RESEARCH NOTE

Check for updates

Tobacco Growth Promotion by the Entomopathogenic Fungus, *Isaria javanica* pf185

Yong-Seong Lee and Young Cheol Kim

Department of Applied Biology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, Republic of Korea

ABSTRACT

Isaria javanica pf185 is an important entomopathogenic fungus with potential for use as an agricultural biocontrol agent. However, the effect of *I. javanica* pf185 on plant growth is unknown. Enhanced tobacco growth was observed when tobacco roots were exposed to spores, cultures, and fungal cell-free culture supernatants of this fungus. Tobacco seedlings were also exposed to the volatiles of *I. javanica* pf185 *in vitro* using I-plates in which the plant and fungus were growing in separate compartments connected only by air space. The length and weight of seedlings, content of leaf chlorophyll, and number of root branches were significantly increased by the fungal volatiles. Heptane, 3-hexanone, 2,4-dimethylhexane, and 2-nonanone were detected, by solid-phase micro-extraction and gas chromatography-mass spectrophotometry, as the key volatile compounds produced by *I. javanica* pf185. These findings illustrate that *I. javanica* pf185 can be used to promote plant growth, and also as a biocontrol agent of insect and plant diseases. Further studies are necessary to elucidate the mechanisms by which *I. javanica* pf185 promotes plant growth.

Entomopathogenic fungi have potential as biocontrol agents against insect pests [1]. These fungi are commercially available and are used primarily as biopesticides to control arthropod pests in various cropping systems [2]. Recent studies have shown that entomopathogenic fungi positively influence plant health through induction of systemic resistance against plant diseases and abiotic stresses, promotion of crop growth [3,4], and direct inhibition of plant pathogens [5,6]. Consequently, entomopathogenic fungi are attractive candidates for the study of integrated pest management. Their cross-kingdom effects indicate that entomopathogenic fungi might have broader roles in crop protection than just insect control.

Several fungal entomopathogens, including *Beauveria* spp., *Metarhizium* spp., and *Lecanicillum* spp., promote plant growth after colonizing the host plant [3,4,7]. Proteomic analysis of palm colonized by the entomopathogenic fungi *Beauveria bassiana* and *Lecanicillium* spp. showed improvement in photosynthesis, energy metabolism, plant defense, and induction of stress-protective proteins [8]. The mechanisms involved in promoting plant growth might include altered plant growth hormone activity and/or improved nutrition. For example, colonization of soybean by *Metarhizium anisopliae* resulted in the induction of salt tolerance by reduction in abscisic acid production and elevation in jasmonic acid levels under stressed conditions [9]. The entomopathogen *Metarhizium robertsii* secretes siderophores under iron-depleted culture conditions [10], which might improve iron nutrition in a host plant. Altered plant nutrition has been reported in plants colonized by *B. bassiana* [11]. Microbial volatile compounds have also been shown to enhance plant growth. For instance, *Fusarium oxysporum* isolates produce organic volatile compounds that differentially affect plant growth [12]. Furthermore, rhizobacteria, such as *Bacillus subtilis* and *Pseudomonas chlororaphis*, produce 2R,3R-butanediol, which enhances plant growth and induces systemic resistance against biotic and abiotic stresses [13–16].

In this study, we investigated the plant growth-promoting effect of the entomopathgenic fungus, *Isaria javanica* isolate pf185 and its metabolites, which have been established to exhibit biocontrol activity against insects and plant diseases [17]. Tobacco was used to determine whether exposure to the fungal spores and mycelia, or secreted and

CONTACT Young Cheol Kim yckimyc@jnu.ac.kr

volatile products improved plant growth. We characterized the volatile compounds produced by the fungus and used authenticated products to determine their effects on tobacco growth. To the best of our knowledge, our finding that the pf185 isolate of *I. javanica* and its volatiles promoted tobacco growth is the first demonstration of the importance of volatiles produced by entomopathogenic fungi in inducing beneficial plant responses.

I. javanica pf185 (KACC93241P) was obtained from the Korean Agricultural Culture Collection, KACC, National Agrobiodiversity Center, Wanju, South Korea). The pf185 strain was grown and maintained on potato dextrose agar (PDA; Difco Inc. Detroit, MI) as described previously [17]. *Nicotiana tabacum* L. "Xanthi" seeds were surface-sterilized using an ethanol and sodium hypochlorite solution as described previously [18]. The sterilized tobacco seeds were planted in Murashige and Skoog (MS; Sigma-Aldrich Inc., St. Louis, MO) salt medium supplemented with 0.5% (w/v) agar and 3% (w/v) sucrose [19] in Petri dishes (SPL Life Science Co., Pocheon, Korea). The tobacco plants were grown for two weeks with a light:dark cycle of 16:8 h under 40-W fluorescent lights (2000 lux, 80 μmol photons m^{-2} s^{-1}). The temperature was maintained at 25 ± 3 °C, with a relative humidity of 50–60%.

