm$s.e.) area eaten from such filters bearing 150 μg of steam distillate (applied in hexane) was 8.5 ($\pm$1.1) mm^2 versus 18.5 ($\pm$0.9) mm^2 for filters treated with hexane alone ($N=10$ arenas, 15 weevils per arena). In a multiple choice dose-response test [9] as little as 40 μg of steam distillate per membrane filter influenced feeding (Fig. 1).

GC/MS examination of the total steam distillate extract revealed the presence of several major and numerous minor components (Fig. 2). Several free carboxylic acids were found, including the major extract constituent, a 16-carbon monounsaturated acid. Phytol and a compound with M_r 196 were the two predominent non-acidic components. Small amounts of (*Z*)-hex-3-enol, 1-octen-3-ol, (*Z*)-hex-3-enyl acetate, linalool, alpha-terpineol, decanal, nerol, and geraniol were detected, and MS evidence for the presence of several oxygenated sesquiterpenes was

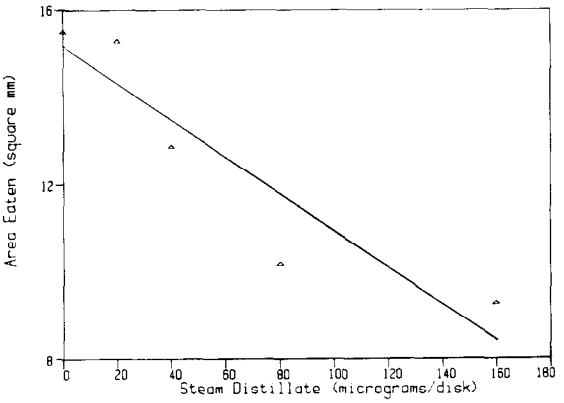

Fig. 1. Feeding of alfalfa weevil adults on phagostimulant-bearing membrane filters treated with several levels of steam distillate from *M. rugosa*. Bioassay was conducted with 10 arenas and 40 weevils per arena. Best fit linear regression line is shown (slope = −0.042, intercept = 15.14, $r^2=0.86$ with 3 d.f.).

