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Could alternative solanaceous hosts act as refuges for the tomato leafminer, *Tuta absoluta*?

Thomas Bawin[1] · David Dujeu[1] · Lara De Backer[1] · Marie-Laure Fauconnier[2] ·
Georges Lognay[3] · Pierre Delaplace[4] · Frédéric Francis[1] · François J. Verheggen[1]

Abstract The tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), is a widespread devastating pest reported to develop on economically important solanaceous plants. The characterization of its effective host range could help to understand and prevent the dispersion behavior of the insect in the environment. In this study, the ability of *T. absoluta* to locate and develop on wild (*Solanum nigrum*, *Atropa belladonna*, *Datura stramonium*) and cultivated (*Solanum tuberosum*) solanaceous plant species under laboratory conditions was assessed. Dual-choice behavioral assays performed in flying tunnels (*S. tuberosum* vs. another plant) revealed that adult distribution and female oviposition did not differ between *Solanum* species, which were preferred to the other tested plants. The volatile molecules released by each tested plant species provide some explanations in the observed behavioral discrimination: *S. nigrum* and *S. tuberosum* volatile profiles were similar and were presenting quantitative and qualitative differences with the other tested solanaceous plants. To determine whether the host plant choice was adaptive or not, we have finally conducted fitness assays, by rearing *T. absoluta* larvae on each plant species and have shown that *Solanum* species allowed higher larval survivability and lower development time (from egg to adult emergence) compared to the other plants. We conclude that *Solanum* species are suitable host plants for *T. absoluta*, but other solanaceous plant species could be opportunistically colonized with fewer incidences.

Keywords *Scrobipalpuloides absoluta* · Solanaceae · Host plant · Potato · Black nightshade · Plant volatile organic compound

Introduction

The tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), is a widespread invasive species originated from South America and damaging economically

✉ François J. Verheggen
fverheggen@ulg.ac.be

Thomas Bawin
thomas.bawin@doct.ulg.ac.be

David Dujeu
david.dujeu@aigx.be

Lara De Backer
ldebacker@doct.ulg.ac.be

Marie-Laure Fauconnier
marie-laure.fauconnier@ulg.ac.be

Georges Lognay
georges.lognay@ulg.ac.be

Pierre Delaplace
pierre.delaplace@ulg.ac.be

Frédéric Francis
frederic.francis@ulg.ac.be

[1] Entomologie fonctionnelle et évolutive, Gembloux Agro-Bio Tech, Université de Liège, Passage des Déportés 2, 5030 Gembloux, Belgium

[2] Chimie générale et organique, Gembloux Agro-Bio Tech, Université de Liège, Passage des Déportés 2, 5030 Gembloux, Belgium

[3] Chimie Analytique, Gembloux Agro-Bio Tech, Université de Liège, Passage des Déportés 2, 5030 Gembloux, Belgium

[4] Biologie végétale, Gembloux Agro-Bio Tech, Université de Liège, Passage des Déportés 2, 5030 Gembloux, Belgium

important tomato crops (*Solanum lycopersicum* L., Solanaceae) (Desneux et al. 2010). Larvae feed on the mesophyll of all aerial parts of the plants, as well as on the fruits, resulting in significant yield losses, cosmetic damages, and rot diseases to fresh market tomatoes. During the last decade, the pest has spread worldwide and is now a serious threat to commercial tomato production in South and Central America as well as in Europe (Desneux et al. 2010, 2011; Tropea Garzia et al. 2012). Since its introduction in Europe in 2006, the moth is reported on the EPPO A2 list of species recommended for regulation as quarantine pests (EPPO/OEPP 2014).

The emergence of an alien invasive phytophagous insect within a geographical range is determined by its ability to localize and successfully develop on new hosts. Host range is constrained by the behavioral and physiological traits of the insect (Suckling et al. 2014). Although causing large injuries on tomatoes, *T. absoluta* is considered as a polyphagous leafminer that can also develop on alternative hosts such as cultivated Solanaceae including eggplant (*Solanum melongena* L.), sweet pepino (*Solanum muricatum* L.), potato (*Solanum tuberosum* L.), and tobacco (*Nicotiana tabacum* L.) (Desneux et al. 2010, 2011). Wild plant species have also been cited to be potential hosts for the development of the pest, such as deadly nightshade (*Atropa belladonna* L.), Jimson weed (*Datura stramonium* L.), and black nightshade (*Solanum nigrum* L.) (Desneux et al. 2010, 2011; Tropea Garzia et al. 2012), that can be found indifferently in croplands, wastelands, logging, and urban areas (Lambinon et al. 2004). In this context, host plant species in the wild could provide reservoirs or refuges for *T. absoluta*.

Host range prediction is a key step for risk assessment that determines whether an invasive alien species should be eradicated, managed, or tolerated (Suckling et al. 2014). However, little is known about the effective developmental and reproductive capacity of *T. absoluta* on most of the agricultural and non-cultivated plants that are reported as potential hosts in the literature. The characterization of its host range could help to understand and prevent the dispersion behavior of the insect in the environment for more efficient integrated management strategies.

In the present study, we investigated whether non-cultivated Solanaceae could act as refuges or reservoirs for *T. absoluta*. Previous works having demonstrated the ability of *T. absoluta* to efficiently develop and reproduce on *S. tuberosum* (Caparros Megido et al. 2013), we have first conducted dual-choice bioassays to evaluate the attractiveness of different wild solanaceous plant species. Plant volatile organic compounds (VOCs) were characterized for each tested wild Solanaceae species to provide explanations regarding host location preferences. Finally, the ability of each plant species to sustain *T. absoluta* larvae was assessed.