Two-week-old tobacco seedlings were planted in pots containing 500 cm^3 of sterile soil-less medium (peat moss:vermiculite:perlite, 7:3:3, v/v) with one seedling per pot. The pots were supplied with 10 mL of sterile water every 2 days. After 2 weeks of growth, the seedlings were drenched with fungal products. To obtain intact cultures, *I. javanica* pf185 cells were grown in potato dextrose broth (PDB, Difco Inc.) at 25 °C in a shaking incubator at 150 rpm for 7 days. These cultures were diluted 1:1 v/v with sterile water. To obtain cell-free extracellular metabolites, the 7-day-old fungal cultures were centrifuged at 15,000g for 10 min to remove fungal debris, and the supernatant was passed through a 0.2 μm filter (Millipore Filter Corp., Bedford, MA). To obtain spores for plant application, fungal colonies from the 7-day-old PDA plates were harvested by suspension in sterile water. The suspension was filtered through two layers of sterile cheese cloth to remove hyphal debris. Spore concentration in the filtrate was determined and adjusted to 1×10^8 spores/mL under a microscope (Olympus BX41, Tokyo, Japan) using a hemocytometer (Marienfeld Superior, Lauda-Königshofen, Germany). The tobacco plants were treated with 15 mL aliquots of the spore suspension or supernatant four times at weekly intervals. The intact culture (20 mL) was applied only once. For the control treatments, sterile water or PDB was used. The experiment was repeated twice with six plants per treatment. The plant growth

parameters in the treatment groups were measured one week after the final treatment.

A previous study indicated that chlorophyll meter can be used to evaluate the correlation between chlorophyll content and nitrogen content in tobacco [20]. To determine the effects of treatments on tobacco plants, the leaf chlorophyll content was measured in at least 10 different locations of the third and fourth fully developed tobacco leaves, using a chlorophyll meter (SPAD-502Plus, Minolta Camera Co., Osaka, Japan). Each plant was subsequently harvested. To measure the fresh weight of shoot and root, the seedlings were cut 1 cm above the root with a sterile knife. The fresh weight of the roots and shoots were immediately measured using an analytical balance (A&D Korea Limited, Seoul, Korea). The length and diameter of the plant stems were measured using a digital ruler (Mitutoyo Corp., Kanagawa, Japan). The lateral root density was measured using the image analysis software ASSESS 2.0 (APS Press, St. Paul, MN). The experiment was repeated twice with six plants per treatment. In this study, the experimental data from the assay of plant growth effects of *I. javanica* pf185 preparations were analyzed by Student's *t*-test using Statistical Package for Social Sciences (SPSS Inc., Armonk, NY), ver. 23. Significance was set at $p < .05$.

In the pot experiments, tobacco plant growth was promoted by the treatment with the fungus and its metabolites. The growth of tobacco seedlings treated with a root drench of *I. javanica* pf185 fungal cultures was significantly higher than that of seedlings treated with non-inoculated half-strength PDB (Figure 1). Treatment with the culture of *I. javanica* pf185 by root drenching significantly enhanced the shoot height ($p = .001$), root length ($p = .004$), and shoot weight ($p = .02$), but there was no significant effect on the root weight at 28 days post-inoculation (dpi). When tobacco plant seedlings were treated with the spore suspension, the shoot weight ($p = .02$) and root weight ($p = .008$) were enhanced (Figure 1). Cell-free supernatants of *I. javanica* pf185 also enhanced shoot growth, shoot height ($p = .006$), and shoot weight ($p = .003$) (Figure 1). These results showed that *I. javanica* pf185 promoted tobacco plant growth.

After 2 weeks, three tobacco seedlings were transplanted to one half of an I plate (100 × 15 mm^2, SPL Life Science Co.) containing solid MS medium. The other half of the plate contained PDA inoculated with 0.1 mL of spore suspension (1×10^7 spores/mL) in sterile water 24 h prior to the experiment. As a control, the PDA was treated with only 0.1 mL sterile water. The inoculated I-plates were sealed with Parafilm and placed in a growth room under fluorescent light for two weeks and used to measure

