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Blackberry (Rubus laciniatus) seeds were air-dried at room temperature for 48 hours, scarified for varying lengths of time in concentrated sulphuric acid and then subjected to treatment solutions of sodium hypochlorite, hydrogen cyanamide or gibberellic acid and followed by stratification at 5°C for varying lengths of time from 0 to 4 months. Overall germination percentage ranged from 0 to 54%. The optimal time of acid scarification was found to be 1 hour. Scarification in excess of 1 hour was detrimental. Of the three chemicals used for germination treatments, sodium hypochlorite was superior. Overcoming endocarp and seed coat dormancy via acid scarification was more critical than cold stratification for Rubus seed germination. Seeds which had been stratified at 5°C for up to 4 months did not germinate unless they were acid scarified. Cold statification enhanced germination slightly and then only after the seeds were acid scarified.

In vitro seed germination of 'Cherokee' blackberry resulted in 77% germination when fresh, undried seeds were cut in half. This method of germination could be used to bypass the need for both acid scarification and cold stratification. Air drying fresh seeds for 24, 48 and 72 hours, reduced the percentage germination of these halved seeds.

In vitro asexual propagation of *R. leucodermis* using axillary buds resulted in the production of both shoots and roots in shoot multiplication medium. The greatest number of shoots and roots proliferated was with 3.0 mg/l Benzyladenine (BA) and 0.3 mg/l Indolebutyric acid (IBA). Leaf wedge cuttings of *R. simplex*, *R. illecebrosus*, *R. rigidus* and *R.* spp. (OSC 884 X OSC 743) were able to regenerate both roots and shoots. Of the 4 clones, *R. simplex* was found to be superior to the others in terms of ease and speed of regeneration. Regeneration via leaf disks was also found to be possible with *R. simplex*.

Sexual And Asexual Propagation Of *Rubus*

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SEXUAL AND ASEXUAL PROPAGATION OF *RUBUS*

INTRODUCTION

Rubus includes about 400 species of deciduous or evergreen shrubs or vines, mainly the blackberry, dewberry, raspberry and salmonberry. It can be propagated by sexual and asexual methods. Sexual propagation, though not used for cultivars in commercial plantings, is important for breeding purposes. Low germinability and erratic seedling emergence are the major problems in *Rubus* seedling production. This low seed germination rate is mainly due to an impermeable seed coat and a dormant embryo. Lasheen and Blackhurst (1956) indicated that a growth inhibitor was present in the freshly harvested blackberry seeds which caused a delay in germination. This inhibitor would gradually disappear following moist cold storage. They also suggested that embryo dormancy was due partly to the seed coverings which chemically affect the embryo. By and large, most workers found that even though the *Rubus* seeds may have undergone chemical treatments or seed scarification process, it was necessary to subject the seeds to a period of moist chilling. This stratification process often takes from two to four months.

For asexual propagation, the most common commercial practice, the use of tip layers or stem cuttings is widely

adopted. However, a major constraint of stem propagation is that a relatively limited number of plants can be produced from each stock plant. For in vitro micropropagation, both shoots and roots can be proliferated in different multiplication media. This method of propagation will be especially important as virus free cultivars are developed. With the discovery of nitrogen fixing ability of Rubus ellipticus (Becking, 1979), there was an interest in screening many other Rubus species for their nitrogen fixing abilities. This interest provided the impetus to develop a technique of rapid multiplication of members of this genus.

In view of the above mentioned interest and difficulties, various studies were designed to investigate ways to improve both sexual and asexual propagation of Rubus.

The objectives of these studies are as follows:

I. Sexual Propagation

1. To determine the efficacy of concentrated sulphuric acid (H_2SO_4), sodium hypochlorite (NaOCl), hydrogen cyanamide (H_2CN_2), and gibberellic acid (GA_3) as germination enhancing chemicals.
2. To evaluate their effectiveness at various concentrations.
3. To evaluate the effectiveness of stratification ($5^{\circ}C$) on germination.
4. To evaluate an in vitro technique of seed germination.

II. Asexual Propagation

1. To evaluate the rooting and budding potential of a wide range of Rubus species and clones.
2. To study the effect of growth regulators on plant regeneration.
3. To study the potential and to maximise the production of plant materials via leaf disks.
4. To investigate the possibility of producing shoots and roots in one multiplication medium using tissue culture.

LITERATURE REVIEW

I. Sexual Propagation

Seeds of many species of Rubus are slow to germinate because of their hard and impermeable endocarp in combination with a dormant embryo (Schopmeyer, 1974). In germinating Rubus seeds, many workers have found that pre-germination treatment is required to soften the hard endocarp or to breakdown some of the inhibitors. Following this pre-treatment, it is also necessary to break embryo dormancy.

A. Pre-germination Treatments

Concentrated sulphuric acid is the most common chemical used for the scarification of Rubus seeds. Heit and Slate (1950) reported that stratified blackberry seeds would not germinate unless they were pre-treated with concentrated sulphuric acid. Raspberry seeds which were treated with concentrated sulphuric acid for 50 minutes improved the germination rate: 39.5% for the treated seeds as compared to 10.3% for the untreated seeds (Fejer and Spangelo, 1970). A germination rate of 70% was obtained with blackberry seeds by treating them in concentrated sulphuric acid for 30 minutes followed by leaching for 6 days in 1% aqueous solution of calcium hypochlorite and followed by stratification for 6 months at 2°to 4°C (Wenzel and Smith, 1975).