Materials and methods

Plant and insect material

Tomato (*Solanum lycopersicum* cv. Moneymaker), potato (*Solanum tuberosum* cv. Charlotte), and wild solanaceous plants (*Atropa belladonna*, *Datura stramonium*, *Solanum nigrum*) were cultivated in a greenhouse (25 ± 5 °C) in plastic pots (20 cm diameter × 20 cm height) filled with loam (VP113BIO; Peltracom, Belgium) and grown with a 16L:8D photoperiod. The plants were watered once every 2 days.

In July 2011, 200 third-instar larvae of the tomato leafminer, *Tuta absoluta*, were collected from a commercial tomato plantation located in Saint-Andiol (France) and were subsequently kept under laboratory conditions at 24 ± 1 °C, 60–70 % RH, and with a 16L:8D photoperiod. *T. absoluta* colony was maintained on tomato in net cages (46.5 × 46.5 × 46.5 cm). Caterpillars were provided with fresh plants three times a week until pupation.

Behavioral assays

Flying tunnels (232.5 cm × 46.5 cm × 46.5 cm) were used to study the preferences of *T. absoluta* reared on tomato plants toward different solanaceous plants (*Solanum tuberosum* cv. Charlotte, versus *Atropa belladonna*, *Datura stramonium* or *Solanum nigrum*; 4 weeks old). The tunnels were divided into three areas (Caparros Megido et al. 2014): a central area for insect release and two areas at opposite sites containing the plants. Twenty newly emerged *T. absoluta* adults were placed in the neutral area at a distance of 116.5 cm from each plant. After 48 h, the number of males, females, and eggs laid on each plant was counted. Forty-eight hours allow the couples to explore the tunnel and to mate, and also allow the females to lay eggs in sufficient numbers on one of the two plants. Each plant combination was randomly tested five times (for a total of 100 insects tested per dual-choice assay). The experiments were carried out under laboratory conditions (20 ± 1 °C, 65 ± 5 % RH, and a 16L:8D photoperiod under cool white LED lights (77 μmol/sqm/s)). These conditions were monitored using an automatic datalogger (HOBO RH/TEMP 8 K; Onset Computer Corporation, Bourne, MA, USA).

Collection of plant volatiles

Entire 4-week old plants (*n* = 4 for each species) were separately placed into 40-L glass jars that were closed with a glass lid. Before volatile collection, each glass and Teflon® pipe used to connect the experimental setup was

washed with Extran® solution (MA 01; Merck, Darmstadt, Germany). In addition, each plant pot was wrapped in aluminum foil to avoid contamination from the pot and the soil. A pull air pump (Escort Elf® Pump, MSA, Pittsburgh, PA, USA) provided constant airflow from the bottom to the top of the jar at 1 L/min, after being charcoal-filtered with a carbon trap (Carbon Cap 75, Whatman, Maidstone, England). Volatiles were collected from each plant during 24 h, after a 45-min acclimation period. To trap the VOCs released into the plant headspace, a 45 mg HayeSep Q glass trap (80/100 mesh; Hayes Separation Inc., Houston, TX, USA) was connected to the setup with a Teflon® pipe connecting the pump and the glass jar. The VOCs collections were conducted in a laboratory at 20 ± 1 °C, 65 ± 5 % RH, and a 16L:8D photoperiod under cool white light emitting diode (LED) lights (77 μmol/sqm/s). All conditions were monitored using an automatic datalogger (HOBO RH/TEMP 8 K; Onset Computer Corporation, Bourne, MA, USA).

Before use, all traps were washed with 600 μL dichloromethane and 600 μL *n*-hexane (95 % purity; Sigma-Aldrich, St. Louis, MO, USA). After volatile collection, the traps were immediately eluted with 200 μL *n*-hexane, and 10 μL of a hexanic solution of *n*-butyl-benzene (750 μg/mL) was added to each sample as the internal standard. The samples were stored in closed vials wrapped with a Teflon® strip at −80 °C before analysis.

Extracts were analyzed by gas chromatography coupled with mass spectrometry (GC–MS) using an Agilent Technologies (Santa Clara, CA, USA) model 6890n GC System/5973 Mass Selective Detector. Aliquots (1 μL) were injected with a splitless injector maintained at 220 °C. The column (HP-5MS, 30 m × 0.25 mm × 0.25 μm) was maintained at 35 °C for 2 min, before being heated to 100 °C at a constant rate of 5 °C/min. The column was then heated to 260 °C at a constant rate of 20 °C/min. The quantification of compounds was realized by comparing their areas with the internal standard (applying a factor response ratio of 1:1 for each compound), using Chemstation software (Agilent Technologies, Palo Alto, CA, USA). The components were identified by comparing the recorded mass spectra with the National Institute of Standards and Technology (NIST) and Wiley spectral databases. Further identification was carried out by calculating non-isothermal retention indices under identical chromatographic conditions. Identification was finally confirmed by the injection of standards (when commercially available) and comparison of retention times. Volatile chemicals identified at least in two replicates were considered for analytical purposes. Compounds commonly associated with the earth's atmosphere (e.g., toluene and benzene), as well as compounds associated with the analytical system (e.g., siloxanes or phthalates), were excluded (Jansen et al. 2009).

