愛媛大学農学部農場報告
(Bull. Exp. Farm Fac. Agr., Ehime Univ.) 28 : 9 −15 (2006)

Peach Root-Bark Extracts Inhibit Tomato Root Growth *In Vitro*

Kipkoriony L. Rutto [1, 2] and Fusao Mizutani [1]

Summary

An experiment was carried out to study the effect of crude peach root-bark extracts on tomato root growth *in vitro*. Crude extracts were prepared by repeated extraction of a 10g ground peach-root-bark sample in 80% ethanol. The crude ethanolic extracts were dried *in vacuo*, taken up in water and used to design experiments to assay the effect of different concentrations on the growth of excised tomato roots *in vitro*. There was a significant reduction in root growth at extract concentrations above 1.0mg L^{-1} root-bark (dry weight equivalent). Tomato root growth was completely inhibited at 1000mg L^{-1}. Results from this study show that phytochemicals in peach root-bark have a negative impact on root growth.

Introduction

Allelopathy is the inhibition of one plant species by another due to the release of chemical substances. It is a common phenomenon in nature and many plant species have been shown to inhibit the growth of neighboring or successional plants through the release of chemicals into the soil either as exudates from living tissues or after the decomposition of plant residues[13].

Among the cultivated species that have been confirmed to express allelopathy, or autotoxicity, (a form of allelopathy where the donor plant and the inhibited recipient are of the same species) include ; barley, alfalfa, strawberry, taro and cucumber among others. It is also likely that autotoxicity may be a root cause of the replant problem as observed in some cultivated tree species. Peach is among the tree species that suffer from replant failure though research has failed to identify a specific cause for seedling inhibition in replant soils. It appears that multiple factors, working in tandem are responsible for the replant failure in peach. Proebsting and Gilmore[9] implicated the decomposition products of peach root residue, but recent studies indicate a more

1) The Experimental Farm, Faculty of Agriculture, Ehime University, Hattanji 498, Matsuyama, Ehime 799-2424, Japan
2) Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology (JKUAT), PO Box 62000, Nairobi 00200, Kenya

complex relationship involving plant residues, soil microflora and other factors that interact to precipitate plant growth suppression[2].

Bioassays are convenient for testing the allelopathic properties of plant products. Visual observations suggesting inhibition of one species by another can be easily followed up by sampling and extraction of potentially active compounds that can then be assayed on test plants in pot or *in vitro* experiments. Interesting reports on the activity and mode of action of allelopathic extracts have been published after such bioassays, *e.g.* lettuce seedling growth inhibition by a steroidal glycoalkaloid isolated from *Solanum arundo* root-bark extracts[4], and the reduced leaf transpiration, leaf stomatal conductance and intracellular CO_2 in cucumber after exposure to autotoxic compounds in root exudates and aqueous root extracts[13].

In our study, we assayed the effect of peach root-bark extracts on the growth of excised tomato roots *in vitro*.

Materials and Methods

Preparation of peach-root-bark crude extracts and fractions

Root bark collected from 10-year-old peach trees was dried in an oven to constant weight then ground to a fine powder. A 10g sample of the root powder was extracted three times in 1000mL of 80% ethanol for 24hrs at 2°C. The filtrate was bulked and the ethanol fraction removed by evaporation *in vacuo*. The extract was made up to 100ml with distilled water and used to assay the effect of peach root-bark extracts on the growth of excised tomato roots *in vitro*.

Effect of peach-root-bark extracts on root growth in solid media

Tomato (*Lycopersicon esculentum* Mill. cv. 'Kyoryokubeiju') seed was sterilized consecutively in 15% sodium hypochlorite and 95% ethanol, rinsed in sterile distilled water and germinated *in vitro* in 4% agar (Wako, Japan). The roots were used to set up experiments to study the effect of peach-root-bark extracts on root growth *in vitro*. In the solid media experiment, five levels (0, 1, 10, 100 and 1000 mg L^{-1} DW equivalent) of root-bark extract were taken up in sterile M-medium in 75mm ø Petri dishes[1]. The composition of the medium (mg L^{-1}) was as follows : $MgSO_4 \cdot 7H_2O$, 731 ; KNO_3, 80 ; KCl, 65 ; KH_2PO_4, 4.8, $Ca(NO_3)_2 \cdot 4H_2O$, 288 ; NaFeEDTA, 8 ; KI, 0.75 ; $MnCl_2 \cdot 4H_2O$), 6 ; $ZnSO_4 \cdot 7H_2O$, 2.65 ; H_3BO_3, 1.5 ; $CuSO_4 \cdot 5H_2O$, 0.13 ; $Na_2MoO_4 \cdot 2H_2O$, 0.0024 ; glycine, 3 ; thiamine, 0.1 ; pyridoxine, 0.1 ; nicotinic acid, 0.5 ; myo-inositol, 50 ; sucrose 10000 and Gelrite®, 4000. Media pH was adjusted to 5.5 and the different concentrations of bark extract treatments added before autoclaving. In the clean bench, 5-7cm lengths of excised tomato root were transferred into the Petri dishes before sealing with film. There were 10 replications (dishes) per treatment including a blank control and the dishes were incubated in the dark at 25°C.