The effectiveness of sulphuric acid and chemical pre-germination treatment agents may be partly due to

their erosion or softening of the endocarp to facilitate gaseous exchange and partly to their oxidizing properties for promoting chemical changes to enable germination (Jennings and Tulloch, 1956).

The duration of acid treatment played an important role in seed germination. Heit and Slate (1950) reported that sulphuric acid treatments longer than one hour always resulted in injury and in lowering germination of blackberry seeds. They also noted that the acid temperature sometimes reached 50°C during treatment. Such a high temperature could result in embryo injury. Use of an ice-bath prevents outside of the treatment solution, its heating, but reduces acid activity so that longer treatment periods are required for satisfactory scarification (Moore, Brown and Lundergan, 1974). These authors reported that blackberry seed germination was enhanced by extending the duration of concentrated sulphuric acid scarification to 3 hours, prior to the moist cold stratification process. The mean percentage of germination was 17.4% in the 3 hour acid scarification treatment while it was only 0.8% when the seeds were acid scarified for ½ hour. Longer acid treatment would decrease endocarp thickness and seed weight. There was a negative correlation between the endocarp thickness and percentage germination.

Besides concentrated sulphuric acid treatment, Scott and Ink (1957) found that a six day pre-germination treatment with 1.0% sodium hypochlorite resulted in 56% germination

and this was superior to the acid treatment with only 26%. Soolova and Kichira (1971) showed that stratification of raspberry seeds for 6 months followed by soaking in 1.0% sodium hypochlorite and calcium hydroxide for 4 days greatly improved germination. Tomanova (1971) reported that the application of 0.06% potassium iodide appreciably increased raspberry seed germination.

B. Stratification

Under natural conditions, Rubus seeds mature early in the summer and then are followed by a warm and cold period. This effect helps the seed to germinate. For sowing seeds in the spring, Heit (1967) recommended a 50 to 60 minute treatment with sulphuric acid, followed by 30 to 90 days cold stratification. The cold treatment can be eliminated for the fall sown seeds.

In studies of physiological changes occurring during moist chilling(3°C) of blackberry seeds, Lasheen and Blackhurst (1956) indicated that a growth inhibitor was present in the endocarp, testa and embryo. The inhibitor gradually disappeared during moist chilling and there was evidence of the presence of growth promoter after 4 months of such treatment. The disappearance of the inhibitor was correlated with the breaking of embryo dormancy giving the seeds the ability to germinate.

Scott and Ink (1957) showed that warm and cold

stratification improved the germination of Rubus seeds. They reported that by keeping seeds at temperatures of 21° to 24°C for 2 months prior to 3 months at 2°C improved germination. However, the stratification process could possibly be replaced by chemical pre-germination treatments. Jennings and Tulloch (1956) reported that the most useful chemical for pre-germination was 20 minutes with concentrated sulphuric acid followed by 6 days with 1.0% calcium hypochlorite containing an excess of calcium hydroxide. It was indicated that such treatment could eliminate the need for a subsequent period of moist chilling. Lundergan and Carlisi (1984) showed that 75% of the scarified blackberry seeds germinated without stratification when oxygenated in distilled water. They further reported that germination was not improved by addition of 3.46 mg/l of gibberellic acid (GA_3) or 0.02 mg/l of benzyladenine (BA).

Light also plays an important role in Rubus seed germination. With blackberry seeds, after-ripened at 2° to 5°C for 3 months and exposed to light, germination was 21.6%. When the seeds were only partially exposed to light, germination was 1.8%. Total darkness almost completely inhibit germination (Scott and Draper, 1967).

C. In Vitro Germination

In vitro germination of Rubus had been reported to be useful in securing high germination (Ke, Skirvin, McPheeters,

and Otterbacher, 1985). These authors reported that the in vitro method resulted in 57% to 81% germination and that this technique of propagation could bypass the need for cold stratification and drying of seeds.

II. Asexual Propagation

A. Traditional Methods

The traditional methods of asexual propagation of Rubus are tip layering, root and stem cuttings. Tip layering is commercially carried out in blackberry, loganberry, dewberry and some varieties of purple fruited raspberry (Bailey, 1941; Kaine and Mc Questen, 1956). The tips of mature shoots are buried in order to produce roots and new shoots. Plants of blackberry which are propagated by tip layering can be ready for planting after a year in the nursery (Kaine and Mc Questen, 1956). However, tip layering was reported to be unsatisfactory under conditions where the growing season was short or the soil was too heavy (poorly drained or aerated) to produce a good rooting system (Angelo, 1937).

Regeneration of blackberry and raspberry plants by root cuttings was reported by Joley and Close (1940), Butterfield (1947), Hudson (1954, 1955) and Kaine and Mc Questen (1956). The roots are cut in pieces usually 2" or 3" long and planted in the propagation beds. In the cool climates, the rooting was done with bottom heat or in a hot

bed to ensure better rooting and to shorten propagation time (Kaine and Mc Questen, 1956). However, one of the problems in root cutting propagation is that some species of Rubus regenerate readily whereas other species show a marked seasonal fluctuation in capacity to grow (Hudson, 1955).

