Crop Protection xxx (2010) 1–7

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Volatile organic compounds as a diagnostic marker of late blight infected potato plants: A pilot study

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ARTICLE INFO

Article history:
Received 3 November 2009
Received in revised form
7 March 2010
Accepted 8 March 2010

Keywords:
Volatile
Potato
Phytophthora infestans
(*E*)-2-hexenal
5-ethyl-2(5H)-furanone
Benzene-ethanol

ABSTRACT

Volatiles from potato plants infected with *Phytophthora infestans* (Mont.) de Bary were monitored by *in situ* headspace sampling. The sampling was done in four periods *i.e.* 28–42, 52–66, 76–90, and 100–114 h after inoculation (HAI). The headspace samples were analyzed by a gas chromatography–flame ionization detector (GC–FID) to assess the differences in volatile fingerprints between the infected-plant group and control groups, *i.e.* non-inoculated-plant and empty-vessel groups. The samples were subsequently analyzed by gas chromatography–mass spectrometry to identify specific peaks observed by GC–FID. Spore germination, infection, symptom development and sporulation were also monitored to ascertain the disease developmental stage when marker volatiles were first generated. The first symptoms of infection were visible after two days. Three marker volatiles *i.e.* (*E*)-2-hexenal, 5-ethyl-2(5H)-furanone and benzene-ethanol were found in the third and fourth trapping periods (3–4 days after inoculation) when sporangiophores were already formed. The volatile metabolites from blighted plants could be applied for sensor development to detect the occurrence of the disease in the field as well as for investigation of volatile production in relation to plant responses to infection.

1. Introduction

Phytophthora infestans (Mont.) de Bary is an oomycete pathogen that causes the destructive plant disease, late blight in potato (*Solanum tuberosum* L.) and other plants in the family Solanaceae. Late blight is considered a re-emerging disease in Europe, North America and Russia due to the recent occurrence of sexual reproduction of *P. infestans* in these regions. This led to an increase in diversity in the pathogen population and severity of the disease. As a result of more aggressive genotypes and easier adaptation to resistance genes and systemic fungicides, the control of the disease has become more difficult (Turkensteen and Flier, 2002).

Many different management strategies have been integrated and applied (Turkensteen and Flier, 2002) such as monitoring and disease forecasting systems, new chemical control methods, resistant or tolerant cultivars and adapted agronomic practices (Taylor et al., 2003; Turkensteen and Flier, 2002; Zwankhuizen et al., 1998). To monitor disease progress in the field, microbiological and molecular tests have been used besides visual observations (Schütz et al., 1999; Taylor et al., 2003; Weingartner, 1997). However, these new methods are still very labour intensive.

Recently, a novel non-destructive method for detection of grey mould disease in tomato crops has been developed, namely the detection of specific plant emitted volatiles in air (Jansen et al., 2009a,b). This method is not necessarily labour intensive since plant volatile analysis can be fully automated (Vercammen et al., 2001). These observations have suggested a potential solution for online monitoring of arable fields for potential disease outbreak.

Although several marker volatiles of infected tomato plants were identified (Laothawornkitkul et al., 2008) and applied to detect infection of tomato, specific volatiles have not been identified for infected potato. All research on volatiles emitted from potato plants were devoted to healthy, herbivore infested or artificially damaged plants (Bolter et al., 1997; Schütz et al., 1996; Visser et al., 1979; Weißbecker et al., 1997).

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doi:10.1016/j.cropro.2010.03.003

Knowledge of specific volatiles of late blight-infested potato plants is not only important for establishing an online monitoring system. It can also contribute to an understanding of various potential ecological functions of the volatiles, namely in plant-pathogen interactions such as immune responses, in plant–plant communication, in control of tritrophic interactions, or as plant hormones (Laothawornkitkul et al., 2009). Therefore volatiles of diseased potato plants are an important subject to be explored especially the volatiles induced by a devastating pathogen such as *P. infestans*.

Plant volatiles have been determined on detached and intact plants (Maes and Debergh, 2003; Maes et al., 2001). Detached tissues have been analyzed by solvent extraction and steam distillation (Visser et al., 1979). However the volatiles analyzed from detached tissues are considered as biologically and ecologically insignificant due to the invasive extraction process. Volatile analysis of intact tissues can be carried out by *ex situ*, and *in situ* headspace volatile sampling. In the *ex situ* method, the volatile trapping is performed on plant organs cut from the mother plant, whereas the *in situ* method whole living plants are used (Vercammen et al., 2001). The main disadvantages of *ex situ* headspace sampling are potential changes in volatile composition due to plant stress and changes in the physical environment during volatile trapping (Vercammen et al., 2001). Therefore, volatiles obtained from the *ex situ* method might not represent the actual volatiles emitted by plants in the original state (Schmelz et al., 2001).