(A)
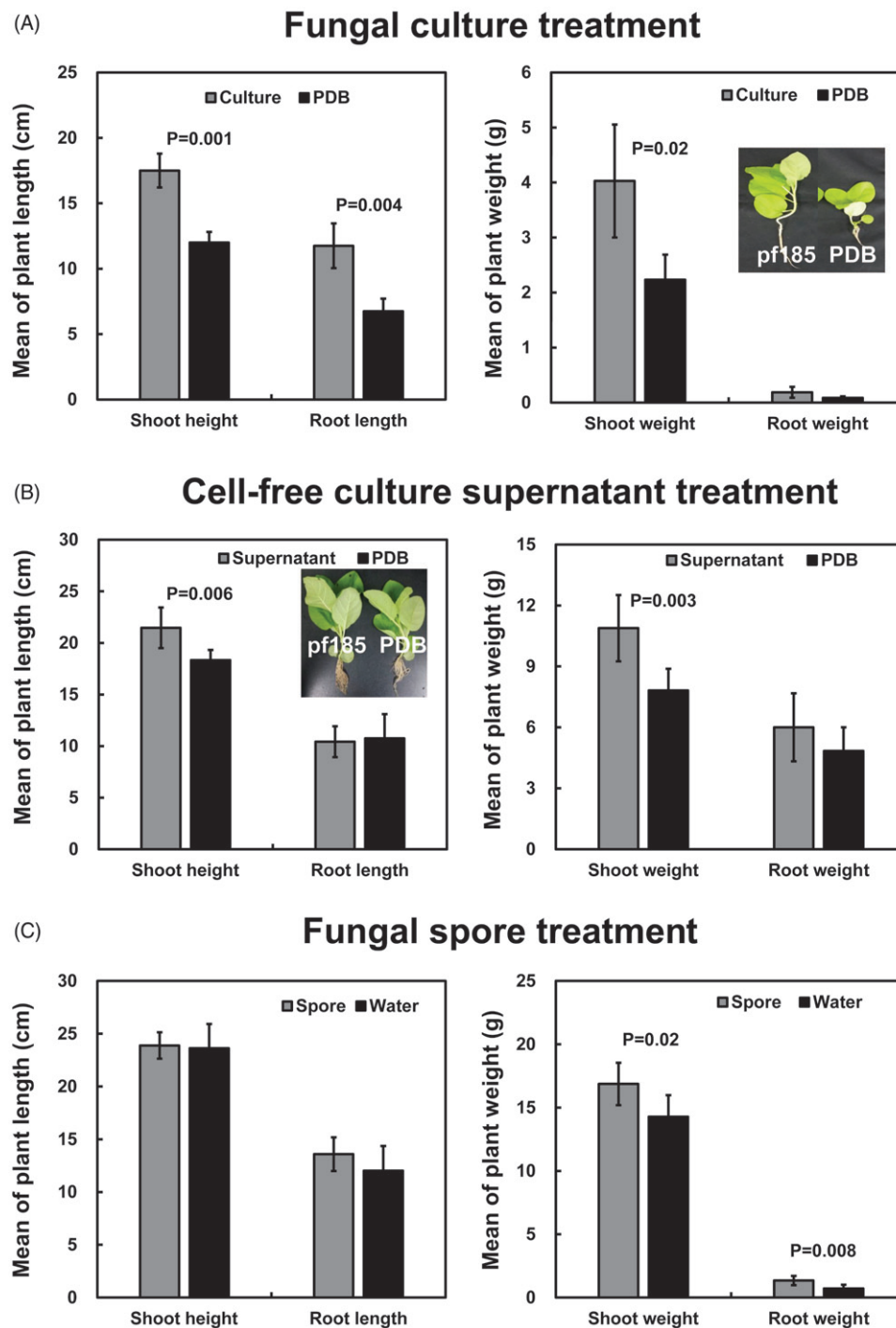
Figure 1. Effect of *Isaria javanica* pf185 on growth of tobacco (*Nicotiana tabaccum* "Xanthi") seedlings grown on potting mix. Tobacco seedlings (14-day-old) were treated for 5 weeks by root drenching with an intact fungal culture (A), cell-free supernatant (B), or spore suspensions (C). Equal volumes of half strength potato dextrose broth or water were used as the control. Means and standard errors of two independent experiments are shown, with six seedlings per replicate. The actual *p* values are provided for significant differences in growth under different treatments (Student's *t*-test, $\alpha < 0.05$). Insert: images of tobacco seedlings.

the tobacco growth parameters. Root development of the tobacco plants in the treatment and control groups was observed using a microscope at ×40 magnification (M165 FC; Leica Microsystems, Tokyo, Japan).

The use of I plates enabled the measurement of the tobacco plant responses to the volatiles produced by *I. javanica* pf185 (Figure 2). Compared