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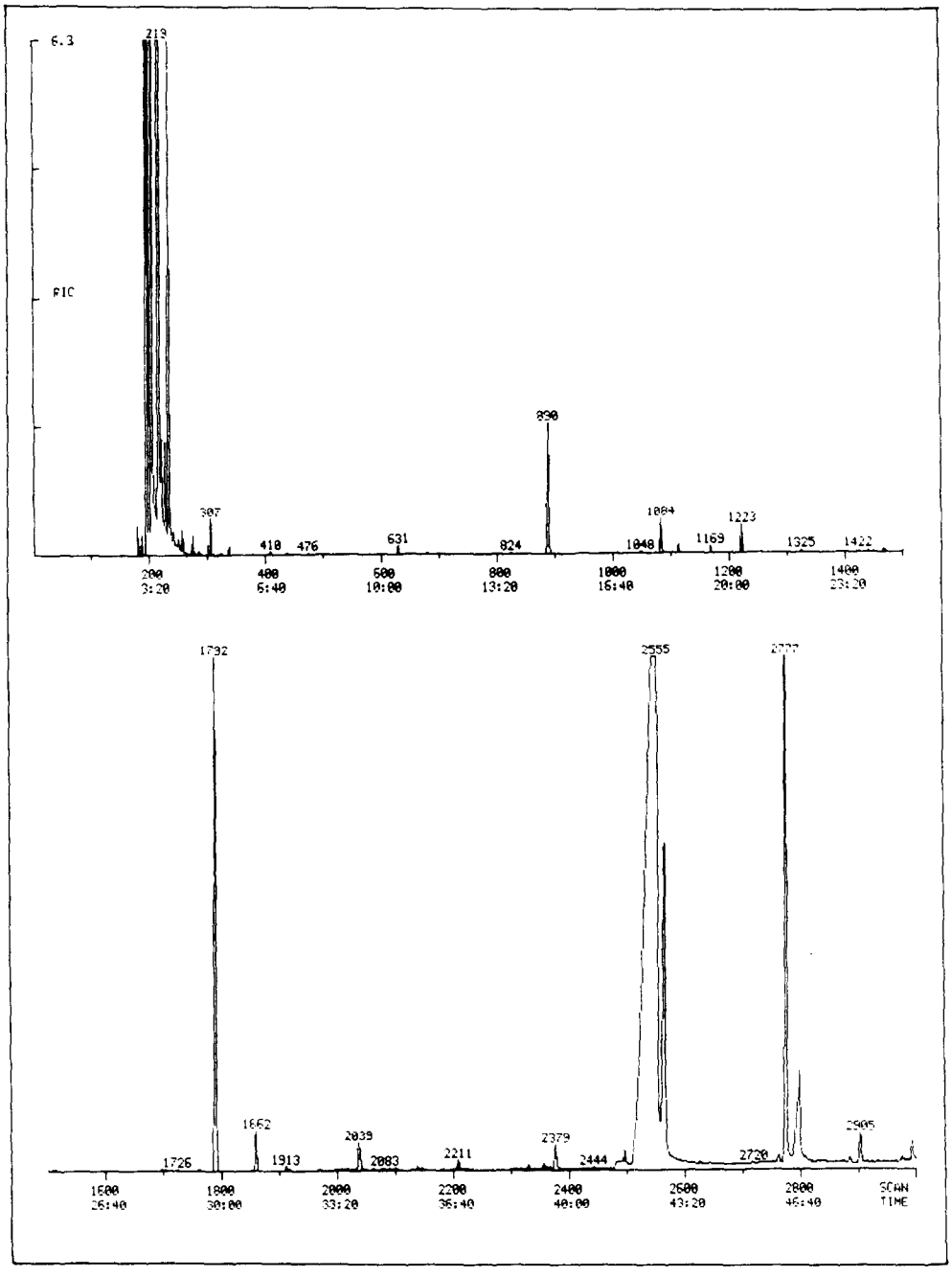


Fig. 2. GLC trace (WCOT column) of steam volatile fraction from *M. rugosa*. The probable identities of materials given with retention times are as follows: (Z)-hex-3-enol 410; 1-octen-3-ol 631; linalool 890; α terpineal 1084; nerol 1169; unknown M_r 196 1792; nerolidol 1862; tetradecanoic acid 2211; pentadecanoic acid 2379; palmitoleic acid 2555; phytol 2777.

obtained. No satisfactory MS reference library match was obtained for the M_r 196 component.

Antifeedant activity was present in a neutral fraction of the steam distillate, but not in a free-acid fraction. Liquid chromatography of the neutral fraction on silica gel was used to purify a material that inhibited weevil feeding (Fig. 3). This material co-chromatographed with the unidentified component of M_r 196 in the steam distillate on a packed GC column (SP2100 liquid phase).

The structure **1** was deduced from the high field ^1H and ^{13}C NMR spectra. The latter revealed two vinyl carbons (δ132.28 and 127.11) and an ester or lactone carbonyl (δ174.68), one carbon with an attached oxygen (δ64.15) and eight additional aliphatic carbons. The DEPT spectrum contained only methines (the vinyl carbons) and methylenes, suggesting compound **1** to be a 13-membered lactone with one double bond. The ^1H NMR spectrum was consistent with this, and the COSY spec-