Evaluation of *Tuta absoluta* fitness

For each tested plant species, twenty newly emerged *T. absoluta* adults (ten males and ten females) were placed in a net cage (46.5 × 46.5 × 46.5 cm) in the presence of a single non-flowering host plant (4 weeks old) until the females death. Fifty eggs were then taken from each host plant species. Eggs were individually placed in a Petri dish (5 cm diameter) lined with a moistened filter paper and containing an excised leaf taken from the host plant species on which they were laid. Each Petri dish was considered to be a replicate (i.e., *n* = 50 for each tested plant species). The emerging larvae were fed with a single piece of leaf (2 × 3 cm), which was changed every day. Water was also added to humidify the filter paper until pupation. Individuals were sexed according to the methods of Coelho and França (1987), and male and female pupae were grouped in couples. Each couple of pupae was placed in an individual Petri dish containing a moistened filter paper and a single corresponding host plant leaf until adult emergence. Following adult emergence, males and females were left together in the Petri dishes for their entire lives. Following the visual observation of the first mating (occurring rapidly after adult emergence), the number of eggs laid on a single plant leaf was evaluated daily until the female died. Water was supplied on the filter paper covering each Petri dish, every 2 days, to optimize adult survival. During the development of the insects, egg-laying date, hatching date, pupation date, adult emergence date, and sex were recorded for each individual. Pupal weight was not recorded, since the ratio between fecundity and pupal weight may not be reliable and should be treated with caution when attempting to understand oviposition (Leather 1988). Experiments were carried out under laboratory conditions (24 ± 1 °C, 65 ± 5 % RH, and 16L:8D photoperiod) and were monitored using an automatic datalogger (HOBO RH/TEMP 8 K; Onset Computer Corporation, Bourne, Massachusetts, USA).

Statistical analyses

Binomial proportion tests (equal distribution hypothesized) were used to compare the number of males, females, and eggs laid on each studied plant observed during the dual-choice behavioral assays conducted in the flying tunnel.

Principal component analysis was performed on a correlation matrix for the visual comparison of the VOC profiles from the different solanaceous plants. One-way ANOVAs were applied to compare the mean quantities of each VOC released by the tested plants.

One-way ANOVAs were applied to compare the mean development times of each life stage of *T. absoluta* on the various host plant species. When assumptions of the one-

Table 1 Dual-choice bio-assays evaluating the preference of *T. absoluta* for *Solanum tuberosum* cv. Charlotte versus *Atropa belladonna*, *Datura stramonium*, or *Solanum nigrum*

Number of tested insects	Responding insects[a]	Two choice assay			*P* value
99	67		*S. tuberosum*	*A. belladonna*	
		Males	26	6	0.001
		Females	25	10	0.017
		Eggs	161	13	<0.001
94	72		*S. tuberosum*	*D. stramonium*	
		Males	27	8	0.002
		Females	30	7	<0.001
		Eggs	154	0	<0.001
87	65		*S. tuberosum*	*S. nigrum*	
		Males	12	15	0.701
		Females	15	23	0.256
		Eggs	74	131	<0.001

Values are total numbers of individuals for each combination (five replicates)

[a] Responding insects include living individuals present in one of the two side areas of the tunnel

way ANOVA (normality of populations and homoscedasticity) were not met, Kruskall–Wallis tests were applied followed by Mann–Whitney comparisons of specific samples pairs. Chi-square tests of independence were applied to compare survival percentages at each life stage and sex ratios on each plant species studied.

All tests were performed using Minitab® version 16 software (http://www.minitab.com/fr-FR/default.aspx).

Results

Behavioral assays

Table 1 lists the total number of males, females, and eggs on each side of the flying tunnels (*S. tuberosum* versus another plant). Adult males and females attraction did not differ between the two *Solanum* species ($P = 0.701$ and $P = 0.256$), but female oviposition occurred preferentially on *S. nigrum* ($P < 0.001$). By contrast, *S. tuberosum* was preferred to the other tested plants, i.e., *Atropa belladonna* and *Datura stramonium*, considering both adult attraction and female oviposition.

Plant volatiles analysis

A total of 34 compounds were found in the headspace of all tested plants (Table 2; Fig. 1). The total amount of VOCs varied among the four tested Solanaceae species, ranging from 5.59 ± 4.93 (*S. nigrum*) to 50.70 ± 15.07 ng (*D. stramonium*) per gram of fresh plant (Table 2). Terpenoids (mono- and sesquiterpenes) were the most abundant identified compounds. Monoterpenes represented 32.5, 84.26, 93.14, and 18.19 % of the total VOCs collected,

respectively, for *S. tuberosum*, *S. nigrum*, *A. belladonna*, and *D. stramonium*. Sesquiterpenes represented 66.82, 5.01, 0.00, and 81.81 % of the total emission, respectively, for *S. tuberosum*, *S. nigrum*, *A. belladonna*, and *D. stramonium*. Related percentages were 0.68, 10.73, 6.86, and 0.00 % for aldehydes.

The principal component analysis (Fig. 2) showed that the volatile compound profiles differed between the four Solanaceae species. All tested plants released α-pinene (1), δ-3-carene (5), limonene (7) and nonanal (12) in equivalent amounts. *S. tuberosum* was the only plant to release β-phellandrene (2), α-cubebene (18), α-copaene (19), β-gurjunene (21), (E)-β-farnesene (25), germacrene-D (28), and bicyclogermacrene (31). In comparison, only *S. nigrum* released β-pinene (3) and myrcene (4). Only *A. belladonna* released 1,4-cineole (6), 1,8- cineole (8), α-terpinolene (11), terpinene-1-ol (13), α- terpineol (16), β-terpineol (14), borneol (15), and tridecanal (30). Only *D. stramonium* released (E)-β-ocimene (9), longifolene (20), valencene (29), α-Muurolene (32), and caryophyllene oxide (35).

Evaluation of *Tuta absoluta* fitness

Table 3 lists survivability rates and sex ratios, while Table 4 lists the development times and fecundity rates of *T. absoluta* reared on the different tested solanaceous host plants.

Mean development times (±SE) of *T. absoluta* eggs were not statistically different according to the host plants on which they were laid ($H_3 = 5.25$; $P = 0.155$). Egg viability differed according to the host plant species ($\chi^2_{(3)} = 8.15$; $P = 0.043$) and was statistically higher on *S. tuberosum* (80 %) than on *D. stramonium* (56 %).