with that of the seedlings grown in the absence of the fungus (control), the seedlings grown with the fungal volatiles showed significantly enhanced growth ($p < .000001$). The shoot height and root length both increased by approximately 1.5-fold when the seedlings were grown with the volatiles, compared with those of the controls, whereas the shoot and root weights increased by more than two-
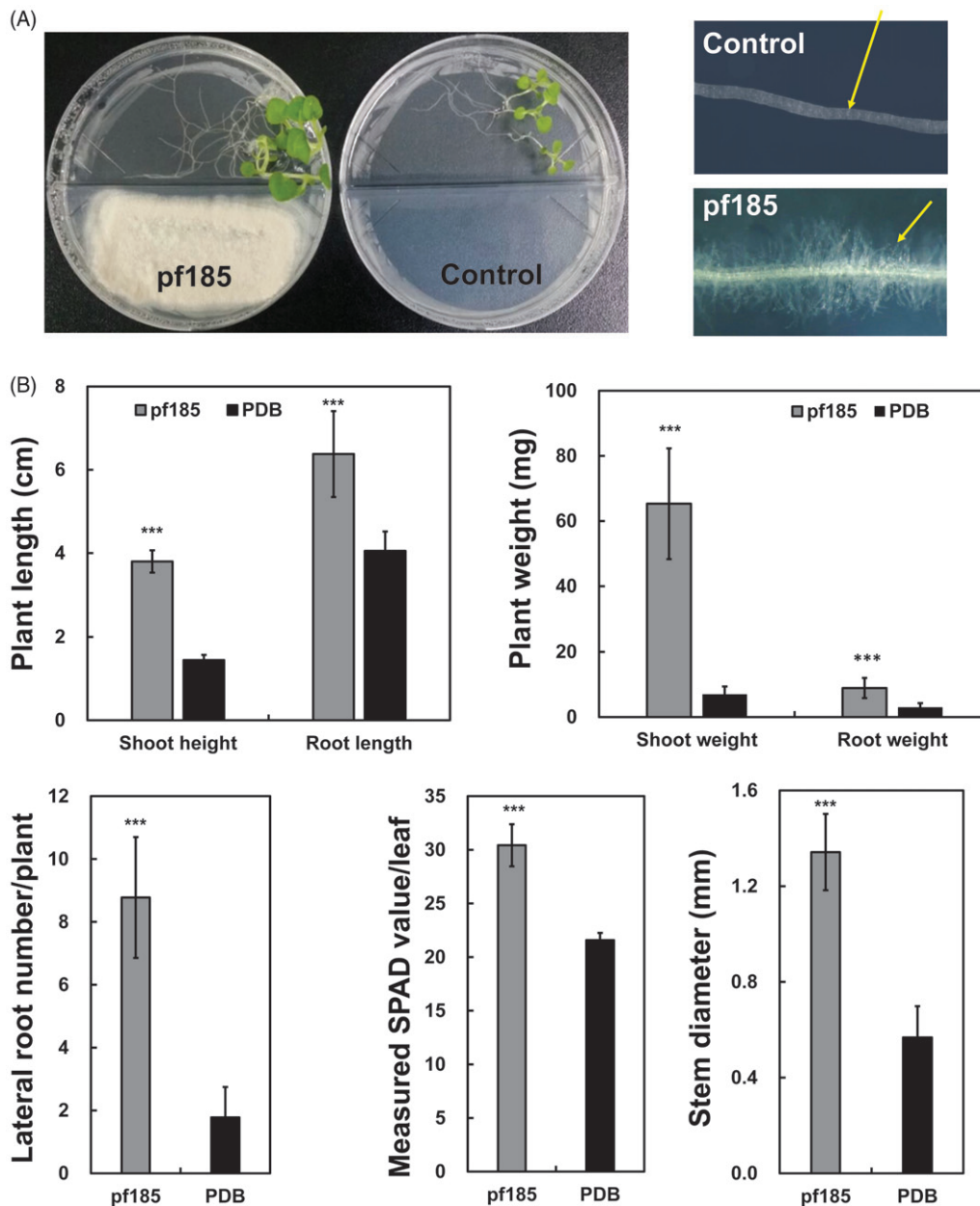
Figure 2. Effect of fungal volatiles on tobacco plant growth. The data shown are of plants 2 weeks after treatment in I plates. (A) Images of tobacco seedlings and tobacco root hair formation after co-inoculation with spore suspension of *Isaria javanica* pf185. Images of root hair formation were captured under a microscope. (B) Mean growth parameters of tobacco cultivated with or without *I. javanica* pf185 (potato dextrose broth) in I plates. A significant difference in responses between treatments and controls, as assessed by Student's *t*-test ($\alpha < 0.01$), are indicated with three asterisks. Means and standard errors of two independent experiments are shown, with six seedlings per replicate.

fold (Figure 2). These changes correlated with the increase in leaf chlorophyll content ($p = .000001$) and stem diameter ($p = .000001$). When the seedlings were grown with the fungal volatiles, both lateral root and root hair formation were enhanced when compared with those of the control. These results suggest that the volatiles from *I. javanica* pf185 have growth-promoting effects that are accompanied by altered root architecture and increased fresh weight biomass in tobacco.

Spores of *I. javanica* pf185 (20 μL suspension) were cultured on 1 mL of PDA at 28 °C for 36 h in closed 20 mL glass headspace bottles (Supelco, Bellefonte, PA) before the collection of volatiles.

Commercially available solid phase microextraction (SPME) fiber (50/30 μm DVB/Carboxen/PDMS; Supelco, Bellefonte, PA) was used to analyze the volatiles produced by *I. javanica* pf185. The volatiles were analyzed by headspace SPME and gas chromatography-mass spectrophotometry (GC-MS) as described previously [21], with minor modifications. The volatile organic compounds (VOCs) from the airspace of the bottles containing 1 mL of PDA without inoculation were used as the control. After extraction, the SPME fiber was desorbed at 30 °C for 30 min in the injection port of an Agilent 6890 GC Plus (Agilent Technologies, Santa Clara, CA) coupled to Pegasus HT (GC-TOF-MS) equipped

Table 1. GC/MS analysis of the volatiles produced by *Isaria javanica* pf185 grown on potato dextrose agar.