This is the first work in which volatiles of late blight infected plants are determined by means of *in situ* volatile trapping. The objectives of the current studies were: (1) to identify specific volatiles produced by potato plants cultivar Agria infected by *P. infestans* isolate No. 98014 in comparison with non-inoculated control plants, and (2) to determine the stage of disease development when the specific volatiles can first be detected in relation to the development of macroscopic and microscopic symptoms of late blight.

2. Materials and methods

2.1. Plant materials

Potato tubers, cultivar Agria, were grown in a greenhouse at 20 °C, 60% relative humidity and L16:D8 photo regime. Tubers were grown in potting mix (Lentse potgrond nr 4) for two weeks. The shoots were separated from the mother tubers and placed into individual pots containing 60 g of moist potting mix. Four-week-old plants, on average 20 cm high, were used in the experiment. Two hours before transferring the plants with potting soil into glass vessels, the plants were watered with 20 ml of water per pot in order to prevent plant desiccation during the experimental period. After transplanting, the soil surrounding each plant was wrapped in aluminium foil to minimize water and volatile evaporation from soil.

2.2. Pathogen cultures

P. infestans (isolate No. 98014 obtained from the Laboratory of Phytopathology, Wageningen UR, the Netherlands) was cultured in 9 cm diameter Petri dishes containing Rye Sucrose Agar (Caten and Jink, 1968). Cultures were incubated at 18 °C and a L16:D8 photo regime for 7–10 days.

2.3. Inoculation

Ten ml of cold distilled water (4 °C) was added onto the pathogen colony to induce sporangium formation. The sporangium suspension was incubated at 4 °C for 2 h to induce zoospore formation, and then filtered through 50 μm nylon mesh. The concentration of zoospores in the suspension was counted and adjusted to 1.8×10^5 zoospores ml^{-1}. The ventral leaf surfaces were spot-inoculated with 10 μl diluted suspension per spot for a total of 10 spots per plant (at this stage, plants were already in the vessels). Control plants were spotted by sterile distilled water. Inoculated and control plants were placed separately in 2.5 l glass vessels, 2 plants per vessel. The vessels were placed in a growth chamber adjusted for volatile collection. Separate plants were used for detailed disease observation. Terminal leaflets were spot-inoculated with 10 μl of zoospore suspension and plants were placed in a growth chamber with the same environmental conditions as the plants used for volatile collection (see below). Each experiment was repeated twice.

2.4. Disease progression and microscopic observation

Lesion areas were measured and microscopic observations were made at 28, 52, 76 and 100 h after inoculation (HAI). Three plants per treatment, each with five inoculation spots (divided over 3–4 leaves) were used per sampling period. For microscopic observation, the leaf areas inoculated with *P. infestans* tissues were stained with trypan blue (Philips and Hayman, 1970). The procedure can be shortly described as: infected leaves were cross sectioned and boiled in 10% KOH for 15 min at 90 °C following by four times flushing with tap water. The tissues were incubated in 0.1 N HCl at room temperature for 1 min and four times flushed with tap water. The samples were incubated in 0.05% trypan blue for 24 h. The excess stain was removed by flushing twice with tap water. Discolouring of non-oomycete tissues was done by 70% glycerol. The samples were subsequently observed by light microscope.

2.5. Collection of headspace volatiles

The volatile trapping unit is depicted in Fig. 1. There were three experimental groups *i.e.* the infected-plant group, non-inoculated-plant group, and empty-vessels group. Within each group, there were three replications (vessels). The vessels were placed under light intensity of 300 μmol m^{-2} s^{-1}, at temperatures of 18 °C at night and 20 °C during the day, with a 14L:10D photoperiod. Both non-inoculated and inoculated plants were incubated at 100% R.H. for 26 h under a constant airflow of 25 ml min^{-1}. A sensor (HMP130Y Series, Vaisala, Finland) was used to measure the temperature and humidity inside one of the vessels. Volatile sampling was performed during the light period. There were four trapping periods *i.e.* 28–42 HAI (first trapping period), 52–66 HAI (second trapping period), 76–90 HAI (third trapping period) and 100–114 HAI (fourth trapping period). Thus, each trapping period was 14 h. In between each trapping period, humid air was supplied through each vessel at a constant airflow of 25 ml min^{-1} per vessel. During day time the humidity was about 40–60% RH and during the night time, the humidity was constant at 100%. Before each volatile trapping period, the humidifier was replaced by trapping filters consisting of 250 ml silica gel, 250 ml molecular sieve 4 Å, and 1000 ml activated charcoal (Merck, Darmstadt, Germany). All of the treatments were purged and trapped in parallel with an airflow of 66 ml min^{-1} per vessel. The purging was started 1.5 h before every trapping period. Then the volatile traps, ORBO 402 disposable Tenax (Supelco, Bellefonte, USA) were placed at the air outlet of the vessels.

