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Plant Odor Analysis of Potato: Response of Guatemalan Moth to Above- and Belowground Potato Volatiles

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The Guatemalan moth *Tecia solanivora* is an invasive pest of potato in Central and South America. The larvae infest potato tubers in the field as well as in storage facilities. The headspace of potato foliage and potato tubers was studied with regard to volatiles that mediate host-finding and oviposition in the Guatemalan moth. Foliage of three phenological stages, from sprouting to tuberization and flowering, released more than 30 sesquiterpenes. The main compounds were β-caryophyllene, germacrene-D-4-ol, germacrene-D, kunzeaol, and (*E*,*E*)-α-farnesene. Sesquiterpenes accounted for >90% of the headspace of green plants, whereas fresh potato tubers emitted only trace amounts of a few sesquiterpenes. Screening of headspace collections with antennae of Guatemalan moth females showed a strong response to several sesquiterpenes and monoterpenes that were emitted from foliage only. In addition, antennae responded to methyl phenylacetate, a floral fragrance that was released in large amounts from flowering plants and that was also present in tuber headspace. Female and male moths were attracted to methyl phenylacetate; this compound may accordingly contribute to female attraction to tuber-bearing potato plants in the field as well as to potato tubers in storage. Oviposition tests showed that females lay eggs near mature flowering plants. Eggs were laid in soil close to the plant and not on potato stems and foliage, which may be due to avoidance of terpenoid compounds released from green plant parts at close range. The results support the concept that potato volatiles mediate host-finding and oviposition behavior and that these compounds may become useful tools for management of the Guatemalan moth.

KEYWORDS: ***Solanum tuberosum*; potato volatiles; *Tecia solanivora*; olfaction; oviposition; semiochemicals; headspace; chemical analysis**

INTRODUCTION

Plant volatile compounds play multiple roles as communication signals and defense agents, mediating interactions with other plants, microorganisms, fungi, and animals (*1*, *2*). These principal biological functions of plant volatiles are established, but the headspace of green plants contains hundred and more compounds, and it is still unclear whether they all are essential and biologically active compounds or whether some of them are merely biosynthetic waste products (*3*). Assigning biological functions to plant volatiles is therefore a current research challenge.

Herbivorous insects exploit plant volatiles for host-finding, and recent studies in lepidopteran species support the concept that blends of only a few key compounds encode specific host attraction (*4*−*7*). Knowledge of the chemicals that guide gravid females to suitable egg-laying sites is essential for the understanding of plant−insect interactions and for the development of safe plant protection strategies (*8*, *9*).

The Guatemalan moth *Tecia* (*Scrobipalpopsis*) *solanivora* Povolny (Lepidoptera: Gelechiidae) is native to Guatemala and has only recently invaded Central America and adjacent South American countries, where it has become a key pest of potato *Solanum tuberosum* L. (Solanaceae) (*10*, *11*). The larvae of *T. solanivora* mine inside potato tubers, and the adult moths hide in soil crevices during daytime. Control by insecticide sprays is therefore not efficient, and substantial crop losses in the field and in storage facilities require the development of alternative methods (*12*).

Air permeation of potato fields with synthetic pheromone has shown to efficiently disrupt sexual communication and matings. Mating disruption may accordingly become an efficient control method in the field (*13*). Infestations in storage facilities, on the other hand, are largely caused by immigrating, already mated, females so mating disruption is accordingly not feasible. Attractant or repellent plant volatiles for behavioral manipulation of mated females may instead become efficacious tools for management of the Guatemalan potato moth.

The Guatemalan moth *T. solanivora* has been recorded from potato *S. tuberosum* and one related species, *S. phureja*. Moths oviposit in soil close to potato plants in the tuberization stage and on potato tubers (*13*), and it is conceivable that host-finding in this nocturnal species is mediated by plant volatiles. We here

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report a comparative analysis of the volatiles from different phenological stages of potato and from fresh tubers toward an identification of the behaviorally active compounds.

MATERIALS AND METHODS

Plant and Insect Material. The aerial parts and tubers of potato *S. tuberosum* L. cv. Princess (Solanaceae) were used for volatile collections. Plants were 2−3 weeks old in the sprouting stage, 5 weeks at the onset of tuberization, and 8 weeks in the flowering stage. Tubers were collected from 11-week-old plants, after the onset of senescence. Potatoes were planted in fertilized and limed peat soil (NPK 180:90:195 g/m^3; Hydro Agri AB, Landskrona, Sweden) in 5-L plastic pots. Plants where fertilized with 15 g of NPK 8:7:16 (Weibulls Trädgård AB, Hammenhög) at three occasions during the complete development. They were kept in a greenhouse at 15 ± 2 °C under a 14:10 L:D photoperiod. Daylight was supplemented with high-pressure sodium lamps (SON-T, 200 W/m^3; Osram, Munich, Germany), at 1 m above pot level.

