COMPARATIVE EFFECTS OF FRESH AND COMPOSTED HARDWOOD BARK EXTRACTS ON PLANT GROWTH

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Abstract. Mung bean (*Phaseolus aureus* Roxb.) cuttings were cultured in water extracts of silver maple (*Acer saccharinum* L.), hackberry (*Celtis occidentalis* L.), sycamore (*Platanus occidentalis* L.) and cottonwood (*Populus deltoides* Marsh.) barks. Extracts of fresh silver maple inhibited adventitious rooting of mung beans whereas rooting in other bark extracts was similar to the control. Composting the silver maple bark prior to preparing the water extracts reduced the inhibition. Pretreatment of the silver maple extracts with polyvinylpyrrolidone (PVP) reduced inhibition and indicated that the compound was phenolic in nature. Chromatography and spectral analysis of common phenolic compounds and silver maple extracts revealed the toxic substance was tannic acid-like.

REVIEW OF LITERATURE

Successful growth of ornamentals in hardwood bark media has been well demonstrated (14, 16, 17, 21, 22, 24). This hardwood bark decomposes over the period of plant growth and since the microorganisms involved in this decomposition are more efficient than higher plants in N absorption and assimilation (1), N must be added to bark media to ensure an adequate supply for plant growth (16, 17, 22). Plant growth inhibition not remedied by supplemental N has also been observed (7, 23, 27). One example (23) is the retardation of chrysanthemum root development in media containing fresh silver maple bark. In this case, a simple 30 day composting of the bark prior to planting apparently inactivated the inhibitor. Recognition that hardwood bark may contain growth inhibitors and knowledge of their identity are important considerations in the future use of hardwood bark as a growth medium.

The occurrence of growth inhibitors in leaves and barks of woody plants is well documented (4, 7, 10, 18, 27) but the chemical identity of these materials has rarely been determined (10, 11). In this case, phenolic compounds are logical choices since they commonly occur in bark (6, 8). The purposes of this study were to identify possible growth inhibitors and to determine differential effects of tree bark species and composting time on plant growth.

MATERIALS AND METHODS

Preparation of bark extract. Fresh (winter) and composted bark of silver maple, hackberry, cottonwood and sycamore were studied. A 0.3 m^3 sample of bark at 65% water holding capacity

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was composted for 30 days in air permeable plastic sacks. Twenty g of each bark was ground to pass a 0.84 mm mesh, mixed with 250 ml of distilled water, and homogenized for 3 min in a Waring blender. The suspension was filtered through 3 layers of cheesecloth and the filtrate centrifuged at 5000 g for 10 min. The supernatant was condensed to 50 ml in a vacuum flash evaporator at 40°C. Since silver maple showed the greatest inhibition in previous reseach (23) PVP[2] was added in saturating quantities to fresh silver maple extracts, shaken for 5 min and centrifuged for 5 min at 5000 g.

Plant culture. Mung beans were grown in vermiculite for 7-10 days on a 16 hr photoperiod at 25°C day and 20°C night temp at 3000 ft-c. Three cuttings were placed in shell vials (15 x 60 mm) containing 3 ml of indole-3-acetic acid (IAA) (5×10^{-6} M) and 2 ml of each concentrated bark extract. To other vials, 1 or 2 ml of 16 different phenolic compounds (1×10^{-3} M) were added. Controls were cultured in IAA plus 2 ml distilled water. Roots 3 mm or longer were counted after 6 days.

Chromatography. Water extracts (50 ml) of each fresh and composted silver maple bark were further reduced to a vol of 5 ml. The extracts of fresh silver maple bark, fresh silver maple bark plus PVP, composted silver maple bark and the 16 phenolic compounds were spotted on 3 MM Whatman chromatography paper (20 x 55 mm) and developed by descending chromatography in butanol:acetic acid:water (4:1:5 v/v/v) (5). Fluorescent or colorimetric inspections of chromatograms were accomplished under long-wave UV light or with 0.1% w/v $FeCl_3$ and 0.2% w/v $FeNH_4(SO_4)_2 \bullet 12\ H_2O$, saturated KIO_3 and Gibbs reagent sprayed onto the chromatograms (15).

Spots of the silver maple chromatogram corresponding to the Rf values of known tannic acid were eluted with 70% EtOH and biological activity determined with the mung bean bioassay. Absorbancies of fresh or composted silver maple eluates, tannic acid eluate and freshly prepared tannic acid were determined on a Beckman DB-G Spectrophotometer.

