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To direct, fund, promote and communicate horticultural research, which increases the quality and value of ornamental plants, improves the productivity and profitability of the nursery and landscape industry, and protects and enhances the environment.

Greenhouse Rose Production in Media Containing Coal Bottom Ash[1]

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Abstract

Coal bottom ash was mixed with composted hardwood bark fines in proportions of 3:1, 2:1 and 1:1 (by vol) and with soil and peat in proportions of 1:1:1 (by vol). A mix of soil, sand and peat 1:1:1 (by vol) was used as the control root medium. *Rosa* x *hybrida* L. 'Samantha' plants were planted and cultured for one year and production indices recorded for four harvests. Flower stem lengths, flower bud diameters, production times, and numbers of flowers produced in media composed of ash and bark were equivalent to those in the control medium. Stem fresh weights of flowers produced in ash:bark media exceeded those in the control during third and fourth harvest. Foliar analysis showed increased B but decreased Mn and Cu in plants grown in the ash:bark media. Values of physical characteristics of all media were within acceptable ranges. Requirements for irrigation and fertilization were higher in the ash:bark media. The coal bottom ash:hardwood bark combinations showed potential as components of artificial root media for growing greenhouse roses.

Index words: greenhouse roses, coal bottom ash, composted hardwood bark fines, cut rose flower production.

Species used in this study: greenhouse rose (*Rosa hybrida* L. 'Samantha').

Significance to the Nursery Industry

Coal bottom ash and hardwood bark are industrial byproducts which are currently utilized in limited quantities. These products are often disposed of in solid waste landfills, but can be purchased at lower costs than comparable quantities of topsoil, sphagnum peat or sand. Utilization of these materials as components of artificial rose root media by the greenhouse and nursery industry could lower crop start up costs, reduce disposal costs and ease pressures for mining of more finite materials such as sphagnum peat.

Introduction

Soils used for growing roses are commonly amended to improve structure, aeration, and drainage (11). Sand is added to improve aeration; sphagnum peat increases water retention. A mixture of composted sandy loam, peat moss, and perlite or sand (3:2:1, by vol) is widely used as a root medium for greenhouse roses (1). The incorporation of coal bottom ash into pine bark in various ratios improved moisture retention (10). The coal bottom ash used in this study was found to contain all essential plant elements, and released measurable amounts of all of these but P in a DTPA extract (15). The same ash tended to fix P when it was used as a component of nutriculture root media. Neal and Wagner (10) found higher amounts of essential elements and Ba, Co, Cr, Ni, Pb, and Sr in water extracts from fresh ash compared to ash stockpiled for several years. Composting of hardwood bark fines increased water holding capacity and reduced N fixation (8) compared to fresh bark. Hardwood bark supplies Ca and Mg as well as many micronutrients (13) but may release toxic amounts of Mn (9).

The objective of this study was to determine suitability of coal bottom ash and composted hardwood bark for components of greenhouse rose root media.

Materials and Methods

The rose media were prepared during January 1992. The coal bottom ash (CBA) was obtained from a local power plant on April 26, 1991, and placed in an uncovered stockpile. Prior to incorporation in the media, the ash was screened through a 1.27 cm (0.5 in) mesh. The composted hardwood bark (HB) was purchased from Paygro, Inc., South Charleston, OH, on December 13, 1991. The soil was analyzed for texture by hygrometer and found to be a clay loam with a pH of 7.28 as measured by 1:1 saturated paste method (14). The soil was screened through a 1.27 cm (0.5 in) mesh before use. Sphagnum peat was Fison Sunshine Peat Moss (Vancouver, B.C.). Sand was U.S. Silica (Berkeley Springs, WV) sharp sand circa 1–2 mm (0.04–0.08 in) in diameter. Five media were prepared; CBA:HB 1:1, 2:1 or 3:1 (by vol), CBA:soil:peat and sand:soil:peat 1:1:1 (by vol). The media were pasteurized for 30 minutes at 75C (165F), and placed into 19 liter (#5) plastic containers. The containers were placed in polyethylene covered greenhouse, pot to pot, in four rows aligned east-west. Each row contained six containers of each medium randomized within the row.