The experiment was terminated after 8 weeks (Fig. 1A), and the dishes kept in a deep freezer at −20°C for 12h to separate the gelling agent from the liquid media. After thawing, roots were separated from the media and fresh weight was measured after blotting with filter paper.

Effect of peach-root-bark extracts on root growth in liquid media

The media used and the treatment levels were as described for the experiment using solid media,

with each treatment having five replications. Two-week old tomato rootlets were transferred into 50ml flasks containing 30mL of liquid M media (minus the gelling agent but with sucrose) and incubated at 22°C with continuous agitation on a rotary shaker set at 100 rpm. The experiment was terminated 8 weeks (Fig. 1B), and fresh root weight determined as described above. Media pH was measured using a Horiba pH meter, and root length (Fig. 1C) was estimated by summing up respective lengths of root pieces arrayed on a grid.

Statistical analysis

Descriptive statistics were done on Ms Excel (2000). Some of the data was subjected to analysis of variance using a trial version of the statistical software 'Analyze-it®' (http://www.analyse-it.com/). Pair-wise comparisons were carried out and means separated by the Bonferroni test.

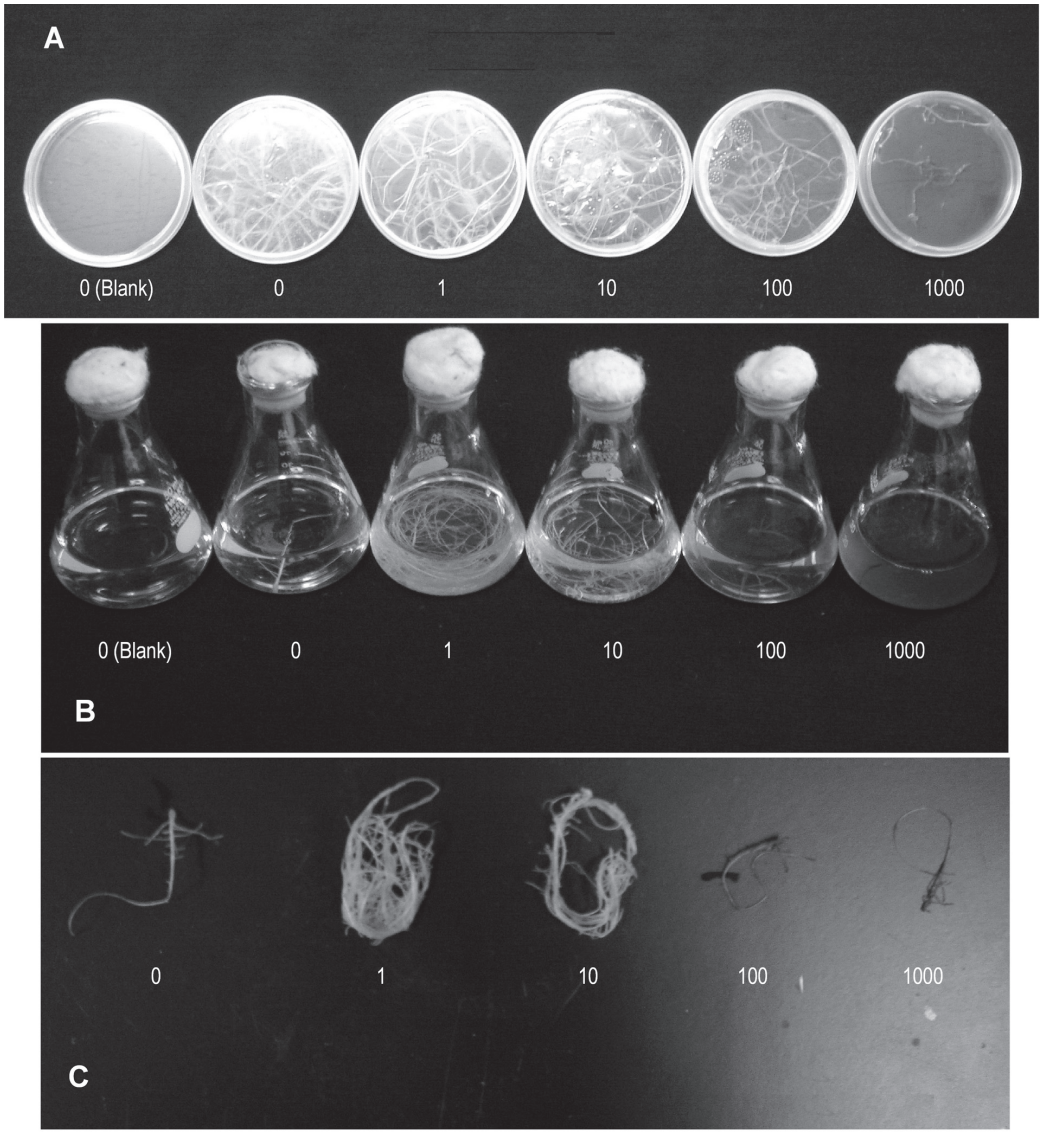

Fig. 1. Tomato root growth in solid (A) or liquid (B, C) M-media amended with different concentrations of peach root-bark extracts. Dishes were incubated in the dark at 25℃ for 8 weeks, while flasks were covered with aluminum foil and shaken at 100rpm for a similar period with room temperature being maintained at 22℃.