A 1 mg/ml stock solution of phenol in hexane was prepared as the internal standard. One hundred and twenty μl of this stock solution was added into 200 ml redistilled pentane:ether (4:1). Of this final solution, three times 1 ml was injected into each trap to extract the volatiles. The obtained samples were concentrated from

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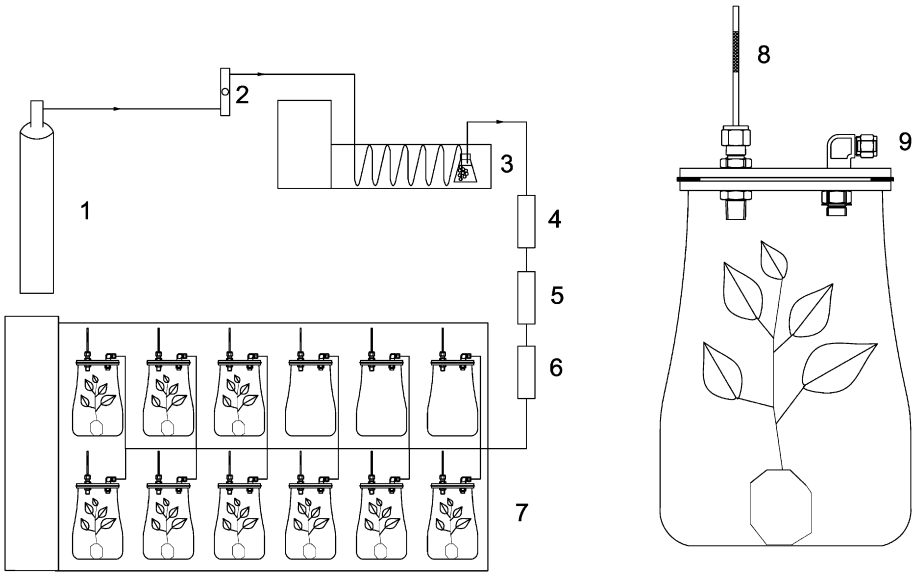

Fig. 1. Diagram of the volatile trapping unit: (1) air supply, (2) flow meter, (3) cooler and humidifier, (4) silica gel, (5) molecular sieve, (6) activated charcoal, (7) temperature and light controlled housing, (8) adsorption trap and (9) air inlet.

3 ml to 150 μl under a slow flow of nitrogen, assuming that the target volatiles had a higher boiling point than the solvent pentane: ether, so that likely only some of the solvent got lost during the concentration procedure. One hundred μl of the eluted samples was used in GC–FID analysis and the other 50 μl was conserved for GC–MS analysis. The experiments were repeated twice.

2.6. Gas chromatography–flame ionization detector (GC–FID) analysis

The GC–FID analyses were made with the samples collected from the non-inoculated-plant group, the infected-plant group and the empty-vessel group collected 28–42, 52–66 and 76–90 HAI. The analysis by GC–FID was aimed at detecting differences in volatile fingerprints to evaluate the need to continue with GC–MS. Hundred μl of each sample were further concentrated to 10 μl by evaporation on a warm plate at 25–30 °C. Two μl out of 10 μl was used for GC–FID analysis. The system was based on a Carlo-Erba series 8000 gas chromatograph (Carlo Erba, Rodano, Italy) with a cold-on-column injector (45 °C), a 1.5 m retention gap pre-column and a 30 m, 0.25 μm internal diameter, DB-5 column with 0.25 μm film thickness (J&W Scientific, Folsom, California). Helium was used as carrier gas at a flow rate of 1.3 ml min^{-1}. The effluent of the column was mixed with 30 ml min^{-1} make-up helium. The GC column temperature was programmed as: injection at 45 °C with secondary cooling for 20 s, the temperature was held at 45 °C for 2 min, and then increased by 10 °C min^{-1} to 225 °C and held at 225 °C for 5 min.