RESULTS AND DISCUSSION

Root growth of mung bean cuttings in extracts of fresh silver maple bark was suppressed (Table 1). The plants cultures in fresh silver maple bark had significantly fewer roots than the control whereas the plants in sycamore, cottonwood and hackberry had significantly more. Enhanced rooting of plants in these 3 bark ex-

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[2] The insoluble form was used. The trade name of PVP is Polyclar AT (GAF Corporation Dyestuff and Chemical Division, 140 West 51 Street, New York, N.Y., 10020).

tracts was probably the result of root promoting compounds. Identity of these promoters was not determined.

Table 1. Effect of water extracts from fresh or 30 day composted barks of 4 hardwood species on adventitious rooting of mung bean cuttings.

Treatment	Number of roots longer than 3 mm		
	Fresh [z]	Fresh	Composted
	1 ml [y]	2 ml	2 ml
IAA	7.8	7.9	7.9
Silver maple	2.3	0.0	25.7
Hackberry	13.1	15.4	11.9
Sycamore	21.4	20.3	17.8
Cottonwood	13.9	16.4	10.4
LSD			
.05	3.5	8.4	8.4

z Degree of compost.
y Amount of bark extract in each vial.

Cuttings grown in treatments containing 1 ml of fresh silver maple extract averaged 2.3 roots per cutting, but those in 2 ml treatments exhibited complete inhibition of adventitious roots (Table 1). Rooting of cuttings in the other 3 bark extracts was not significantly affected by increasing the amount of extract. Several authors have reported similar findings (3, 25, 26). Tourneau (25) showed that increasing the amount of material extracted from weeds or crops resulted in increased inhibition to wheat germination. The number of roots per cutting in silver maple extract increased significantly after composting. It is probable that the inhibitor in fresh silver maple bark was reduced or eliminated by composting. Gartner et al. (13) recommended that fresh bark be composted for 30 days to reduce phytotoxins. Pattrick et al. (19) reported that phytotoxicity to lettuce seed germination was most severe after plant residues had decomposed for 10 to 25 days but decreased with further decomposition and extracts with stimulatory properties were often obtained after 30 days. Rooting of mung bean cuttings in PVP treated fresh silver maple extract was greater than rooting in the composted extract. An average of 12 roots occurred on cuttings cultured in composted bark extract and 17 on cuttings cultured in PVP treated extract. Application of PVP and subsequent centrifugation resulted in a supernatant apparently free of toxic compounds. Elimination of inhibition by the addition of PVP suggested that the inhibitor(s) were phenolic type compounds for PVP complexes phenolic substances (2).

Tests with 16 known and commonly occurring plant phenolic compounds were used in an attempt to establish the chemical na-

ture of the inhibitor(s). Application of 1 ml of a 1 x 10^{-3} M phenolic solution was insufficient to cause a significant reduction in rooting (Table 2). However, when 2 ml of each compound were added to the vials, ellagic acid, juglone, quercetin, rutin, scopoletin and tannic acid exhibited inhibitory properties. Adventitious rooting was eliminated by tannic acid (Table 2). Other researchers (9, 12, 20) reported phenolic compounds at low concentrations combined with IAA to promote growth whereas the same phenolics at higher concentrations greatly inhibited growth. Apparently this occurred with the above phenolic compounds, particularly tannic acid.

Table 2. Adventitious rooting of mung bean cuttings as affected by various phenolic compounds.

	Number of roots longer than 3 mm	
Treatment	1 ml	2 ml
IAA (1 x 10^{-3} M)	10.8	10.8
Catechin	10.1	10.5
Chlorogenic Acid	10.0	9.7
Coumaric Acid	8.3	14.4
Ellagic Acid	13.6	4.7
Ferulic Acid	11.3	9.1
Gentisic Acid	12.9	14.5
Hydroquinone	14.7	21.5
p-Hydroxybenzoic Acid	10.9	9.7
Juglone	19.3	7.5
Quercetin	10.9	6.6
Rutin	11.2	5.7
Salicylic Acid	17.7	21.6
Scopoletin	8.9	3.6
Shikimic Acid	12.8	14.7
Tannic Acid	7.2	0.0
Vanillic Acid	13.7	13.5
LDS		
.05	5.2	6.1

Co-chromatography of fresh silver maple extract with tannic acid gave spots with identical Rfs as tannic acid (.45 and .67). These spots fluoresced under UV light and gave positive colorimetric tests with ferric salts, KIO_3 and Gibbs reagent. Chromatographed extracts of fresh silver maple plus PVP showed no response to the above tests. No compounds were found in the silver maple extract corresponding to the other 5 phenolics. Eluates from strips corresponding to the Rf values of tannic acid and silver maple completely inhibited growth. However, cuttings cultured in extracts obtained from eluates of composted silver maple bark rooted readily (Fig. 1) indicating degradation or loss of the inhibitor during composting. Eluates of strips of fresh silver

maple and tannic acid and freshly prepared tannic acid exhibited an absorption maximum between 270 and 280 nm (Fig. 2). Chromatographed extracts of composted silver maple or PVP treated fresh silver maple extracts did not exhibit this absorption maximum indicating the tannic acid was degraded or precipitated by the respective treatments (Fig. 3).