Leaf-bud cuttings as one method of Rubus propagation was reported by Stoutemyer (1973), Joley and Close (1940), Angelo (1937) and Thomas (1935). A leaf-bud cutting consists of a leaf and a bud with a bit of bark attached. Thomas (1935) reported 90 to 100% success in leaf-bud propagation of Rubus armeniaca, R. ellipticus, R. fraxinifolia, R. glaucus and R. parvifolia.

Blackberry could also be easily propagated from one-node softwood cuttings (Zimmerman, Galletta and Broome, 1980). These authors reported that the one-node softwood cuttings rooted better than hardwood cuttings. 83% of the one-node softwood cuttings rooted compared to 61% in the hardwood cuttings.

An increased number of buds on stem cuttings increased the percentage of rooting. As reported by Rosati and Faedi (1978) for blackberry, stem cuttings with 5 buds produced the highest percentage of rooting, as compared to 1,2,3 or 4 buds.

In comparing the rooting media for blackberry cuttings, Zimmerman, Galletta and Brown (1980) reported that 56% to 65% of the cuttings rooted equally well in sand, perlite, peat-perlite, sand-peat and 'Oasis' root cubes. The cuttings which

rooted in sand had the most extensive root systems, but the roots were found to be coarse and brittle with a tendency to break when the cuttings were extracted. A fibrous rooting system was obtained when the cuttings were rooted in peat-perlite and sand-peat media, and the roots could withstand handling better.

Growth regulators enhanced rooting in stem cuttings. Spriovska (1982) obtained 60% success by treating the blackberry cuttings with a quick dip of 800 mg/l indolebutyric acid (IBA) in perlite or peat and sand at 25° C under mist. Schimmelpfeng (1974) reported that by soaking one-node cuttings in 25, 50 and 100 mg/l of indoleacetic acid (IAA) for 22 hours increased the number of rooted cuttings, rate of root formation and the size of root mass.

B. Tissue Culture of Rubus

In vitro micropropagation has been successful in the propagation of blackberry and raspberry (Broome and Zimmerman, 1978; James, 1979; Carrillo and Mendoza, 1979; James, Knight and Thurbon, 1980; Anderson, 1980; Skirvin, Chu and Gomez, 1981; Snir, 1981; Pyott and Converse, 1981; Slivinski, Preece and Myer, 1983).

For shoot proliferation, different workers used different culture media and varying concentrations and combinations of hormones and nutrients. Anderson (1980) reported a two fold increase in shoot multiplication with

red raspberry on Anderson Inorganics (8.6 shoots) as compared with Murashige and Skoog Inorganics (4.1 shoots). The optimal concentration of hormones for shoot proliferation was 0.02 to 0.5 mg/l IBA and 1.0 to 2.0 mg/l BA. In contrast, Broome and Zimmerman (1980) successfully propagated thornless blackberry on Murashige and Skoog medium with 1.0 mg/l BA and 0.1 mg/l GA_3 and 1.0 mg/l IBA. James, Knight and Thurbon (1980) reported that using Linsmaier and Skoog medium with BA at 0.1 mg/l and IBA at 0.1 mg/l was optimal for red raspberry. They also reported that phloroglucinol at 162 mg/l could enhance the shoot number.

In thornless blackberry, Slivinski, Preece and Myer (1983) reported that BA at 1.0, 2.0 and 5.0 mg/l did not significantly affect shoot proliferation. However, GA_3 at 0.5 mg/l and 1.0 mg/l significantly increased the shoot number. Approximately 2 shoots were formed per explant in the medium containing 0.5 or 1.0 mg/l GA_3 as compared to less than 1 shoot formed with 0.1 mg/l GA_3. Skirvin, Chu and Gomez (1981) reported that rapid proliferation of axillary buds of trailing blackberry cvs. Thornless Boyesnberry and Thornless Youngberry was achieved after 4 to 6 weeks on a modified Murashige and Skoog medium, containing 2 mg/l BA and 0.1 mg/l of naphthalene acetic acid (NAA).

In studying the effect of natural products on *in vitro* development of *Rubus*, Carrillo and Mendoza (1979) reported that 5% of pure honey resulted in 69% of well formed plants.

In investigating the rooting ability of the shoots, Anderson (1980) recommended that no BA should be included in the medium, but IBA at 1.0 mg/l and activated charcoal at 600 mg/l were optimal for red and black raspberry. The proliferated shoots did not root at all in the medium if charcoal was omitted (Snir, 1981). James, Knight and Thurbon (1980) suggested that removing cytokinin and increasing IBA to 1.0 mg/l resulted in adventitious root formation. Skirvin, Chu and Gomez (1981) reported that shoots were induced to root on Murashige and Skoog medium consisting of higher mineral salts and stable vitamins plus myoinositol at 100 mg/l.

Many workers also reported that taking the shoots out of the test tubes and planting them in peat pellets could ensure good rooting. Snir (1981) reported 100% rooting effeciency by planting the shoots in Jiffy 7 pellets compared to 10% rooting in agar medium. Slivinski, Preece and Myer (1983) achieved over 90% rooting success in peat-lite medium under high humidity condition. Pyott and Converse (1981) reported over 75% of shoots rooted when dusted with rooting powder (0.1% IBA) and placed in sand with mist.

Anderson (1980) suggested that tissue culture of Rubus could be used to: 1) rapidly increase varieties to commercial quantities; 2) maintain germplasm 3) develop disease-free planting stocks.