The Guatemalan moth *T. solanivora* Povolny (Lepidoptera: Gelechiidae) was reared on potato tubers in the laboratory. The laboratory population originated from insects collected near Bogota, Colombia. Insects were kept in 15 cm ∅ × 20 cm plastic cylinders coated with filter paper for mating. Egg batches on filter paper were placed on potato tubers in 20 × 30 × 15 cm^3 plastic containers. Tubers infested with larvae were kept at 22 ± 3 °C and 55 ± 5% relative humidity, under a 14:10 L:D photoperiod. Last-instar larvae pupated in corrugated cardboard, and the pupae were separated by sex. Females and males for electrophysiological and behavioral experiments were kept separately in Plexiglas cages (33 × 33 × 33 cm^3) under a 14:10 L:D photoperiod and fed with a sugar solution.

Volatile Collections. Potato foliage in three developmental stages and tubers (n = 8) were enclosed in a 2-L glass jar, which was closed with a ground glass fitting. Plants were cut at the base of the stem, which was held in a 10-mL vial of water. A charcoal-filtered airstream (150 mL/min) was pulled over the plant material from the bottom to the top of the jar, over an air filter (see below) during 24 h, at 20−22 °C and 10−30 lx. The charcoal filter for incoming air and the trap for outcoming air were connected with glass fittings to the jar. All glassware was heated to 375 °C for 8 h before use.

The filters for collection of potato volatiles were made of 4 × 40 mm glass tubes containing 35 mg of Super Q (80/100 mesh; Alltech, Deerfield, IL) held between glass wool plugs. Before use, the traps were rinsed sequentially with 3 mL of methanol, ether, and redistilled *n*-hexane (Labscan, Malmö, Sweden), after 15-min treatments in ultrasonic baths in ether and hexane, respectively. After sample collection, the traps were rinsed with 0.3 mL of redistilled hexane (LabScan), and 500 ng of heptyl acetate (99.8% chemical purity; Aldrich) was added as an internal standard to the filter eluent. The sample volumes were reduced under a stream of nitrogen to ca. 60 μL, at ambient temperature in Francke vials with an elongated tip (5 cm × 2 mm i.d.). The samples were stored in sealed glass capillary tubes at −19 °C (*14*).

Chemical Analysis. Headspace samples were analyzed on a gas chromatograph with a flame ionization detector (GC-FID; HP-5890, Agilent Technologies Inc., Santa Clara, CA) and a gas chromatograph coupled to a mass spectrometer (GC-MS; 6890 GC and 5975 MS, Agilent). The gas chromatographs were equipped with a DB-Wax or a HP-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm; J&W, Agilent).

Samples were injected manually in aliquots of 2 μL in splitless mode, and the inlet temperature was 200 °C. The carrier gas was hydrogen at a linear flow of 45 cm/s (GC-FID) or helium at 35 cm/s (GC-MS). For the GC-FID analysis, the oven temperature was 30 °C (5-min hold), followed by an increase of 8 °C/min to 230 °C (10-min hold). The FID temperature was 250 °C. The GC-MS temperature program was the same as above. The transfer line between the GC and MS was programmed to hold at 200 °C and to track in synchrony with the GC oven temperature above 200 °C. Mass spectral data were obtained with electron impact ionization at 70 eV and were acquired in scan mode, from m/z 29 to 330, at 2 scans/s.

Compound identification was based on comparison of mass spectra and retention times with those from synthetic and authentic standards and by comparison with published Kovats retention indices (KI), obtained on the same or a similar type of column. Enantiomers of chiral compounds were not available. Limonene and α-pinene were purchased from Janssen Pharmaceutica (Beerse, Belgium), (*E,E*)-α-farnesene and (*E*)-β-farnesene were from Bedoukian (Danbury, CT), and 1-octen-3-ol was from Acros Organics (Geel, Belgium). Germacrene-D, β-elemene, δ-elemene, α-cubebene, β-cubebene, and γ-cadinene were gifts from Anna-Karin Borg-Karlsson (Stockholm, Sweden), and (*Z*)-β-ocimene was from Wittko Francke (Hamburg, Germany). All other standards were from Sigma-Aldrich and Fluka.

Compounds in the aeration extracts were quantified by their ion abundances relative to those of the added internal standard heptyl acetate and were corrected by response factors calculated for each compound with standards. Compounds for which pure standards were not available were quantified according to the response factor of a structurally similar compound. The amounts of compounds released from different phenological stages were compared by a one-way analysis of variance, followed by a Tukey test.