Results indicated that lack of N is not always the limiting factor in utilization of hardwood bark. Toxic compounds contained in bark exhibit phytotoxic properties. Growth inhibition by fresh silver maple bark extracts has been previously reported[3] but the

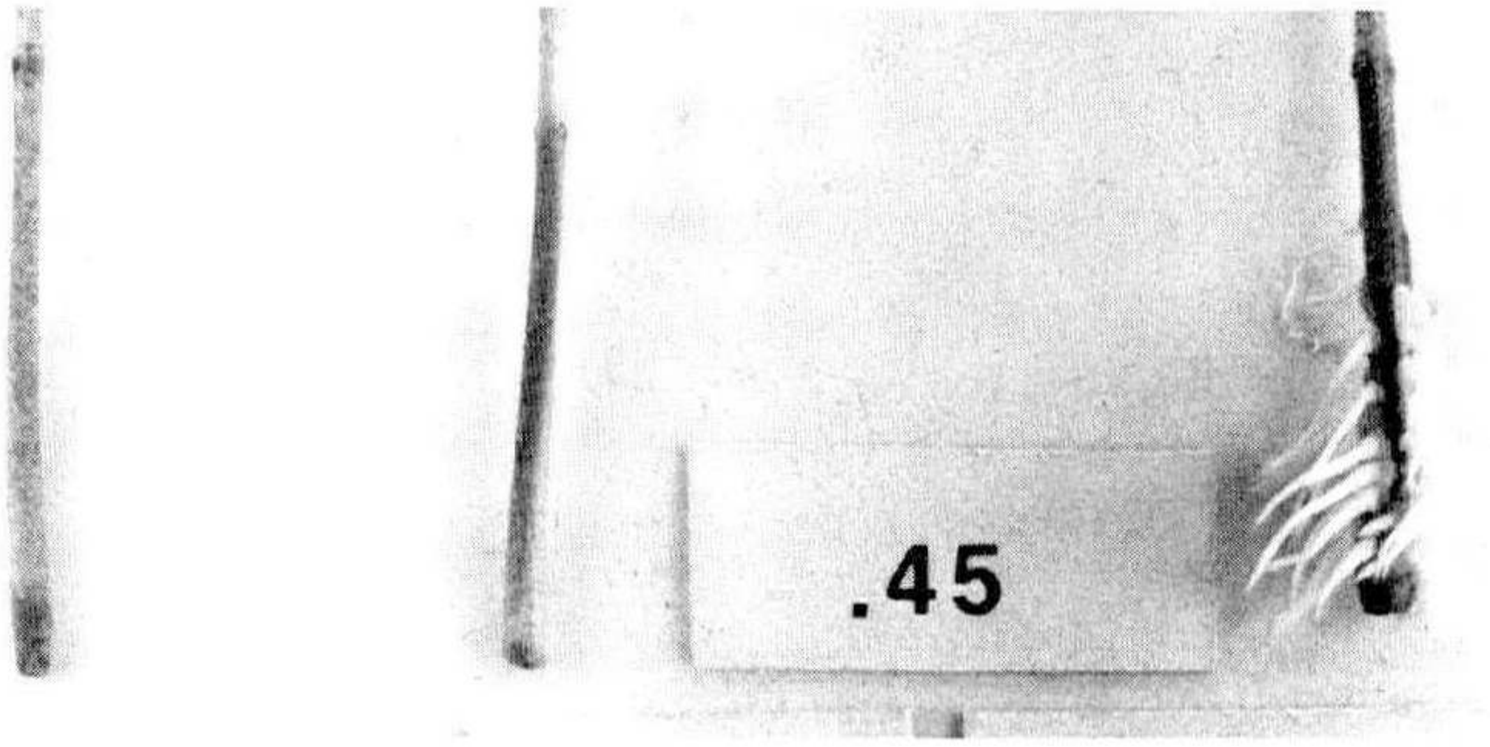

Figure 1. Adventitious rooting of mung bean cuttings cultured in eluates obtained from chromatography strips corresponding to Rf 0.45. Left, **Tannic acid.** Center, **Fresh silver maple bark.** Right, **Aged silver maple bark.**