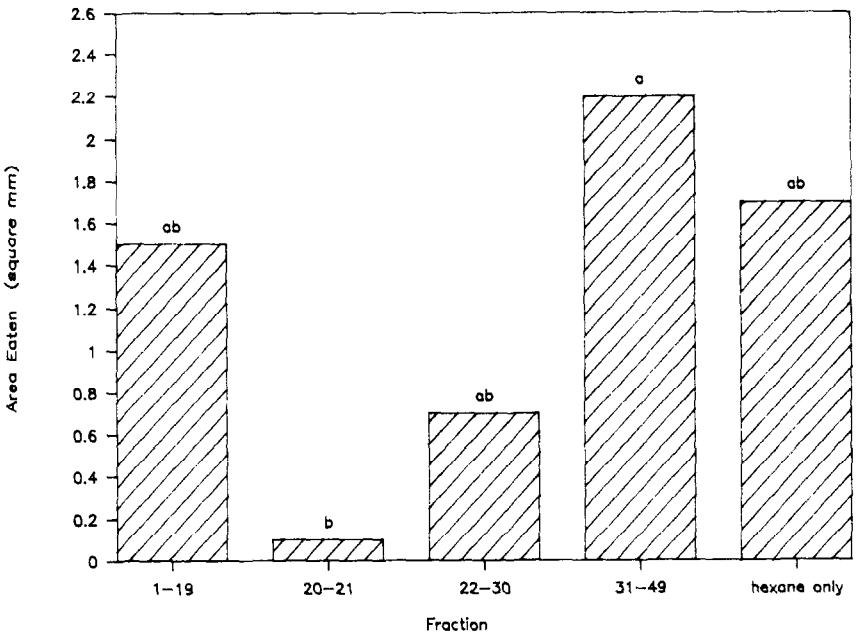
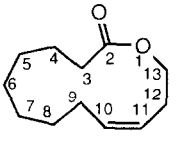


Fig. 3. Feeding of alfalfa weevil adults on phagostimulant bearing membrane filters treated with HPLC fractions. Each filter (except for one treated only with hexane) bore 50 μg. Bioassay was conducted with 10 arenas and 40 weevils per arena. Bars bearing different letters represent significantly ($p < 0.05$) different amounts of feeding as determined using Tukey's Studentized Range Test. Note: Light feeding in this bioassay resulted from using weevils whose feeding activity was declining [11 and Experimental].



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trum (Fig. 4) revealed a double bond between positions 10 and 11. Thus, the vinyl hydrogens (δ5.40) showed correlations to δ2.43 and 2.10, while the latter was correlated with the oxygen-bearing methylene at δ4.24. Comparison of the ^{1}H NMR, IR and MS data with the literature [10, 11] established the stereochemistry as *cis*-(*Z*)-oxacyclotridec-10-en-2-one (**1**) the lactone of (*Z*)-hydroxydodec-9-enoic acid, which has previously been reported as a fragrance element of citron [12].

As little as 12.5 μg of this material inhibited adult alfalfa weevil feeding on phagostimulant treated membrane filters (4.4 ± 1.0 mm^2 eaten from deterrent treated filter versus 8.3 ± 1.0 mm^2 from filter treated with hexane alone, $\bar{X} \pm$ s.e. for $N = 10$ arenas). Further work will be required to establish where (*Z*)-oxacyclotridec-10-en-2-one is found in the *M. rugosa* plant, and to determine to what extent this compound is responsible for the strong resistance to adult weevil feeding exhibited by this species.

EXPERIMENTAL

Plant material. Seeds of *M. rugosa* Desr. provided by Dr E. L. Sorensen (U. S. Dept. Agriculture, Agricultural Research Service, Manhattan, KS, U. S. A.) were used to grow 20 to 30 plants from which seed for growing of experimental plants was collected. Germination took place on moist filter paper after nicking the seed coat with a razor blade. Seedlings in the cotyledon stage were dusted with a *Rhizobium* inoculum, planted into a commercial peat–vermiculite potting medium, and grown in a greenhouse maintained with a 21° max. and 16° min. temperature. Supplemental light was provided by high intensity Na discharge lamps from 0600 until 2200 hr daily. Plants were fertilized weekly using a modified Hoaglands solution [13]. *Medicago sativa* L. cv Ranger was grown under conditions identical to those used for *M. rugosa*.

Insects. Adult alfalfa weevils, *Hypera postica* Gyllenhal, collected from commercial alfalfa fields in Eastern Oregon, U.S.A., were used for some bioassays. For other bioassays adult weevils reared from larvae collected in Eastern Oregon were used. Consistent with earlier studies [14], feeding by weevils was sufficiently heavy for reliable bioassay only for several weeks after resumption of feeding activity for overwintered insects; or for several weeks after emergence, for newly hatched insects. Weevil colonies were maintained in 20 l plastic buckets in a growth chamber at $21 \pm 2°$ under 16(8) photoperiodic cycles. Larvae and adults were fed freshly harvested sprigs of field- or greenhouse-grown alfalfa, and were provided with moist dental wicks as a source of water.