Table 2 Amounts (ng ± SE/g of plant) of the different volatile compounds of four solanaceous plants

	Compound name	CAS	IUPAC name	Identification	Retention Index	Literature	S. tuberosum	S. nigrum	A. belladonna	D. stramonium	$F_{(3,15)}$	P value
1	alpha-pinene	000080-56-8	(1S,5S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	MS, RI, STD	929	933[1]	2.83 ± 1.03a	1.34 ± 1.28a	3.25 ± 1.76a	0.56 ± 0.17a	1.08	0.394
2	beta-phellandrene	000555-10-2	3-Isopropyl-6-methylenecyclohexene	MS, RI	964	1026[3]	0.06 ± 0.04a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	2.72	0.091
3	beta-pinene	000127-91-3	6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane	MS, RI	966	964[1]	0.00 ± 0.00a	0.19 ± 0.14a	0.00 ± 0.00a	0.00 ± 0.00a	1.69	0.222
4	beta-myrcene	000123-35-3	7-Methyl-3-methylene-1,6-octadiene	MS, RI, STD	987	991[1]	0.00 ± 0.00a	0.13 ± 0.09a	0.00 ± 0.00a	0.00 ± 0.00a	1.96	0.174
5	delta-3-carene	013466-78-9	3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene	MS, RI, STD	1010	1009[2]	1.83 ± 0.68a	0.98 ± 0.91a	2.57 ± 1.61a	1.31 ± 0.57a	0.46	0.716
6	1,4-cineole	000470-67-7	1-Isopropyl-4-methyl-7-oxabicyclo[2.2.1]heptane	MS, RI	1013	1016[1]	0.00 ± 0.00a	0.00 ± 0.00a	3.52 ± 1.71b	0.00 ± 0.00a	4.25	0.029
7	limonene	000138-86-3	1-Methyl-4-(1-methylethenyl)-cyclohexene	MS, RI	1028	1030[4]	4.41 ± 1.96a	2.07 ± 1.98a	7.51 ± 4.61a	1.24 ± 0.44a	1.08	0.394
8	1,8-cineole	000470-82-6	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane	MS, RI, STD	1030	1030[2]	0.00 ± 0.00a	0.00 ± 0.00a	2.00 ± 1.34a	0.00 ± 0.00a	2.22	0.139
9	trans-beta-ocimene	003779-61-1	(E)-3,7-Dimethyl-1,3,6-octatriene	MS, RI, STD	1046	1050[1]	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	6.11 ± 0.45b	182.84	<0.001
IS	n-Butyl-Benzene	000104-51-8	Butylbenzene	MS, RI	1056	–	–	–	–	–	–	–
10	alpha-terpinolene	000586-62-9	1-Methyl-4-(1-methylethylidene)-cyclohexene	MS, RI, STD	1085	1084[1]	0.00 ± 0.00a	0.00 ± 0.00a	6.80 ± 2.33b	0.00 ± 0.00a	8.49	0.003
11	nonanal	000124-19-6	Nonanal	MS, RI, STD	1103	1104[2]	0.06 ± 0.04a	0.37 ± 0.25a	2.50 ± 1.56a	0.00 ± 0.00a	2.27	0.133
12	terpinene-1-ol	000586-82-3	4-Isopropyl-1-methyl-3-cyclohexen-1-ol	MS, RI	1140	1137[3]	0.00 ± 0.00a	0.00 ± 0.00a	2.50 ± 1.13b	0.00 ± 0.00a	4.89	0.019
13	beta-terpineol	000138-87-4	4-Isopropenyl-1-methylcyclohexanol	MS, RI	1151	1159[1]	0.00 ± 0.00a	0.00 ± 0.00a	8.32 ± 4.14b	0.00 ± 0.00a	4.04	0.034
14	borneol	000507-70-0	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol	MS, RI	1184	1165[1]	0.00 ± 0.00a	0.00 ± 0.00a	2.84 ± 1.25b	0.00 ± 0.00a	5.16	0.016
15	alpha-terpineol	000098-55-5	2-(4-Methylcyclohex-3-enyl)-propan-2-ol	MS, RI	1192	1189[1]	0.00 ± 0.00a	0.00 ± 0.00a	4.26 ± 1.74b	0.00 ± 0.00a	5.99	0.010
16	decanal	000112-31-2	Decanal	MS, RI	1203	1204[1]	0.13 ± 0.09a	0.23 ± 0.15a	0.34 ± 0.17a	0.00 ± 0.00a	1.37	0.299
17	alpha-cubebene	017699-14-8	(3aS,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyl-3a,3b,4,5,6,7-hexahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzene	MS, RI	1357	1351[1]	0.06 ± 0.02a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	6.42	0.008
18	alpha-copaene	003856-25-5	8-Isopropyl-1,3-dimethyl-tricyclo[4.4.0.0(2,7)]dec-3-ene	MS, RI	1386	1391[1]	0.80 ± 0.35a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	5.15	0.016
19	longifolene	000475-20-7	(1R,2R,7S,9S)-3,3,7-Trimethyl-8-methylenetricyclo[5.4.0.0(2,9)]undecane	MS, RI, STD	1418	1416[1]	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.33 ± 0.01b	586.45	<0.001
20	alpha-gurjunene	000489-40-7	(1aR,4R,4aR,7bR)-1,1,4-trimethyl-7-methylidene-2,3,4,4a,5,6,7a,7b-octahydro-1aH-cyclopropa[e]azulene	MS, RI	1422	1407[1]	0.10 ± 0.07a	0.10 ± 0.06a	0.00 ± 0.00a	0.00 ± 0.00a	1.44	0.280

