

## NEW PHEROMONE COMPONENTS OF THE GRAPEVINE MOTH *Lobesia botrana*

PETER WITZGALL,<sup>1,\*</sup> MARCO TASIN,<sup>1,2</sup> HANS-RUEDI BUSER,<sup>3</sup>  
GERTRUD WEGNER-KIß,<sup>4</sup> VICENTE S. MARCO MANCEBÓN,<sup>5</sup>  
CLAUDIO IORIATTI,<sup>2</sup> ANNA-CARIN BÄCKMAN,<sup>1</sup> MARIE BENGTSSON,<sup>1</sup>  
LUTZ LEHMANN,<sup>6</sup> and WITTKO FRANCKE<sup>6</sup>

<sup>1</sup>Chemical Ecology Group, Swedish University of Agricultural Sciences, P.O. Box 44230 53  
Alnarp, Sweden

<sup>2</sup>Agricultural Institute of S. Michele a/Adige, 38010 San Michele a/Adige, Trento, Italy

<sup>3</sup>Swiss Federal Research Station, 8820 Wädenswil, Switzerland

<sup>4</sup>Department of Biology, State Institute of Viticulture and Enology, 79100 Freiburg im  
Breisgau, Germany

<sup>5</sup>Department of Agriculture, University of La Rioja, 26006-Logroño, Spain

<sup>6</sup>Institute of Organic Chemistry, University of Hamburg, 20146 Hamburg, Germany

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**Abstract**—Analysis of extracts of sex pheromone glands of grapevine moth females *Lobesia botrana* showed three previously unidentified compounds, (*E*)-7-dodecenyl acetate and the (*E,E*)- and (*Z,E*)-isomers of 7,9,11-dodecatrienyl acetate. This is the first account of a triply unsaturated pheromone component in a tortricid moth. The monoenic acetate (*E*)-7-dodecenyl acetate and the trienic acetate (7*Z*,9*E*,11)-dodecatrienyl acetate significantly enhanced responses of males to the main pheromone compound, (7*E*,9*Z*)-7,9-dodecadienyl acetate, in the wind tunnel. The identification of sex pheromone synergists in *L. botrana* may be of practical importance for the development of integrated pest management systems.

**Key Words**—Grapevine moth, *Lobesia botrana*, Tortricidae, (*E*)-7-dodecenyl acetate, (7*E*,9*E*,11)-7,9,11-dodecatrienyl acetate, (7*Z*,9*E*,11)-7,9,11-dodecatrienyl acetate, sex pheromone, synergist

\* To whom correspondence should be addressed. E-mail: peter.witzgall@phero.net

## INTRODUCTION

The grapevine moth *Lobesia botrana* Denis and Schiffermüller (Lepidoptera, Tortricidae) is among the economically most important insects in Europe. It occurs throughout the wine-growing area of ca. 4 million hectares and completes two to four generations per year. The larvae feed on grapes, where *Botrytis cinerea* and other fungi rapidly develop, causing the entire grape cluster to rot (Fermaud and Giboulot, 1992). Pheromone-based control of *L. botrana* has been successfully adopted in wine-growing areas in Germany, Italy, and Switzerland. However, the cost effectiveness of the mating disruption technique needs to be improved (Arn and Louis, 1996).

The sex pheromone of *L. botrana*, which has been studied since the 1970s, consists of the main compound (7*E*,9*Z*)-7,9-dodecadienyl acetate (E7,Z9-12Ac) plus at least four other compounds: (7*E*,9*Z*)-7,9-dodecadien-1-ol (E7,Z9-12OH), (Z)-9-dodecenyl acetate (Z9-12Ac), (E)-9-dodecenyl acetate (Z9-12Ac), and 11-dodecenyl acetate (11-12Ac; Roelofs et al., 1973; Arn et al., 1988; El-Sayed et al., 1999). Here, we show that *L. botrana* pheromone glands contain further compounds, which are of potential interest for the development of integrated pest management systems.

## METHODS AND MATERIALS

*Insects.* Insects were collected near Fribourg (Germany), San Michele (Italy), and La Rioja (Spain) and were reared in the laboratory on an artificial diet under a 16-hr light/8-hr dark photoperiod. Pupae were separated by sex, and adult moths were kept in 36-l Plexiglas cages.