Bioassays. Extracts and fractions were bioassayed using a membrane filter bioassay technique [9]. In order to promote weevil feeding, 13 mm diameter membrane filters were pretreated with 50 mg-equivalents of an exhaustive ethanolic extract prepared using leaves of *M. sativa* L. cv Ranger. Materials to be bioassayed for feeding deterrent activity were applied in hexane. Insects used in bioassays were starved for 24 hr. Bioassays were conducted for 6 hr at a temp. of $21 \pm 2°$. A single

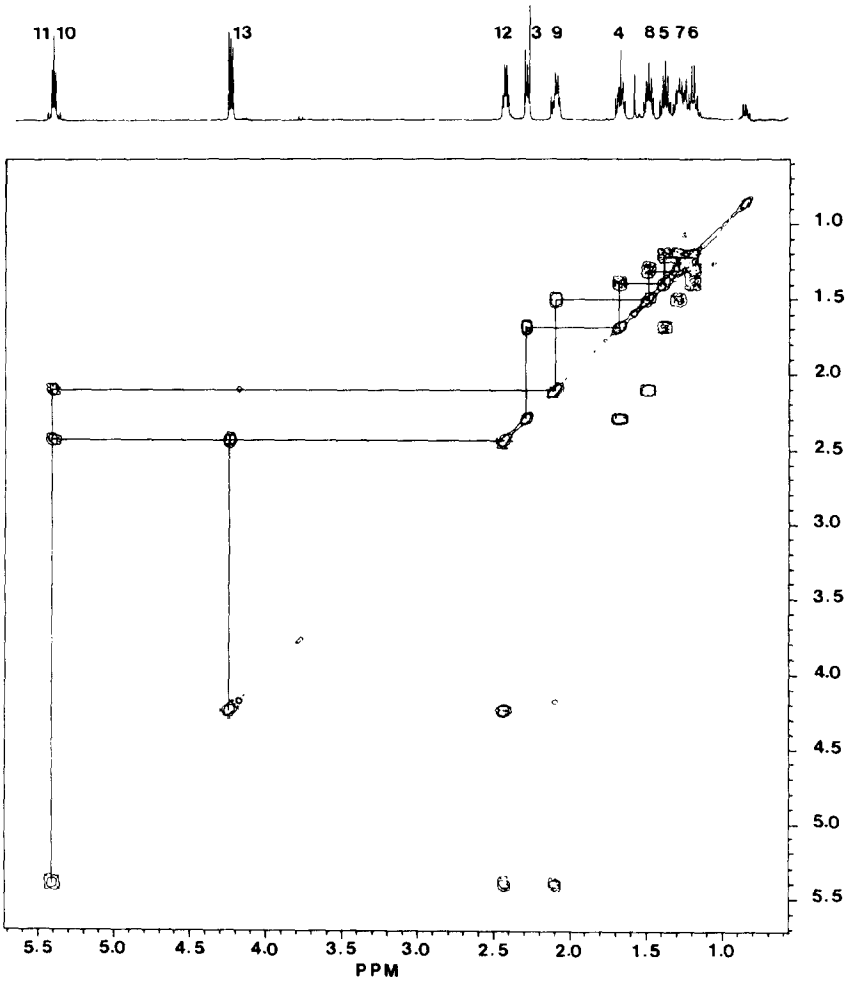

Fig. 4. $^1H/^1H$ COSY spectrum of **1**. Spectral aquisition parameters: 2066 Hz sweep width in the F_2 dimension; 256 spectra (8 scans each) were accumulated in 0.48 msec increments; resolution 2.0 Hz/pt.

moist dental wick was placed in each 5 cm high × 9 cm diameter cylindrical cardboard carton used as a bioassay arena. The number of weevils used per arena depended on the number of fractions under evaluation (see text and figure legends).

