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Ginsenosides stimulate the growth of soilborne pathogens of American ginseng

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

Ginseng saponins (ginsenosides) were isolated from soil associated with the roots of commercially grown American ginseng (*Panax quinquefolius* L.), identified via LC-MS and quantified via analytical HPLC. The ginsenosides, including F_{11}, Rb_1, Rb_2, Rc, Rd, Re and Rg_1, represented between 0.02 and 0.098% (average 0.06%) of the mass of the soil collected from roots annually between 1999 and 2002. The same ginsenosides were also isolated from run-off of undisturbed plants grown in pots in a greenhouse using a root exudate trapping system. To investigate (1) whether these saponins could influence the growth of pythiaceous fungi pathogenic to ginseng, and (2) whether soil levels of ginsenosides were sufficient to account for any effects, bioassays were completed using a crude saponin extract and an ecologically relevant concentration of purified ginsenosides. Thus, when cultured on media containing crude saponins, the colony weight of both *Phytophthora cactorum* and *Pythium irregulare* was significantly greater than that of control, indicating a strong growth stimulation by ginsenosides. The growth of *Pythium irregulare* was also significantly stimulated after addition of an ecologically relevant, low concentration (i.e. 0.06%) of purified ginsenosides to culture medium. By contrast, growth of the saprotrophic fungus *Trichoderma hamatum* was slightly (but not significantly) inhibited under the same conditions. These results imply that ginsenosides can act as allelopathic stimulators of the growth of pythiaceous fungi in the rhizosphere, and this may contribute to the disease(s) of this crop.
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Keywords: *Panax quinquefolius*; American ginseng; Araliaceae; Pythiaceae; Allelopathy; Saponin; Ginsenosides

1. Introduction

"...not all plant ecologists accept the concept of allelopathy as a significant factor in competition... There is clearly a problem in that conclusive proof of allelopathy occurring in any given ecological situation is extremely difficult to obtain... Having said this...[and] considering the enormous capacity of angiosperms to synthesize such a wide range of generalized highly toxic compounds, it would be very surprising if no such interactions ever occurred." —J. B. Harborne (1993)

In part due to the extensive work completed by Jeffrey Harborne and his collaborators (Kokubun et al., 1994; Kokubun and Harborne, 1995; Marshall et al., 1987; Robeson et al., 1980; Senevirante and Harborne, 1992), and as reviewed by Grayer and Harborne (1994), it is well established that a diverse array of secondary phytochemicals are potent antifungal agents. Amongst these are the saponins (Levy et al., 1986; Nicol et al., 2002; Takechi and Tanaka, 1990) and research with triterpenoid saponins in oat roots (Bowyer et al., 1995; Papadopoulou et al., 1999; Turner, 1956) has led to the conclusion that these compounds function as preformed chemical defenses against fungal infection (Osbourn, 1996). Furthermore, there has been speculation that saponins may also act as allelopathic mediators of plant-fungal interactions (Carter et al., 1999; Levanon et al., 1982), but in order to do so they must be present in sufficient concentration in the rhizosphere. At present there is an absence of information about the concentration of plant saponins, as well as secondary phytochemicals in general, in soil. Nevertheless, roots are known to influence the composition of soil microbes (Grayston et al., 2001; Kandeler et al., 2002; Marschner

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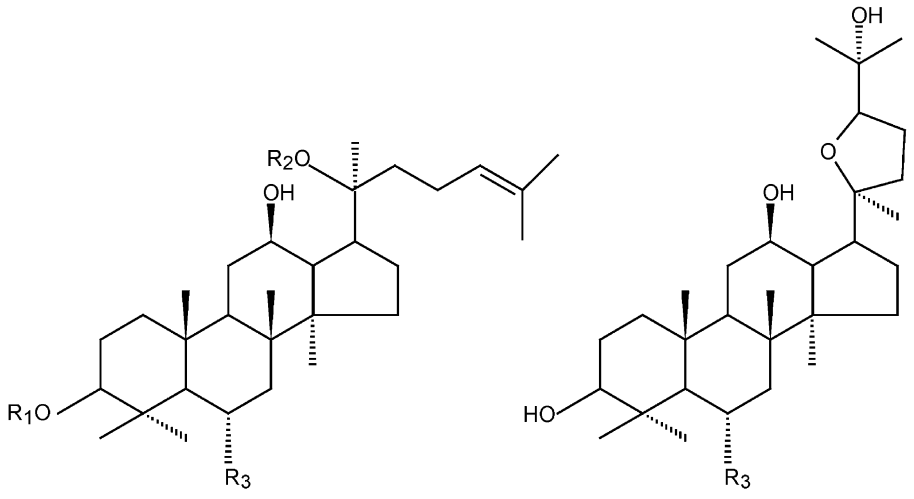
et al., 2001; Miethling et al., 2000) and it is generally thought that various soil physico-chemical factors plus the presence of plant compounds combine to affect the growth of soil microbes, although the exact mechanism(s) remains elusive. Different species of fungi can be differently susceptible to secondary chemicals (Arneson and Durbin, 1968; Nicol et al., 2002; Sandrock and VanEtten, 1998) and the antifungal activity of specific compounds is therefore not necessarily universal. For example, the potential chemical defenses of American ginseng, i.e. ginseng saponins, or ginsenosides (Fig. 1), inhibit the in vitro growth of some fungi (especially *Trichoderma* spp.) while stimulating that of *Cylindrocarpon destructans*, a major soilborne ginseng pathogen (Nicol et al., 2002). Therefore, it follows that if ginseng secondary compounds are present in the rhizosphere, they could influence the growth and/or species composition of the soil fungal community: an allelopathic interaction that could help explain the often extensive fungal diseases observed in ginseng crops (Punja, 1997; Reeleder and Brammall, 1994). It was in this context that we investigated the potential for ginsenosides to be released from the roots of American ginseng, *Panax quinquefolius* L., and subsequently influence the growth of important ginseng root pathogens in the Pythiaceae.