On April 20, 1992, one 3X bareroot 'Samantha' rose bush (Jackson and Perkins, Medford, OR) was planted in each container and irrigated twice. First flower buds were removed circa May 11 and all plants were hand pinched on June 5 in order to establish the time for the first harvest. Harvests were conducted June 29–July 24, September 28–October 22, December 4–January 19 and March 19–April 14. Flowers were cut above the second node up from flower stem origins when two outer petals began to unfurl. Days from pinch to harvest, number of flowers per plant, fresh weight per stem, stem length, and flower bud diameters were recorded. Flowers with bud diameters less than 1.0 cm (0.4 in) and/or stem lengths less than 30.0 cm (12 in) were not recorded. At the fourth harvest, 24 five-leaflet leaves were collected at random within each treatment from the harvested flower stems and sent for elemental analysis to the Research-Extension Analytical Laboratory, Ohio State University, Wooster.

Media electrical conductivity (EC) and pH were measured weekly during crop time. A 160 ml (5.7 oz) sample of me-

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Table 1. Stem mean fresh weights (g) at four harvests of 'Samantha' roses grown in root media containing coal bottom ash (CBA), composted hardwood bark (HB), topsoil (T), sphagnum peat (P) and/or sand (S).

Root medium/ Volume ratio	Harvest periods			
	June 29 to July 24, 1992	September 28 to October 22, 1992	December 4, 1992 to January 19, 1993	March 19 to April 14, 1993
CBA:HB/1:1	8.4b[y]	15.7a	20.9ab	22.2ab
CBA:HB/2:1	9.8ab	14.9ab	21.7a	28.1a
CBA:HB/3:1	9.9a	13.5b	22.0a	22.1ab
T:P:CBA/1:1:1	8.7ab	13.7ab	20.3ab	22.6ab
T:P:S/1:1:1	8.6ab	14.6ab	18.2b	21.5b
Soilless vs. soil-containing[z]	NS	NS	*	*

[z]*, NS: Significant at P = 0.05, not significant, respectively.

[y]Mean separation in columns by Duncan's multiple range test, P = 0.05.

dium was drawn from a randomly selected container of each medium and mixed 1:2 (by vol) with distilled water. EC and pH were then measured with a Myron L AG6/pH Agrimeter (Myron L Co., Carlsbad, CA). As long as leachate EC remained < 2.0 dS m, that treatment was irrigated weekly with a (0.2N–0.08P–0.18K) fertilizer solution, 1.2 g/liter (0.16 oz/gal). When pH was > 7.0, the treatment was irrigated with ammonium nitrate, 1.2 g/liter; when pH was < 5.5, calcium nitrate was used.

Physical and chemical properties of the media were measured immediately after formulation and after one year of growing the roses. Four replications of each medium were packed uniformly in 7.8 cm (3.1 in) diameter × 7.6 cm (3.0 in) high polyvinylchloride cylinders. Media-filled containers were placed under water in dessicators, a vacuum was applied until bubbling on top of the media ceased, and the cylinders were drained for 4 hours. The quantity of water drained was used as an estimate of air capacity (AC) (5). Moisture retention curves for each medium were established by placing cylinders on porous pressure plate extractors (Soil Moisture Equipment Co., Santa Barbara, CA) (6) and recording moisture retained at pressures of 0.5 to 10 kPa.

Wet bulk density equaled weight of the drained medium divided by medium volume. Dry bulk density equaled weight of the medium after drying at 70C (158F) for 24 hours. divided by medium volume. Container capacity was defined as the moisture content after drainage expressed as a percentage of medium volume (4).

Macro- and micronutrients were extracted from four replications of each root medium by the modified DTPA-saturated medium extraction method (14). Calcium, Na, and K were measured with an atomic absorption spectrophotometer (Model 5000; Perkin-Elmer, Pittsburgh). Phosphorus was measured with a Perkin-Elmer spectrophotometer 55B. Nitrate was measured with a nitrate-specific ion electrode attached to an expandable ion analyzer (model EA920; Orion Research, Boston). All other elements were measured by ICP analysis with a Perkin-Elmer Emission Spectrophotometer 400.

Data were analyzed by a General Linear Model, using a SAS program.