Table 2 continued

	Compound name	CAS	IUPAC name	Identification	Retention Index	Literature	S. tuberosum	S. nigrum	A. belladonna	D. stramonium	$F_{(3,15)}$	P value
21	beta-caryophyl-lene	000087-44-5	(1R,4Z,9S)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	MS, RI, STD	1434	1467[1]	8.76 ± 2.84ab	0.00 ± 0.00a	0.00 ± 0.00a	25.79 ± 9.05b	6.57	0.007
22	beta-cubebene	013744-15-5	(3aS,3bR,4S,7R,7aR)-4-Isopropyl-7-methyl-3-methylene octahydro -1H-cyclopenta[1,3] cyclopropa[1,2]benzene	MS, RI	1444	1470[5]	0.05 ± 0.04a	0.00 ± 0.00a	0.00 ± 0.00a	7.61 ± 1.48b	26.48	<0.001
23	beta-gurjunene	073464-47-8	(1aR,4R,4aR,7aR,7bR)-1,1,4-Trimethyl-7-methylenedecahydro-1H-cyclopropa[e]azulene	MS, RI	1449	1433[7]	0.07 ± 0.03a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	5.43	0.014
24	trans-beta-farnesene	018794-84-8	(6E)-7,11-Dimethyl-3-methylene-1,6,10-dodecatriene	MS, RI	1461	1458[1]	0.75 ± 0.39a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	3.69	0.043
25	alpha-humulene	006753-98-6	(1E,4E,8E)-2,6,6,9-Tetramethyl-1,4,8-cycloundecatriene	MS, RI, STD	1470	1467[2]	0.42 ± 0.17a	0.00 ± 0.00a	0.00 ± 0.00a	1.05 ± 0.67a	2.07	0.158
26	alpha-amorphene	000483-75-0	1,2,4alpha,5,6,8alpha-Hexahydro-1-isopropyl-4,7-dimethylnaphthalene	MS, RI	1489	1487[4]	0.07 ± 0.05a	0.00 ± 0.00a	0.00 ± 0.00a	0.78 ± 0.29b	6.39	0.008
27	germacrene-d	023986-74-5	(1E,6E,8S)-8-Isopropyl-1-methyl-5-methylene-1,6-cyclodecadiene	MS, RI	1497	1499[1]	6.36 ± 2.54a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	6.24	0.008
28	valencene	004630-07-3	(2R)-8,8,8a-trimethyl-2-prop-1-en-2-yl-1,2,3,4,6,7-hexahydronaphthalene	MS, RI	1503	1491[1]	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	1.08 ± 0.39b	7.70	0.004
29	tridecanal	010486-19-8	Tridecanal	MS, RI	1511	1511[1]	0.00 ± 0.00a	0.00 ± 0.00a	0.37 ± 0.20a	0.00 ± 0.00a	3.61	0.046
30	bicyclogermacrene	024703-35-3	(4E,8E)-4,8,11,11-tetramethylbicyclo[8.1.0]undeca-4,8-diene	MS, RI	1513	1507[7]	0.90 ± 0.28a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b	10.45	0.001
31	alpha-muurolene	031983-22-9	(1S,4aS,8aR)-4,7-dimethyl-1-propan-2-yl-1,2,4a,5,6,8a-hexahydronaphthalene	MS, RI	1513	1500[6]	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	1.25 ± 0.46b	7.55	0.004
32	delta-cadinene	000483-76-1	4,7-dimethyl-1-propan-2-yl-1,2,3,5,6,8a-hexahydronaphthalene	MS, RI	1537	1526[4]	0.18 ± 0.09a	0.02 ± 0.01a	0.00 ± 0.00a	1.69 ± 0.25b	38.18	<0.001
33	palustrol	005986-49-2	1,1,4,7-tetramethyl-2,3,4,5,6,7,7a,7b-octahydro-1aH-cyclopropa[h]azulen-4a-ol	MS, RI	1590	1569[6]	0.25 ± 0.09a	0.16 ± 0.06ab	0.00 ± 0.00b	0.00 ± 0.00b	5.54	0.013
34	caryophyllene oxyde	001139-30-6	(1R,4R,6R,10S)-4,12,12-Trimethyl-9-methylene-5-oxatricyclo[8.2.0.0(4,6)]dodecane	MS, RI	1609	1606[1]	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	1.90 ± 0.84b	5.17	0.016
	Total	–	–	–	–	–	28.09 ± 10.80	5.59 ± 4.93	46.78 ± 23.55	50.70 ± 15.07	–	–

[1] Pherobase (2014)

[2] Flavornet (2014)

[3] ChemSpider (2014)

[4] Maggio et al. (2012)

[5] Ferraz et al. (2014)

[6] Gbolade et al. (2009)

[7] Adams (2007)