2. Results and discussion

2.1. Isolation, purification and identification of ginsenosides from roots, soil and root exudates

A crude ginseng saponin fraction (GSF) was prepared from three-year-old American ginseng roots, and the presence of ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 (Fig. 1) tentatively determined by co-chromatography with authentic standards (data not shown). After further purification using C_{18} and aminopropyl Extract-Clean™ solid phase extraction columns, a purified (>95% by HPLC) ginsenoside fraction (GSF-Sep) was obtained (Fig. 2). HPLC-MS data collected at low (20 V) cone voltage, which yields $[M-H]^-$ parent ions and little fragmentation, provided evidence for seven ginsenosides (Fig. 2), including ginsenoside F_{11}, (RT 27.4 min) that was not evident in the UV trace. Re-analysis of the purified GSF-Sep by HPLC-MS using a higher cone voltage (100 V), yielded characteristic fragmentation patterns (Fuzzati et al., 1999) including evidence for protopanaxadiol (*m*/*z* 459) and protopanaxatriol (*m*/*z* 475) aglycones (Fig. 3). Based on these data, ginsenosides F_{11}, Rb_1, Rb_2, Rc, Rd, Re and Rg_1 were unequivocally identified in the crude and purified ginsenoside fractions used for the bioassays in this study. A seventh



Rb_1: R_1 = glc[2→1]glc, R_2 = glc[6→1]glc, R_3 = H
Rb_2: R_1 = glc[2→1]glc, R_2 = glc[6→1]ara(*p*), R_3 = H
Rc: R_1 = glc[2→1]glc, R_2 = glc[6→1]ara(*f*), R_3 = H
Rd: R_1 = glc[2→1]glc, R_2 = glc, R_3 = H
Re: R_1 = H, R_2 = glc, R_3 = O-glc[2→1]rha
Rg_1: R_1 = H, R_2 = glc, R_3 = O-glc

F_{11}: R_3 = O-glc[2→1]rha

Fig. 1. Structures of ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1 and pseudoginsenoside F_{11} from *Panax quinquefolius*. glc = glucose, ara(*p*) = arabinose (pyranose form), ara(*f*) = arabinose (furanose form), rha = rhamnose.

protopanaxadiol-derived ginsenoside (RT = 38.2 min) that showed a MS spectrum similar to Rd, remains unidentified.

Ginsenosides were also recovered from root-associated soil collected at five different times between 1999 and 2002. Co-chromatography of soil extracts with authentic ginsenosides (data not shown), as well as HPLC-MS analysis (Fig. 4) as described above for GSF-Sep confirmed the presence of ginsenosides F_{11}, Rb_1, Rb_2, Rc, Rd, Re and Rg_1 in the soil extracts. The quantities of soil ginsenosides were within the same order of magnitude for all sampling times and ranged from 0.02 to 0.098% of the dry mass of the soil extracted (average = 0.06 ± 0.03%).

In order to confirm that the ginsenosides recovered from root-associated soil were present in the exudate of intact ginseng roots and not isolated from residual root tissue in our soil preparations, we collected root exudates from pot-grown ginseng plants using a root exudate trapping system (Tang and Young, 1982). HPLC and HPLC-MS analysis of the trapped exudate confirmed the presence of the same suite of ginsenosides as found in the soil (data not shown). In total, approximately 12 mg of ginsenosides were recovered over 22 days and this equaled approximately 25 μg of ginsenosides produced per plant per day. Taken together, these data indicate that ginsenosides are released from the roots of American ginseng, and are present in the rhizosphere at levels approximating 0.06% of the soil dry weight.

2.2. Growth stimulation of pathogens

The crude ginsenoside fraction prepared from ginseng roots (GSF) caused a dose dependent increase in the biomass of both *P. cactorum* (Fig. 5a) and *Py. irregulare* (Fig. 5b). The growth increase in *P. cactorum* was significantly different from the control only at the highest concentration (0.5%) whereas the growth of *Py. irregulare* was significantly stimulated at all concentrations of crude ginsenosides tested. At the highest concentration of saponins, *P. cactorum* weighed over 3 times, and *Py. irregulare* almost 4 times more than control (Fig. 5). Therefore, both *P. cactorum* and *Py. irregulare* appear to be uninhibited by the putative chemical defenses of American ginseng.

2.3. Interaction between ginsenosides and ergosterol

The mode of action for the antifungal activity of saponins against higher fungi is thought to be via an interaction with fungal membrane sterols that leads to a

