proliferation medium described with the following exceptions: full strength MS was used, BA and agar were omitted, and 0.1 mg/liter NAA was added. The culture tubes were held at 20°C. About 85% of the shoots rooted within 1 month (Fig. 2). The fact that rooting was better on the well-aerated paper bridges agrees with the widely accepted notion that proteaceous roots require a well aerated environment (1).

The in vitro propagation method described may be used to study proteoid root formation (2, 3, 6, 7) and for rapid propagation for this woody shrub as well as for other proteaceous species (5).

Successful tissue cultures have been reported for many members of the genus Rubus involving callus cultures (2, 3, 4, 5, 9, 13), shoot tip growth and/or axillary bud proliferation (1, 4, 7, 8, 11, 12, 13), parthenocarpic fruit development (6, 15), and root development (1, 4, 7, 8, 11, 13). Despite these studies, no good system for the rapid proliferation of the trailing blackberry has been developed. This paper discusses a system for the propagation of 3 trailing blackberry cultivars. Shoot tips of 'Thornless Boysenberry', 'Thornless Youngberry', and virus-free 'Thornless Evergreen' trailing blackberries were obtained from greenhouse grown plants at the University of Illinois. These tips (about 1-2 cm in length) were stripped of expanded leaves and disinfected in 0.5% sodium hypochlorite (10% Clorox) with 0.1% Triton X-100 for 10 min followed by two 5-min rinses in sterile distilled water. The explants were aseptically transferred to shoot proliferation medium which consisted of modified MS medium (10) = MS high mineral salts, myo-inositol, and thiamine-HCl diluted to 1/16 to 1/2 strength and supplemented with full strength sucrose and agar. Rooted plants have been successfully moved to soil and grown in the greenhouse.


In Vitro Propagation of Thornless Trailing Blackberries

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Abstract. Rapid proliferation of axillary buds of 'Thornless Boysenberry' and 'Thornless Youngberry' (Rubus sp.) in tissue culture has been achieved on a modified Murashige and Skoog (MS) medium containing 6-benzylaminopurine (BA) and α-naphthaleneacetic acid (NAA). Shoots were induced to root on medium consisting of MS high mineral salts, myo-inositol, and thiamine-HCl diluted to 1/16 to 1/2 strength and supplemented with full strength sucrose and agar. Rooted plants have been successfully moved to soil and grown in the greenhouse.

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mineral salts, Staba vitamins, myoinositol (100 g/liter), sucrose (30 g/liter), agar (10 g/liter) and various levels of NAA; 2) rooting medium 2 (RM-2) = White’s mineral salts (14) with the Fe$_2$(SO$_4$)$_3$ replaced with Na$_2$EDTA (37.3 mg/liter) and FeSO$_4$	extsubscript{7}H$_2$O (27.8 mg/liter), White’s vitamins, sucrose (20 g/liter), agar (6 g/liter), and various levels of NAA; 3) rooting medium 3 (RM-3) = half strength MS high mineral salts, myo-inositol and thiamine-HCl, sucrose (30 g/liter) and agar (10 g/liter). In later experiments dilutions of 1/4, 1/8, 1/16 strength MS salts were also made. The pH of all media was adjusted to 5.7, agar was added, and media dispensed into 25 x 150 mm culture tubes and autoclaved at 1 kg/cm$^2$ for 15 min. All cultures were grown under 16-hr day length at about 21.6 klx (Cool White fluorescent light) at about 23°C.

All uncontaminated cultures were transferred to fresh media after 4 to 6 weeks and were evaluated at that time for callus, axillary shoot development, and root formation.

In some cases, sub-cultured axillary shoots which had developed from axillary buds were transferred either to soil or to Jiffy-7 pots after being dipped in a commercial rooting compound containing naphtylacetamide (0.067%), 2-methyl-1-naphthylacetic acid (0.033%), 2-methyl-1-naphthylacetamide (0.013%), indole-3-butyric acid (0.057%), and thiram (tetramethylthiuramsulfide) (4.000%) (Rootone F). The potted shoots were placed in loosely sealed plastic bags to prevent shoot desiccation during the root induction period.

All cultures that survived explanting produced axillary shoots on shoot proliferation medium (Table 1). Axillary buds of 'Thornless Evergreen' and 'Thornless Boysenberry' produced multiple shoots at a very rapid rate (Fig. 1) and spontaneous rooting of 'Thornless Boysenberry' and 'Thornless Youngberry' was observed. Sub-cultured shoots have been utilized for the various rooting experiments described below.

Broome and Zimmerman (1) reported that thornless blackberry shoots dipped in Rootone F and inserted into Jiffy-7 peat pots rooted well under mist to yield intact plants. In vitro proliferated shoots of 'Thornless Boysenberry' and 'Thornless Youngberry' trailing blackberries were dipped in Rootone F and either placed in Jiffy-7 peat pots or in autoclaved soil and covered with plastic for several days to prevent desiccation. Only 1 of the 23 shoots planted in Jiffy-7 peat pots survived transfer while 15 of the 21 shoots planted directly in soil survived.