Electroantennography. A HP 5890 GC with a DB-Wax column, using the same temperature program as for GC-FID and GC-MS (see above), was interfaced with an electroantennogram apparatus for simultaneous recordings by FID and electroantennographic detection (EAD). The antenna was cut at the base and was held in electroconductive gel (Cefar, Lund, Sweden) in a forked antenna holder. The effluent from the GC column was split equally in a four-way splitter, with nitrogen as makeup gas (20 mL/min), dividing the GC effluent to the FID and EAD into two deactivated fused silica capillaries (90 cm × 0.25 mm), between the FID and a cut antenna of a *T. solanivora* female. The EAD capillary effluent was delivered to the antennal preparation in a stream of humidified and carbon-filtered air in a glass tube (8 mm × 150 mm; 1.5 L/min). The antennal signal and the FID signal were amplified and recorded simultaneously on a Syntech IDAC-4 (Hilversum, The Netherlands). Moths were 2−3 days old; recordings of foliage (n = 40) and tuber headspace (n = 7) were made during the first part of the scotophase. Antennal responses with a signal-to-noise ratio of > 3.0 were considered to be significant.

Behavioral Assays. Attraction and oviposition behavior of the Guatemalan potato moth in response to potted potato plants in three developmental stages was studied in a mesh house (6 × 6 × 5 m^3) with walls made of metal mesh (0.5 × 0.5 mm^2). Plants were in the sprouting (3 weeks), tuberization (5 weeks), and flowering stages (8 weeks old). Three plants, one of each stage, were placed at 2 m distances from each other. Twenty mated females were released, and the eggs laid on the plant (stem, leaves) and on the soil, at a 20-cm radius from the stem, were counted after 2 days (n = 6). Data were analyzed with a one-way analysis of variance, followed by a Tukey test. For egg counts in the soil, the top 1-cm layer was removed and the soil grains were examined under a light microscope, where the eggs are visible due to their bright yellow color.

A two-choice oviposition experiment was done to further examine the effect of green potato foliage on oviposition. Five mated males and females were placed in plastic cages (20 × 30 × 15 cm^3, n = 14) during 48 h under a 16:8 L:D photoperiod and fed with sugar solution. Two circles (7 cm ∅) excised at the bottom of the cage were covered with a black screen. Green potato foliage from mature plants, held in a 2-mL vial with water, and a 2-mL water vial alone were placed in cylinders (7 cm ∅ × 10 cm) underneath the screen. The number of eggs laid on the screen above the cylinders, with and without potato foliage, was compared with a Wilcoxon test.

The attraction of Guatemalan moth to methyl phenylacetate was tested with water traps in a mesh house (see above) with 25 potted potato plants in the tuberization stage. Water traps made of white plastic bottles (16 cm ∅ × 23 cm), with two holes (15 cm ∅), contained water and a red rubber septum (Phero.Net, Lund, Sweden) baited with 1 mg of methyl phenylacetate. Three blank traps and three baited traps, spaced equally in the mesh house, were placed with the trap opening at ca. 60 cm from the ground, at canopy height, at > 50 cm distance from surrounding potato plants. Thirty freshly eclosed males and 30 females were released into the mesh house, and trap captures were recorded during 2 days. The number of trapped males and females in baited and blank traps was analyzed with a t test.

RESULTS

Chemical Analysis of Potato Headspace. Green potato plants released 32 sesquiterpenes, accounting for 86, 93, and 55% of the volatiles released in the sprouting, tuberization, and flowering

Table 1. Volatile Compounds in Headspace Collections from Potato Foliage and Tubers (Cv. Princess) and Their Antennal Activities in Guatemalan Moth Females