*Chemical Analysis.* Excised glands of 2- to 3-d-old calling females were extracted for 1 min in 7  $\mu$ l of redistilled hexane (Labscan, Malmö, Sweden). These extracts were analyzed on a Hewlett-Packard (HP) 5970B mass spectrometer (MS) with electron impact ionization, which was interfaced with a HP 5890 gas chromatograph (GC), using a polar DB-Wax column (30 m  $\times$  0.25 mm; J&W Scientific, Folsom, CA, USA). Gland extracts were further studied on an HP 6890 GC, using a DB-Wax column and a nonpolar SE-54 column (25 m  $\times$  0.32 mm; Kupper, Bonaduz, Switzerland). The oven temperature was programmed from 60°C/2 min, then at 10°C/min to 100°C, at 1.5°C/min to 150°C, and at 10°C/min to 230°C.

Gland extracts were also injected on an HP 6890 GC equipped with an HP-INNOWax capillary column (30 m  $\times$  0.25 mm), which was coupled to an electroantennographic detector (GC-EAD; Syntech, Hilversum, The Netherlands). The oven was programmed from 50°C (2 min hold) at 10°C/min to 230°C. The column outlet was split in a 1:1 ratio for simultaneous recordings by

EAD and the flame ionization detector of the GC. Excised antennae were mounted in a holder, which was 0.5 cm away from the GC outlet.

**Synthesis.** The syntheses of all compounds are described in detail in the online supplement (Appendix) (Electronic Supplementary Material is available for this article at <http://dx.doi.org/10.1007/s10886-005-8404-1> and is accessible for authorized users). 3-Octyn-1-ol (Aldrich) was isomerized to 7-octyn-1-ol using the “acetylene zipper” protocol (Abrams and Shaw, 1988). The obtained octynol served as the starting material for the syntheses of (7Z,9E,11)-dodecatrienyl acetate (Scheme 1) as well as for the (7E,9Z,11) and the (7E,9E,11) isomers (Scheme 2). Preparation of the latter two acetates proceeded similarly to the synthesis of the corresponding 9,11,13-tetradecatrienyl acetates (Tellier, 1991; Tellier et al., 1991), whereas the approach to (7Z,9E,11)-dodecatrienyl acetate was essentially the same as the sequences described for (9Z,11E,13)-tetradecatrienyl (Millar, 1990) and for (11Z,13E,15)-hexadecatrienyl acetate (Gries et al., 2004).

Hydrogenation of conjugated triple bonds with activated zinc in methanol produced (*Z*)-double bonds (Boland et al., 1987), whereas  $\text{LiAlH}_4$  reduction yielded (*E*)-double bonds (Porter et al., 1985). All reactions were carried out under argon. Intermediates were purified by distillation *in vacuo* or by column chromatography on silica gel. In all three syntheses, the alcohols corresponding to the target acetates were carefully recrystallized from hexane/toluene at  $-25^\circ\text{C}$  to furnish products with stereochemical purity  $>98\%$ .

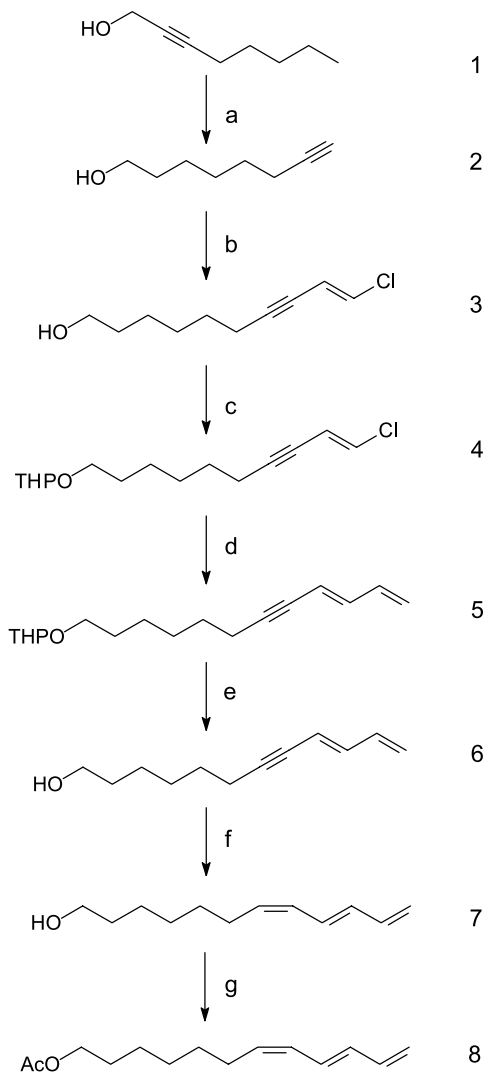
For the synthesis of (7Z,9E,11)-dodecatrienyl acetate, 7-octyn-1-ol was chain-elongated with (*E*)-1,2-dichloroethane (Sonogashira, 1991). The resulting  $\omega$ -chloroalcohol was protected and, again, chain-elongated with vinyl magnesium bromide (Millar, 1990). The  $\text{C}_{14}$  chain obtained was easily transformed into the final product.