Results

Tomato root growth

There was a decline in root growth with increasing concentrations of the root bark extract beyond 1.0mg L^{-1}. Growth was completely inhibited at 100 mg L^{-1} (Fig. 3), and 1000mg L^{-1} (Fig. 2) dry weight equivalent of the root bark extract, in the liquid and solid media experiment, respectively. At the termination of the experiment, root fresh weight was highest in the 1.0mg L^{-1} treatment in both experiments, and significantly lower than in all the other treatments in the 1000mg L^{-1} solid media treatment. Root length in the liquid media experiment followed a trend similar to that of fresh weight with length being significantly higher at 1 and 10 mg L^{-1} dry weight root bark equivalent (Fig. 4).

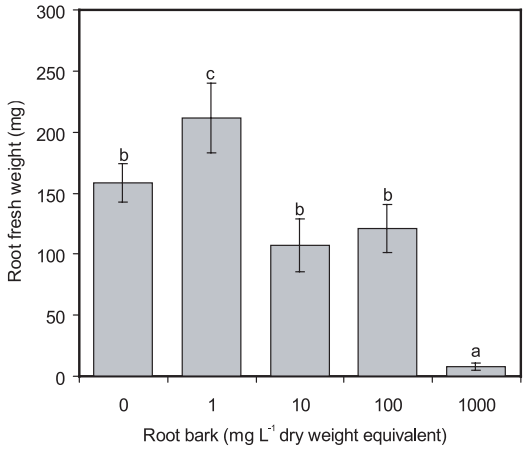

Fig. 2. Tomato root fresh weight after culturing for 8 weeks in solid M-media amended with different concentrations of peach root bark extract. Roots were grown *in vitro* in the dark at 25℃. Different column letters denote significant differences at $p<0.05$ (Bonferroni test) and bars represent SE of means (n=10).

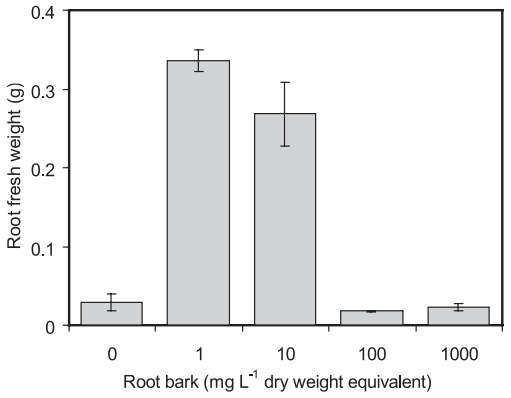

Fig. 3. Tomato root fresh weight after culturing for 8 weeks at 22℃ in liquid M-media amended with different concentrations of peach-root-bark extracts. Bars represent SE (n=5).