	phenological stage									Kovats index[e]		
	sprouting		tuberization		flowering		tubers			column		
compound[a–c]	ng/g/h	%	ng/g/h	%	ng/g/h	%	ng/kg/h	%	refs[d]	DB-Wax	HP-5MS	refs
aliphatics												
alcohols												
3-hexanol	0.5 b	0.6	0.4 ab	0.1	0.8 a	0.2	1.9	2.6		1190		*25*
2-hexanol	0.3 a	0.4	0.3 a	0.1	0.5 a	0.1	1.7	2.3		1217		
(*Z*)-3-hexen-1-ol	0.5 a	0.6	0.7 a	0.3	0.7 a	0.2			(*16*–*18*)	1379		*25*
1-octen-3-ol							10.7	14.4	*19*	1445		*26*
2-ethyl-1-hexanol							2.1	2.8	*20*	1483		
alkenes												
heneicosane							4.8	6.5		2100		
aldehydes												
(*E*)-2-hexenal	0.2 a	0.2	0.8 a	0.3	0.5 a	0.1			(*16*, *17*)	1209		*25*
nonanal	0.5 a	0.6	0.2 a	0.1	0.4 a	0.1	1.3	1.7	(*16*–*20*)	1391		*25*
decanal	tr[f]		tr		tr		1.8	2.4	(*16*, *18*–*20*)	1500		*27*
phenylacetaldehyde	1.2 a	1.4	0.8 a	0.3	0.9 a	0.2	1.2	1.6		1632		
tetradecanal							**15.8**	**21.3**		2022		
ketones												
2-hexanone			0.2 b	0.1	0.9 a	0.2	2.5	3.4		1075		
3-hexanone*	0.5 a	0.6	0.4 a	0.1	0.8 a	0.2	2.1	2.8		1047		
3-octanone			0.2 a	0.1	0.2 a	0.0	5.8	7.8		1253		*25*
1-octen-3-one							1.2	1.6		1310		*27*
2,5-hexanedione*	1.3 a	1.5	tr		0.7 b	0.2	2.1	2.8		1515		
benzenoids and phenylpropanoids												
hydrocarbons												
1,3-dimethylbenzene	0.2 a	0.2	0.2 a	0.1	0.2 a	0.0	1.2	1.6		1136		
1,2-dimethylbenzene	0.4 a	0.5	0.4 a	0.1	0.4 a	0.1	2	2.7		1177		*25*
alcohols												
2-phenylethanol	0.2 b	0.2	0.5 b	0.2	2.1 a	0.5	1.2	1.6		1905		*26*
aldehydes												
phenylacetaldehyde	1.2 a	1.4	0.8 a	0.3	0.9 a	0.2	1.2	1.6		1632		
esters												
methyl 2-methylbutanoate	0.1 b	0.1	1.1 b	0.4	1.8 a	0.4				1004		
methyl phenylacetate					**157.2**	**38.3**	**3.2**	**4.3**		1754	1180	*28*
monoterpenes												
hydrocarbons												
α-pinene	0.5 b	0.6	0.4 b	0.1	2.3 a	0.6	1.3	1.7	(*18*, *19*)	1017	939	*27*
β-pinene	0.5 a	0.6	0.6 a	0.2						1104	981	*27*
sabinene	**0.4 a**	**0.5**	**0.7 a**	**0.3**	**0.6 a**	**0.1**			*18*	1115	978	(*29*, *30*)
β-myrcene	**2.5 a**	**3.0**	**9.7 a**	**3.6**	**8.3 a**	**2.0**			*18*	1160	992	(*25*, *30*)
limonene	0.4 a	0.5	0.4 a	0.1	0.9 a	0.2			(*18*–*20*)	1194	1034	*27*
(*Z*)-β-ocimene							5.1	6.9	*19*	1246		*29*
p-cymene					0.1	0.0				1261		*27*
acids												
(*E*)-rose oxide			0.1 a	0.0	0.2 a	0.0	0.9	1.2		1350	1115	*28*
sesquiterpenes												
alcohols												
ledol*	**3.5 b**	**4.2**	**10.8 a**	**4.0**	**7.7 b**	**1.9**			(*18*, *21*, *22*)	2030	1627	*25*
germacrene D-4-ol	**10.8 b**	**12.8**	**40.9 a**	**15.2**	**26.9 b**	**6.6**			(*21*, *22*)	2044	1595	*30*
τ-cadinol	**0.7 a**	**0.8**	**1.9 a**	**0.7**	**1.9 a**	**0.5**				2169	1660	
α-cadinol	0.7 b	0.8	2.3 a	0.9	2.3 a	0.6			*21*	2232	1674	(*29*, *30*)
kunzeaol	7.6 b	9.0	19.6 a	7.3	10.7 a	2.6			*23*	2326	1711	
(*E*,*E*)-farnesol			0.1 b		4.9 a	1.2	1.2	1.6		2348		*28*
hydrocarbons												
α-cubebene	**0.7 a**	**0.8**	**1.7 a**	**0.6**	**1.7 a**	**0.4**	**tr**		(*18*, *21*)	1460	1361	(*25*, *30*)
∂-elemene	**0.0 b**	**0**	**0.8 a**	**0.3**	**1 a**	**0.2**				1480		*25*
α-copaene	**1.4 a**	**1.7**	**1.7 a**	**0.6**	**1.2 a**	**0.3**			(*18*–*21*, *24*)	1491	1390	(*25*, *30*)
β-bourbonene	**0.3 b**	**0.4**	**0.6 b**	**0.2**	**1.5 a**	**0.4**			*24*	1521		*25*
α-gurjunene	3.2 a	3.8	7.7 a	2.9	6.4 a	1.6			*21*	1534		*25*
β-cubebene	0.5 b	0.6	1.5 ab	0.6	1.6 a	0.4			*21*	1540		*25*