Results and Discussion

Most production indices were quantitatively equal among media within each harvest with the exception of flower bud diameter and stem fresh weight (Table 1). Mean stem lengths for all harvests ranged from 36.5 to 68.3 cm (14.4 to 26.9 in), flower bud diameters 2 to 2.5 cm (0.8 to 1.0 in), days from pinch or cut to harvest 36.4 (summer) to 62.8 (winter), and blooms per plant 17 to 22.8. Flower bud diameter was larger in soilless media in the second harvest. Stem fresh weight appeared to increase with increased CBA in the first harvest while this trend seemed to reverse itself in the second and third harvest. Stem fresh weight from plants in T:P:S/1:1:1 media was significantly lower than that of plants in CBA:HB/3:1 in the third harvest and of plants in CBA:HB/2:1 in the fourth harvest. Blooms harvested per plant in all media were less in the third and fourth harvest than in the first and second, probably a result of diminished light intensity from October through March.

Leaf tissue analysis showed all macro-elements to be within recommended ranges (3); N 3.5–3.7%, P 0.3%, K 2.4–2.6%, Ca 0.9–1.0% and Mg 0.3%. Leaf Mn (Table 2) was significantly lower in all CBA:HB roses compared with plants in soil. This was unexpected because of the tendency of hardwood bark to release Mn (9). Boron concentration in leaves of plants in T:P:S/1:1:1 was lower than that in the other media. Woodard et al. (15) found B concentration of this ash to be 170 mg/liter, and this may account for increased levels of B in the media containing CBA. Cu levels were below recommended levels in leaves of roses in all media containing ash, while Zn concentration was low in T:P:S/1:1:1 plant leaves.

In the root media, available NO3 concentration increased during the year of rose cropping in the soil based media and in CBA:HB/1:1 (Table 3). Phosphorus levels were low at the beginning of the year but increased to acceptable levels

Table 2. Micro-elemental status of rose leaves after plants were grown for 11 months in root media containing coal bottom ash (CBA), composted hardwood bark (HB), topsoil (T), sphagnum peat (P), and/or sand (S).

Root medium/ Volume ratio	Parts per million				
	Mn	Fe	B	Cu	Zn
CBA:HB/1:1	45c[z]	55	15ab	3	22ab
CBA:HB/2:1	42c	49	15ab	2	24a
CBA:HB/3:1	41c	50	16a	5	24a
T:P:CBA/1:1:1	109b	47	14b	5	20ab
T:P:S/1:1:1	156a	50	12c	7	18b

[z]Mean separation in columns by Duncan's multiple range test, P = 0.05.

Table 3. Elemental status (ppm) of rose root media DTPA extracts before planting (BP) and after one year of crop production (AP). Media components are coal bottom ash (CBA), composted hardwood bark (HB), topsoil (T), sphagnum peat (P), and/or sand (S).

	Root medium/Volume ratio										Contrast[y]	
	CBA:HB/1:1		CBA:HB/2:1		CBA:HB/3:1		T:P:CBA/1:1:1		T:P:S/1:1:1		soiless vs.	precrop vs.
Element	BP	AP	BP	AP	BP	AP	BP	AP	BP	AP	soil-containing	postcrop
NO_3-N	49.3d[z]	84.3c	40.5d	55.8d	38.0d	42.5d	10.9e	119.8b	10.6e	270.0a	**	**
P	2.2c	6.7a	3.8b	6.4a	0.7d	8.2a	1.0d	4.1b	1.0d	3.6b	**	**
K	202.2b	281.9a	152.2c	179.6b	124.5d	117.6d	8.6e	294.6a	8.5e	245.2ab	NS	**
Ca	201.2c	572.0ab	212.0c	469.3ab	159.4c	346.8b	220.3c	813.8a	255.8bc	668.3a	**	**
Mg	79.2a	15.6d	70.2a	14.1d	50.4ab	9.7d	29.0c	49.4ab	32.0c	38.1b	**	**
Fe	48.9a	37.4a	41.0a	34.9ab	35.6ab	27.2b	17.9c	21.1c	21.3c	23.6bc	**	NS
Mn	27.3ab	8.3d	32.3a	6.8d	36.2a	6.4d	26.4ab	15.0c	35.3a	15.2c	**	**
Zn	2.8a	2.4b	1.99bc	1.73cd	2.24b	1.37def	1.42de	1.10ef	1.44def	1.01f	**	**
Cu	0.2c	7.1b	0.22c	7.51b	0.25c	16.50a	0.8c	16.7a	0.77c	12.58a	**	**
B	2.2a	0.1b	2.05a	0.01b	2.14a	0.02b	1.42a	0.01b	1.43a	0.00b	*	**
Na	1.4d	62.2b	1.4d	51.6b	1.2d	36.1c	3.5c	125.1a	4.8c	106.4a	**	**
Al	2.0d	4.4c	3.9c	8.3b	7.5b	14.3a	0.1e	6.4b	0.1e	4.5c	**	**