Extraction and isolation. Steam volatile materials were exhaustively extracted from leaves and stems of preanthesis plants in the bud stage of growth using a modified Nielson–Kryger apparatus [15]. The yield of steam volatiles was 0.226 ±0.008 mg/g fr. wt ($\bar{X} \pm$ s.e. for 5 extractions).

A neutral fraction was prepared by diluting the steam distillate with Et_2O and partitioning ×4 with 2 M KOH. The organic phase was washed ×3 with satd NaCl, dried over $CaSO_4$ and Molecular Sieve 4a and taken to dryness *in vacuo*. The neutral volatiles were redissolved in hexane. In one case 0.195 g of crude distillate yielded 0.039 g of neutral volatiles. Free acids, if not discarded, were recovered by partitioning into hexane after acidification of the KOH soln with HCl.

Components within the neutral fraction were separated by HPLC on a 250 × 10 mm silica gel column (Alltech, 10 μm). A $CHCl_3$–C_6H_6 (1:1), solvent system was used with a flow rate of 1 ml/min. Consolidation of fractions was based on analysis by TLC on silica gel, with components detected by charring after spraying the plates with H_2SO_4–MeOH (1:1). Purity was assessed using GC. In one separation 9.8 mg of the purified feeding deterrent material was obtained starting with 54.7 mg of the neutral fraction.

General. GC was carried out using a 1.9 m × 2 mm glass column packed with 3% (w:w) SP2100. Injector and FID were held at 260°. Column temp. was programmed from 60 to 220° at a rate of 7°/min, with initial and final holds of 2 min.

A Finnigan MAT 4500 GC/MS/DS was employed for examination of a 0.2 μl aliquot of the total steam distillate extract. A 60 m × 0.32 mm bonded crosslinked methylsilicone fused silica WCOT column (DB-1) was used with a He head pressure of 0.965 bar and a column flow rate of 2.6 ml/min at 50°. Temperature was programmed from 50 to 250° at 4° min^{-1}, with initial and final holds of 0.1 min. The split injector (1/25 split) was held at 225°. The column exit was connected directly to the MS ion source (ion source temp. *ca* 180°). MS was operated in the EI (70 eV, −0.30 mA) mode with repetitive 1 sec scans taken over a 33–350 *m/z* mass range.

^1H and ^{13}C NMR spectra were obtained at 400 and 100.6 MHz, respectively, using a 5 mm probe. The ^{13}C NMR spectrum was broad-band proton decoupled. NMR spectra are reported as ppm downfield from TMS (δ0.0). IR: the sample was evaporated onto an NaCl plate from a $CHCl_3$ solution.

Compound **1**. FT-IR (film) 1735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ1.20 (2H, *m*, H-6), 1.28 (2H, *m*, H-7), 1.39 (2H, *m*, H-5), 1.49 (2H, *tt*, J = 6.2, 6.3 Hz, H-8), 1.68 (2H, *m*, H-4), 2.10 (2H, *dt*, J = 6.3, 5.4 Hz, H-9), 2.29 (2H, *m*, H-3), 2.43 (2H, *dt*, J = 5.6, 4.9 Hz, H-12), 4.24 (2H, *t*, J = 5.3 Hz, H-13), 5.40 (2H, *m*, H-10 and H-11); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ174.68, 132.28, 127.11, 64.15, 35.35, 29.67, 27.52, 27.28, 26.00, 25.89, 24.60, and 23.50; GC-MS 70 eV, *m/z* (rel. int.): 196 $[M]^+$ (19), 178 $[M-H_2O]$ (1), 167 $[M-29]$ (2), 149 $[M-47]$ (6), 136 $[M-60]$ (27), 123 $[M-73]$ (12), 109 $[M-87]$ (22), 98 $[M-98]$ (39), 95 $[M-101]$ (46), 81 $[M-115]$ (70), 68 $[M-128]$ (100), 67 $[M-129]$ (74), 54 $[M-142]$ (61).

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