Table 1. Continued

	phenological stage									Kovats index[e]		
	sprouting		tuberization		flowering		tubers			column		
compound[a–c]	ng/g/h	%	ng/g/h	%	ng/g/h	%	ng/kg/h	%	refs[d]	DB-Wax	HP-5MS	refs
β-elemene	1.8 a	2.1	7.6 b	2.8	8.2 b	2.0			(*18*, *21*, *24*)	1589	1403	(*25*, *29*)
β-caryophyllene	**15.2 a**	**18.1**	**46.4 a**	**17.3**	**54.2 a**	**13.2**			(*16*–*18*, *20*–*22*, *24*)	1608	1439	*29*
alloaromadendrene	tr		0.5 a	0.2	0.7 a	0.2				1650		(*25*, *29*)
(*E*)-β-farnesene	**1.2 b**	**1.4**	**4.3 a**	**1.6**	**5 a**	**1.2**			(*16*, *18*, *21*, *22*)	1660		*29*
α-caryophyllene	1 b	1.2	3.7 a	1.4	4 a	1.0			(*18*, *21*, *22*)	1673	1474	(*25*, *30*)
γ-muurolene	0.5 a	0.6	1.6 a	0.6	1.9 a	0.5			*18*	1689		*29*
germacrene-D	**8.1 b**	**9.6**	**39.9 a**	**14.9**	**46.8 a**	**11.4**	**tr**		(*16*, *18*, *21*, *22*)	1710	1500	(*25*, *27*)
α-zingiberene	0.2 b	0.2	1.0 b	0.4	1.4 a	0.3			*22*	1715		*31*
α-bergamotene	0.8 b	1.0	3.3 ab	1.2	3.3 a	0.8			(*21*, *22*)	1720		
α-muurolene	2.3 a	2.7	9.4 a	3.5	8.2 a	2.0				1725		*31*
isolongifolene*	1.2 b	1.4	6.2 a	2.3	7.2 a	1.8				1732		
bicyclogermacrene	0.1 a	0.1	0.7 a	0.3	0.7 a	0.2			(*21*, *22*)	1734		*25*
α-longipinene	tr		0.7 a	0.3	1.3 a	0.3			*18*	1739		
(*E*,*E*)-α-farnesene	**4.3 b**	**5.1**	**14.9 a**	**5.5**	**n.q.[g]**					1743	1545	(*25*, *30*)
δ-cadinene	**2.8 b**	**3.3**	**12.2 a**	**4.5**	**11.1 a**	**2.7**			(*21*, *24*)	1759	1539	(*27*, *32*)
γ-cadinene	0.7 a	0.8	0.8 a	0.3	0.6 a	0.1			*24*	1762		*25*
β-sesquiphellandrene	0.5 b	0.6	3.2 a	1.2	2.7 a	0.7			(*21*, *22*)	1767		*29*
α-cadinene	1 a	1.2	1 a	0.4	0.5 a	0.1				1793	1556	*30*
cuparene	0.1 a	0.1			0.2 a					1825		*31*
caryophyllene oxide	0.9 a	1.1	2 a	0.7	1.5 a	0.4			*22*	1989	1607	(*27*, *29*)
irregular terpenes												
ketones												
6-methyl-5-hepten-2-one	**0.2 b**	**0.2**			**1.1 a**	**0.3**	**0.4**	**0.5**		1332		*27*
geranyl acetone							0.8	1.1		1817		
6,10,14-trimethylpentadecanone	0.4 a	0.5	0.6 a	0.2	0.6 a	0.1				2112		
miscellaneous cyclic compounds												
furans												
2-pentylfuran							0.8	1.1		1230		

[a] Compounds identified by GC-MS in comparison with standards on two columns. Bold-faced compounds elicited an antennal response in female moths. Asterisks indicate compounds for which standards were unavailable; these were identified according to NIST 05 library. Compounds are listed according to Knudsen et al. (*15*). [b] Volatiles were collected from the aerial part of green potato plants in the sprouting (2–3 weeks; foliage), tuberization (5 weeks; foliage), and flowering stages (8 weeks; foliage and flowers). Tubers were from 11-week-old plants. [c] Release rates (ng/h), per g of foliage or kg of tuber weight, and relative to the sum of all compounds. Mean release rates ($n = 8$) followed by different letters are significantly different at $P < 0.05$ (ANOVA, Tukey test). [d] Compounds previously reported from potato (*16*–*24*). [e] Kovats indices, as measured on DB-Wax and HP-5MS columns (*25*–*32*). [f] Trace amounts. [g] Not quantified.

stages, respectively. The most abundant sesquiterpenes were β-caryophyllene, germacrene-D-4-ol, germacrene-D, kunzeaol, and (*E*,*E*)-α-farnesene (**Table 1**).