2.7. Gas chromatography–mass spectrometer (GC–MS) analysis

The 36 samples from four trapping periods were analyzed by coupled gas chromatography–mass spectrometry (GC–MS). Two μl of each samples was injected into the GC–MS system. This system was based on a gas chromatograph (5890 series II, Hewlett–Packard) equipped with a 30 m × 0.25 mm internal diameter, HP5-MS column with 0.25 μm film thickness (5MS, Hewlett–Packard). The temperature program was initially set at 45 °C for 1 min. The temperature increased with a rate of 10 °C min^{-1} to 250 °C and was kept at the final temperature for 5 min. The injection port (splitless mode), interface and MS source temperature were 250 °C, 290 °C and 180 °C respectively. The helium inlet pressure was controlled at constant column airflow of 1.0 ml min^{-1}. The ionization potential was set at 70 eV, and scanning was performed from 30 to 250 atomic mass units. The volatiles 5-ethyl-2(5H)-furanone, (*E*)-2-hexenal and benzene-ethanol were tentatively identified by comparison of the mass spectrum with the WILEY 138 mass spectra library and the GC retention Kovats index (Adams, 2001). The identity of (*E*)-2-hexenal and benzene-ethanol was confirmed by injection of authentic samples (Sigma, St. Louis, USA) and (Acros, Geel, Belgium).

2.8. Statistical analysis

The areas under each peak were calculated and standardized by dividing these by the area of the internal standard, phenol. To determine the volatile compounds differing between infected and control plants during each trapping period, a MANOVA was carried out in SPSS (SPSS 11.0 for Windows, SPSS Inc., USA) after log transformation of the data. As the transformed data were still not normally distributed, the standardized peak areas of the three treatments, *i.e.* infected-plant, non-inoculated-plant and empty-vessel groups, were compared by the Kruskal Wallis nonparametric test (Corder and Foreman, 2009) for *K* independent samples. The volatile compounds, which had significantly higher concentrations in the infected-plant group than in the other groups according to the Kruskal Wallis test, were subsequently subjected to paired comparisons by the Mann–Whitney U test (Corder and Foreman, 2009); *i.e.* the infected-plant group vs. non-inoculated-plant group, and infected-plant group vs. empty-vessel group.

The effect of time (HAI) on the production of selected volatiles was tested by comparing the standardized areas of the volatiles produced in successive trapping periods by the Kruskal Wallis nonparametric test.

3. Results

3.1. Disease progression and microscopic observation

At 28 HAI, no symptoms were observed. Small brown lesions (Hypersensitive Response, HR) were first observed at 52 HAI. The

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average lesion size was 0.136 cm^2, and sporulation was not observed. The lesion sizes increased progressively thereafter (Fig. 2; Fig. 3 H–K). At 76 HAI, the average lesion size was dramatically increased to 0.790 cm^2, and the colour of lesions changed to darker brown. White mycelium developed on both sides of the leaves. At 100 HAI, the necrotic lesion areas enlarged to 2.193 cm^2, and the lesions were covered with white mycelium, especially at the margins. The lesion sizes in the second experiment were not significantly different from those of the first experiment (Fig. 2). No symptoms were present on leaves of non-inoculated plants used as controls.

Microscopic observations after trypan blue staining confirmed that there was no infection at 28 HAI. Sporangia and zoospores were apparently not attached to the leaf surface, and were removed by the vigorous staining procedure. Cyst germination, appressorium formation and penetration occurred later than 28 HAI but before 56 HAI. At 56 HAI (Fig. 3A, B, C), hyphae had formed haustoria in the mesophyl cells. Mycelium started to emerge from the stomata. Some original zoospore cysts were also still present. This implied that not all cysts developed simultaneously. At 76 HAI, mycelium had spread throughout the leaf tissues, covering the epidermis. Some sporangiophores and sporangia were already found (Fig. 3D). At 100 HAI sporangiophores and sporangia were profusely present and secondary infection started (Fig. 3E, F, G).

3.2. GC–FID detection of volatiles produced by infected plants

The concentrations of volatile metabolites of each experimental group were compared by the Kruskal Wallis test (Table 1). Only one unique volatile with a retention time of 4.13 min was found at significant concentrations in the infected-plant group in the third trapping period. No specific volatiles were detected in the first and the second trapping periods.

3.3. GC–MS detection of volatiles produced by infected plants

The volatile metabolites of the treatment groups were compared by the Kruskal Wallis test (Table 2) and Mann–Whitney U test (results not shown). In the first and second trapping periods volatile metabolites of the inoculated plants were not significantly different from those of the non-inoculated-plant and empty-vessel groups. The absence of a specific volatile could be related to the observation that the pathogen was not yet established in the first trapping period. Thus, induction of specific plant volatiles had not yet been triggered. In the second trapping period, the pathogen already showed a biotrophic interaction with plant tissues

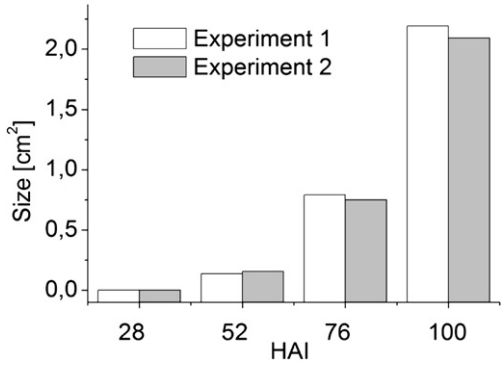

Fig. 2. Average sizes of blighted lesions on potato leaves over time in two experiments at light intensity of 300 μmol m^{-2} s^{-1}, temperature of 18 °C night, 20 °C day, and 14L:10D photoperiod. HAI = hours after inoculation.