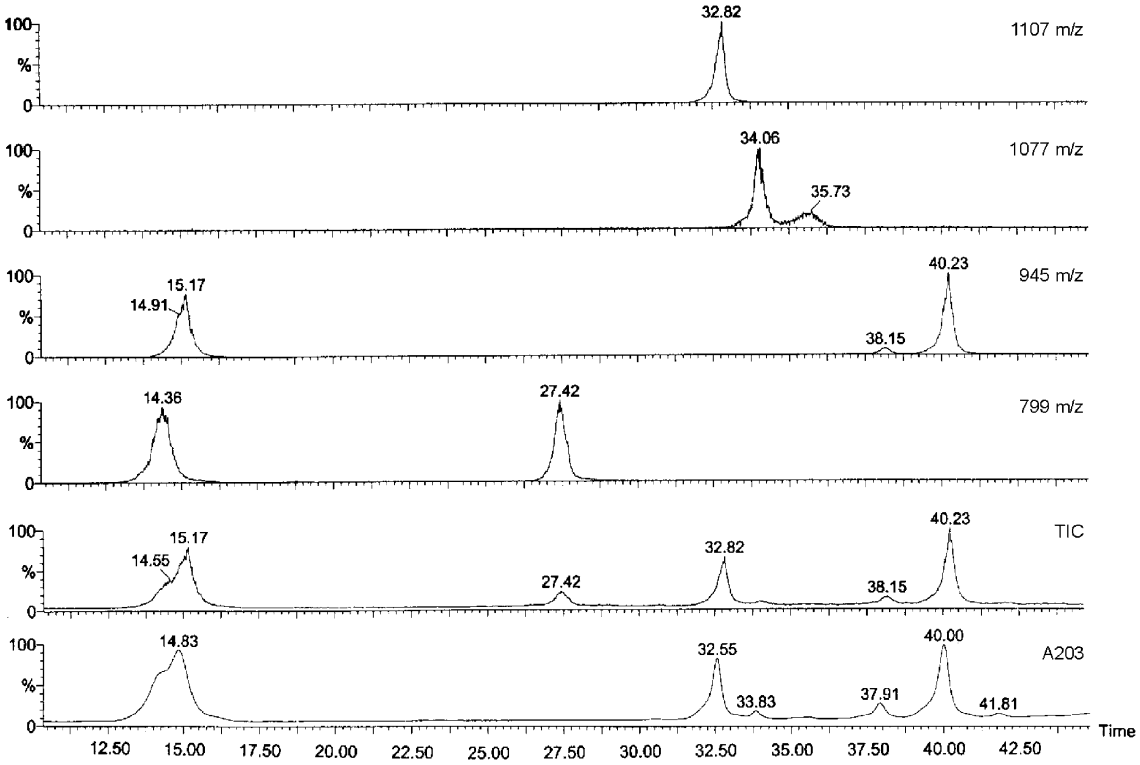


Fig. 2. HPLC-MS analysis of purified ginseng root extracts. A sample of the purified ginsenoside fraction prepared from three-year-old American ginseng roots was analyzed by HPLC-ESI-MS in negative ion mode. Compared with the UV trace (A_{203}), the total ion count (TIC) ionogram reveals the presence of a novel peak (RT = 27.4 min), also present in the 799 *m*/*z* selected ion trace. Selected ion traces for $[M-H]^-$ ions expected for ginsenosides (i.e., 799, 945, 1077 and 1107 *m*/*z*) are shown. Note that the offset in retention time between the UV trace and TIC ionogram is due to the length of capillary tubing linking the two detectors. Ionograms were generated with a cone voltage of 20 V.

compromising of membrane integrity (Morrissey and Osbourn, 1999). Members of the Pythiaceae, however, lack ergosterol (Schlösser et al., 1969; Weete, 1989), the main membrane sterol of higher fungi, and likely lack the ability to synthesize sterols in general (Marshall et al., 2001). Interestingly, when sterols are added to growth media, pythiaceous fungi readily incorporate them into their membranes (Mikes et al., 1997; Panabières et al., 1997). The lack of sterols in the membrane of these fungi has been used to explain previous observations of an absence of antifungal activity of saponins (Arneson and Durbin, 1968; Olsen, 1971) as well as polyene antibiotics (Schlösser et al., 1969). Although some researchers have been able to induce saponin toxicity in members of the Pythiaceae through the addition of sterols to the growth medium (Olsen, 1973; Steel and Drysdale, 1988) we were not able to repeat this finding. By contrast, the addition of ergosterol to the growth medium resulted in an increase in the growth of *Py. irregulare* to the same extent as that observed when ginsenosides were added. The incorporation of both ginsenosides and ergosterol resulted in an additive effect, producing the greatest growth increase overall for *Py. irregulare* and *P. cactorum* (Table 1). Since the tetracyclic ginsenosides (Fig. 1) are structurally similar to phytosterols and are derived from the same cytosolic mevalonate pathway (Chappell, 2002) they may function in a similar manner as phytosterols (i.e. enhancement of growth and spore production, Nes et al., 1982) when utilized by members of the Pythiaceae. These observations suggest that the strategy of enhancing resistance to pythiaceous pathogens by engineering the sterol content of soybean (Marshall et al., 2001) may not prove fruitful as this plant also contains saponins (Gu et al., 2002).

2.4. Effect of purified ginsenosides at a low concentration on fungal growth

Overall, our data are consistent with the leaching/exudation of ginsenosides from the roots of ginseng plants in the field, but the question remains as to whether the quantities measured are sufficient to affect fungal growth. To address this, we tested the effect of the addition of purified saponins (>95% by HPLC) to growth media at an ecologically relevant concentration (i.e., 0.06%) on the growth of *Py. irregulare* and *T. hamatum* (a saponin-sensitive fungus; Nicol et al., 2002). Under these conditions the growth of *Py. irregulare* was significantly stimulated, whereas that of *T. hamatum*