For the synthesis of the two (7E)-configured trienylacetates, 7-octyn-1-ol (**1**) was transformed in two steps into (*E*)-1-*t*-butoxy-8-iodo-7-octene (Alexakis et al., 1988; Alexakis and Duffault, 1988). Chain elongation of this key intermediate used  $\text{Pd}^\circ$ -catalyzed alkynyl coupling (Sonogashira, 1991 and references cited therein). The syntheses of the target acetates were completed by conventional steps (Scheme 2).

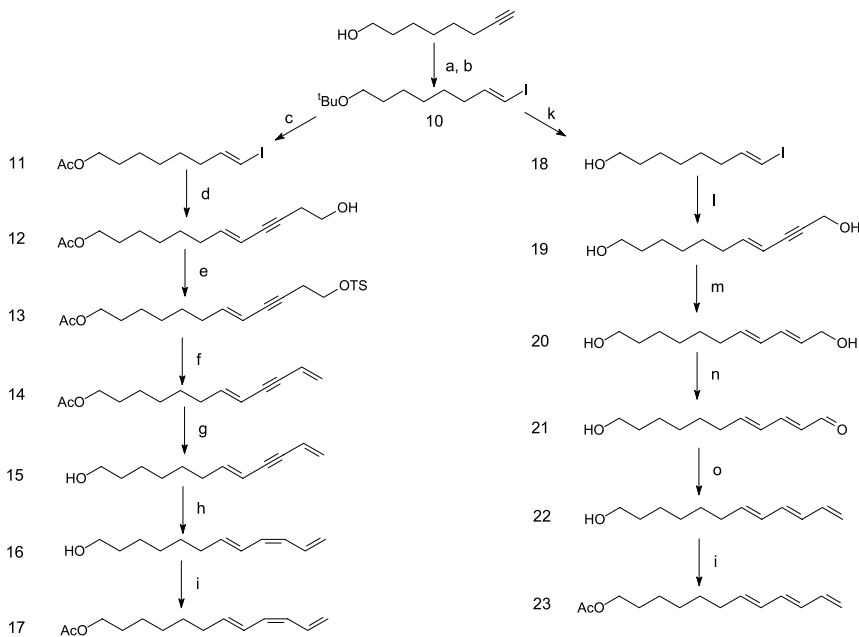
Details of the syntheses are provided in the online supplement.

**Wind Tunnel.** The wind tunnel hardware and the test protocol have been described (Witzgall et al., 2001). The wind tunnel with a flight section of  $63 \times 90 \times 200$  cm was lit diffusely at 6.5 lx. Charcoal-filtered air was blown by a horizontal fan through the tunnel at 30 cm/sec, temperature was  $23 \pm 2^\circ\text{C}$ , and relative humidity was 40–60%. The outcoming air was aspirated by another fan and cleaned by two sets of charcoal filters.

Synthetic pheromone components in redistilled ethanol were released from a piezoelectric sprayer (El-Sayed et al., 1999; Gödde et al., 1999). The



SCHEME 1. a) 1,3-Diaminopropane/Li; KO <sup>t</sup>Bu; b) (*E*)-1,2-dichloroethene, diisopropylamine, PdCl<sub>2</sub> (PPh<sub>3</sub>)<sub>2</sub>, CuI/THF; c) DHP, TsOH/Et<sub>2</sub>O; d) vinylmagnesium bromide/THF; e) TsOH/MeOH; f) Zn/MeOH; g) Ac<sub>2</sub>O/pyridine.