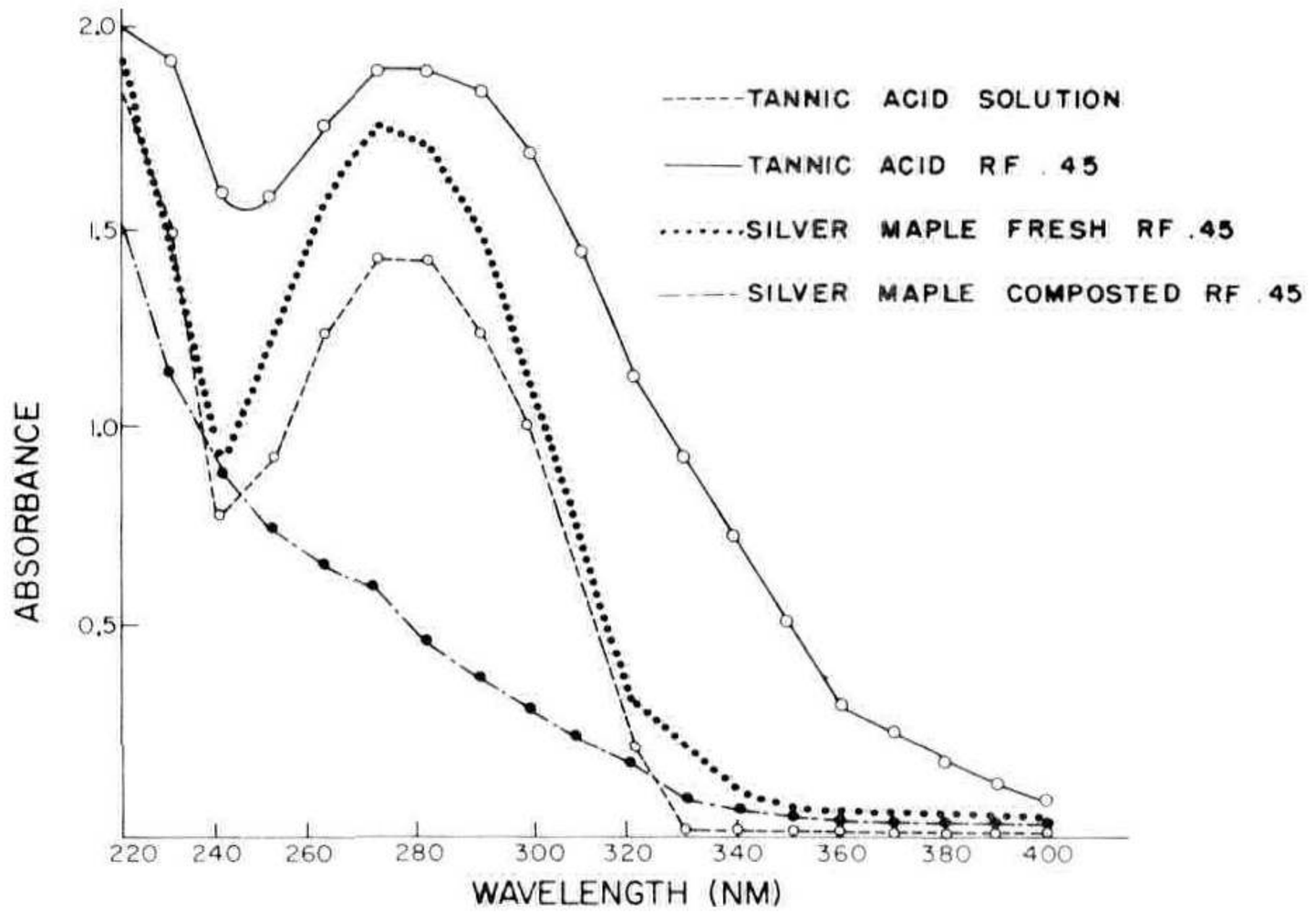

Figure 2. Absorbancies of tannic acid and fresh or 30-day composted, silver maple bark extracts.

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[3] Haramaki, C., R. J. Nuss and C.S. Oliver. 1971. Final report on the study of the feasibility of using deciduous tree bark as a soil substitute and as a mulch material for ornamental plants. Dept. of Hort., Penn State Univ., University Park, Penn. 16 p.

nature of the inhibitor was not determined. The barks studied represent a fraction of those that could be utilized as growth media and, therefore, additional research is required. Although a 30 day compost was sufficient to eliminate the inhibitor from silver maple, other barks might require longer compost periods.