Tissue culture could be a good technique for rapid multiplication, but it may be too expensive for commercial

application and there may be a potential for mutation during *in vitro* culture but this has not yet been fully evaluated (Zimmerman, Galletta and Broome, 1980). So far, in the study of the phenotypic stability and field performance of tissue culture in blackberry, Swartz, Galletta and Zimmerman (1981) reported that the total growth, branching habits, fruit size and yield performance of the tissue cultured plants were similar to that of plants produced from one-node cuttings.

C. Leaf Disk Propagation

Plant regeneration via leaf disks has not been documented so far in *Rubus*. However, it was successful in *Begonia* and *Peperomia* (Schraudolf and Reinert, 1959; Lagerstedt, 1967; Henny, 1978). To be successful in plant regeneration, ability of the disks to both root and to develop adventitious buds is of utmost importance. There are a number of plant regulators and environmental factors which have been successfully reported for the promotion of roots and shoots by various workers.

1).Effect of Plant Growth Regulators

Root initiation is greatly enhanced by auxins. In the case of *Begonia* (Lagerstedt, 1967), 40 to 50 propagules could be obtained from a single leaf. The root initiation of *Begonia* leaf disks was greatly accelerated with IBA concentrations of 50 and 100 mg/l. The rooting response was further enhanced at an IBA concentration exceeding 100 mg/l and treatment longer

than 30 minutes; however, it was detrimental to the bud development. Kinetin at 1 mg/l was found to inhibit rooting. If kinetin concentration up to 10 mg/l was used with IBA at 50 mg/l, root morphology was influenced.With this treatment, a single root was initiated and it grew long and relatively unbranched. IBA at 50 mg/l usually initiated numerous short roots which branched readily. Since rooting always preceded budding, the latter was also accelerated and so a whole plantlet was formed more rapidly than where IBA was not used.

Using leaf squares of Peperomia sandersii, Harris and Hart Ennid (1964) reported that bud initiation does not occur independently of rooting. Promotion of roots by IBA was accompanied by an increase in the number of buds. Excision of roots from the leaf square prevented the formation of buds. Similarly, when the roots were inhibited by kinetin and BA, bud initiation was inhibited. James (1979) showed that IBA and phloroglucinol (PG) had a synergistic effect on the promotion of roots in the Rubus (ursinus X idaeus). On a roots per rooted culture basis, IBA with PG resulted in 20 roots compared with only 8 roots if with IBA alone.

In a study by Wildern and Crily (1975), cytokinin was found to increase shoot production on leaf cuttings of Begonia. IBA at 50 mg/l increased the average number of primary roots and root length per cutting by 10% and 13% respectively over the control. However, the beneficial effects of IBA were outweighed by its detrimental effects in reducing the average

number of shoots and shoot length per cutting by 30% and 20% respectively. On the other hand, the application of a cytokinin, either BA or 6-(benzylamino)-9-(2-tetrahydropyrayl)-9-H-Purine (PBA) at 50 mg/l gave a significant increase in the production of new shoots. PBA at a concentration of 200 mg/l stimulated the greatest number of shoots without reducing the number of roots below the control level. Reduction of root length was experienced in treatments where PBA was 500 and 1000 mg/l. The application of 25 mg/l of kinetin was reported to induce the bud initiation in the basal callus of leaf cuttings of Saintpaulia ionantha (Plummer and Leopold, 1957).

Further studies on combinations of growth regulators showed that a high kinetin/NAA ratio promoted shoot initiation in Peperomia leaf disks (Henny, 1978). Treatments of 10, 5 and 2.5 mg/l kinetin combined with 0.05 mg/l NAA or 10 mg/l kinetin combined with 0.5 mg/l NAA produced numerous (more than 50) shoots at the leaf section, but there was no root production. Conversely, application of NAA at a concentration of 5.0 mg/l caused root production regardless of kinetin level, but the shoot rarely developed.

In regards to adventitious bud formation of Begonia leaf cuttings, it appeared that kinetin alone had no appreciable effect at 0.5 mg/l, but promoted bud formation at 8.6 mg/l (Heide, 1965a). Indoleacetic acid (IAA) did not inhibit bud formation at concentrations of 0.4, 1.8 or 7.0 mg/l, but

suppressed it entirely at a concentration of 28 mg/l. With the combination of the two substances, IAA at 0.4 to 7.0 mg/l was not antagonistic to the promoting effect of kinetin, but at 28 mg/l, it was. The opposite effect of the two substances was found on root formation. IAA at 7.00 and 28 mg/l increased the number of roots and kinetin at 1.8 mg/l reduced it.

Other plant growth regulators also have profound effects on vegetative propagation. In leaf cutting of Sedum, 2-chloroethyl-phosphonic acid (ethephon) at 500 mg/l and 1000 mg/l produced the most shoots and NAA at 25, 50 and 250 mg/l formed the most roots (Boe, Steward and Banko, 1972). Inhibitory results on root formation were shown by Zych (1965) whereby 10 mg/l, 100 mg/l and 1000 mg/l of potassium salt of gibberellin (GA_3) reduced the percentage of rooted cuttings. Root initiation of Begonia leaf disks was reported to be accelerated by 2,4-dichlorophenoxy-acetic acid (2,4-D) at a concentrations of 10^{-5} and 10^{-2} mg/l and was delayed at lower concentrations (up to 10^{-4} mg/l) (Schraudolf and Reinert, 1959). In the absence of auxin, kinetin at 5×10^{-3} mg/l increased shoot formation.