SCHEME 2. a) Amberlyst 15/isobutene–heptane  $\rightarrow$  9; b) DIBALH/I<sub>2</sub>; c) Ac<sub>2</sub>O/FeCl<sub>3</sub>/Et<sub>2</sub>O; d) 3-butyn-1-ol, Pd (PPh<sub>3</sub>)<sub>4</sub>, *n*-C<sub>3</sub>H<sub>7</sub>NH<sub>2</sub>, CuI/toluene; e) TsCl/pyridine; f) K–O <sup>t</sup>Bu, 18-crown-6/toluene; g) KOH/MeOH; h) Zn/MeOH; i) Ac<sub>2</sub>O/pyridine; k) i: TMS/(1.3 eq)/CCl<sub>4</sub> rt, ii: MeOH; l) as in (d) but using propynol; m) LiAlH<sub>4</sub>/THF; n) MnO<sub>2</sub>/CH<sub>2</sub>Cl<sub>2</sub>; o) Ph<sub>3</sub>PCH<sub>3</sub>Br, BuLi/THF, –78°C.

solution was delivered at a rate of 10  $\mu$ l/min through Teflon tubing to a 20- $\mu$ l glass capillary tube with a drawn-out tip. The capillary was vibrated by a piezo-ceramic disk at ca. 100 kHz to produce an aerosol, which evaporated within a few centimeters from its tip. This “sprayer” allows application of pheromone chemicals at a constant rate and known chemical purity. Isomeric purity of the test compounds was  $\geq$ 99%, except for 7*E*,9*E*,11-12Ac (98%) and 7*E*,9*Z*,11-12Ac (95%). Single females were held in glass tubes (2.5  $\times$  5 cm) covered with gauze on both sides. Females were kept in the wind tunnel for at least 30 min before experiments until they showed the typical calling behavior.

Wind tunnel tests were carried out from 1 to 4 hr after onset of the scotophase. Four batches of 15 2- to 3-d-old males (*N* = 60) were tested per pheromone blend on four different days. Single males were placed in glass tubes (2.5  $\times$  12.5 cm), which were stoppered with gauze on both sides ca. 15 min before testing. Males were tested individually and were given 2 min to respond. The number of males landing at the source, following upwind-oriented flight, in

each batch of 15 males was transformed to  $\log(x + 1)$  and submitted to a one-way ANOVA followed by Duncan's multiple range test ( $P < 0.05$ ).

#### RESULTS AND DISCUSSION

Chemical analysis of sex pheromone gland extracts of *L. botrana* females confirmed the presence of previously identified compounds. The main pheromone compound is *E7,Z9*-12Ac. The analogous *E7,Z9*-12OH and two saturated alcohols were present, in addition to several saturated, mono-, and diunsaturated acetates (Table 1; Arn et al., 1988; El-Sayed et al., 1999).

GC-EAD recordings (Figure 1) showed an additional, active compound eluting after *E7,Z9*-12OH. GC-MS studies indicated a conjugated trienylacetate because of characteristic fragments and its retention time. Co-occurrence of  $\Delta 7$ ,  $\Delta 9$ -12Ac and 11-12Ac in *L. botrana* pheromone glands made  $\Delta 7$ ,  $\Delta 9$ ,11-12Ac a likely candidate. Synthesis of 7,9,11-12Ac isomers confirmed the presence of *E7,E9*,11-12Ac in glands of Italian females (Table 1). The *EZ* and *ZE* isomers were found in smaller amounts. These three trienyl acetates also were present in gland extracts of females from Germany and Spain (not shown in Table 1). This is the first report of a triply unsaturated compound in a tortricid moth (Arn et al., 2000). The extremely unstable (7*Z*,9*Z*,11)-dodecatrienyl acetate or corresponding products of its rearrangements (Näf et al., 1975; Rockach et al., 1980) was not detected. No attempts were made to synthesize this isomer.

Another compound that elicited antennal responses, which could not be associated with known *L. botrana* pheromone compounds, eluted shortly before *E*- and *Z9*-12Ac. This new compound was shown to be *E7*-12Ac. It was present in gland extracts of females from Italy (Table 1; Figure 1) and from Spain. However, *E7*-12Ac was not detected in insects from Germany (this study, data not shown), which corroborates analysis of insects from Switzerland (Arn et al., 1988). This suggests the occurrence of *L. botrana* pheromone dialects north and south of the Alps.