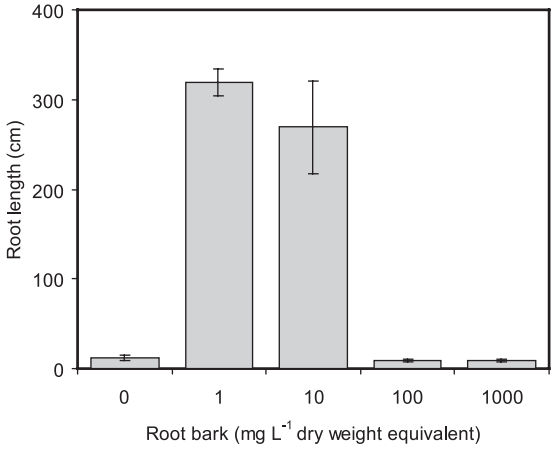

Fig. 4. Tomato root length after culturing for 8 weeks at 22℃ in liquid M-media amended with different concentrations of peach root bark extract. Bars represent SE (n=5).

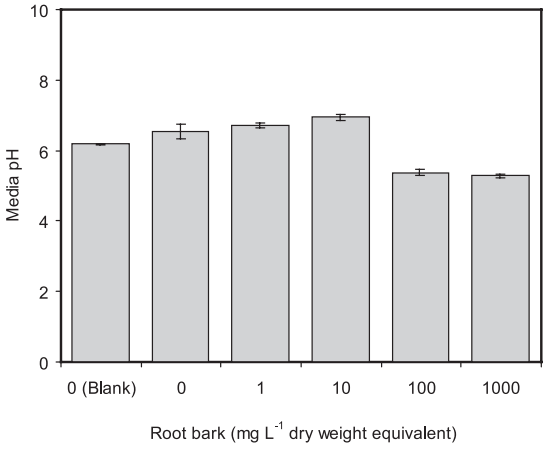

Fig. 5. Media pH after 8 weeks in an experiment where tomato roots were cultured in liquid M-media amended with different concentrations of peach root bark extract. Bars represent SE (n=5).

Change in media properties after root growth

There was no significant change in liquid media pH over the duration of the experiment. The addition of peach-root-bark extracts did not affect media pH, though the media became more alkaline with increasing root growth, with the highest increase being observed in the 10 mg L^{-1} treatment. (Fig. 5)

Discussion

The results presented in this study show that compounds found in peach root-bark extracts inhibit tomato root growth *in vitro*. However, the mechanism by which the inhibition is accomplished cannot be explained from the results of this study.

Several identified and putative compounds with allelopathic properties have been isolated from different plant parts and species. For example, in alfalfa, toxic substances capable of delaying seed germination and reducing root elongation were extracted from leaves[3]. Compounds with similar allelopathic properties were also isolated from pea (*Pisum sativum*) residue[7], *Solanum arundo* root bark[4], cucumber roots[13], cattail (*Typhus domingensis*) roots[5], and the aerial parts of *Prunus armeniaca*[10]. Further, it was found that *Prunus armeniaca* trees retarded germination, growth and yield in nearby *Triticum aestivum* plants. The observation by Rawat et al,[10] that the magnitude of interference decreased away from the tree agrees with our results for peach seedlings grown within and outside the drip line of mature peach trees[12].

The compounds responsible for the allelopathic properties in *Prunus* are likely to be by-products of prunasin and amygdalin, both found in relatively high quantities in *Prunus* spp. Prunasin and amygdalin are readily converted into hydrogen cyanide(HCN) by soil microbes during decomposition[2, 6]. The mechanism by which HCN could affect plant growth may be through interference with physiological processes. Hydrogen cyanide is a well-known inhibitor of mitochondrial function, and exogenous HCN may inhibit plant mitochondrial activity by blocking cytochrome oxidase activity. Endogenous HCN may also disrupt plant metabolism if enzyme mediated detoxification of the HCN released during ethylene biosynthesis is impaired. Mizutani et al.[8] extracted a compound capable of suppressing plant cyanide metabolism from peach tannins.