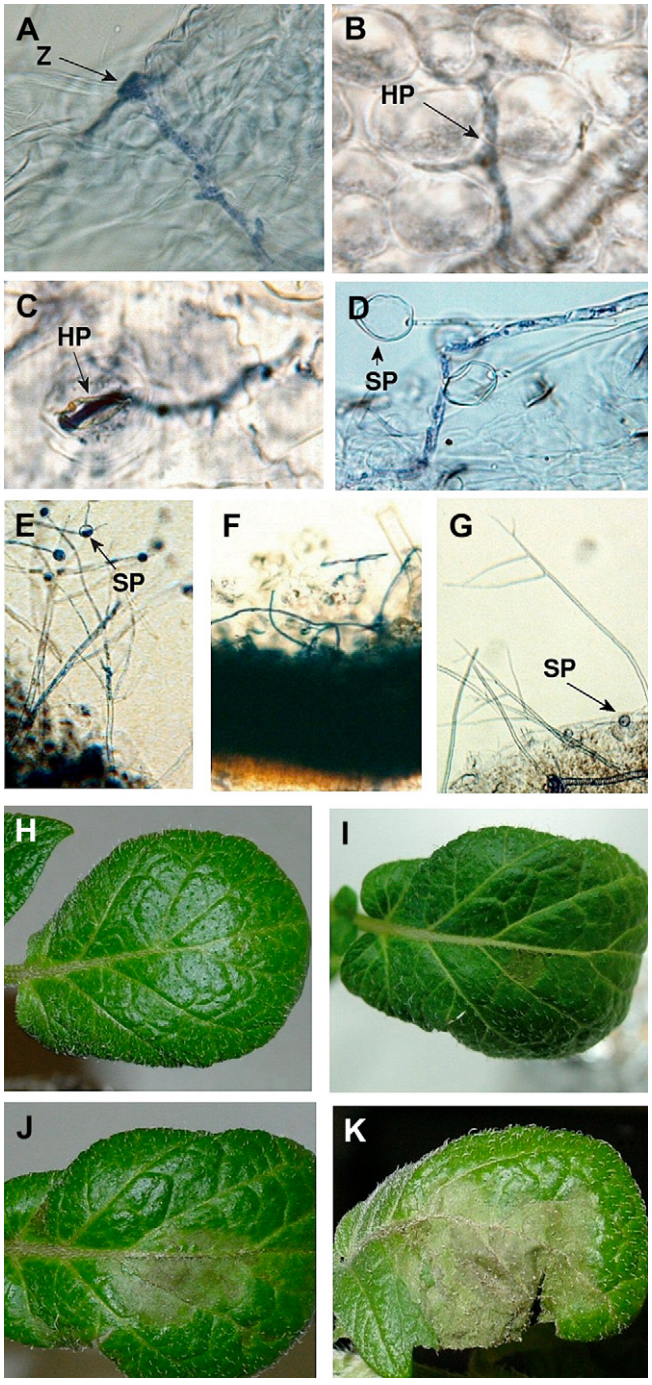

Fig. 3. Observations on late blight progression on potato leaves cv. Agria. The tissue of *Phytophthora infestans* isolate 98014 invading potato leaves under a light microscope (A–G): at 52 h after inoculation (HAI), an encysted zoospore germinating and penetrating leaf tissues (A); hyphae formed after biotrophic interaction (B) and growing through a stoma to form a sporangiophore (C); at 76 HAI, sporangia (SP) present on the leaf surface (D); at 100 HAI, profuse sporangia production (E), intense mycelium covering the leaf surface (F), and some sporangia shed and ready for secondary infection (G). Lesion progression (H = 28 HAI, I = 52 HAI, J = 76 HAI and K = 100 HAI).

(Fig. 3A–C). Yet, no significantly different pattern was detected in volatile composition compared to that from the control plants (Table 3).

In the third trapping period, two specific volatiles, (*E*)-2-hexenal and 5-ethyl-2(5H)-furanone were found at significantly higher concentrations in the infected-plant group than in the control

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Table 1
Asymptotic significance of the Kruskal Wallis Test for volatiles detected at significantly greater concentrations above potato plants cv. Agria infected by *Phytophthora infestans* isolate 98014 than above control plants. The volatile headspace samples were analyzed by gas chromatography–flame ionization detection (RT = retention time).