Wind tunnel tests were performed with the newly identified compounds, *E7*-12Ac, and the *EE*, *EZ*, and *ZE* isomers of 7,9,11-12Ac (Table 2). These compounds were tested in two-component blends with the main compound, using males of a lab population originating from northern Italy. A 10% addition of *Z7,E9*,11-12Ac significantly increased attraction followed by landing at the source from 24% of the test males with the main compound alone to 65% ( $N = 60$ ; Table 2). The two other isomers, *E7,E9*,11-12Ac and *E7,Z9*,11-12Ac, were less active. A four-component blend of the main compound plus 10% of each of the three triene isomers attracted 61% of males to the source. The trienic *E7,Z9*,11-12Ac may mimic the main pheromone compound *E7,Z9*-12Ac because some males were attracted to this compound as a single component.

TABLE 1. COMPONENTS OF THE SEX PHEROMONE GLAND OF *L. Botrana* FEMALES FROM NORTHERN ITALY

Compound	Short form	Identification <sup>a</sup>	Amount <sup>b</sup>		GC-EAD <sup>c</sup> (mV/pg × 100)
			(pg/Female)	(%)	
Dodecyl acetate	12Ac	GC, MS	28 ± 12	3	–
( <i>E</i> )-7-Dodecenyl acetate <sup>d</sup>	<i>E7</i> -12Ac	GC, MS	106 ± 16	12	0.28 ± 0.24
( <i>E</i> )-9-Dodecenyl acetate	<i>E9</i> -12Ac	GC, MS	27 ± 30	3	0.43 ± 0.31
( <i>Z</i> )-9-Dodecenyl acetate	<i>Z9</i> -12Ac	GC, MS	113 ± 46	13	0.89 ± 0.33
11-Dodecenyl acetate	11-12Ac	GC	Trace <sup>e</sup>	–	–
( <i>E,E</i> )-7,9-Dodecadienyl acetate	<i>E7,E9</i> -12Ac	GC	28 ± 23	3	– <sup>f</sup>
( <i>E,Z</i> )-7,9-Dodecadienyl acetate	<i>E7,Z9</i> -12Ac	GC, MS	867 ± 472	100	0.46 ± 0.29
( <i>Z,E</i> )-7,9-Dodecadienyl acetate	<i>Z7,E9</i> -12Ac	GC, MS	25 ± 11	3	1.41 ± 0.64
( <i>Z,Z</i> )-7,9-Dodecadienyl acetate	<i>Z7,Z9</i> -12Ac	GC	Trace <sup>e</sup>	–	– <sup>f</sup>
( <i>E,Z</i> )-7,9-Dodecadien-1-ol	<i>E7,Z9</i> -12OH	GC, MS	88 ± 35	10	0.12 ± 0.18
( <i>E,E</i> )-7,9,11-Dodecatrienyl acetate <sup>d</sup>	<i>E7,E9,11</i> -12Ac	GC, MS	65 ± 27	7	0.73 ± 0.30
( <i>E,Z</i> )-7,9,11-Dodecatrienyl acetate <sup>d</sup>	<i>E7,Z9,11</i> -12Ac	GC	Trace <sup>e</sup>	–	–
( <i>Z,E</i> )-7,9,11-Dodecatrienyl acetate <sup>d</sup>	<i>Z7,E9,11</i> -12Ac	GC	1 ± 2	<1	–
Tetradecyl acetate	14Ac	GC	1 ± 2	<1	–
Octadecan-1-ol	18OH	GC, MS	73 ± 15	8	–
Eicosan-1-ol	20OH	GC, MS	109 ± 127	13	–

<sup>a</sup> Identification method: gas chromatography (GC) on two different columns, and mass spectrometry (MS).

<sup>b</sup> Absolute and relative amounts (mean ± SD) in batch extracts of 20–75 female pheromone glands (*N* = 7).

<sup>c</sup> Antennal response (mean ± SD) during GC–electroantennographic detection (EAD) recordings (*N* = 5).

<sup>d</sup> Newly identified compound.

<sup>e</sup> Trace amounts <1 pg/female.