Most compounds were present in all three phenological stages, but the release rates of many sesquiterpenes increased significantly between sprouting and tuberization. Flowering plants released in addition large amounts of methyl phenylacetate, and this compound has a strong honey-like floral fragrance with earth tones. It was emitted at a rate of 157 ± 52 ng/g/h. Interestingly, tubers also released methyl phenylacetate, at 3 ± 1 ng/kg/h (**Table 1**; **Figure 1**).

Tuber headspace was otherwise distinguished from foliage headspace by the near absence of sesquiterpenes, only three of which were found in trace amounts. Tubers released predominately alipathic alcohols, aldehydes, and ketones together with several benzenoids and monoterpenes. Most of these compounds were also found in foliage headspace. However, several abundant compounds, such as tetradecanal, octen-3-ol, (*Z*)-β-ocimene, and heneicosane, were characteristic for tuber headspace (**Table 1**).

Antennal Recordings. Antennae of Guatemalan moth females *T. solanivora* consistently responded to two compounds from tuber headspace, methyl phenylacetate, which was also released from flowering plants, and tetradecanal. A third compound, 6-methyl-5-hepten-2-one (sulcatone), elicited a response in 4 of 7 recordings of tuber headspace. In addition, the headspace of flowering plants contained 2 monoterpenes and 12 sesquiterpenes that elicited a strong antennal response in all recordings ($n = 40$; **Table 1**; **Figure 1**). The identity of the compounds eliciting an antennal response was corroborated by recordings with synthetic compounds, with the exception of τ-cadinol and β-bourbonene.

Behavioral Assays. Mated *T. solanivora* females, released into a mesh house, were attracted to potted potato plants for oviposition. Significantly more eggs were laid near flowering plants than near younger, preflowering plants. Eggs were deposited only on soil, close to the stems of potato plants, but not directly on stems or leaves (**Table 2**).

In a choice test, females avoided laying eggs near green potato foliage. Significantly fewer eggs were laid in response to green potato leaves than in response to blank (38.9 ± 24.8 and 18.1 ± 15.1 eggs, respectively; $P = 0.004$; $n = 14$).

Traps baited with 1 mg of methyl phenylacetate captured 15.33 of 30 released males and 3.67 of 30 released females in a mesh house with potted potato plants. Captures were significantly different from blank ($n = 3$, $P = 0.0011$, $t = 8.356$; and $P = 0.241$, $t = 3.536$, df = 4).