The allelopathic properties of peach root bark extracts are further confirmed in this study with the implication that autotoxicity may partly be responsible for the peach replant problem. It is clear from both studies that the growth limiting effect of the root bark extract increases at higher concentrations, and reaches a maximum at between 10 and 100mg L^{-1}, and between 100 and 1000mg L^{-1} (root bark dry weight equivalent) in the case of liquid and solid media, respectively.

The reason for the lack of growth in the liquid media control treatment (0mg L^{-1} root bark dry weight equivalent) is not clear and repeat experiments will be carried out to clarify this point. Additionally, the possibility of treatment related changes in nutrient availability, and osmotic properties of the media will be examined.

Literature Cited

(1) Bécard, G. and J. A. Fortin. 1988. Early events of vesicular-arbuscular mycorrhizal formation of Ri T-DNA transformed roots. New Phytol. 108 : 211-218.

(2) Benizri, E., S. Piutti, S. Verger, L. Pagès, G. Vercambre, J. L. Poessel and P. Michelot. 2005. Replant diseases : Bacterial community structure and diversity in peach rhizosphere as determined by metabolic and genetic fingerprinting. Soil Biol. Biochem. 37 : 1738-1746.

(3) Chon, S-U., C. J. Nelson and J. H. Couts. 2004. Osmotic and autotoxic effects of leaf extracts on germination and seedling growth of alfalfa. Agron. J. 96 : 1673-1679.

(4) Fukuhara, K., K. Shimizu and I. Kubo. 2004. Arudonine, an allelopathic steroidal glycoalkaloid from the root bark of *Solanum arundo* Mattei. Phytochemistry 65 : 1283-1286.

(5) Gallardo, M. T., B. B. Martin and D. F. Martin. 1998. Inhibition of water fern *Salvinia minima* by cattail (*Typha domingensis*) extracts and by 2-chlorophenol and salicylaldehyde. J. Chem. Ecol. 24 : 1483-1490.

(6) Gur, A. and Y. Cohen. 1988. Causes of soil sickness in replanted peaches. 1. The role of cyanogenesis in peach soil sickness. Acta Hort. 233 : 25-31.

(7) Kato-Noguchi, H. 2003. Isolation and identification of an allelopathic substance in *Pisum sativum*. Phytochemistry 62 : 1141-1144.

(8) Mizutani, F., R. Hirota and K. Kadoya. 1988. Growth inhibiting substances from peach roots and their possible involvement in peach replant problems. Acta Hort. 233 : 37-43.

(9) Proebsting, E. and A. Gilmore. 1941. The relation of peach root toxicity to the re-establishing of peach orchards. Proc. Amer. Soc. Hort. Sci. 38 : 21-26.

(10) Rawat, M. S. M., G. Pant, D. Prasad, R. K. Joshi and C. B. Pande. 1998. Plant growth inhibitors (proanthocyanidins) from *Prunus armeniaca*. Biochem. Syst. Ecol. 26 : 13-23.

(11) Rice, E. L. 1984. Allelopathy, 2nd ed. Academic Press, Orlando, Florida.

(12) Rutto, K. L. and F. Mizutani. 2005. Replant soil and peach detritus inhibit arbuscular mycorrhizal activity and retard peach seedling growth. Bull. Exp. Farm Fac. Agr., Ehime Univ. 27 : 1-9.

(13) Yu, J. Q., S. F. Ye, M. F. Zhang and W. H. Hu. 2003. Effects of root exudates and aqueous root extracts of cucumber (*Cucumis sativus*) and allelochemicals, on photosynthesis and antioxidant enzymes in cucumber. Biochem. Syst. Ecol. 31 : 129-139.

モモの根樹皮の抽出物による
試験管内におけるトマトの根の生長抑制

キプコリオニー　L. ルット・水谷房雄

摘要

モモの根樹皮の抽出物が試験管内において、トマトの根の生長に及ぼす効果について試験を行った。乾燥したモモ根樹皮の粉末サンプル10ｇを80％のエタノールで抽出し、エタノールを蒸発させた抽出液について、濃度を変えて試験管内でトマトの根の生長を調査した。モモの根１ｍｇ/ｌの濃度で、根の生長の抑制が見られた。1000ｍｇ/ｌの濃度では完全に根の生長が抑えられた。これらの結果は、モモの根の化学物質が植物の根の生長に対して抑制効果を持つことを示している。