Water Soluble Extracts from Peach Plant Parts and Their Affect on Growth of Seedlings of Peach, Apple and Bean¹

Sung Do Oh² and Robert F. Carlson³ Department of Horticulture, Michigan State University, East Lansing, MI 48824

Additional index words. amygdalin, leaf mineral nutrition, hydrogen cyanide

Abstract. Water suspensions from seeds, root and shoots of peach (Prunus persica (L.) Batsch) influenced growth of peach, apple and bean seedlings when applied to soil of potted plants. Different levels of amygdalin were found in plant parts of peach and apple. Synthetic amygdalin applied to potted peach seedlings was not toxic. Certain nutrient elements were altered due to the soil treatment. Disposal of plant parts is suggested as a practical sanitation practice to possibly reduce peach tree decline on old soil.

Peach trees planted on old peach sites often grow poorly or not at all. Considerable research has been done on peach transplant problems which has shown that several factors are responsible for tree decline. Some authors have suggested nutritional deficiencies (4, 10, 11); others have stated that soil organisms and/or an unfavorable soil microflora are causal factors (3, 8, 9, 9)14, 28, 32); whereas, some mentioned soil nematodes being associated with the malady (15, 16, 19, 20, 21, 22, 24, 29, 31). The accumulation of toxic substances in soils have been reported as factors causing poor tree growth (6, 12, 13, 23, 26, 27, 30). One report demonstrated that toxic substances were formed during microbial decomposition of peach root residue and that amygdalin was involved (23). That is, hydrolysis of amygdalin produces hydrogen cyanide (HCN) which is toxic to roots. The amount of amygdalin is high in stone fruit seed and roots but low in stem of peach (2, 30).

The purpose of this research was to determine to what degree leachates from peach plant parts would affect growth of young seedlings.

Materials and Methods

Two-year-old dormant 'Redhaven' peach trees were used for preparing the water soluble plant suspension for treating soil in which test plants were grown. The trees were separated into tops and roots, cut up and oven dried for 48 hr. The dried plant material was ground in a Wiley mill. 'Halford' peach pits were cracked and separated into stony pericarp and seed for same use. The stony pericarp was crushed with a hammer and the seed cut into fine pieces; 50 g of the dried plant material was added to 200 ml distilled water and mixed in a blender.

'Halford' peach and 'McIntosh' apple seed were stratified and germinated, and when 10 cm in height, were transplanted into plastic pots using a prepared sterilized sand-loam soil mixture and grown in the greenhouse. When these seedlings were 35 cm high, soil treatments were begun by adding 25 ml of the prepared suspension to each plant 2 times per week, for 2 months. Control plants received same amounts of distilled water. Equal nutrient solution and water were given all plants. Fifteen plants were used per treatment, randomized 3 times, and data analyzed by complete randomized design.

In another experiment synthetic amygdalin was used to determine if it would cause retardation of growth of peach seedlings similar to that of prepared seed and pericarp suspensions. Two concentrations (500 and 1000 ppm) of amygdalin were made and peach seedlings treated as previously described by adding 2 ml amygdalin solution to each plant 2 times/week.

For further bioassays, snap beans were germinated in sterilized soil and treated with same amount of peach seed suspension.

Leaf analyses to determine nutrient level of seedlings exposed to different treatments were made 60 and 70 days following initial soil treatment. The N analyses were made by the Kjeldahl method, and K by the atomic absorption spectrophotometric method. Other elements were determined by the spectrograph.

Since amygdalin is present in some plants, analyses were made to determine relative amounts present in seeds, shoots, and roots of apple and peach. The steam distillation method for

Water suspension	Seedling ht (cm)		Root dry wt (g)		Avg no. shoots/sdlg.	Total length (cm) shoots/sdlg	Plant survival (%)	
from:	Apple	Peach	Apple	Peach	Peach	Peach	Apple	Peach
Seed	19.0	41.5	1.5	14.7	4	87.4	60	46
Root		50.5	_	15.2	7	143.5	100	100
Shoot	_	53.9	—	16.7	6	162.5		100
Pericarp	54.7		4.5		_	_		
Water	50.7	55.1	4.1	19.1	10	202.5	100	100
LSD 5%	3.6	8.87	0.49	2.89	1.44	21.31	_	_
LSD 1%	4.4	NS	0.74	NS	2.08	31.01	_	_

Table 1. Growth responses of peach and apple seedlings following soil treatment with water suspensions from peach seeds, roots, and shoots.