2). Effect of temperature, photoperiod, light and their interactions with plant growth regulators.

Shoot formation was stimulated at temperatures of 13°C to 21°C and suppressed at high temperature of 27°C. The reverse effect was observed for root initiation. Good root formation was found at 24°C (Heide, 1964, 1965). The appearance

of roots was delayed at 15°C compared with 24°C.

High percentage of adventitious bud formation was obtained at low temperature(15°C) even without kinetin treatment. However, with kinetin treatment (28 mg/l), the number of buds forming per cutting increased greatly.

Short days (9 hours) applied during the propagation period stimulated the formation of buds compared with continuous light. In contrast, short days (8 to 10 hours) suppressed root formation as compared to 16 hour days or continuous light (Heide, 1964, 1965).

Light intensity has proved to be a critical environmental factor in leaf disk propagation. Lagerstedt (1968) indicated that a light intensity of 500 foot candles was inhibitory to growth of Begonia. The growth of Geranium callus was observed to be poor at 10 klx of continuous cool white fluorescent light compared to lower light intensities of 1 and 5 klx (Hammerschlag, 1978).

3). Varieties and age of leaf cuttings

The ease of regeneration of leaf cutting of blackberry differed among varieties. In one-node leaf cuttings, Zimmerman, Galletta and Broome (1980) found tha 'Black Satin', S1-US-68-6-6 and S1-US-68-6-17 were the easiest to root as compared to 'Smooth Stem'. The ability of leaf cuttings to form buds and roots depends on the age of the leaf. Heide (1965a)

showed that the young expanding leaves had a greater ability to form buds than older leaves. Root formation was more easily inhibited by cytokinin application in very young or very old leaves as compared to newly expanded leaves. The old leaves also showed less budding stimulation after cytokinin treatment than the younger leaves.

MATERIALS AND METHODS

I. Sexual Propagation

A. Germination Trials

Ripe, frozen fruits of Rubus laciniatus 'Thornless Evergreen' were soaked overnight, placed in a bladder and cleaned to obtain the seeds. The seeds which sank in the water were used for this trial and seeds which floated were discarded. The cleaned seeds were then air dried for 48 hours at 25°C and subjected to the following sequence of treatments:

1. Scarification with concentrated sulphuric acid for 0, ½, 1 or 1½ hours.
2. Chemical treatment with GA or NaOCl or H_2CN_2 for 24 hours.
3. Cold stratification at 5°C for 0, 1, 2, 3, or 4 months.

For scarification treatments, the seeds were soaked in concentrated sulphuric acid in a cold water bath (8° to 10°C) for ½, 1 or 1½ hours. The scarified seeds were then rinsed with 1.0% sodium bicarbonate solution followed by running water for 10 minutes. To overcome the rest period, seeds were then treated with GA_3 or NaOCl or H_2CN_2. The seeds were soaked in the treatment solutions for 24 hours and then rinsed with tap water before placing them on a moist piece of germination paper in a quadratic petri dish (9 cm diameter).

Five similar sets of GA_3, NaOCl and H_2CN_2 treatments

were prepared and subjected to stratification at 5°C for 0,1,2,3 or 4 months. The germination paper moisture was maintained by periodically adding a 0.2% Captan solution which also prevented fungal contamination.

Germination was carried out in a germinator with 25°C, day; 15°C night temperature and a light intensity of 330 foot candles for 12 hours each day.

The experiment was a 4x5x5 factorial i.e., 4 different time of acid scarification, 5 different time of stratification and 5 levels of either GA_3 or NaOCl or H_2CN_2 concentrations.

Each treatment consisted of 100 seeds and the experiments were arranged in a completely randomized design with 4 replicates. The treatment numbers were arranged and listed on the following pages:

GA_3 Trial

Stratification	Scarification	GA_3(mg/l)				
5°C (month)	H_2SO_4(hour)	0	100	200	300	400
0	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20
1	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20
2	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20
3	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20
4	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20

NaOCl Trial

Stratification 5°C (month)	Scarification H_2SO_4 (hour)	NaOCl (%)				
		0	1	2	3	4
0	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20
1	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20
2	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20
3	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20
4	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20

H_2CN_2 Trial

Stratification 5°C (month)	Scarification H_2SO_4 (mg/l)	H_2CN_2 (mg/l)				
		0	100	200	300	400
0	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20
1	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20
2	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20
3	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20
4	0	1	5	9	13	17
	½	2	6	10	14	18
	1	3	7	11	15	19
	1½	4	8	12	16	20

The number of seeds that germinated was recorded weekly for 3 months. Seeds were considered to be germinated when the plumule or radicle emerged. At the end of the 3 month period, sample seed viablibilty of those that had not germinated was determined by tetrazolium test (TZ). Fifty seeds were clipped on one side and treated with a 1.0% TZ solution sufficient to cover the seeds. The seeds were then incubated at 38°C for 12 hours. Viable seeds produced a red stain and the non-viable seeds were white or only partially stained.

A separate group of seeds were subjected to an X-ray scanning test to determine the percentage of normal, abnormal and empty seeds. Two hundred seeds were placed in templates and the templates were put on a clipboard which was then placed in the X-ray machine. The seeds were exposed to X-ray for 5 minutes at 18 kilovolts. Normal and abnormal seeds could be distinguished from the X-ray film. The cotyledons of normal seeds fully filled the seed as compared to the abnormal seed which was only partly filled or empty.