[z]Mean separation in rows by Duncan's multiple range test, P = 0.05.

[y]**,*, ns: Significant at P = 0.10, 0.05, not significant, respectively.

Table 4. Elemental composition of coal bottom ash from the electrical power plant at Fort Martin, WV.

Element	Mass percent
Si Oxide	47.8
Al Oxide	19.9
Fe Oxide	17.7
Ca Oxide	10.6
K Oxide	1.9
Ti Oxide	1.3
S Oxide	0.2
V Oxide	0.1
Total	99.5

(14) by year's end. Potassium decreased with increase in CBA in spite of the 1.5 percent K contained in the ash (Table 4). Calcium in all media increased from near optimal to very high levels by year's end. Sources of this increase may have been CBA (Table 4), HB (8) and the topsoil. Magnesium levels declined from optimal to low in all CBA:HB media during the year, perhaps due to the increase in Ca (14). Iron and Mn remained within optimum levels (14), while Zn remained low throughout the crop period. Copper increased in all media. Boron declined from adequate to very low levels in all media. Sodium increased to greater than the maximum suggested (11) and Al increased more as proportions of CBA increased.

All media were similar in particle size distribution (Table 5) except T:P:S, in which the percentage of 1–2 mm (0.04–0.08 in) particles greatly exceeded that of the other media, due to the 33% proportion of sand in this mix. Percentages of 0–0.5 (0.02 in) and 2–5 mm (0.08–0.2 in) particles were correspondingly reduced. Little change occurred in any medium during crop time.

Wet and dry bulk densities differed little among the media (Table 6) and little change occurred from beginning to end of the crop. A considerable increase in air capacity took place in both soil based media from values less than that of the CBA:HB media to similar values at end of crop. This may have been caused by aggregation of soil particles (7). Container capacity increased in all HB media due perhaps to increased humic materials (12) from decomposition of the bark media but slightly declined in T:P:S. Large pores may have formed at the expense of small pores through aggregation. Easily available water (6) showed a similar trend

Table 5. Particle size distribution of rose root media before planting (BP) and after one year of crop production (AP). Media components are coal bottom ash (CBA), composted hardwood bark (HB), topsoil (T), sphagnum peat (P), and/or sand (S).

Root medium/ Volume ratio	Time	Particle size (mm)					
		0–0.5	0.5–1	1–2	2–3	3–4	4–5
		Mass Fraction (%)					
CBA:HB/1:1	BP	27.9a[z]	12.5b	17.9c	16.0b	10.2b	15.6bc
	AP	26.3a	12.7b	18.7c	16.2b	10.4b	15.7bc
CBA:HB/2:1	BP	24.3b	12.2b	19.4bc	16.4b	11.3ab	16.4b
	AP	25.5a	11.5c	19.6bc	17.5a	10.8b	15.1bc
CBA:HB/3:1	BP	22.7bc	10.5c	19.2bc	18.0a	11.6a	18.0ab
	AP	25.5a	11.3c	20.3b	17.5a	11.0ab	14.5c
T:P:CBA/1:1:1	BP	20.5c	12.5b	19.0c	16.9ab	11.6a	19.5a
	AP	18.6c	15.1a	20.3b	16.7ab	11.7a	17.4ab
T:P:S/1:1:1	BP	11.1d	12.9b	54.4a	8.1c	5.0c	8.6d
	AP	10.8d	12.3b	53.5a	8.3c	5.5c	9.7d

[z]Mean separation in columns by Duncan's multiple range test, P = 0.05.