Trapping Period	Statistical value	Compounds produced by infected plants
		(RT = 4.13 min)
1st trapping period (28–42 HAI)	Chi-Square	0
	df	2
	Asymp. Sig.	1
2nd trapping period (52–66 HAI)	Chi-Square	1.67
	df	2
	Asymp. Sig.	0.43
3rd trapping period (76–90 HAI)	Chi-Square	7.62
	df	2
	Asymp. Sig.	0.02[a]

[a] Asymptotic significance level < 0.05.

groups. Benzene-ethanol was also detected but the amount produced was not significantly different from that in the control treatment (Table 2 and Fig. 5). No benzene-ethanol was present in headspace gases from the control groups.

In the fourth trapping period, the concentrations of three volatiles were significantly higher in the infected-plant group than in the controls, *i.e.* (*E*)-2-hexenal, 5-ethyl-2(5H)-furanone and benzene-ethanol. Chromatographic profiles of volatiles from a control plant and an infected plant are provided in Fig. 4. (*E*)-2-hexenal was produced at the highest concentration while 5-ethyl-2(5H)-furanone was produced at the lowest concentration (Fig. 5). The amount of specific volatiles produced was not significantly higher in the fourth than in the third trapping period (Table 1).

4. Discussion

Three marker volatiles, *i.e.* (*E*)-2-hexenal, benzene-ethanol and 5-ethyl-2(5H)-furanone were detected by GC–MS for the first time as a result of the interaction between potato foliage and *P. infestans*. Using GC–FID analysis, only one specific volatile ((*E*)-2-hexenal) was identified in the third trapping period when two volatiles were already found using GC–MS analysis. This confirmed that the detection power of GC–MS is higher than that of GC–FID.

Table 3
Mann–Whitney U Test, Wilcoxon W test, and Z test for the effect of time on volatile production of potato plants cv. Agria infected by *Phytophthora infestans* isolate 98014. The marker volatiles produce in the third (76–90 HAI) and the fourth (100–114 HAI) trapping period were compared (RT = retention time).

Statistical value	Compounds produced by infected-plant		
	RT = 4.87 min	RT = 6.50 min	RT = 8.98 min
	(*E*)-2-hexenal	5-ethyl-2(5H)-furanone	benzene-ethanol
Mann–Whitney U	0	2	1
Wilcoxon W	6	8	7
Z	−1.96	−1.09	−1.53
Asymp. Sig. (2-tailed)	0.05	0.28	0.13

Visser et al. (1979) found (*E*)-2-hexenal as a component of healthy potato leaves, but the method of volatile analysis was considered unreliable, because the extraction process likely resulted in changes in volatile production (Visser et al., 1979). Recent *in situ* headspace analysis of healthy potato leaves cv. Desireé revealed that β-caryophyllene, (*E*)-β–farnesene, (*Z,Z*)-α-farnesene, germacrene-D and β–bisabolene were produced in significant amounts (Agelopoulos et al., 2000). This supports our results that the three volatiles released by blighted plants in our experiments are not produced by undamaged potato plants.

Blighted tubers of cultivar Maris Piper produced benzothiazole, 2-ethyl-1-hexanol, hexanal, 2-methylpropanoic acid-2,2-dimethyl-1-(2-hydroxy-1-methylethyl)-propyl ester, 2-methylpropanoic acid-3-hydroxy-2,4,4-trimethyl-pentyl ester and phenol (Costello et al., 2001). However, these volatiles were also found in tubers infected with *Fusarium coeruleum*. The volatiles specifically produced by blighted tubers were butanal, 3-methylbutanal, undecane and verbanone (Costello et al., 2001). None of these volatiles was detected in the current experiments, indicating that differences in plant cultivar and cell physiology, *i.e.*, leaf and tuber cells, may result in different volatiles being produced.

(*E*)-2-hexenal had also been found in mechanically damaged and in beetle-damaged plants of potato cultivar Granola (Schütz et al., 1996). The concentration was higher after mechanical damage than after damage by Colorado potato beetles. However,

Table 2
The analysis of volatile samples by gas chromatography–mass spectrometry. Asymptotic significance of the Kruskal Wallis Test for volatiles found at significantly higher concentrations above potato plant cv. Agria infected by *Phytophthora infestans* isolate 98014 than above control plants and empty vessels (RT = retention time).