MS, comparison of mass spectra with those of Nist08 and Wiley 275 libraries; RI, comparison of retention indices with those reported in the literature; STD, comparison of retention time and mass spectra of available standards
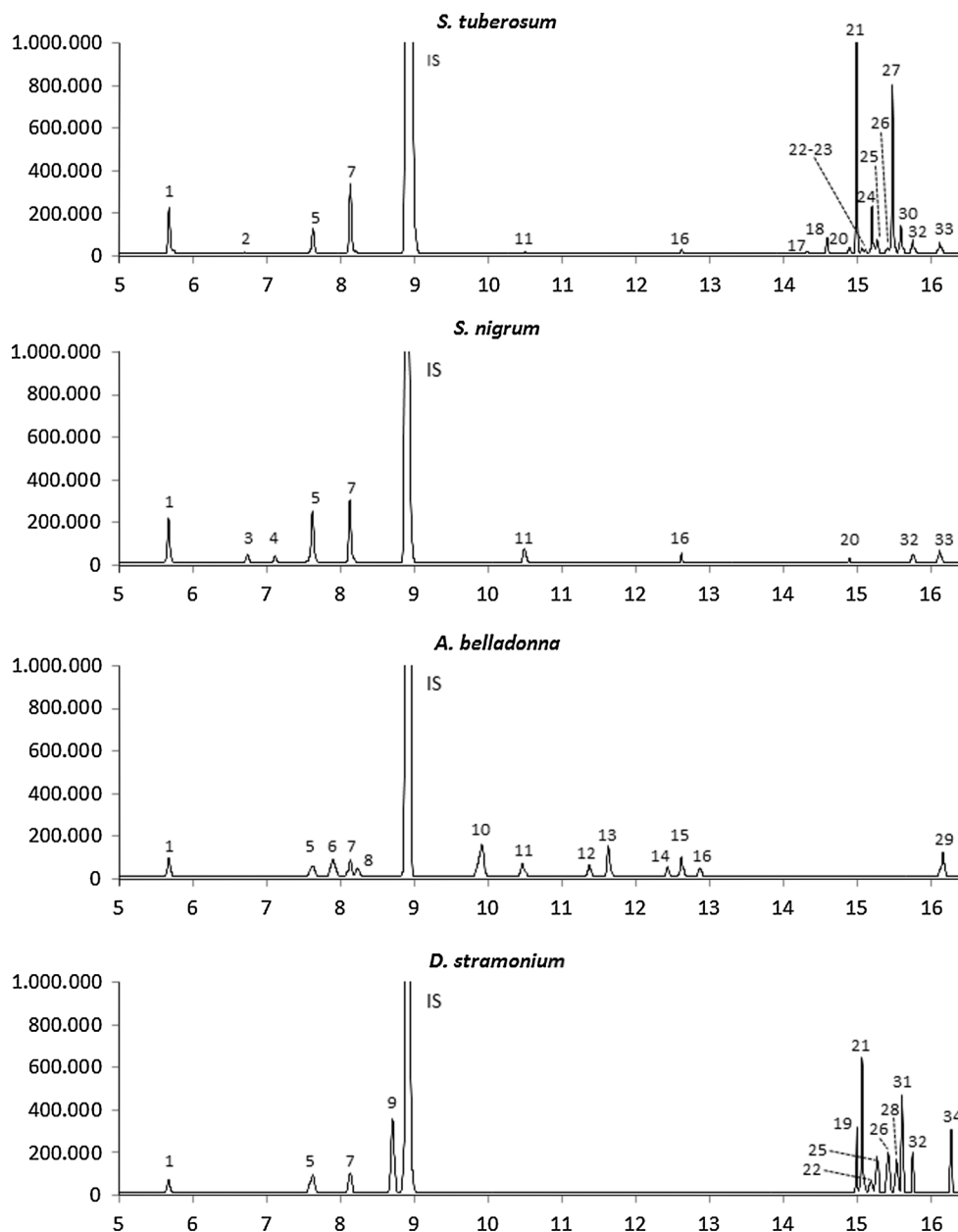
Fig. 1 Chromatograms of VOCs emitted by *S. tuberosum*, *S. nigrum*, *A. belladonna*, and *D. stramonium*. The number on peaks refers to the compound number given in Table 2

Larval development times differed according to the host plant ($H_3 = 45.96$; $P < 0.001$). Larvae fed on *S. tuberosum* developed faster (8.5 ± 0.2 days) than those fed on *S. nigrum* (11.7 ± 0.3 days), *D. stramonium* (11.8 ± 1.3 days), and *A. belladonna* (13.0 ± 0.5 days). Moreover, differences in terms of larvae survival were observed between the four tested host plants ($\chi^2_{(3)} = 25.81$; $P < 0.001$). Survivability of larvae fed on *S. nigrum* (79 %) did not differ from *S. tuberosum* (67 %), but was higher than on *A. belladonna* (50 %) and *D. stramonium* (20 %). Survivability of larvae fed on *D. stramonium* was significantly lower than those fed on any of the other host plants.

Pupae development times were not statistically different according to the host plants on which pupae were reared ($H_3 = 5.78$; $P = 0.123$). However, association tests performed on sample pairs indicated a significant difference between *S. nigrum* (7.4 ± 0.2 days) and *D. stramonium* (8.8 ± 0.4 days). There were no significant differences in pupae survival percentages ($\chi^2_{(3)} = 0.37$; $P = 0.946$), which ranged from 83 % on *A. belladonna* and *D. stramonium* to 88 % on *S. nigrum*.

Development times from egg to adult emergence were significantly lower for insects reared on *S. tuberosum* (20.4 ± 0.4 days) than those reared on *S. nigrum*
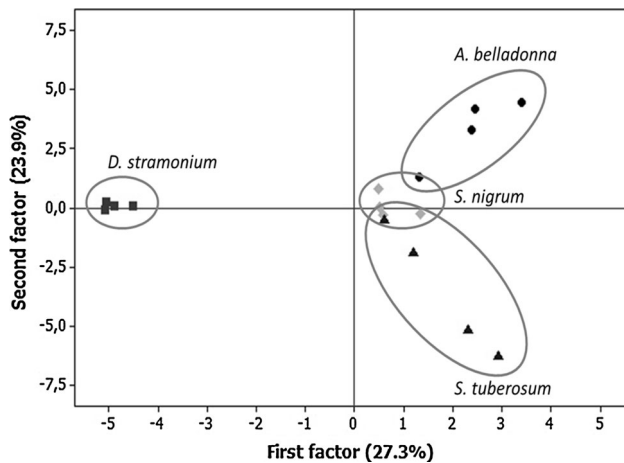
Fig. 2 Spatial distribution of the volatile blends released by *S. tuberosum*, *S. nigrum*, *A. belladonna*, and *D. stramonium* (*n* = 4)

(22.9 ± 0.3 days), *A. belladonna* (24.7 ± 0.6 days), and *D. stramonium* (24.4 ± 1.4 days) ($H_3 = 33.77$; $P < 0.001$). Survival rates from egg to adult emergence were lower with insects reared on *D. stramonium* (9 %) than those fed on *S. tuberosum* (45 %), *S. nigrum* (45 %), and *A. belladonna* (39 %).