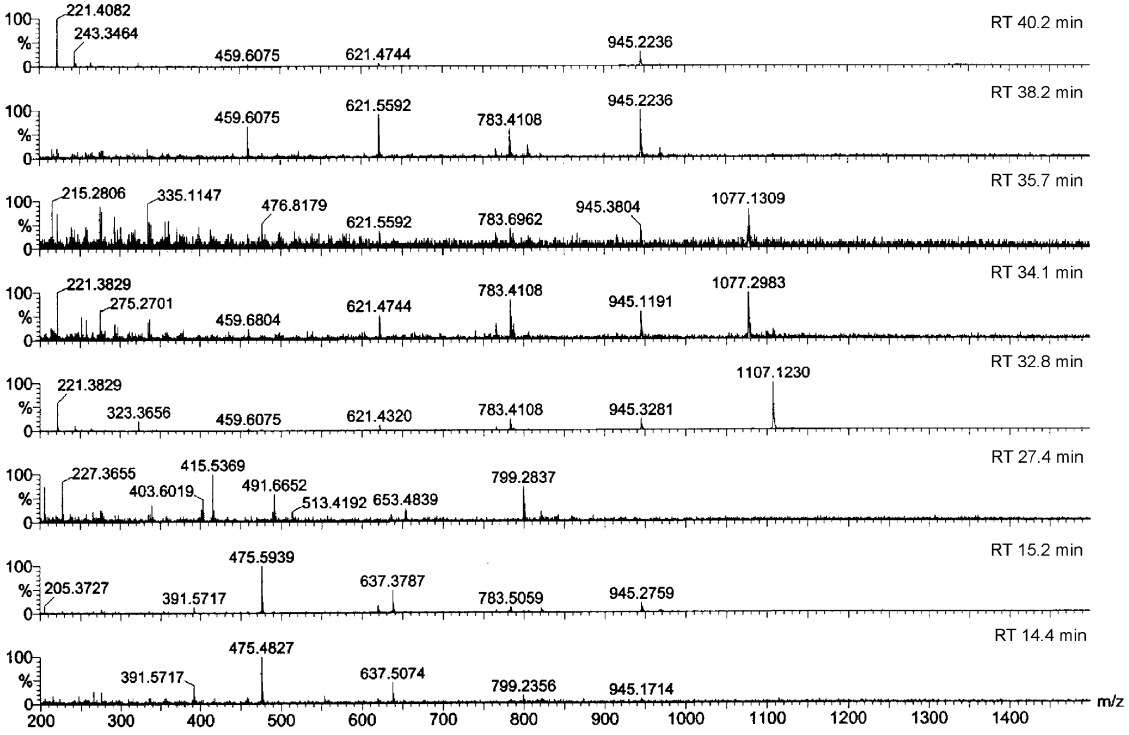


Fig. 3. ESI-Mass spectra of ginsenosides at high cone voltage. A sample of the purified ginsenoside fraction prepared from three-year-old American ginseng roots shown in Fig. 2 was re-analyzed by HPLC-ESI-MS in negative ion mode, using a high cone voltage (100 V) to promote in-source collision-induced fragmentation. Spectra were collected at the retention times (RT) shown. Characteristic protopanaxatriol aglycone peaks (475 *m*/*z*) for ginsenosides Rg_1 (RT 14.4 min) and Re (RT 15.2 min) are evident, along with small amounts of the $[M-H]^-$ peak and appropriate fragments. Similarly, the protopanaxadiol aglycone (459 *m*/*z*) is evident in the spectra for Rb_1 (RT 32.8 min), Rc (RT 34.1 min), Rb_2 (RT 35.7 min) Rd (RT 40.2 min) and an unidentified ginsenoside (RT 38.2 min), along with appropriate fragments and a significant $[M\text{-}H]^-$ peak. Pseudoginsenoside F_{11} is evident at RT 27.4 min.

was slightly (but not significantly) inhibited (Table 2). These results are consistent with our earlier report that a crude ginsenosides preparation inhibited the in vitro growth of some fungi (especially *Trichoderma* spp.) in a dose dependent manner, while stimulating that of *Cylindrocarpon destructans*, a major soilborne ginseng pathogen (Nicol et al., 2002).

At this stage, it is not possible to distinguish how ginsenosides stimulate the growth of pythiaceous fungi. That is, it is not known if the added ginsenosides (1) served as a carbon source (2) exerted an effect on the structure or function of the fungal membrane (Marshall et al., 2001) or (3) acted as precursors or analogues of unidentified hormones in the Pythiaceae (Elliott, 1994). Preliminary results in our lab suggest that pythiaceous fungi metabolize ginsenosides differently than saponin-sensitive fungi, but more work needs to be done on the precise mode of action of saponins upon pythiaceous pathogens.

3. Conclusions

The discovery of ginsenosides in the rhizosphere at biologically active concentrations coupled with the differential response of soilborne fungi to them (Tables 1 and 2; Nicol et al., 2002), provides evidence that secondary phytochemicals could influence the species composition of the soil fungal community. This allelopathic effect may be a factor in the fungal diseases of ginseng as the chemical environment of the rhizosphere would likely favour the growth of *Py. irregulare* as well