Trapping period		Compounds produced by infected plants		
	RT (min)	4.87	6.5	8.98
		(*E*)-2-hexenal	5-ethyl–2(5H)-furanone	benzene-ethanol
	Statistical value			
1st trapping period (28–42 HAI)	Chi-Square	0	0	0
	df	2	2	2
	Asymp. Sig.	1	1	1
2nd trapping period (52–66 HAI)	Chi-Square	0	0	0
	df	2	2	2
	Asymp. Sig.	1	1	1
3rd trapping period (76–90 HAI)	Chi-Square	7.62	7.62	4.5
	df	2	2	2
	Asymp. Sig.	0.02[a]	0.02[a]	0.11
4th trapping period (100–114 HAI)	Chi-Square	7.62	7.62	7.62
	df	2	2	2
	Asymp. Sig.	0.02[a]	0.02[a]	0.02[a]

[a] Asymptotic significance level < 0.05.

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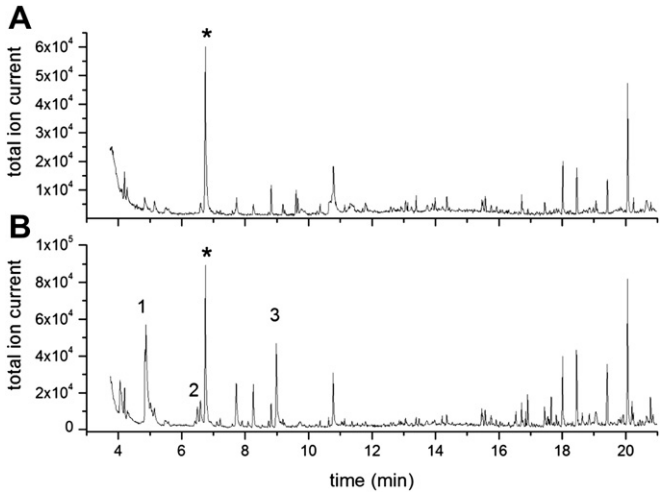

Fig. 4. Chromatographic profiles of volatiles from potato plants cv. Agria. At 100–114 HAI (hours after inoculation; fourth trapping period), the volatile profiles of control plants (A) were compared with the volatile profile of plants infected by *Phytophthora infestans* strain 98014 (B). The infected plants emitted three volatiles which were significantly different from those of control plants by the Kruskal Wallis Test. 1, (*E*)-2-hexenal; 2, 5-ethyl-2(5H)-furanone, 3, benzene-ethanol; *, internal standard. Note the different *y*-axis scales.

cultivar Surprise did not produce (*E*)-2-hexenal after beetle damage (Bolter et al., 1997). Cultivar Granola also produced benzene-ethanol when infested by Colorado beetles (Weißbecker et al., 1997), similar to our findings after infection by *P. infestans*. These research results indicate that the types and amounts of volatiles produced may vary according to the plant cultivars, the plant parts, as well as the damage types. Therefore, the volatile profiles may also vary dependent on specific interactions between plant cultivars and *P. infestans* isolates. It has already been shown that compatible and incompatible interactions give rise to different volatile profiles (Croft et al., 1993).

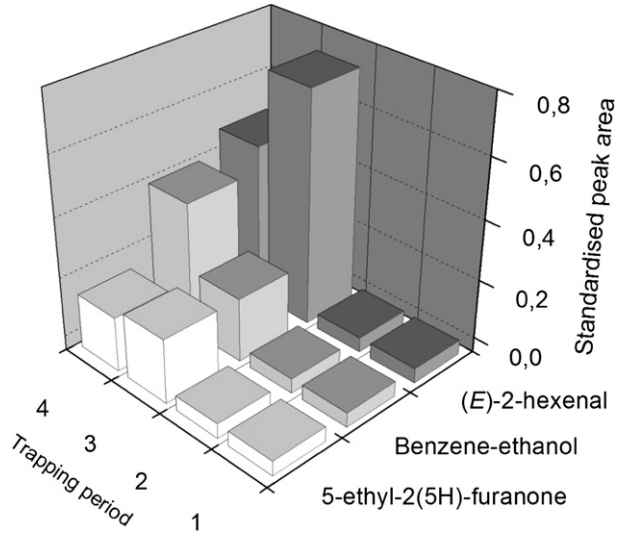

Fig. 5. The mean concentrations of volatiles significantly produced by potato plants cv. Agria infected by *Phytophthora infestans* isolate 98014. Volatiles were trapped in 4 trapping periods *i.e* 28–42 HAI (hours after inoculation; first trapping period), 52–66 HAI (second trapping period), 76–90 HAI (third trapping period), and 100–114 HAI (fourth trapping period). The volatile headspaces were analyzed by gas chromatography–mass spectrometry. The identification of volatile species was done by library, index and authentic samples.