For anatomical studies, the sides of the seeds were clipped off to permit penetration of the fixatives FAA (Formaldehyde, Acetic acid, Alcohol). After 24 hours of FAA treatment, the seeds were run through a series of tertiary butyl alcohol and then embeded in paraffin for sectioning. Cross sections, 15 μm thick were cut and then stained with

safranin and fast green and photographed at 125X.

B. In Vitro Germination

The germination ability of both intact and halved seeds of 'Cherokee' blackberry was investigated. The methods of obtaining the cleaned seeds were similar to that of Experiment A. The seeds were divided int 8 lots as follows:

Intact seeds	Halved seeds
Fresh (Control)	Fresh (Control)
24 hours air-dried	24 hours air-dried
48 hours air-dried	48 hours air-dried
72 hours air-dried	72 hours air-dried

Except for the fresh treatment, all seeds were air-dried at 25°C for varying length of time. Before placing onto the culture medium, all seeds were disinfected in 1.0% NaOCl solution for 15 minutes, then rinsed 2 times with sterilized distilled water. Following surface sterilization, halved seeds were cut in cross section and both halved were placed onto the culture medium. The endocarp remained attached to the halved seed.

All seeds were placed in Anderson medium(II) (Anderson, 1980), salts only, no plant hormones. The pH was adjusted to 5.7 before autoclaving. The autoclaved medium (20 minutes) was then suspended in sterilized quadratic Petri dishes.

The culture room temperature was constant at 25°C ± 2°C with 16 hours of light. The experiment was conducted in

a completely randomized design. Each treatment consisted of 100 seeds with 4 replications.

The germination was recorded weekly for 6 weeks.

II. Asexual Propagation

A. Leaf Wedge Cuttings - Screening Trial

The experiment was initiated in July, 1984 in the greenhouse of the National Clonal Germplasm Repository in Corvallis. The preliminary trials consisted of screening some of the Rubus accessions to determine if they possessed the capacity of regenerating from leaf cuttings. A total of 25 species and clones were screened (See list page 27). The leaf was cut into half and each half was used as a leaf wedge cutting. The leaf cuttings were divided into 3 lots. In one lot, the control cuttings were dipped in water. The other two lots were dipped in Hormodin #1 (0.1% IBA) and Hormodin #2 (0.3% IBA) respectively. The leaf cuttings were then planted in a sand bed with intermittant mist. Each treatment consisted of 24 cuttings which were replicated 4 times. The experiment was arranged in a completely randomized design.

The number of cuttings which rooted was recorded periodically and the rooted cuttings were transplanted into a 10 x 10 cm pot with a mixture of sand:perlite:peat moss (1:1:1). The number of cuttings that subsequently also gave rise to adventitious buds and shoots was recorded periodically for 6 months.

Leaf Wedge Cuttings Trial

List of National Clonal Germplasm Repository Rubus Plants Used In Leaf Wedge Trial

Local No	Rubus Plant Name	Pedigree
31	R. illecebrosus	
39	R. scanicus	
40	R. scheutzii	
41	R. shankii	
42	R. wahlbergii	
43	R. radula	
44	R. plicatus	
45	R. picetorus	
48	R. thyrsoideus	
49	R. flosculosus	
51	R. hirtus	
144	R. rigidus	
178	R. calycinoides	
181	R. lambertianus	
218	R. moluccanus	
264	R. biflorus	
344	R. spp.	Olallie X OSC 878
346	R. spp.	OSC 743 X Chehalem
351	R. spp.	Jenner X Eldorado
353	R. spp.	Chehalem X Olallie
360	R. spp.	Chehalem X Cascade
361	R. spp.	Rote Reisen X Willamette
368	R. spp.	OSC 884 X OSC 743
394	R. ellipticus	
428	R. simplex	

B. Leaf Disk Propagation

From the leaf wedge cutting study, one specie, Rubus simplex responded well and was selected for further investigation, i.e., propagation by leaf disks. The leaf disk experiment was started in May, 1985. Mature, expanded leaves were collected in the morning to ensure good turgidity. A leaf disk was then carefully obtained by using a 2 cm diameter cork borer. Generally, 3 to 7 leaf disks could be obtained from a single leaf. The leaf disks were thoroughly mixed to form a composite sample. From this composite sample, the leaf disks were randomly selected and treated with the appropriate solutions on filter paper in a petri dish for 24 hours. The thorns underneath the leaf disk were cut to ensure a flat surface for good absorption. Each treatment consisted of 32 leaf disks which were replicated 4 times. The experiment was a 4x4 factorial and it was arranged in a completely randomized design. Following the 24 hours treatment, the leaf disks were planted in a sand bed with an intermittant mist system.

The treatments were as follows:

IBA (mg/l)	BA (mg/l)			
	0	2.5	5.0	10.0
0	1	5	9	13
25	2	6	10	14
50	3	7	11	15
100	4	8	12	16

IBA and BA were prepared as a stock solution of 1000 mg/l and 100 mg/l respectively in 95% ethyl alcohol and then diluted with distilled water. The pH of the solution was adjusted to 7.0.

The number of cuttings developing roots and shoots were recorded periodically for 3 months.