Adult longevity was not affected by the host plants, ranging from 8.6 ± 1.4 days on *D. stramonium* to 15.0 ± 1.5 days on *A. belladonna* ($F_{3,73} = 2.05$; $P = 0.114$). Similarly, the sex ratio (male/female) ($\chi^2_{(3)} = 7.45$; $P = 0.059$) and female fecundity rates ($H_2 = 1.03$; $P = 0.598$) did not differ significantly between *S. tuberosum*, *S. nigrum*, and *A. belladonna*. Only adult males have emerged from pupae reared on *D. stramonium*.

Discussion

The insect ability to localize suitable host plants in a given environment determines its propensity to persist and spread to new areas (Suckling et al. 2014). In Europe, the tomato leafminer is abundantly present in the Mediterranean countries, where it mainly infests tomato production sites. But recent studies have suggested that alternative cultivated and non-cultivated host plants could be used by *T. absoluta* to spread and reproduce in Northern Europe (Desneux et al. 2010, 2011). In the present study, we have shown that *T. absoluta* females preferentially orientate in dual-choice assays to *Solanum* species rather than to other wild Solanaceae. However, oviposition has occurred on each of the tested plants, suggesting that all of these plants could be localized and identified as a potential host for the offspring development. During host selection, female insects are exposed to a variety of cues, including plant volatiles, contact chemicals, and visual signals related to

plant morphological characteristics, which help to determine the suitability of a host plant (Awmack and Leather 2002; Faria et al. 2008). That *T. absoluta* females were preferentially attracted by *Solanum* species (i.e., potato and black nightshade) was expected because these plants are related to the tomato (Lambinon et al. 2004), which is the primary host for the insect development (Desneux et al. 2010, 2011). Male preferences are rather related to the female choice as most of lepidopteran males are primarily attracted by female sex pheromones and rarely exhibit adaptive behavior in response to host plant volatiles (Ramaswamy 1988). All *T. absoluta* tested individuals were reared on tomato prior to dual-choice assays, and this fact should be underlined because larval experience has been reported to have an impact on the host finding behavior of the subsequent adults (Caparros Megido et al. 2014; Facknath and Wright 2007). The host plant preferences of individuals reared for one or more generations on wild solanaceous host plants have to be described in further studies in order to better understand their dispersion behavior in the environment.

Recent advances in chemical ecology demonstrated that the attraction and oviposition of *T. absoluta* females are mediated by the volatile signature of their host plant. VOCs released by leaves provide capital information to the females on the suitability of the plant as a larval substrate. It was found that tomato leaf odors mainly include volatile terpenoid compounds which elicit in mated females upwind orientation flight followed by landing as well as egg-laying (Proffit et al. 2011). Tomato volatiles are principally made of monoterpenes including α-pinene, β-pinene, α-phellandrene, β-phellandrene, δ-2-carene, δ-3-carene, limonene, myrcene, and sabinene, as well as the sesquiterpene (E)-β-caryophyllene (Caparros Megido et al. 2014; Proffit et al. 2011). Similar observations were made for potato plants: the volatile emissions of *S. tuberosum*, principally made of sesquiterpenes as (E)-β-caryophyllene (E)-β-farnesene, and germacrene-D, are implicated in the host selection by *T. absoluta* females (Caparros Megido et al. 2014). Our results are consistent with these previous headspace analyses regarding the latter species. Despite presenting similarities, each solanaceous plant species released a qualitatively and quantitatively specific volatile blend, as visually demonstrated by the principal component analysis. The fact that the insect has laid eggs on each plant suggests that one or more combinations of volatile compounds are responsible for the attraction and oviposition behavior, some of which being more attractive than others. By example, even both are principally made of monoterpenes, *A. belladonna* emissions differed from those of *S. nigrum* which are mainly constituted with tomato-related compounds such as α-pinene, β-pinene, δ-3-carene, limonene and myrcene. The total quantity of terpenoids is likely

Table 3 Survivability and sex ratio (m/f) of *T. absoluta* on different solanaceous host plant species

	S. tuberosum	*S. nigrum*	*A. belladonna*	*D. stramonium*	Statistical analyses	*P* value
Survivability						
Eggs	80 % a	65 % ab	65 % ab	56 % b	$\chi^2_{(3)} = 8.15$	0.043
Larvae	67 % ab	79 % b	50 % a	20 % c	$\chi^2_{(3)} = 25.81$	<0.001
Pupae	85 % a	88 % a	83 % a	83 % a	$\chi^2_{(3)} = 0.37$	0.946
Egg to adult	45 % a	45 % a	39 % a	9 % b	$\chi^2_{(3)} = 21.69$	<0.001
Sex ratio	0.53 a	0.90 a	0.85 a	–	$\chi^2_{(3)} = 7.45$	0.059

Values that do not share a common letter are significantly different

Table 4 Mean development times (days) and fecundity rates (eggs laid per female) of *T. absoluta* (±standard error) on different solanaceous host plant species

	S. tuberosum	*S. nigrum*	*A. belladonna*	*D. stramonium*	Statistical analyses	*P* value
Development times						
Eggs	4.3 ± 0.2a	3.9 ± 0.1a	3.8 ± 0.1a	3.8 ± 0.4a	$H_{(3)} = 5.25$	0.155
Larvae	8.5 ± 0.2a	11.7 ± 0.3b	13.0 ± 0.5c	11.8 ± 1.3bc	$H_{(3)} = 45.96$	<0.001
Pupae	7.6 ± 0.3ab	7.4 ± 0.2b	7.9 ± 0.4ab	8.8 ± 0.4a	$H_{(3)} = 5.78$	0.123
Egg to adult	20.4 ± 0.4a	22.9 ± 0.3b	24.7 ± 0.6b	24.4 ± 1.4b	$H_{(3)} = 33.77$	<0.001
Adults	11.8 ± 1.2a	11.1 ± 1.5a	15.0 ± 1.5a	8.6 ± 1.4a	$F_{(3,73)} = 2.05$	0.114
Fecundity rate	24.9 ± 5.5a	25.4 ± 15.0a	15.0 ± 8.0a	–	$H_{(2)} = 1.03$	0.598