The blighted plants produced (*E*)-2-hexenal in relatively high amounts during the initial stages of infection when a typical hypersensitive response (HR) was observed in the moderately resistant cultivar Agria (this paper). (*E*)-2-hexenal is known for its anti-fungal activity (Hamilton-Kemp et al., 1992; Kobaisy et al., 2001). Thus, this volatile may have been involved in the initial defence mechanism activated by the plants. However, although (*E*)-2-hexenal has antimicrobial activity in *in vitro* experiments, the *in vivo* activity of this compound is still difficult to assess, because the bioactivity depends on the compound concentration in a single cell that has direct contact with the pathogen.

(*E*)-2-hexenal can also have a function as communication signal between plants to trigger resistance genes (Arimura et al., 2001) or systemic terpene production (Farag and Paré, 2002). Application of (*E*)-2-hexenal on healthy lima bean leaves (*Phaseolus lunatus*) induced the expression of defence genes (Arimura et al., 2001), and application on tomato plants (*Lycopersicon esculentum*) triggered local and systemic terpene production. It would be interesting to investigate the effect of this compound on the defence response in potato plants against *P. infestans*, although it is likely not specific.

Two of the three volatiles identified in our study had been documented for plants damaged mechanically or by Colorado potato beetles. The third volatile, tentatively identified as 5-ethyl-2(5H)-furanone, has never been reported for potato plants artificially damaged or damaged by beetles. Yet, this volatile was found in leaves of *Aesculus hippocastanum* infested by butterflies *Cameraria ohridella* and opportunistic fungi (*Guignardia aesculi*) (Johne et al., 2004). In addition, 5-ethyl-2(5H)-furanone was found in undamaged tomato, raspberry, asparagus leaves (Buttery and Takeoka, 2004). Transgenic tomato leaves with the yeast Δ9 desaturase gene gave rise to a large production of this compound (Wang et al., 2001). However, this compound was not detected in either non-inoculated potato plants, nor in damaged potato plants, but was produced in *P. infestans* infected plants. Therefore, it is worth investigating if 5-ethyl-2(5H)-furanone could be used as a marker volatile for developing a late blight sensor. Although the matching factor of 5-ethyl-2(5H)-furanone was high (93%), its identity should be confirmed by injection of an authentic standard; preferably on columns of different polarity.

Two days after inoculation, small lesions were observed, which were considered to be ineffective HR (Cuypers and Hahlbrock, 1988; Vleeshouwer et al., 2000). HR is the result of induced plant responses including many events *e.g.*, the production of active oxygen radicals and disruption of the cell membrane, which provides initial substrates (linolenic and linoleic acid) for the lipoxygenase pathway (Agrios, 1997). Within this pathway, several volatile compounds are intermediates (Siedow, 1991). The absence of these intermediate volatiles in our experiments might due to a high degree of specificity of enzymes involved in the cascade as well as the detection limit of the method. To improve the detection limit, thermal desorption might be used. However in the current research, the solvent desorption method was selected instead of thermal desorption because of the advantage that GC–FID could be performed prior to GC–MS and this leads to the possibility of using GC-EAG to detect the marker volatiles for future sensor development.

Nevertheless, our experimental set-up resulted in detection of realistic volatiles with minimal artifacts, unlike results obtained from *ex situ* headspace trapping (Vercammen et al., 2001). The current trapping system was designed to trap the different treatments simultaneously to ensure that unsystematic variation was evenly allocated to all treatments. By *in situ* headspace sampling, stress volatiles were avoided. Moreover, the drought stress which might have come from the long dry-air-flushing period was compensated by applying humidified air in between trapping

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periods. Thus, the volatiles trapped depicted the actual volatile composition induced by the plant–pathogen interaction.

The knowledge gained from the experiment could contribute to two kinds of applications: sensor development and volatile therapy. The marker volatiles detected, particularly 5-ethyl-2(5H)-furanone, could be further tested for the development of sensors to detect the occurrence of late blight in the field. With respect to volatile therapy, there is evidence that volatiles from attacked plants can trigger plant defense responses in healthy plants. Thus, it is important to investigate whether the volatiles from blighted plants can trigger the defense against *P. infestans* in healthy potato plants. This might lead to a novel way of plant vaccination.

Acknowledgement

The authors thank the Laboratory of Phytopathology of Wageningen UR for providing the *P. infestans* strain as well as P. van de Vondervoort and F.P.M. Govers for their suggestions about maintaining and inoculating *P. infestans*. We really appreciate the help of D. Volker and B. Essenstam for providing and taking care of the potato plants. We are thankful to F.W.A. Verstappen and M.A. Posthumus for useful suggestions on GC–MS analysis.

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