C. In Vitro Propagation

Rubus leucodermis was used for this experiment conducted in the tissue culture laboratory in the National Clonal Germplasm Repository, Corvallis. The explants were obtained from a stock culture maintained in Anderson medium (stage II). Axillary buds (2 to 4 mm) were excised from the stock plants. The Anderson II medium was used omitting the plant hormones. Instead, a combination of different concentrations of IBA and BA were used as the treatments for this experiment. It was a 4x4 factorial experiment and the treatments were arranged as follows:

BA (mg/l)	IBA (mg/l)			
	0	0.1	0.2	0.3
0	1	5	9	13
1	2	6	10	14
2	3	7	11	15
3	4	8	12	16

The test propagules were randomly selected and planted in test tubes perpendicular to surface of the culture medium. Each treatment consisted of 40 propagules replicated 4 times in a completely randomized design. The culture room temperature was maintained at $25^{\circ} \pm 2^{\circ}$C with 16 hours of light.

Uncontaminated test propagules were transferred to fresh media after 4 weeks and were evaluated at that time for shoot and root development. The evaluation was carried out over a 12 week period. The rate of shoot multiplication was determined by counting the number of shoots produced by each propagule after each 4 weeks of incubation. The rooting index was based on: 1) no roots 2) initial roots 3) more than 1 long and branched root.

RESULTS AND DISCUSSION

I. Sexual Propagation

A. Germination Trials

1. X-ray, viability tests and anatomical study

Only cleaned Rubus laciniatus seeds which sank in the water were used for the germination experiments. From the X-ray scanning tests, it was found that 16% of the seeds that sank were either empty or abnormal (Plate 1). Thus, it became obvious that this sizeable percentage of empty and abnormal seeds would make it impossible to achieve 100% germination in Rubus. Kerr (1954) also reported that blackberry seeds which sank in water contained as high as 20.5% defective seeds.

From the seed viability tests, an average of 67% of the non-germinated seeds in the non-stratified treatments remained viable (Table 1). Acid scarification reduced seed viability about 23%. Similar results were reported by Moore, Broown and Lundergan (1974) where 3 hours of acid scarification allowed the acid to penetrate and kill the embryo of 'Arkansas 578' blackberry seed.

A cross-section of the seed shows that the embryo is enclosed by a thick endocarp (Plate 2). As reported by Lasheen and Blackhurst (1956), the endocarp is composed of thick-walled, stone-like cells packed tightly together. This

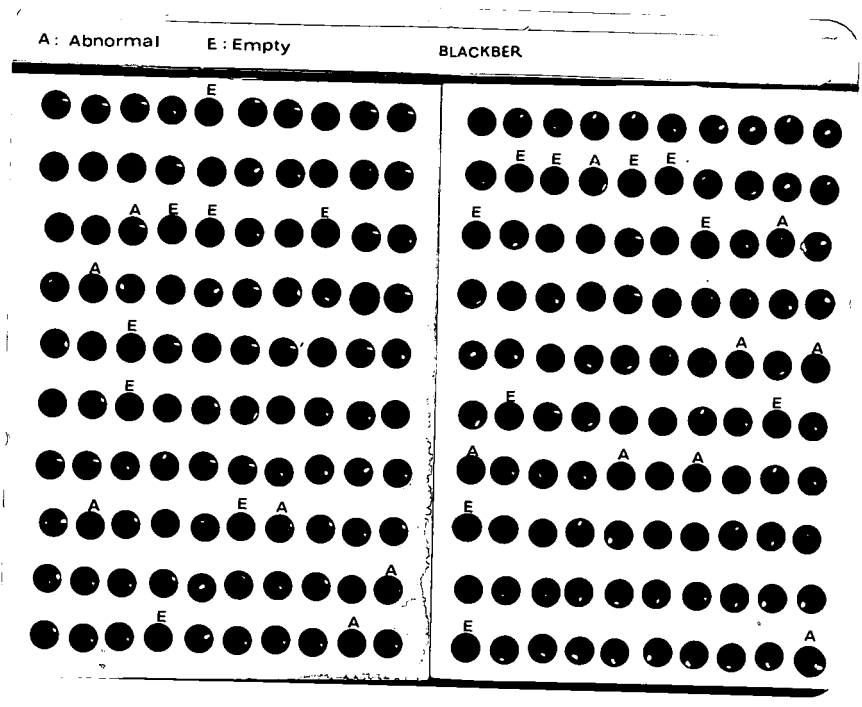

Plate 1. X-ray test of Rubus laciniatus seeds.

Table 1. Viability test of non-germinated, non-stratified *R. laciniatus* seeds.

Treatment	Mean Percentage Viability
Water (control)*	78
½ hour H_2SO_4	68
1 hour H_2SO_4	65
1½ hour H_2SO_4	55
Mean	66.5
L.S.D. (1%)	8.6