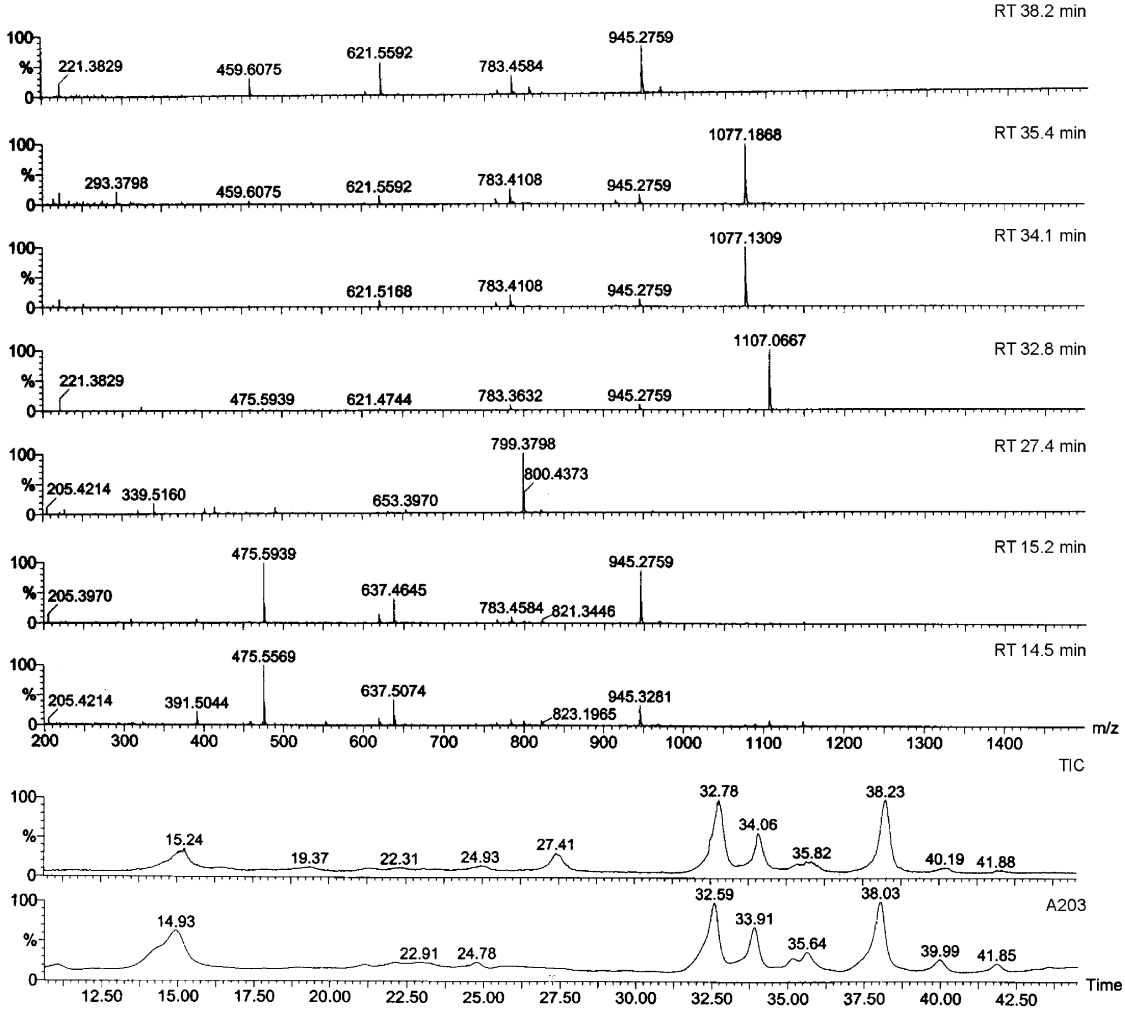


Fig. 4. HPLC-MS analysis of ginseng root-associated soil extracts. A sample of purified ginsenosides isolated from root-associated soil extracts was analyzed by HPLC-ESI-MS in negative ion mode, using a high cone voltage (100 V) to promote in-source collision-induced fragmentation. The lower two traces show the UV (203 nm) chromatogram and TIC ionogram, respectively. Mass data were collected at the retention times (RT) shown for the upper seven spectra. Characteristic protopanaxatriol aglycone peaks (475 *m*/*z*) for ginsenosides Rg_1 (RT 14.5 min) and Re (RT 15.2 min) are evident, along with small amounts of the $[M-H]^-$ peak and appropriate fragments. Note that the spectrum for Rg_1 (RT 14.5 min) contains *m*/*z* peaks from the more abundant, overlapping Re peak (RT 15.2 min). The protopanaxadiol aglycone (459 *m*/*z*) is evident in the spectra for Rb_1 (RT 32.8 min), Rc (RT 34.1 min), Rb_2 (RT 35.8 min) an Rd-like compound (RT 38.2 min) and Rd (RT 40.2 min; not shown), along with appropriate fragments and a significant $[M-H]^-$ peak. Pseudoginsenoside F_{11} is evident at RT 27.4 min.

as other fungal pathogens while inhibiting or leaving the growth of other fungi unaltered (Nicol et al., 2002).

The identification and testing of phytochemicals as potential allelopathic compounds presented here complements observations that different plants have a characteristic community of soil microbes growing around them (Azad et al., 1987; Carter et al., 1999; Grayston et al., 2001; Kandeler et al., 2002; Marschner et al., 2001; Miethling et al., 2000; Miller et al., 1989). Obviously, the influence of phytochemicals is one of many possible factors contributing to microbial growth in soil and complete elucidations of how plant/fungal communities interact should take this into account.

4. Experimental

4.1. Fungal bioassays

Trichoderma hamatum (DAOM 215955), *Phytophthora cactorum* (CCFC 000009) and two isolates of *Pythium irregulare* (CCFC 000299 and CCFC 000245) were obtained from the Canadian Collection of Fungal Cultures (Agriculture and Agri-Food Canada, Ottawa, ON). Preliminary tests using a crude ginseng saponin fraction showed no difference in the growth responses between the two isolates of *Py. irregulare*, so thereafter only one isolate (CCFC 000299) was used. Cultures were maintained on potato dextrose agar (PDA) and incubated in the dark at 25 °C.