Table 6. Moisture and aeration properties of rose root media before planting (BP) and after one year of crop production (AP). Media components are coal bottom ash (CBA), composted hardwood bark (HB), topsoil (T), sphagnum peat (P), and/or sand (S).

Volume ratio	Root medium/ Time	Wet bulk density (g/ml^{-1})	Dry bulk density (g/ml^{-1})	Air capacity (cm^3/cm^{-3})	Container capacity (cm^3/cm^{-3})	Easily available water (cm^3/cm^{-3})
CBA:HB/1:1	BP	1.12c[z]	0.58d	20.66a	53.57c	8.53b
	AP	1.13c	0.54e	19.39a	58.47b	18.78a
CBA:HB/2:1	BP	1.24b	0.71b	18.57a	53.01c	10.62b
	AP	1.18c	0.63c	22.24a	55.06b	19.43a
CBA:HB/3:1	BP	1.27b	0.80a	20.67a	46.36d	9.07b
	AP	1.24b	0.71b	26.78a	53.42c	18.37a
T:P:CBA/1:1:1	BP	1.26b	0.65c	13.71b	61.00b	17.51a
	AP	1.22b	0.57d	22.95a	64.68a	21.98a
T:P:S/1:1:1	BP	1.49a	0.85a	11.69b	63.43a	16.83a
	AP	1.34b	0.74b	20.95a	60.55b	21.45a

[z]Mean separation in columns by Duncan's multiple range test, P = 0.05.

(See also Fig. 1, in which the two media with greatest divergence in moisture release characteristics are shown). All values fell within recommended ranges (11).

Initial soluble salt levels (EC) were highest in the bark media (Table 7), no doubt due to fertilizer residues from composting, but decreased in all media except T:P:CBA from January to December during crop time. In CBA:HB media, pH increased while it decreased in both topsoil:peat based media. Both CBA and HB may increase pH by release of Ca (15) (Table 4) while the presence of sphagnum peat may have lowered the pH (4) in the topsoil:peat based media.

Irrigation frequency for CBA:HB media was higher than that for T:P based media (6 to 10 times a month vs. 3 to 6 times), and fertilization frequency showed a similar pattern (6 vs. 3 times a month). The initial lower container capacities and easily available water of the CBA:HB media (Table 6, Fig. 1) probably increased the irrigation requirement while the topsoil and peat may have provided superior exchange capacity for nutrient retention.

The potential of CBA and HB for use as components of artificial root media for greenhouse rose growing seems promising. The use of recycling systems could reduce additional costs from more frequent fertilization and irrigation. The improvements in physical characteristics of the CBA:HB media during crop time may indicate that these additional costs may be temporary.

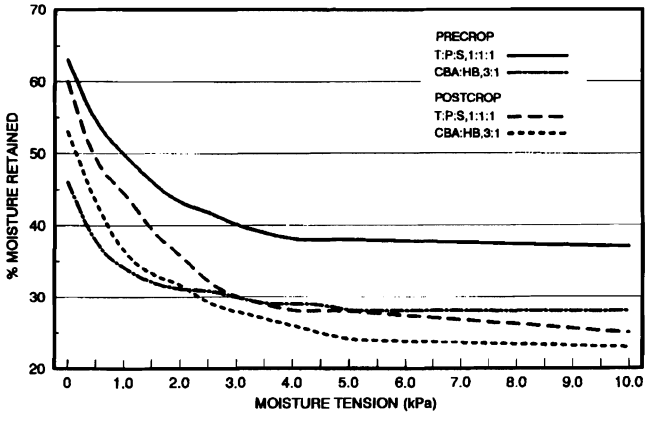

Fig. 1. Moisture release characteristics before and after one year of growing a rose crop of root media containing coal bottom ash (CBA), composted hardwood bark (HB), topsoil (T), spagnum peat (P), and/or sand (S).

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Table 7. Electrical conductivities (EC) and pH values of leachates recorded beginning and end of a one year rose crop cycle from root media containing coal bottom ash (CBA), composted hardwood bark (HB), topsoil (T), sphagnum peat (P), and/or sand (S).