Identified compound	Retention time (min:s)	Mass	Relative area[a]
O-(Carboxymethyl) hydroxylamine	1:24	45	7.9
2-Methyl propanal	1:54	72	4.7
3-Methyl-furan	2:02	81	24.8
Hexamethyl-disiloxane	2:33	147	5.5
Heptane (peak 1)	2:50	57	–*
3-Hexanone (peak 2)	4:24	57	–*
2,4-Dimethyl-hexane (peak 3)	4:46	85	–*
2-Nonanone (peak 4)	12:22	58	–*

[a]Relative area values of each compound detected in the head space of *I. javanica* pf185-inoculated potato dextrose agar (PDA) bottles versus non-inoculated PDA bottles. These values are from one of the two repetitions that showed similar results (Supplemental Figure 1). Four volatiles (*) were detected only when the fungus was present. The peak numbers refer to the compounds separated by GC (see Figure 3).

with a Combi-Pal Autosampler. The chromatographic separation was performed with a DB-WAX (30 m × 0.25 μm, 0.25 μm film thickness) column using helium as the carrier gas at a constant flow rate of 1 mL/min. The column was held at 45 °C for 3 min, and then the temperature was increased to 240 °C using the program; (3 min) → 5 °C/min → 80 °C → 20 °C/min → 150 °C (5 min) → 30 °C/min → 240 °C (15 min). The LECO Pegasus 4D-TOFMS detector (LECO Corp., Saint Joseph, MI) was programmed with an electron ion (EI) source operating at 70 eV, and the acquisition range was between *m/z* 50 and 500 (Supplemental Table 1). The temperature of the transfer line and ion trap was 230 °C. The volatile compounds were identified by comparing them with those in the GC-MS system data banks [National Institute of Standards and Technology (NIST) Main EI MS Library, 2014]. Each sample was tested twice.

The GC-MS analysis of the volatile compounds produced by *I. javanica* pf185 showed the presence of several compounds (Table 1 and Figure 3). Volatiles were also detected in the headspace of the non-inoculated PDA bottles. However, fungal growth changed the composition of the volatiles. The level of *O*-(carboxymethyl) hydroxylamine, 2-methyl propanal, 3-methyl-furan, and hexamethyl-disiloxane was 5–25 times higher in the fungus inoculated PDA bottles than in the non-inoculated PDA bottles. Additionally, four volatiles (heptane, 3-hexanone, 2,4-methyl-hexane, and 2-nonanone) were only present in the headspace of *I. javanica* pf185 inoculated PDA bottles (Table 1 and Figure 3).

This study extends the documented of the growth-promoting effects of fungal genera to include an entomopathogenic strain of *I. javanica* and establishes a role for the metabolites that are volatile. The findings add to similar findings in isolates of *Aspergillus*, *Fusarium*, *Penicillium*, and *Trichoderma* [12,22–25], as well as other genera of entomopathogenic fungi such as *Beauveria*, *Metarhizium*, *Purpureocillium*, and *Lecanicillium* [3,4,26]. For example, *B. bassiana*, *I. fumosorosea*, and *M. brunneum* stimulated cabbage

growth under water stress [27], and foliar applications of the endophytes *B. brongniartii* and *M. brunneum* increased *Vicia faba* growth [4].

In this study, the importance of volatiles produced by the insect pathogen *I. javanica* pf185 in promoting tobacco plant growth was demonstrated. The ability of microbial metabolites, including volatiles, to directly and indirectly promote plant health is well-documented [13–15,28–32]. In this study, we detected four new volatiles produced by *I. javanica* pf185 grown on PDA medium. However, we are yet to confirm which of the identified volatile compounds from *I. javanica* pf185 cells were responsible for the improvement in plant growth. The active metabolites secreted or released as volatiles by *I. javanica* pf185 are currently under investigation.

The volatiles produced by *I. javanica* pf185 increased root branching and root hair formation. Changes in root morphology have also been observed with entomopathogenic *Metarhizium* isolates; for instance, *M. robertsii* stimulated root hair development [26], and *M. anisopliae* improved root development [33]. A recent study showed that pathogenic strains of *Fusarium oxysporum* produced organic volatiles that can enhance plant growth and lateral root development [12], while volatiles from a nonpathogenic strain of *F. oxysporum* also promoted plant growth [34]. Root colonization and changes in lateral root development promoted by *I. javanica* pf185 can enhance its rhizosphere competency by acting as a competitor, limiting both penetration of fungal pathogens and their access to nutrients. It is known that the primary and lateral roots are the main penetration sites for several soil-borne fungal pathogens [35].