'Thornless Boysenberry' shoots rooted on all root-induction media (Fig. 2). In subsequent studies with MS salt dilutions of 1/2, 1/4, 1/8, and 1/16 strength supplemented with various levels of NAA, we have found the percentage of rooted shoots was above 80% regardless of hormone or salt strength. Individuals interested in rooting 'Thornless Boysenberry' in vitro should choose the medium that is the easiest and most economical to prepare, i.e., 1/16 strength MS salts with no hormones. Since the completion of this study, Kiss and Zatyko (5) have reported ease of rooting for a blackberry-raspberry hybrid on Nitsch and Nitsch medium with 3% sucrose and 1.0 mg/liter IBA.

Although 'Thornless Evergreen' did not root from primary explants, sub-cultures rooted spontaneously on shoot proliferation medium after about 6-8 weeks (Fig. 3). Rooted 'Thornless Youngberry', 'Thornless Boysenberry', and 'Thornless Evergreen' have been moved to soil and have grown well in the greenhouse (Fig. 4).

We have continued to proliferate 'Thornless Boysenberry' and 'Thornless Evergreen' for over 2 years in vitro. Although we have noted a few variegated plantlets of 'Thornless Evergreen', there does not seem to be a deterioration in the ability of these cultures to produce large numbers of axillary buds or for these cultures to root on the appropriate medium.

**Relationship of Prior Heat Treatment to Growth and Fruiting of 'Thornless Oregon Evergreen' Blackberry**

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Additional index words. thermotherapy, Rubus lacinatus

Abstract. Propagules of 'Thornless Oregon Evergreen' blackberry (Rubus lacinatus Willd.) were obtained from mother plants grown at 37°C for 3 to 8 months. Subsequent field performance indicated that the number of floricanes per plant and the number of fruit per fruiting lateral of these propagules were not influenced by the length of the heat-exposure period. A subclone of this cultivar that has been heat-treated for 245 days and is free of known viruses is being released as 'Thornless Oregon Evergreen-80'.

Heat treatment has been widely used to secure clones of Rubus cultivars that are free of known viruses (1, 2, 3, 4, 8, 10). Plants undergoing thermotherapy are often kept for months at a constant 37°C before propagules are taken. This study was conducted to establish whether somatic mutations could be detected after prolonged exposure of Rubus lacinatus Willd. cv. 'Thornless Oregon Evergreen' to 37°C.

Four mother plants of the 'Thornless Oregon Evergreen-72', a USDA clone found by indexing to be free of known viruses (5), were well established in 19-cm pulp pots in a pasteurized soil mix (3 soil:2 peat:1 perlite) placed with peat moss packing into 23-cm pulp pots. The mother plants were placed in a growth chamber with 16 hr at 10,000 lux daily from a combination of incandescent and fluorescent lights. In the growth chamber pots were kept well watered only with ¼ x Hoagland's solution. The temperature in the growth chamber was raised from ambient to 37°C over a period of 14 days. One-to-two node shoots from new growth were removed from each mother plant at about monthly intervals (from 3 to 8 months) to be used as cuttings. In each case an average of 5 plants (range, 1 to 11) were rooted under intermittent mist in the greenhouse. Subclones were grown, and one set of plants from each propagule was kept in the greenhouse, while the second set was planted in the field at Oregon State University in October 1977.

Propagules from a given original mother plant were planted at random in the row (3.5 x 1.5 m spacing). The plants were given standard horticultural care and were trained onto two wires for the 1979 fruiting season. Vigor and fruitfulness were evaluated during the 1979 growth season by inspection, by measuring the number of floricanes that were 15 cm or less from ground level, and by counting the number of red or ripe fruits at one time on six randomly selected major fruiting laterals per plant.

No significant differences between mother plant lines were found in number of floricanes per plant or in number of fruit per lateral. The number of floricanes per plant (Table 1) did not vary significantly during the heat treatment. The coefficients for the linear regression of floricanes numbers on days at 37°C for each of the 4 original mother plants were: b = 0.0009, 0.0210, --0.0280, and --0.0129 (none significant); and, for data from all 103 propagules pooled, b = --0.0003 (not significant). The extrapolated number of floricanes per plant for propagules taken on day 0 (before heat treatment) was 6.1; the average number on day 245 was 6.3.

The number of fruits per fruiting lateral (Table 1) likewise did not vary significantly with treatment duration. For these data, b values for the regression of fruit counts on time at 37°C for each of the 4 mother plants were b = 0.0044, 0.0034, and --0.0239 (none significant), and for all data pooled, b = --0.0020 (not significant). The extrapolated number of fruits per lateral for propagules taken on day 0 (before heat treatment) was 21; the average number on day 245 was 20.

During the 1979 fruiting season 14 'Thornless Oregon Evergreen' clones were selected in the test plots as being unusually vigorous and fruitful. They were equally representative of all 4 original mother plants and were drawn in about equal numbers from propagules taken from the heat chamber at 122, 157, 214, and 245 days.

Exposure to high temperature has been found to increase the incidence of visible somatic mutations in several plant species (6, 7). Thermotherapy for virus elimination was thought to have caused somatic mutations in potato tubers (9, 11) but these results were interpreted by later workers (8) as effects of virus reinfection. No data have been obtained on the possible effects of prolonged high-temperature growth on the spontaneous mutation rate of vegetatively propagated crop plants.

**Literature Cited**