¹Received for publication February 7, 1975. Michigan Agr. Expt. Station Journal Article No. 7114.

²Present address: Horticulture Expt. Station, Suwon, Korea.

³Professor of Horticulture.



Fig. 1. Peach seedlings (A) given soil treatment of water suspension from current peach shoot growth, from peach roots and from peach seeds. Both root and seed suspension caused stunting, chlorosis and defoliation. Root growth (B) was retarded from the addition to the soil of water suspension of roots and seed.

cyanogenetic glucosides was used to determine amygdalin, and calculated on the basis of 1 mole AgNO₃=1 mole HCN=1 mole amygdalin (17). These determinations were made on the assumption that all HCN came from amygdalin.

Results

Plant response. The growth response of peach seedlings treated with water suspension varied with plant parts used. The water suspension from peach seed significantly reduced growth, followed by root and shoot suspension (Table 1, Fig. 1). Thirty days following treatment the leaves of the adversely affected plants showed severe chlorosis; plants later defoliated and 46% died. Plants treated with root and shoot suspension were chlorotic and stunted when compared to water-treated plants. Root growth of peach seedlings also was affected by the seed and root suspension treatments (Fig. 1B).

Since seed suspension treatment severely affected peach seedling growth, a water suspension from crushed stony peach pits (pericarps) was used to treat apple seedlings and bean plants.

Table 2. Growth of peach seedlings following soil treatment with synthetic amygdalin and water extract of stony pericarp.

Treatment	Seedling ht (cm)	Avg shoot no. per/sdlg.	Total shoot length per sdlg. (cm)	Dry wt/sdlg. (g)
Amygdalin				
(1000 ppm)	58.5	179.5	10.3	3.27
Amygdalin				
(500 ppm)	58.5	186.5	8.7	3.98
Stony pericarp				
extracts	58.7	182.8	8.9	3.65
Water	56.7	181.0	9.4	3.47
LSD 5%	NS	NS	NS	NS





Fig. 2. Apple seedlings (A) showing loss of leaves and poor root growth from soil treatment with water suspension from peach seed. Suspension from stony pericarp was not as effective. Bean seedlings (B) died from the seed treatment and were only slightly effected by the stony pericarp.

Peach seed suspension treatment was included for comparison. Apple and bean seedlings showed plant stunting, chlorosis, and defoliation from peach seed suspension treatment. Bean seedlings treated with seed suspension died. The peach stony pericarp treatment did not effect plants as much as did the seed suspension (Table 1, Fig. 2).

Synthetic amygdalin applied to potted peach seedlings did not significantly influence subsequent growth (Table 2). Synthetic amydalin may be chemically bonded and thus have no effects on growth.

Amygdalin content. The amygdalin content in different plant parts of peach and apple varied significantly (Table 3). Peach

Table 3. Amygdalin extracts (in mg/10g) from different plant part tissues of peach and apple trees, as determined by 3 samplings using steam distillation methods. Data are means of 3 samplings.

Plant	Plant part	Amydalin (mg/10 g dry wt)
Redhaven peach	Seeds	5.94
*	Leaves	2.32
	Shoots	3.29
	Roots	4.51
	LSD 5%	0.46
	LSD 1%	0.70
McIntosh apple	Seeds	2.48
	Leaves	1.23
	Shoots	0.36
	Roots	0.66
	LSD 5%	0.24
	LSD 1%	0.36

Table 4. Amygdalin extracts from various peach fruit parts removed from tree at different dates. Data are means of 2 samplings.

Peach fruit sampled	Amygdalin (mg/10 g dry wt)
Natural June drop (entire fruit)	0.10
First thinned fruit 6/28 (entire fruit)	0.35
First thinned fruit 6/28 (seed removed)	0.25
First thinned fruit $6/28$ (seed only)	2.15
Stony pericarp (fruits removed from tree 7/12)	0.20
Seed (fruits removed from tree $7/12$)	4.55
Fleshy pericarp (fruits removed from tree 7/12)	0.20
LSD 5%	0.36
LSD 1%	0.55

tissues were higher in amygdalin than apple, and the seeds of these plants had more than the leaves, shoots, and roots. Amygdalin extracted from different fruit parts from seed collected at various times during the season showed the highest content in the naked seed (Table 4). The entire fruit from natural "June drop," the fleshy and stony pericarps, and the thinned fruit were low in amygdalin.