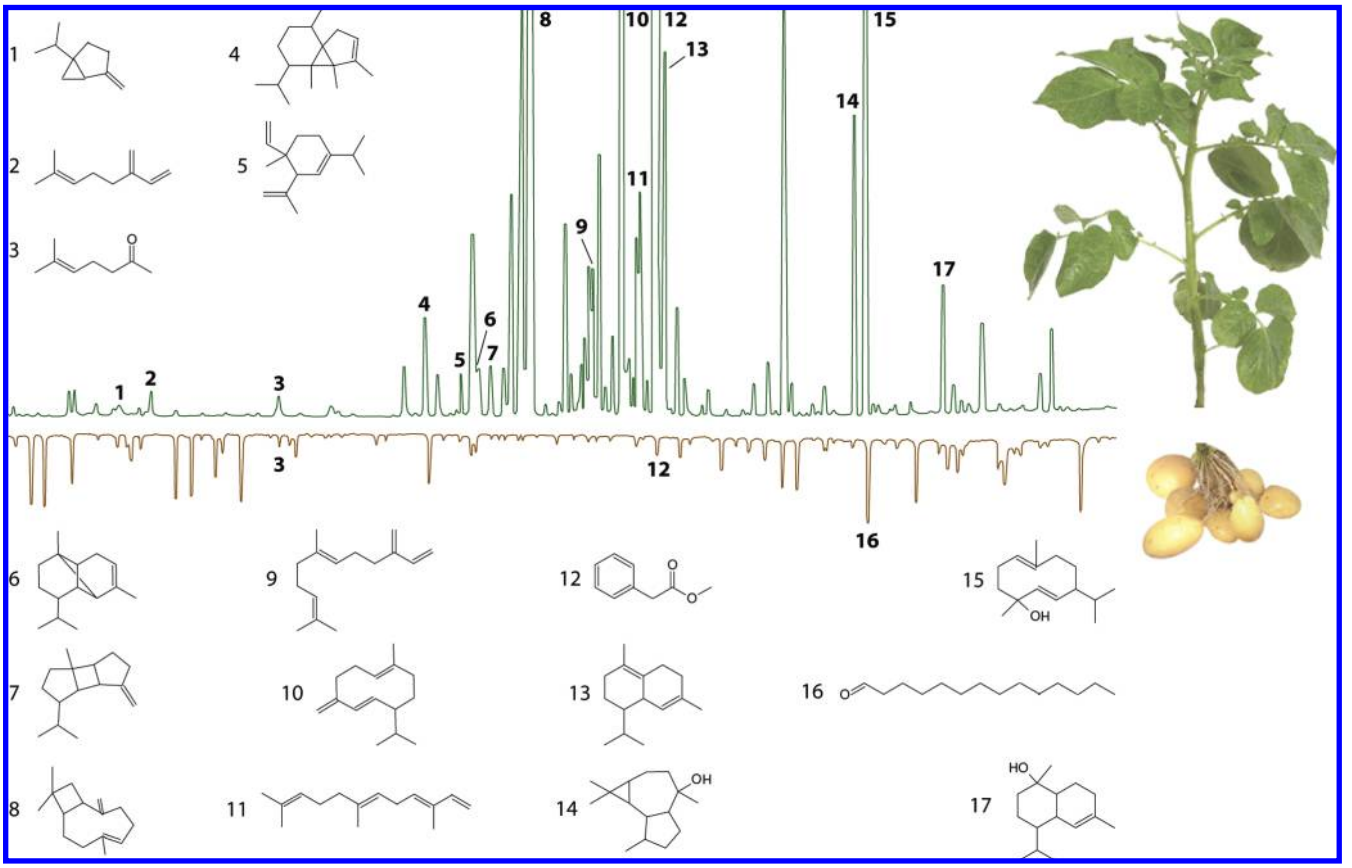

Figure 1. Volatile compounds from potato foliage with flowers (top GC trace) and potato tubers (bottom trace) eliciting an antennal response in Guatemalan moth females *Tecia solanivora*, as analyzed by GC-MS and GC-EAD. The active compounds are sabinene (**1**), myrcene (**2**), 6-methyl-5-hepten-2-one (**3**), α-cubebene (**4**), δ-elemene (**5**), α-copaene (**6**), β-bourbonene (**7**), β-caryophyllene (**8**), (*E*)-β-farnesene (**9**), germacrene-D (**10**), (*E*,*E*)-α-farnesene (**11**), methyl phenylacetate (**12**), δ-cadinene (**13**), ledol (**14**), germacrene-D-4-ol (**15**), tetradecanal (**16**), and τ-cadinol (**17**).

Table 2. Oviposition of Guatemalan Moth *T. solanivora* Females in Soil Surrounding Green Potato Plants

	potato phenology[a]		
no. of eggs on	sprouting	tuberization	flowering
stem and foliage	0	0	0
soil[b]	60 b	67 b	256 a

[a] Potted plants were in the sprouting (3 weeks), tuberization (5 weeks), and flowering stages (8 weeks). [b] Significantly more eggs are laid in soil close to mature, flowering plants ($P = 0.0475$, Tukey test; $n = 6$).

DISCUSSION

Analysis of Potato Headspace. The odor of green potato foliage is distinguished by the abundance of sesquiterpene hydrocarbons and alcohols, comprising up to 93% of the total volatile release. Most of these sesquiterpenes have been found also in other potato cultivars. Eight sesquiterpenes, including τ-cadinol, δ-elemene, α-muurolene, and (*E*,*E*)-α-farnesene, have been newly identified from potato, according to mass spectra and retention times in comparison with authentic compounds (**Table 1**) (*16*, *19*, *21*, *22*).

Sesquiterpenes are characteristic for potato foliage, but were only released in trace amounts from tubers. The headspace of fresh tubers contains mainly aliphatic compounds, along with several benzenoids and monoterpenes (**Table 1**) (*23*). Several of the compounds that are released from fresh tubers have even been found in the aroma of baked potatoes, such as octen-3-ol, nonanal, decanal, and (*Z*)-β-ocimene (**Table 1**) (*24*).

Interestingly, methyl phenylacetate, the principal odor compound of flowering potatoes, was also present in tuber headspace (**Table 1**; **Figure 1**). This odor-active compound has a strong honey-like floral flavor and has been identified for the first time from potato. It elicits a strong antennal response in Guatemalan moth *T. solanivora*, and a first trap test showed that it probably contributes to the attraction of egg-laying females to flowering plants and tubers. Methyl phenylacetate is an important component of the floral scent of Japanese privet *Ligustrum japonicum*, which is attractive to foraging cabbage butterflies *Pieris rapae* (*33*). Further behavioral work with more complex volatile blends is needed to completely identify the chemical signal eliciting attraction of the Guatemalan moth to potato.