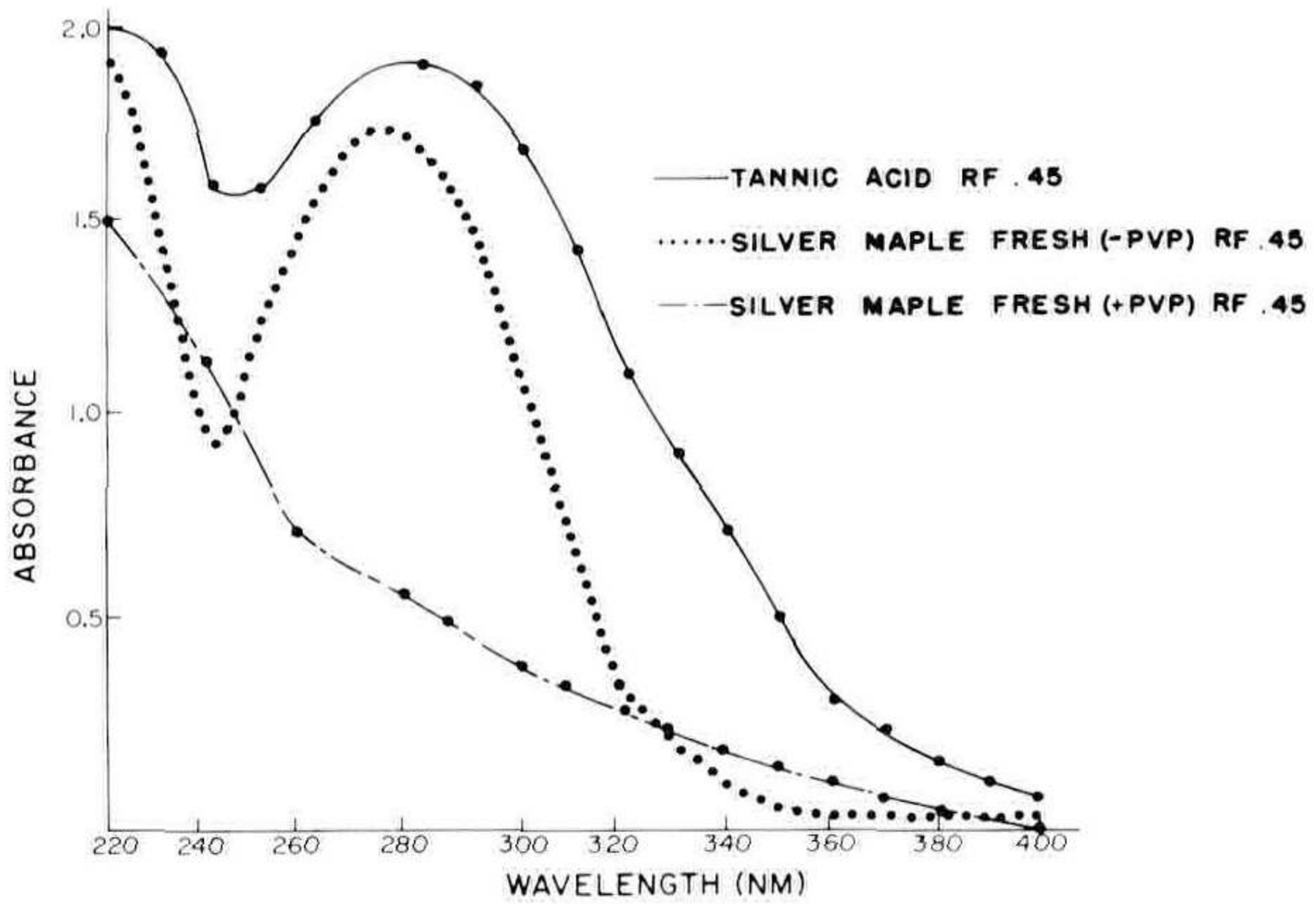

Figure 3. Absorbancies of tannic acid, fresh silver maple bark extract, and fresh silver maple bark extract treated with PVP.

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LARRY CARVILLE: Thank you, Mr. Still; that was a stimulating talk on the use of composted hardwood bark. At this time I'll turn the podium over to Chiko Haramaki who will serve as moderator for the education session of this afternoon's program.

MODERATOR HARAMAKI: For this session of the program, we have three speakers who will present some quite different ideas concerning the teaching of horticulture. Our first speaker is the President of the G.B.&I. Region, Dr. Richard Martyr, from Pershore, England.