Nutritional effects of treated plants. Leaves from treated plants were analyzed for 12 major and minor mineral elements, but only 4 showed any significant change in leaf composition (Table 5). The P and K content was reduced in peach leaves treated with the seed suspension. The Al and Na level was increased (nearly to a toxic concentration) in leaves from both peach and apple leaves so treated.

Discussion

In this study water soluble extracts (suspensions) of peach seeds were toxic to some plants when incorporated with the soil. The glycoside amygdalin present in stone fruit seed has been noted to yield toxic hydrogen cyanide (HCN) upon hydrolysis (2). If HCN is stable and is annually added to the soil from peach fruit drop and peach plant decomposition, then this accumulation could lead to a partial cause of peach tree decline on old peach sites. A study is suggested wherein a new soil site is used and all drops are removed versus typical culture, thus allowing monitoring of differences in HCN levels.

Amygdalin (50 mg/g dry wt) has been found in peach roots (30). However, our study did not give that high level of amygdalin. Other reports indicate that when amygdalin is added to the soil in which peach seedlings were growing, no toxic cyanide was produced in the soil after 14 days (13). This result indicates that the toxic factor is not soil stable, or that it may take some time to develop.

Sodium has been reported to be associated with peach transplant problems (1, 18). In our study peach and apple leaves from plants treated with peach seed suspension gained in Na content (Table 4). Under orchard conditions decomposition of large amounts of peach seed may be one of many factors contributing to poor tree growth.

Calcium content in the soil has been reported to be another associated factor in peach tree growth (5, 25). Soil nutrition apparently has not been observed as a direct causal condition of poor tree growth (7). The Ca content in leaves in this study did not change with the treatments.

Correcting peach tree replant problems and increasing tree longevity is not simple. As this report shows, temporary toxic levels of some substance may be introduced to the soil. Since these toxins apparently are by-products of decaying plant parts (seed included) it is conceivable that soil contamination is a major part of the tree decline cause. Soil sanitation by avoiding returning plant parts (roots, fruit, leaves, stems, etc.) to the prospective peach site would be a good practice.

Table 5. Leaf mineral (P, K, Na, Al) composition from peach and apple seedlings treated with water suspensions from peach tree roots, shoots and extracts from stony pericarp, 60 and 70 days after initial soil treatment.

Leaves from	Р ((%)	K (%)	Na (p	opm)	Al (p	opm)
treated plants	60 ^z	70	60	70	60	70	60	70
		Pea	ch leaf	minera	al comp	osition	1	
Control	0.467	0.532	1.46	1.46	666	963	641	656
Shoot suspension	0.604	0.572	1.52	1.43	625	864	690	586
Root suspension	0.680	0.555	1.38	1.35	2165	1186	967	931
Seed suspension	0.470	0.361	0.67	0.62	4360	1737	969	991
LSD 5%	0.154	0.020	0.31	0.34	231	208	150	40
LSD 1%	NS	0.029	0.46	0.49	383	345	249	66
		Ap	ple leat	^c miner	al comp	ositior	1	
Control	0.532		1.46	_	699	_	146	
Seed suspension	0.660		1.31	_	1416	_	304	-
Stony pericarp	0.617	-	1.48	-	778	-	395	
LSD 5%	0.049		NS	_	104	-	122	
LSD 1%	0.089		NS	-	171		223	

^zLeaf analyses were made 60 and 70 days following initial soil treatment.