Two sets of bioassays were completed using medium prepared with pluronic F-127 (100 g per liter water) as the solidifying agent (Gardener and Jones, 1984). This solidifying agent has the unusual property of liquefying when cooled, allowing for the recovery of hyphae via filtration (see below). For the first set of bioassays, a crude ginsenoside fraction obtained from American ginseng root (Nicol et al., 2002) was used as the saponin treatment, and the pluronic F-127 growth medium was supplemented with nutrients (17 g/l Czapek-Dox broth, or for the *P. cactorum* saponin-ergosterol bioassay only, 3% dextrose plus 0.3% casamino acids and tap water). For the second set of assays, a purified ginsenoside treatment (see below) was used as the sole carbon source for *Py. irregulare* and *T. hamatum*. Since not enough ginsenosides were isolated from soil to enable

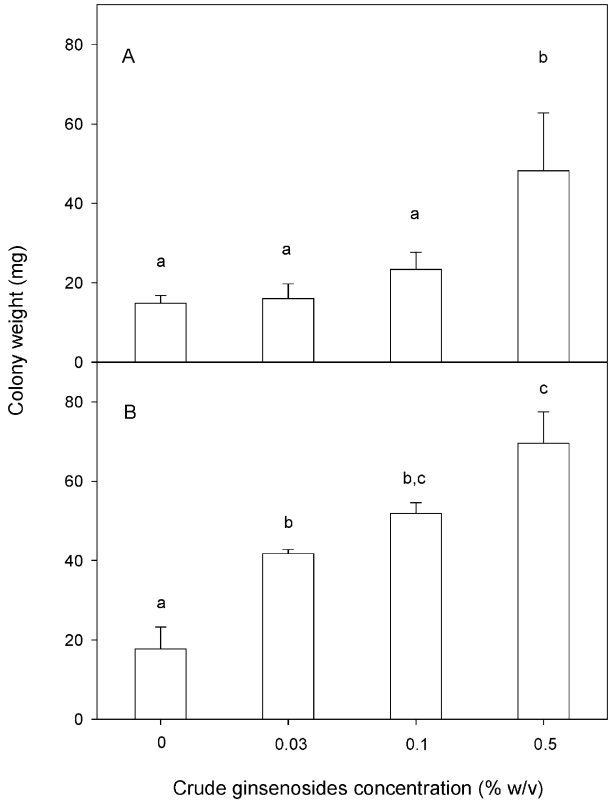


Fig. 5. Dose response of *Phytophthora cactorum* and *Pythium irregulare* to ginsenosides. Fungi were cultured on media supplemented with a crude ginsenoside preparation at concentrations ranging from 0% of the final volume of medium (control) to 0.5% (5 mg ml^{-1}). The growth of *Phytophthora cactorum* (A) and *Pythium irregulare* (B) was measured by weighing fungal hyphae trapped on a 0.45 μm filter (see Experimental). The bars are means±standard deviation ($n=5$). Bars labelled with the same letter were not significantly different via a Tukey's test.

Table 1
Effect of ginsenosides and ergosterol on the growth of *Pythium irregulare* and *Phytophthora cactorum* in vitro. Mean colony weights were measured for fungi cultured on medium alone (control) or media supplemented with 1 mg ml^{-1} crude ginsenosides, 20 μg ml^{-1} ergosterol or a combination of both

Treatment	Mean colony weight (mg)[a]	
	Pythium irregulare	*Phytophthora cactorum*
Control	8.4±2.7a	26.7±13.0a
Ergosterol	31.3±7.0b	38.4±5.3a
Ginsenosides	27.5±8.9b	31.3±5.1a
Ergosterol + ginsenosides	75.2±30.3c	50.5±9.1b

[a] Values are a mean ($n=5$)±standard deviation. Means followed by the same letter are not significantly different from each other (within species) by way of a Tukey's test.

Table 2
Effect of ginsenosides on the growth of *Pythium irregulare* and *Trichoderma hamatum* in vitro. The fungi were cultured on an artificial medium (control) or artificial medium supplemented with an ecologically relevant concentration (0.06% w/v) of purified (>95%) ginsenosides

Treatment	*Py. irregulare* weight (mg)	*T. hamatum* weight (mg)
Control	1.1±0.5[a]	6.4±1.3
Ginsenosides 0.06%	4.0±1.9*	6.0±2.3

[a] Values are a mean ($n=3$–5)±standard deviation. Treatments followed by * are significantly different from control by way of a U-test.

replicated testing, the crude root extract used for the first set of assays was further purified using C_{18} and aminopropyl Extract-Clean™ solid phase extraction tubes (Alltech Canada, Guelph, ON) according to Li et al. (1996) and added to the medium at a concentration (0.06% w/v) that simulated saponin levels found in soil.

For both sets of assays, saponins were dissolved in deionized or tap (no added nutrients bioassay) water, filter-sterilized through a 0.22 μm PVDF syringe tip membrane and added to cold (i.e. liquid) pluronic F-127 growth medium. Plates were inoculated with 5.6 mm plugs cut with a cork bore from the edge of actively growing fungal cultures on PDA (crude ginsenosides bioassay) or 1.5% water agar (purified ginsenosides bioassay), and incubated in the dark at 25 C. Experiments were terminated after 4–7 days by placing the Petri dishes in a refrigerator (which caused the medium to liquefy) followed by dilution with 50 ml of ice-cold deionized water and filtration through a pre-weighed 0.45 μm nylon membrane using a vacuum filtration unit. Hyphae caught on the surface of the membrane were gently washed with ice-cold deionized water and the membranes plus hyphae dried overnight at 72 °C and weighed.

4.2. Soil collection and extraction

Three-year-old American ginseng plants were collected in late August or September of 1999, 2000, 2001 and 2002 from a ginseng farm near Delhi, Ontario, Canada by digging up the roots and shaking off loose soil. Root-associated soil was removed with a brush and sieved through a 208 μm screen (Dalton et al., 1987) to remove plant debris. The soil was dried at 45 °C for 24 h and then extracted for 24 h with MeOH:H_2O (80:20) on a gyratory shaker. The liquid extract was filtered under vacuum and the MeOH removed by rotary evaporation. The remaining aqueous extract was partitioned three times with $CHCl_3$ and three times with water-saturated *n*-BuOH (Ma et al., 1999). Ginsenosides were measured in the *n*-BuOH fraction.