In the present study, the volatiles produced by *I. javanica* pf185 grown on PDA were identified as heptane, 3-hexanone, 2,4-methyl-hexane, and 2-nonanone. Although their biological function is largely uncharacterized, such volatiles are known to have varied effects on plants and microbes. Production of 2-nonanone by the root-colonizing bacterium, *Paenibacillus polymyxa*, inhibited nematode growth
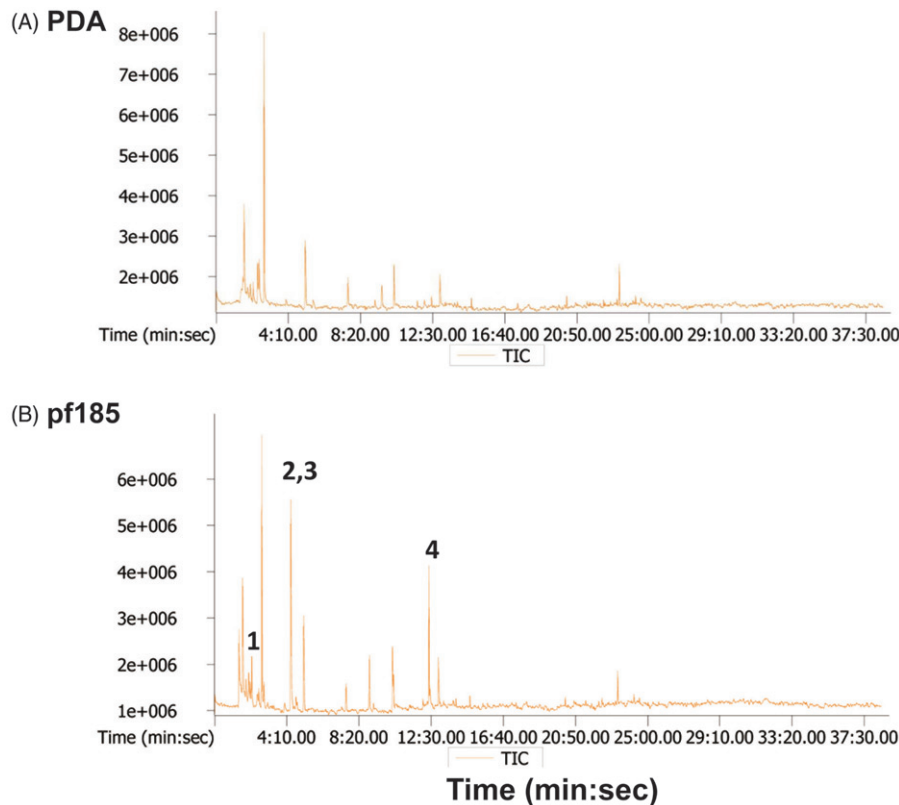
Figure 3. GC profile of volatiles produced by the fungus *Isaria javanica* pf185 grown on potato dextrose agar (PDA). The volatiles were absorbed onto a solid phase microextraction (SPME) and analyzed by gas chromatography-mass spectrophotometry (GC-MS). GC-MS profiles of volatiles from a non-inoculated PDA bottle (A) and from *I. javanica* pf185-inoculated PDA bottle (B). The peaks unique to the volatiles detected in the *I. javanica* pf185-inoculated PDA bottle are labeled 1–4. These GC-MS profiles are representative of two independent experiments.

[30]. Heptane produced by *Burkholderia ambifaria* promoted plant growth and productivity [36–38]. The 2,4-dimethyl hexane extracted from the leaves of *Tragia involucrata* exhibited weak *in vitro* antibacterial activity against *Staphylococcus aureus* [39]. 2-Nonanone is a male-specific pheromone for nitidulid beetles and mealworms [40,41]. Other cocktails of volatiles that improve plant growth are also reported for other fungi [42].

Our previous study indicated that dibutyl succinate, also produced by *I. javanica* pf185, was an aphicide and inhibited growth of *Colletotrichum acutatum* causing anthracnose disease in red-pepper [43]. We are currently monitoring the insecticidal or insect-repellent activities of the volatiles produced by *I. javanica* pf185 using an olfactometer, following previously described protocols with aphids, mites, and root-knot nematodes as targets [44]. These studies will be complemented by using single or mixed application(s) of the authenticated volatiles to compare the potency of the array of metabolites derived from the fungus.

Our findings that *I. javanica* pf185 produces plant-active volatiles *in vitro* are the first steps in our understanding of the beneficial effects of this entomopathogen on plants. Diverse microbial groups exist in the rhizosphere, which serves as a battleground for soil-borne plant pathogens and beneficial microbes [45]. The rhizosphere competency of biocontrol microbes is promoted by biocontrol mechanisms for acquisition of space and the nutrients required for growth and production of active metabolites. The emerging concept that microbial volatiles are part of the successful plant root-microbe interaction will lead to development of these products as eco-friendly chemicals for aiding plant health and productivity [46]. Our findings expand our knowledge of probiotic microbes, particularly when fungal studies are scarcer than those on bacteria. The ability of *I. javanica* pf185 to promote crop growth, as well as biocontrol for plant damage by microbial pathogens and insects, makes this strain an attractive candidate for inclusion in integrated crop protection measures.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

ORCID

Young Cheol Kim http://orcid.org/0000-0002-7661-7600

References

[1] Vega FE, Goettel MS, Blackwell M, et al. Fungal entomopathogens: new insights on their ecology. Fungal Ecol. 2009;2:149–159.