<sup>f</sup> Recording not possible during antennal recovery phase after stimulation by preceding compound.

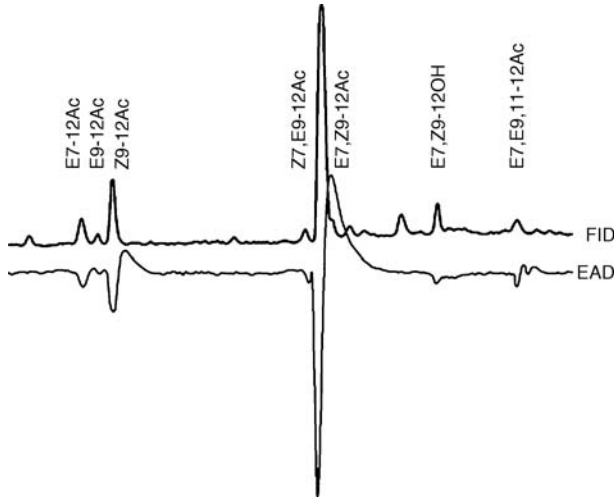


FIG. 1. Analysis of pheromone gland extracts of grapevine moth *L. botrana* females from Northern Italy ( $N = 5$ ) by GC-EAD. FID: response of the flame ionization detector; EAD: response of a *L. botrana* male antenna.

*E7-12Ac* had a similar effect as *Z7,E9,11-12Ac*, with addition of 10% increasing landings to 67% ( $N = 60$ ; Table 2). Male attraction to the best synthetic blends, at a release rate of 10 pg/min of the main compound, was not different from attraction to calling females. The female release rate is on the order of 10 pg/min (El-Sayed et al., 1999).

The monoene acetates *E9-12Ac*, *Z9-12Ac*, and *11-12Ac*, and the alcohol corresponding to the main compound, *E7,Z9-12OH*, are known pheromone synergists of *L. botrana* (Arn et al., 1988; El-Sayed et al., 1999). Here, we show that *E7-12Ac* and *Z7,E9,11-12Ac* are additional, strong pheromone synergists. Partial blends are as attractive as calling females (Table 2; El-Sayed et al., 1999), and this may indicate redundancy in the pheromone signal of *L. botrana*, as described for the cabbage looper moth *Trichoplusia ni* (Linn et al., 1984).

Availability of new pheromone synergists is nonetheless of practical importance. Mating disruption of *L. botrana* by aerial dissemination of pheromone can be effective, but there are many reports of unexpected failures (Arn and Louis, 1996). For example, geographic pheromone races may contribute to inconsistent results. Studies of *L. botrana* sex pheromone have all relied on insects from Switzerland (Roelofs et al., 1973; Arn et al., 1988; El-Sayed et al., 1999), whereas pheromone-mediated mating disruption is used in vineyards both north and south of the Alps (Kast, 2001; Louis and Schirra, 2001; Varner



TABLE 2. WIND TUNNEL RESPONSE OF *L. Botrana* MALES FROM NORTHERN ITALY TO BLENDS OF THE MAIN PHEROMONE COMPOUND *E7,Z9-12Ac* AND MINOR COMPOUNDS FROM FEMALE PHEROMONE GLANDS

Compound	Blend composition (pg/min) <sup>a</sup>							
	10	10	10	10	10	10	10	10
<i>E7,Z9-12Ac</i>	10	10	10	10	10	10	10	10
<i>Z7,E9,11-12Ac</i>		1				1		
<i>E7,Z9,11-12Ac</i>			1			1	10	
<i>E7,E9,11-12Ac</i>				1	1			
<i>E7-12Ac</i>							1	
Calling female <sup>b</sup>								1 <sup>b</sup>
Attraction to source (%) <sup>c</sup>	24 c	65 a	48 b	38 bc	61 ab	11 d	67 a	71 a

<sup>a</sup>Release rate (pg/min).

<sup>b</sup>One live calling female.

<sup>c</sup>Percentage of males (*N* = 60) landing at the odor source. Numbers followed by different letters are significantly different according to ANOVA followed by Duncan's test (*P* < 0.05).

et al., 2001; Zingg, 2001). The availability of a behavioral synergist, *E7-12Ac*, which can be made economically, merits a reinvestigation of the effect of pheromone synergists on *L. botrana* mating disruption.

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