4.3. Root exudate trapping system

A root exudate trapping system was built based loosely on the design of Tang and Young (1982). Under greenhouse conditions, two two-year-old plants were placed in washed coarse silica sand and watered approximately four times per week. Water that drained from the pots first passed through a glass column (1.5 cm i.d.×20 cm long) packed with washed Amberlite XAD-4 resin (Sigma-Aldrich Canada Limited, Oakville, ON). After 22 days, the XAD-4 columns were eluted with 100 ml MeOH followed by 50 ml dichloromethane and the eluants combined, filtered and concentrated under reduced pressure.

4.4. Chromatographic analysis

Root extracts, root exudates and soil extracts were analyzed via HPLC as described earlier (Nicol et al., 2002). For co-chromatography and calibration, authentic standards of ginsenosides Rg_1, Re, Rb_1, Rc, Rb_2 and Rd, were obtained from INDOFINE Chemical Company Inc (Somerville, NJ). For HPLC-MS, samples were processed as above except that one-fifth of the eluent (0.2 ml min^{-1}) was mixed with 1% aqueous ammonia (0.05 ml min^{-1}) and introduced into the electrospray ionization source of a Micromass Model LCT mass spectrometer operating in the negative ion mode with a desolvation gas flow of 400 l h^{-1} and a cone gas flow of 12 l h^{-1}. Samples were ionized at a capillary voltage of 3000 V. Mass spectra were recorded between 200 and 1500 m/z with the cone voltage either at 20 V to maximize production of $[M-H]^-$ ions or at 100 V to promote the formation of in-source collision-induced fragment ions.

4.5. Statistical analysis

Using SYSTAT™ (SPSS Science, Chicago IL), the fungal growth response to increasing concentrations of crude ginsenosides was analyzed via an ANOVA followed by a Tukey's means comparison. The fungal response to soil ginsenoside levels was analyzed using a *t*-test or the nonparametric equivalent. Data that violated the assumptions of equal variance and normal distribution were log or square root transformed before analysis.

Acknowledgements

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References

Arneson, P.A., Durbin, R.D., 1968. The sensitivity of fungi to α-tomatine. Phytopathology 58, 536–537.

Azad, H.R., Davis, J.R., Schnathorst, W.C., Kado, C.I., 1987. Influence of verticillium wilt resistant and susceptible potato genotypes on populations of antagonistic rhizosphere and rhizoplane bacteria and free nitrogen fixers. Appl. Microbiol. Biotech. 26, 99–104.

Bowyer, P., Clarke, B.R., Lunness, P., Daniels, M.J., Osbourn, A.E., 1995. Host range of a plant pathogenic fungus determined by a saponin detoxifying enzyme. Science 267, 371–374.

Carter, J.P., Spink, J., Cannon, P.F., Daniels, M.J., Osbourn, A.E., 1999. Isolation, characterization, and avenacin sensitivity of a diverse collection of cereal root-colonizing fungi. Appl. Env. Microbiol. 65, 3364–3372.

Chappell, J., 2002. The genetics and molecular genetics of sterol and terpene origami. Curr. Op. Plant Biol. 5, 151–157.

Dalton, B.R., Sterling, B.W., Blum, U., 1987. Plant phenolic acids in soils: a comparison of extraction procedures. Soil Sci. Soc. Am. J. 51, 1515–1521.

Elliott, C.G., 1994. Reproduction in Fungi. Chapman and Hall, London.

Fuzzati, N., Gabette, B., Jayakar, K., Pace, R., Peterlongo, F., 1999. Liquid chromatography-electrospray mass spectrometric identification of ginsenosides in Panax ginseng roots. J. Chromatography A 854, 69–79.

Gardener, S., Jones, J.G., 1984. A new solidifying agent for culture media which liquefies on cooling. J. Gen. Microbiol 130, 731–733.

Grayer, R.J., Harborne, J.B., 1994. A survey of antifungal compounds from higher plants, 1982-1993. Phytochemistry 37, 19–42.

Grayston, S.J., Griffith, G.S., Mawdsley, J.L., Campbell, C.D., Bardgett, R.D., 2001. Accounting for variability in soil microbial. communities of temperate upland grassland ecosystems. Soil Biol. Biochem. 33, 533–551.

Gu, L., Tao, G., Gu, W., Prior, R.L., 2002. Determination of soya saponins in soy with LC-MS following structural unification by partial alkaline degradation. J. Agric. Food Chem. 50, 6951–6959.

Harborne, J.B., 1993. Introduction to Ecological Biochemistry, 4th Edition. Academic Press, London. 244–245.

Kandeler, E., Marschner, P., Tscherko, D., Gahoonia, T.S., Nielsen, N.E., 2002. Microbial community composition and functional diversity in the rhizosphere of maize. Plant and Soil 238, 301–312.

Kokubun, T., Harborne, J.B., Eagles, J., 1994. 2′,6′-dihydroxy-4′-methoxyacetophenone, a phytoalexin from the roots of Sanguisorba minor. Phytochemistry 35, 331–333.

Kokubun, T., Harborne, J.B., 1995. Phytoalexin induction in the sapwood of plants of the Maloideae (Rosaceae): biphenyls or dibenzofurans. Phytochemistry 40, 1649–1654.

Levanon, D., Henis, Y., Okon, Y., Dovrat, A., 1982. Alfalfa saponins and microbial transformations of nitrogen in peat. Soil Biol. Biochem. 14, 501–504.