[2] Jackson MA, Dunlap CA, Jaronski ST. Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. BioControl. 2010;55:129–145.

[3] Jaber LR, Enkerli J. Effect of seed treatment duration on growth and colonization of *Vicia faba* by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. Biol Control. 2016;103:187–195.

[4] Jaber LR, Enkerli J. Fungal entomopathogens as endophytes: can they promote plant growth? Biocontrol Sci Technol. 2017;27:28–41.

[5] Lacey LA, Grzywacz D, Shapiro-Ilan DI, et al. Insect pathogens as biological control agents: back to the future. J Invertebr Pathol. 2015;132:1–41.

[6] Ownley BH, Gwinn KD, Vega FE. Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. In: Roy HE, Vega FE, Chandler D, Goettel MS, Pell J, Wajnberg E, editors. The ecology of fungal entomopathogens. Dordrecht: Springer Netherlands; 2010. p. 113–128.

[7] Sasan RK, Bidochka MJ. Antagonism of the endophytic insect pathogenic fungus *Metarhizium robertsii* against the bean plant pathogen *Fusarium solani* f. sp. *phaseoli*. Can J Plant Pathol. 2013;35:288–293.

[8] Gómez-Vidal S, Salinas J, et al. Proteomic analysis of date palm (*Phoenix dactylifera* L.) responses to endophytic colonization by entomopathogenic fungi. Electrophoresis. 2009;30:2996–3005.

[9] Khan AL, Hamayun M, Khan SA, et al. Pure culture of *Metarhizium anisopliae* LHL07 reprograms soybean to higher growth and mitigates salt stress. World J Microbiol Biotechnol. 2012;28:1483–1494.

[10] Krasnoff SB, Keresztes I, Donzelli BG, et al. Metachelins, mannosylated and N-oxidized coprogen-type siderophores from *Metarhizium robertsii*. J Nat Prod. 2014;77:1685–1692.

[11] Sánchez-Rodríguez AR, Del Campillo MC, Quesada-Moraga E. *Beauveria bassiana*: an entomopathogenic fungus alleviates Fe chlorosis symptoms in plants grown on calcareous substrates. Sci Hortic. 2015;197:193–202.

[12] Bitas V, McCartney N, Li N, et al. *Fusarium oxysporum* volatiles enhance plant growth via affecting auxin transport and signaling. Front Microbiol. 2015;6:1248.

[13] Cho SM, Kang BR, Han SH, et al. 2R, 3R-butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis* O6, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana*. Mol Plant Microbe Interact. 2008;21:1067–1075.

[14] Han SH, Lee SJ, Moon JH, et al. GacS-dependent production of 2R, 3R-butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. tabaci in tobacco. Mol Plant Microbe Interact. 2006;19:924–930.

[15] Ryu C-M, Farag MA, Hu C-H, et al. Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol. 2004;134:1017–1026.

[16] Ryu C-M, Farag MA, Hu C-H, et al. Bacterial volatiles promote growth in *Arabidopsis*. Proc Natl Acad Sci USA. 2003;100:4927–4932.

[17] Kang BR, Han JH, Kim JJ, et al. Dual biocontrol potential of the entomopathogenic fungus, *Isaria javanica*, for both aphids and plant fungal pathogens. Mycobiology. 2018;46:440–447.

[18] Han SH, Anderson AJ, Yang KY, et al. Multiple determinants influence root colonization and induction of induced systemic resistance by *Pseudomonas chlororaphis* O6. Mol Plant Pathol. 2006;7:463–472.

[19] Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant. 1962;15:473–497.

[20] Castelli F, Contillo R. Using a chlorophyll meter to evaluate the nitrogen leaf content in flue-cured tobacco (*Nicotiana tabacum* L.). Ital J Agronomy. 2009;2:3–11.

[21] Farag MA, Ryu C-M, Sumner LW, et al. GC–MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants. Phytochemistry. 2006;67:2262–2268.

[22] Hossain MM, Sultana F, I. S Plant growth-promoting fungi (PGPF): phytostimulation and induced systemic resistance. In: Singh DP, Singh HB, Prabha R, editors. Plant-microbe interactions in agro-ecological perspectives. Volume 2: Microbial interactions and agro-ecological impacts. Singapore: Springer Singapore; 2017. p. 135–191.

[23] Salas-Marina MA, Silva FMA, Cervantes BMG, et al. The plant growth-promoting fungus *Aspergillus ustus* promotes growth and induces resistance against different lifestyle pathogens in *Arabidopsis thaliana*. J Microbiol Biotechnol. 2011;21:686–696.