Literature Cited

- 1. Ballinger, W. E., H. K. Bell, and N. F. Childers. 1966. Peach nutrition p. 276-390. *In* N. F. Childers (ed.) Fruit nutrition. Horticultural Publications, New Brunswick, New Jersey.
- 2. Conn, E. E. and G. W. Butler. 1969. The biosynthesis of cyanogenic glycosides and other simple nitrogen compounds. Perspectives in phytochemistry. Academic Press, N.Y. p. 47-74.
- 3. Gilmore, A. E. 1959. Growth of replanted peach trees. Proc. Amer. Soc. Hort. Sci. 73:99-111.
- 4. _____. 1963. Pot experiments related to the peach replant problem. *Hilgardia* 34:63-78.
- Havis, L. A. 1962. Some effects of old soil treatments on young peach trees in the greenhouse. Proc. Amer. Soc. Hort. Sci. 81:147-152.
- 6. _____ and A. L. Gilkeson. 1947. Toxicity of peach roots. Proc. Amer. Soc. Hort. Sci. 50:203-205.
- , H. F. Morris, R. Manning, and T. E. Denman. 1958. Responses of replanted peach trees to soil treatments in field tests in Texas. *Proc. Amer. Soc. Hort. Sci.* 71:67-76.
- 8. Hendrix, F. F. and W. M. Powell. 1970. Control of root pathogens in peach decline sites. *Phytopathology* 60:18-19.
- 9. _____, W. M. Powell, and J. H. Owen. 1966. Relation of root necrosis caused by *Pythium* species to peach tree decline. *Phytopathology* 56:1226-1232.
- 10. Hewetson, F. N. 1957. Re-establishing the peach orchard: The influence of various nutrient solutions and fertilizers on the growth and development of one-year peach trees. *Proc. Amer. Soc. Hort. Sci.* 69:122-125.
- 11. _____. 1953. Re-establishing the peach orchard. PA Agr. Expt. Sta. Prog. Rpt. 106.
- 12. Hildebrand, E. M. 1945. Peach root toxicity in a New York orchard. *Plant Dis. Rptr.* 26:179.
- 13. Hine, R. B. 1961. The role of amygdalin breakdown in the peach replant problem. *Phytopathology* 51:10-13.
- 14. ______. 1961. The role of fungi in the peach replant problem. *Plant Dis. Rptr.* 45:462-465.
- 15. Hung, C. P. and W. R. Jenkins. 1969. Criconemoides curvatum and the peach tree decline problem. J. Nematol. 1:12. (Abstr.)
- 16. Koch, L. W. 1955. The peach replant problem in Ontario. I. Symptomatology and distribution. *Can. J. Bot.* 33:450-460.
- 17. Lepper, H. A. 1950. Official methods of analysis of the Association of Official Agricultural Chemists. 7th Ed. A.O.A.C., Washington.
- Lilleland, O. 1966. The present status of leaf analysis in relation to fruit tree nutrition. *Blue Anchor* 23(1):14-16, 28-31.
- 19. Lownsbery, B. F., H. English, E. H. Moody, and F. J. Schick. 1973. *Criconemoides xenoplax* experimentally associated with a disease of peach. *Phytopathology* 63:994-997.
- 20. Mountain, W. B. and H. R. Boyce. 1958. The peach replant problem

in Ontario. V. The relation of parasitic nematodes to regional differences in severity of peach replant failure. *Can. J. Bot.* 36:125-134.

- 21. ______ and _____. 1958. The peach replant problem in Ontario. VI. The relation of *Pratylenchus penetrans* to the growth of young peach trees. *Can. J. Bot.* 36:135-151.
- and Z. A. Parick. 1959. The peach replant problem in Ontario. VII. The pathogenicity of *Pratylenchus penetrans* (Cobb, 1917) Filip. & Stek. 1941. *Can. J. Bot.* 37:459-470.
- 23. Patrick, Z. A. 1955. The peach replant problem in Ontario. II. Toxic substances from microbial decomposition products of peach root residues. *Can. J. Bot.* 33:461-486.
- 24. Parker, K. G., W. F. Mai, G. H. Oberly, K. D. Brase, and K. D. Hickey. 1966. Combating replant problems in orchards. *Cornell Extension Bul.* 1169.
- 25. Prince, V. E. and L. Havis. 1955. Effect of soil treatments in a greenhouse study of the peach replant problem. *Proc. Amer. Soc. Hort. Sci.* 65:139-148.
- 26. Proebsting, E. L. 1950. A case history of a "peach replant" situation.

Proc. Amer. Soc. Hort. Sci. 56:46-48.