Levy, M., Zehavi, U., Naim, M., Itzhack, P., 1986. An improved procedure for the isolation of medicagenic acid 3-O-β-D-glucopyranoside from alfalfa roots and its antifungal activity on plant pathogens. J. Agric. Food Chem. 34, 960–963.

Li, T.S.C., Mazza, G., Cottrell, A.C., Gao, L., 1996. Ginsenosides in roots and leaves of American ginseng. J. Agric. Food Chem. 44, 717–720.

Ma, W.G., Mizutani, M., Malterud, K.E., Lu, S.L., Ducrey, B., Tahara, S., 1999. Saponins from the roots of *Panax notoginseng*. Phytochemistry 52, 1133–1139.

Marschner, P., Yang, C.H., Liebereia, R., Crowley, D.E., 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol. Biochem. 33, 1437–1445.

Marshall, P.S., Harborne, J.B., King, G.S., 1987. A spiroketalenol ether phytoalexin from infected leaves and stems of *Coleostephus myconis*. Phytochemistry 26, 2493–2494.

Marshall, J.A., Dennis, A.L., Kumazawa, T., Haynes, A.M., Nes, W.D., 2001. Soybean sterol composition and utilization by *Phytophthora sojae*. Phytochemistry 58, 423–428.

Miethling, R., Wieland, G., Backhaus, H., Tebbe, C.C., 2000. Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L33. Micobiol. Ecol. 40, 43–56.

Mikes, V., Milat, M.L., Ponchet, M., Ricci, P., Blein, J.P., 1997. The fungal elicitor cryptogein is a sterol carrier protein. FEBS Lett. 416, 190–192.

Miller, H.J., Henken, G., van Veen, J.A., 1989. Variation and composition of bacterial populations in the rhizosphere of maize, wheat and grass cultivars. Can. J. Microbiol. 35, 656–660.

Morrissey, J.P., Osbourn, A.E., 1999. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Microbiol. Mol. Biol. Rev. 63, 708–724.

Nes, W.D., Saunders, G.A., Heftmann, E., 1982. Role of steroids and triterpenoids in the growth and reproduction of *Phytophthora cactorum*. Lipids 17, 178–183.

Nicol, R.W., Traquair, J.A., Bernards, M.A., 2002. Ginsenosides as host resistance factors in American ginseng (*Panax quinquefolius*). Can. J. Bot 80, 557–562.

Olsen, R.A., 1971. Triterpeneglycosides as inhibitors of fungal growth and metabolism 1. Effect on growth, endogenous respiration and leakage of UV-absorbing material from various fungi. Physiol. Plant 24, 534–543.

Olsen, R.A., 1973. Triterpeneglycosides as inhibitors of fungal growth and metabolism. 6. The effect of aescin on fungi with reduced sterol contents. Physiol. Plant 29, 145–149.

Osbourn, A.E., 1996. Preformed antimicrobial compounds and plant defense against fungal attack. Plant Cell 8, 1821–1831.

Panabières, F., Ponchet, M., Allasia, V., Cardin, L., Ricci, P., 1997. Characterization of border species among Pythiaceae: several *Pythium* isolates produce elicitins, typical proteins from *Phytophthora* spp. Mycol. Res. 101, 1459–1468.

Papadopoulou, K., Melton, R.E., Leggett, M., Daniels, M.J., Osbourn, A.E., 1999. Compromised disease resistance in saponin-deficient plants. Proc. Nat. Acad. Sci. USA 96, 12923–12928.

Punja, Z.K., 1997. Fungal pathogens of American ginseng (*Panax quinquefolium*) in British Columbia. Can. J. Plant Pathol. 19, 301–306.

Reeleder, R.D., Brammall, R.A., 1994. Pathogenicity of *Pythium* species, *Cylindrocarpon destructans* and *Rhizoctonia solani* to ginseng seedlings in Ontario. Can. J. Plant Pathol. 16, 311–316.

Robeson, D.J., Ingham, J.L., Harborne, J.B., 1980. Identification of two chromone phytoalexins in the sweet pea, *Lathyrus odoratus*. Phytochemistry 19, 2171–2173.

Sandrock, R.W., Van Etten, H.D., 1998. Fungal sensitivity to and enzymatic degradation of the phytoanticipin α-tomatine. Phytopathology 88, 137–143.

Schlösser, E., Shaw, P.D., Gottlieb, D., 1969. Sterols in species of *Pythium*. Arch. Mikrobiol. 66, 147–153.

Senevirante, G.I., Harborne, J.B., 1992. Constitutive flavonoids and induced isoflavonoids as taxonomic markers in the genus *Vigna*. Biochem. Syst. Ecol. 20, 459–467.

Steel, C.C., Drysdale, R.B., 1988. Electrolyte leakage from plant and fungal tissues and disruption of liposome membranes by α-tomatine. Phytochemistry 27, 1025–1030.

Takechi, M., Tanaka, Y., 1990. Structure-activity relationships of the saponin α-hederin. Phytochemistry 29, 451–452.

Tang, C.S., Young, C.S., 1982. Collection and identification of allelopathic compounds from the undisturbed root system of bigalta limpograss (*Hemarthria altissima*). Plant Physiol. 69, 155–160.

Turner, E.M.C., 1956. The nature of the resistance of oats to the take-all fungus. II. Inhibition of growth and respiration of *Ophiobolus graminis* Sacc. and other fungi by a constituent of oat sap. J. Exp. Bot. 7, 80–92.

Weete, J.D., 1989. Structure and function of sterols in fungi. Adv. Lipid Res. 23, 115–167.