Biological Significance of Potato Sesquiterpenes. Sesquiterpenes are essential components of several insect attractants based on plant volatiles (*5*, *7*, *34*). Sesquiterpenes account for the largest part of potato headspace, and 12 of them elicit an antennal response in females of the Guatemalan moth *T. solanivora* (**Table 1**). The Guatemalan moth is monophagous on potato, and it is conceivable that sesquiterpenes are part of the volatile signature that encodes host-finding. Moreover, sesquiterpenes from potato foliage, possibly in combination with other compounds, may allow the Guatemalan moth to discriminate between different phenological stages of potato and between different plant parts (**Table 1**). Females laid more eggs at mature flowering than nonflowering plants (**Table 2**), corroborating the observation that the Guatemalan moth is attracted to flowering potato fields (*12*, *35*).

It is intriguing that Guatemalan moth females are attracted to and oviposit close to flowering plants in soil, but not on their stems and foliage (**Table 2**) (*12*, *35*). This compares to the oviposition behavior of a closely related species, potato tuber

moth *Phthorimaea operculella*. Under field conditions, females lay a high proportion of eggs in the soil adjacent to host plants rather than directly on the leaves, despite the leafmining feeding habit of *P. operculella* larvae (*36*). This dichotomy between attraction to the plant and oviposition in soil, away from the plant, indicates either differences in the cues mediating attraction and oviposition or differential behavioral effects of the same compounds at long and short ranges. In comparison, lepidopteran pheromone blends act as a unit, mediating the entire behavioral sequence from long-range attraction to landing and close-range behavior (*37*).

Potato Secondary Metabolites as Signals of Host Quality. Potatoes produce sesquiterpenoid phytoalexins mainly in response to fungal infections (*38*) and steroidal glycoalkaloids in response to wounding, including insect attack. The concentration of glycoalkaloids in potato cultivars is negatively correlated with their susceptibility to insects (*39*, *40*). Steroidal glycoalkaloids and sesquiterpenes are biosynthesized via different branches of the mevalonate pathway. This facilitates a diversion from antimicrobial sesquiterpenoid phytoalexins to wound-induced glycoalkaloids and adjusts the defense response to pathogens and herbivores (*41*, *42*). Even in undamaged plants, preformed glycoalkaloids are constitutively present in organs with the greatest metabolic activity, such as new leaves, fruits, flowers, and sprouts. Release of large amounts of sesquiterpenes from green plant parts is therefore indicative of the presence of steroidal glycoalkaloids (*40*, *43*).

Sesquiterpenes may accordingly play a dual role in host-finding of the Guatemalan moth. In addition to their putative role in long-range attraction, they may facilitate the choice of oviposition sites at close range. Females were attracted to, but did not oviposit on, green potato plants (**Table 2**), and a subsequent choice test confirmed that females avoided oviposition close to potato foliage. On the other hand, females readily oviposit on soil or on tubers that do not release sesquiterpenes and that contain much lower amounts of glycoalkaloids than leaves (**Table 1**) (*12*, *40*, *43*).

Insects can avoid eating toxic plants or plant parts, as soon as they are able to detect them visually, olfactorily, or via contact. The Colorado beetle, feeding with biting mouthparts on potato leaves, taste glycoalkaloids (*44*), but ovipositing Guatemalan moths cannot directly sense nonvolatile chemicals contained in the plant. Ovipositing females could instead use sesquiterpenes as signals for elevated levels of the biosynthetically correlated glycoalkaloids in green plant parts as well as in damaged tubers that are less suitable for larval development.

Support for the concept that volatiles trigger avoidance mechanisms comes from the sister species *P. operculella*. Females were attracted to volatiles from intact potato tubers and not to volatiles from tubers damaged by conspecific larvae (*45*). The effect of potato glycoalkaloids on larvae of *T. solanivora* has not been studied, but a tomato glycoalkaloid, α-tomatine, is negatively correlated with larval development of *P. operculella* on tomato (*46*).

Future Application of Plant Volatiles for Control of Guatemalan Moth. A plant volatile attractant for female moths, such as methyl phenylacetate, may enable population monitoring and possibly control of Guatemalan moth in potato storage by mass-trapping. Repellent volatiles from potato foliage, on the other hand, might be used to deter oviposition (*47*). Ongoing studies aim at a detailed behavioral analysis of potato volatile compounds in the Guatemalan moth, leading toward the development of efficient and sustainable control methods.

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