- 27. _____ and A. E. Gilmore. 1941. The relation of peach root toxicity to the re-establishing of peach orchards. Proc. Amer. Soc. Hort. Sci. 38:21-26.
- 28. Savage, E. F. and F. F. Cowart. 1954. Factors affecting peach tree longevity in Georgia. Proc. Amer. Soc. Hort. Sci. 64:81-86.
- 29. Shannon, L. M. and E. G. Christ. 1954. Replanting peaches. Amer. Fruit Grower October, p. 14-15.
- 30. Ward, G. M. and A. B. Durkee. 1956. The peach replant problem in Ontario. III. Amygdalin content of peach tree tissues. *Can. J. Bot.* 34:419-422.
- Weaver, D. J. Wehunt, and W. M. Dowler. 1974. Association of tree site, *Pseudomonas syringe, Criconemoides xenoplax*, and pruning date with short life of peach trees in Georgia. *Plant Dis. Rptr.* 58:76-79.
- 32. Wensley, R. M. 1956. The peach replant problem in Ontario. IV. Fungi associated with replant failure and their importance in fumigated and nonfumigated soils. *Can. J. Bot.* 34:967-981.

J. Amer. Soc. Hort. Sci. 101(1):57–59. 1976. Use of Cryoprotectants on Apple and Pear Trees¹

D. O. Ketchie and C. Murren²

Tree Fruit Research Center, Washington State University, Wenatchee, WA 98801

Additional index words. cold resistance, acclimation, glycerol, ethylene glycol, polyvinylpyrolidone, dimethyl sulfoxide, Malus domestica, Pyrus communis

Abstract. The croprotectants, polyvinylpyrolidone, glycerol, ethylene glycol and dimethyl sulfoxide were applied individually or in combination with each other in the form of a spray on whole apple (Malus domestica Borkh.) trees in the greenhouse and by terminal feeding apple and pear (Pyrus communis L.) trees in the field. The trees were tested both by artificial and natural freezing. The cryoprotectants increased cold resistance, however, different cultivars showed different effects with the various protective agents. Factors other than the colligative properties appeared to modify the effects of cryoprotectants.

According to Meryman (12), cryoprotectants have been divided into 2 classes: penetrating agents which at multimolar concentrations protect the cell against injury from slow freezing, and non-penetrating agents which protect in low molar concentrations against rapid rates of freezing and thawing. Doebbler and Rinfret (2) observed some correlation between H-bonding capacities of cryoprotective agents and their protective capacities during hemolyses of erythrocytes by freezing and thawing. Since cryoprotective agents fall in the 2 classes of penetrating and non-penetrating, it is hard to subscribe to the theory that protection comes solely from H-bonding. At such a high molar concentration, penetrating cryoprotectants must penetrate the cell, or the agent would simulate freezing and dehydrate the cell. A penetrating agent must be non-toxic at the higher concentration. The non-penetrating cryoprotectants reduce the maximum cooling rate and increase the percentage of recovery of cells. We define a cryoprotectant as any agent added to living tissue that reduces susceptibility to cold injury but does not act in a regulating capacity, such as a hormone in animals or a growth regulator in plants.

Most cryoprotectant work has been on animal tissue (9, 10, 11), however, Coulter (1) found that N-vinyl-2 pyrolidone, ethylene glycol and glycerol gave some protection to citrus flowers. Kuiper (8) has shown that decenylsuccinic acid increases frost resistance by changing the permeability of the

¹Received for publication February 27, 1975. Scientific Paper No. 3284, Project 1965, College of Agriculture. This work was supported in part by the Washington State Tree Fruit Research Commission.

²Associate Horticulturist and Experimental Aide, respectively.

membrane. Ketchie (5, 6) has shown freeze protection in excised apple bark by soaking the bark in cryoprotectants. Protection was also increased by feeding 1-year-old apple trees the same materials.

The most logical approach to increasing cold resistance would appear to be through the development of a non-toxic cryoprotective agent. The following study was made to determine if these materials would give freeze protection when applied as a spray application in the greenhouse or by terminal feeding in the field.

Materials and Methods

'Delicious' apple trees were grown in the greenhouse until 5 or 6 branches were formed and terminal buds were set. Two weeks after terminal buds were set, the trees were placed in an environmental chamber where they received a 9-hr photoperiod. The temperature was 20° C during day and 10° night. After 2 weeks, the trees were sprayed with an atomizer-type hand

Table 1. Cold resistance of shoots on 'Delicious' apple trees grown in the greenhouse sprayed with PVP and in combination with glycerol (GLY) and DMSO.

Treatment	T ₅₀ (^o C)
Check	- 9dz
10% PVP	-13a
15% PVP	-10cd
15% PVP + 25% GLY	-11bc
15% PVP + 25% GLY + 0.5% DMSO	-12ab

^zMean separation by Duncan's multiple range test, 5% level.