Values that do not share a common letter are significantly different

not affecting host selection, as the total amount of monoterpenes and sesquiterpenes released by the four Solanaceae is not correlated with the attraction results and the number of eggs laid. Rather than the total quantity of VOCs released by a plant, the presence of specific blend of certain chemicals can be involved in the selection of a host and the quantity of eggs deposited. Further electrophysiological and behavioral assays conducted on natural blends as well as on separated identified chemicals are required to confirm the effect of specific chemicals on the choice of the oviposition site in *T. absoluta*.

Oviposition on a suitable host in response to plant cues is of major importance for an insect to maximize its fitness. A significant variability in egg survivability was observed among the tested plants. This discordance may be due to either morphological characteristics of the plants or defense mechanisms in response to egg presence, impacting embryonic development. By example, low humidity on the leaf surface due to stomatal closure or hypersensitive response (i.e., formation of necrotic tissue at the site of egg deposition) may lead to their desiccation (Hilker and Meiners 2011; Woods 2010). Volatile and contact chemicals from plant tissues could also alter the embryonic development (Hilker and Meiners 2011). Another hypothesis could be that females adjust the nutritional content of their eggs in response to a poor quality host plant, thus allocating fewer resources to embryos, which has an

evident impact on the performances of the resulting offspring (Boggs 1992). By example, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) was reported to lay eggs on *Populus tremuloides* Michx. (referred as a good-quality host plant) that contained the greatest amount of vitellogenin compared with *Quercus rubra* L., *Q. prinus* L., and *Pinus rigida* Mill. (referred as lower-quality host plant species) (Rossiter et al. 1993).

Considering herbivorous insects, short development times, high survival rates, and high fecundity rates (number of eggs per female) are related to a suitable food quality (Awmack and Leather 2002; Greenberg et al. 2002; Pereyra and Sánchez 2006). In the present study, the host plant affected the development times of *T. absoluta* from egg to adult emergence: individuals reared on *S. tuberosum* grew faster than the others. Corresponding survival rates were only found to be statistically lower on *D. stramonium*, although those observed on *S. tuberosum* and *S. nigrum* appeared to be numerically equal to each other and higher than on *A. belladonna*. In the absence of abiotic and biotic stresses, survival rates are mainly influenced by the feeding stage (larval instar), suggesting differences in nutritive quality and/or production of plant metabolites that have impaired larval development. By example, *A. belladonna* and *D. stramonium* tissues contain tropane alkaloids (Krenzelok 2010; Pomilio et al. 2008) involved in insect herbivory resistance (Arab et al. 2012; Shonle and

Bergelson 2000). Adult longevity and fecundity rates were similar between the four plants, while no female adults were obtained on *D. stramonium*. In terms of *T. absoluta* fitness, it can be assumed that solanaceous plants are not of equal quality for the development of the insect pest. *S. tuberosum* appears to be the most suitable host among the tested plants considering the biological parameters described above, but *S. nigrum*, *A. belladonna*, and *D. stramonium* can also be exploited as food source with a decreased success in their respective order of citation.

Surprisingly, longer development times (28 days) for similar survivability rates (47 %) were previously observed using an identical protocol for *T. absoluta* reared on its primary host plant, *Solanum lycopersicum* (Caparros Megido et al. 2013). However, adult fecundity rate reported on *S. lycopersicum* (50 eggs per female) was twofold higher than on *S. tuberosum* and *S. nigrum* (which allowed the largest offspring in our study). Therefore, it can be assumed that this decrease in oviposition could be directly related to a lower reproductive capacity of the adults resulting from our bioassays, making tomato more beneficial in terms of fitness. Aside this decrease in performances, it is not excluded that a female strategic choice also has occurred in response to a host plant with a lower quality, strengthening the observed egg lay depression. According to this hypothesis, females could have delayed their oviposition, or resorbed eggs and reallocated the nutriments in order to extend their own longevity, waiting to find a more suitable host plant where the offspring performances will be optimized (Awmack and Leather 2002). High adult longevity, which is classically related to a high fecundity (Leather 1988) and low fecundity rates observed with insects reared on *A. belladonna*, complies with this expectation.

In our experimental design, insect performances were assessed during a single generation, starting with egg-laying adults taken from their primary host plant. The potential effect of food reserves allocated to the eggs on the biological traits of the immature and mature instars should be considered. We might expect that generation time, survivability, and female fecundity will be impacted after several generations on an alternative host plant. Compared to previous results (Caparros Megido et al. 2013), egg to adult development time on *S. tuberosum* cv. Charlotte increases from 20 to 24 days after five generations, being less beneficial in terms of fitness. Additional experiments are needed to assess the impact on insect performances of a long-term infestation on wild solanaceous host plants as reservoirs.

Finally, genetic variability in plants and insects may have a great impact, respectively, on VOCs emitted or perceived (Harrewijn et al. 1995), and insecticidal compounds secretion or plant defense avoidance (Moore et al. 2014). Climatic variations (Agosta 2006), phenological and physiological state of the plants (Ayabe et al. 2014), local selective pressures on plant traits (Castillo et al. 2014), or the presence of plant endophytic symbionts and insect pathogens (Crawford et al. 2010; Gurulingappa et al. 2010) may also have an influence on host plant availability for the insects. Such variability in plant resource may have a significant impact on the spatiotemporal patterns of plant selection for female oviposition.

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