[24] Zhou L, Tang K, Guo S. The plant growth-promoting fungus (PGPF) *Alternaria* sp. A13 markedly enhances *Salvia miltiorrhiza* root growth and active ingredient accumulation under greenhouse and field conditions. Int J Mol Sci. 2018;19:270.

[25] Zhang S, Gan Y, Xu B. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. Front Plant Sci. 2016;7:1405.

[26] Sasan RK, Bidochka MJ. The insect-pathogenic fungus *Metarhizium robertsii* (Clavicipitaceae) is also an endophyte that stimulates plant root development. Am J Bot. 2012;99:101–107.

[27] Dara S, S R Dara S, S Dara S. Impact of entomopathogenic fungi on the growth, development, and

health of cabbage growing under water stress. Am J Plant Sci. 2017;08:1224–1233.

[28] Blom D, Fabbri C, Eberl L, et al. Volatile-mediated killing of *Arabidopsis thaliana* by bacteria is mainly due to hydrogen cyanide. Appl Environ Microbiol. 2011;77:1000–1008.

[29] Chen H, Xiao X, Wang J, et al. Antagonistic effects of volatiles generated by *Bacillus subtilis* on spore germination and hyphal growth of the plant pathogen, *Botrytis cinerea*. Biotechnol Lett. 2008; 30:919–923.

[30] Cheng W, Yang J, Nie Q, et al. Volatile organic compounds from *Paenibacillus polymyxa* KM2501-1 control *Meloidogyne incognita* by multiple strategies. Sci Rep. 2017;7:16213.

[31] Utama IM, Wills RB, Ben-Yehoshua S, et al. *In vitro* efficacy of plant volatiles for inhibiting the growth of fruit and vegetable decay microorganisms. J Agric Food Chem. 2002;50:6371–6377.

[32] Vespermann A, Kai M, Piechulla B. Rhizobacterial volatiles affect the growth of fungi and *Arabidopsis thaliana*. Appl Environ Microbiol. 2007;73: 5639–5641.

[33] Liu S-F, Wang G-J, Nong X-Q, et al. Entomopathogen *Metarhizium anisopliae* promotes the early development of peanut root. Plant Protect Sci. 2017;53:101–107.

[34] Minerdi D, Bossi S, Maffei ME, et al. *Fusarium oxysporum* and its bacterial consortium promote lettuce growth and expansin A5 gene expression through microbial volatile organic compound (MVOC) emission. FEMS Microbiol Ecol. 2011;76: 342–351.

[35] Dean R, Van Kan JAL, Pretorius ZA, et al. The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol. 2012;13:414–430.

[36] Groenhagen U, Baumgartner R, Bailly A, et al. Production of bioactive volatiles by different *Burkholderia ambifaria* strains. J Chem Ecol. 2013; 39:892–906.

[37] Kanchiswamy CN, Malnoy M, Maffei ME. Chemical diversity of microbial volatiles and their potential for plant growth and productivity. Front Plant Sci. 2015;6:151.

[38] Budai Z, Kis-Tamás A, Mezei T, et al. inventors. Bicyclo(2.2.1) heptane oximes used for plant growth regulating. 1984. US patent 4,425,158. doi: 10.1002/ps.5191.

[39] Samy RP, Gopalakrishnakone P, Houghton P, et al. Purification of antibacterial agents from *Tragia involucrata*-a popular tribal medicine for wound healing. J Ethnopharmacol. 2006;107:99–106.

[40] Hassemer MJ, Sant'Ana J, Borges M, et al. Revisiting the male-produced aggregation pheromone of the lesser mealworm, *Alphitobius diaperinus* (Coleoptera, Tenebrionidae): identification of a six-component pheromone from a brazilian population. J Agric Food Chem. 2016;64:6809–6818.

[41] Moliterno AAC, Martins CBC, Szczerbowski D, et al. The male produced aggregation pheromone of a strawberry sap beetle, *Lobiopa insularis* (Coleoptera: Nitidulidae). J Chem Ecol. 2017;43: 550–556.

[42] Naznin HA, Kimura M, Miyazawa M, et al. Analysis of volatile organic compounds emitted by plant growth-promoting fungus *Phoma* sp. GS8-3 for growth promotion effects on tobacco. Microb Environ. 2013;28:42–49.

[43] Lee YS, Han JH, Kang BR, KYC, et al. Dibutyl succinate, produced by an insect-pathogenic fungus, *Isaria javanica* pf185, is a metabolite that controls of aphids and a fungal disease, anthracnose. Pest Manag Sci. 2019. doi: 10.1002/ps.5191.

[44] Ballhorn DJ, Kautz S. How useful are olfactometer experiments in chemical ecology research? Commun Integr Biol. 2013;6:e24787.

[45] Raaijmakers JM, Paulitz TC, Steinberg C, et al. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil. 2009;321:341–361.

[46] Bailly A, Weisskopf L. Mining the volatilomes of plant-associated microbiota for new biocontrol solutions. Front Microbiol. 2017;8:1638.