Root medium/ Volume ratio	1993			
	January[z]		December[y]	
	EC	pH	EC	pH
CBA:HB/1:1	2.3	5.8	0.5bcd	6.7de
CBA:HB/2:1	1.5	6.2	0.5bcd	6.7de
CBA:HB/3:1	1.7	6.5	0.3cde	6.8bcd
T:P:CBA/1:1:1	0.6	6.5	1.5a	6.3fg
T:P:S/1:1:1	0.6	6.8	0.6bc	6.3ef

[z]January EC and pH values recorded from media stockpiles prior to planting roses.

[y]Mean separations within December by Duncan's multiple range test, P = 0.05.

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Development of Embryo Rescue and Shoot Regeneration Techniques in *Ilex*[1]

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Abstract

Studies were conducted on embryo rescue and shoot regeneration from juvenile leaves to develop a system to improve *Ilex* using in vitro techniques. Unlike previous reports for *Ilex*, embryo germination and growth in the eight species tested was not consistently affected by light. Gibberellic acid had little effect on excised embryo germination in August through October; however, embryo germination was completely inhibited by 3 µM GA in November and December.

Shoot regeneration was obtained from juvenile leaves of *Ilex myrtifolia* and *I. opaca*, the two species tested. Thidiazuron, at concentrations from 5 to 50 µM, consistently resulted in the largest percentage shoot regeneration, when compared to benzyl adenine or a lower rate of thidiazuron. Indole butyric acid or pretreatment of source shoots with cytokinin or auxin did not increase regeneration percentages. Colchicine treatment of source shoots had an effect on regeneration; 100 µM was slightly stimulatory while 5 µM was significantly inhibitory. This protocol has resulted in regeneration rates that appear suitable for production of polyploid or transformed plants.

Index words: holly, organogenesis, gibberellin, paclobutrazol, light, thidiazuron.

Species used in this study: *Ilex myrtifolia*; Winterberry (*I. verticillata* (L.) A. Gray); English holly (*I. aquifolium* L.); *I.* x *koehneana*; Yaupon holly (*I. vomitoria* Ait.); American holly (*I. opaca* Ait.); Japanese Winterberry (*I. serrata* Thunb.); and *I. pernyi* x *latifolia.*

Chemicals used in this study: Gibberellin A_3, A_4 and A_7; Paclobutrazol (Bonzi) ß-((4-chlorophenyl)methyl)α-(1,1,-dimethylethyl)-1*H*-triazole-1-ethanol as a 50 WP from Sandoz Crop Protection; Thidiazuron (Dropp), N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) as a 99.6% technical grade sample from E.I. Lilly.

Significance to the Nursery Industry

Two techniques were developed to allow genetic improvement of hollies using both standard sexual hybridization and the tissue culture techniques of genetic engineering and mutagenesis. First, to reduce the time needed for holly seed germination, studies were conducted on embryos rescued from maturing seeds. Unlike previous studies, dark was not required for embryo growth. Gibberellic acid, normally used to promote embryo germination, completely inhibited holly embryo growth in November and December. Further study is required to determine if this GA effect is linked to the inhibition of embryo growth that results in prolonged seed dormancy in the field.

Second, an adventitious shoot regeneration technique was developed using juvenile in vitro-grown leaves. This technique allows researchers to recover novel and usable plants from cells that were intentionally altered. This clears a major obstacle for production of polyploid or mutant hollies or those with improved traits developed through genetic engineering.

Introduction

Until recently, tissue culture manipulation of *Ilex* (Aquifoliceae) was limited to excised embryo culture (10), which aids in reducing the period required for seed germination. In the past few years; propagation by shoot tip culture has been reported for *I. paraguariensis* St. Hillaire (15) and *I. vomitoria* Ait. 'Schillings Dwarf' (1). Woody Plant Medium with 4.4 µM benzyl adenine (BA), 0.05 (*I. myrtifolia* Walter) or 0.5 µM (*I. opaca* Aiton) indole butyric acid (IBA) and 0.22 µM adenine sulfate caused satisfactory prolifera-

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