* non-scarified

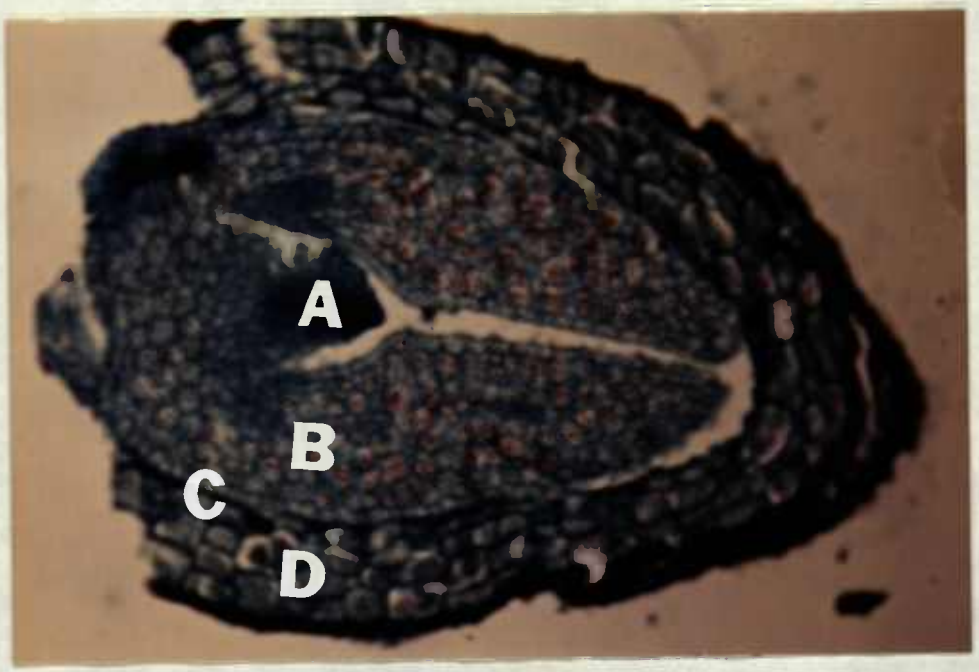

Plate 2. Cross-section of a mature seed of Rubus laciniatus (125 X). A: Embryo, B: Cotyledon, C: Seed Coat, D: Endocarp.

endocarp can restrict seed germination by being a mechanical barrier and by excluding oxygen and water according to Moore, Brown and Lundergan (1974).

2. Analysis of Variance (ANOVA)

The ANOVAs for scarification x stratification x NaOCl, and for scarification x stratification x H_2CN_2, show that these 3 factor interactions were all significantly different at the 1% level, but had a low 'F' value as compared to 2 factor interactions (Tables 2 and 3). The mean percentage of germination for the 2 factor interactions are presented in 2 way tables (Tables 5,6,7,8,9, and 10). The 3 way tables of mean percentage of germination for NaOCl and H_2CN_2 are presented in the appendix. For the GA_3 trial, there was no significant difference in the 3 factor interactions (Table 4).

For each of the three chemicals used, i.e., NaOCl, H_2CN_2 and GA_3, the 2 way interactions between acid scarification and cold stratification were highly significant (Tables 2,3, and 4). This indicates the importance of these two physical treatments.

When cold stratification is not considered, significant 2 way interactions are found between acid scarification and NaOCl or H_2CN_2 treatments, but not between acid scarification and the GA_3 treatment.

Table 2. ANOVA of Scarification x Stratification x NaOCl

Source	df	SS	MS	F
Total	399	128961.56		
NaOCl	4	2279.76	569.94	47.14**
Scarification	3	106847.16	35615.72	2945.88**
NaOCl x Scarification	12	1568.24	130.69	10.81**
Stratification	4	5976.56	1494.14	123.58**
NaOCl x Stratification	16	314.24	19.64	1.62
Scarification x Stratific.	12	7229.84	602.40	49.83**
NaOCl x Scar. x Strat.	48	1117.76	23.29	1.93**
Error	300	3628.00	12.09	

** significant at 1% level

Table 3. ANOVA of Scarification x Stratification x H_2CN_2

Source	df	SS	MS	F
Total	399	97606.79		
H_2CN_2	4	223.84	55.96	5.11**
Scarification	3	82834.83	27611.61	2521.61**
H_2CN_2 x Scarification	12	331.52	27.63	2.52**
Stratification	4	4565.24	1141.31	104.23**
H_2CN_2 x Stratification	16	617.96	38.62	3.53**
Scar. x Strat.	12	4714.12	392.84	35.88**
H_2CN_2 x Scar. x Strat.	48	1033.28	21.53	1.97**
Error	300	3285.99	10.95	

** significant at 1% level

Table 4. ANOVA of Scarification x Stratification x GA_3

Source	df	SS	MS	F
Total	399	86402.39		
GA_3	4	82.84	20.71	2.50*
Scarification	3	74623.23	24874.41	2996.92**
GA_3 x Scarification	12	108.52	9.04	1.09
Stratification	4	5327.84	1331.96	160.48**
GA_3 x Stratification	16	123.96	7.75	0.93
Scar. x Strat.	12	3335.52	277.96	33.49**
GA_3 x Scar. x Strat.	48	309.48	6.45	0.78
Error	300	2491.00	8.30	

* Significant at 5% level

** Significant at 1% level

3. Non-scarified seeds.

There was no germination for non-scarified seeds regardless of other treatments such as NaOCl, H_2CN_2 or GA_3 at various concentrations for 24 hours (Tables 6,9 and 12). It may be possible that 24 hours of soaking was insufficient because Scott and Ink (1957) reported that one or two weeks of soaking seeds in 1% sodium hypochlorite was necessary. Jennings and Tulloch (1965) reported that 6 days of seed soaking in 0.5% solution of sodium hypochlorite improved germination.

For non-scarified seeds, even stratification at 5°C for one to four months did not facilitate germination regardless of NaOCl, H_2CN_2 or GA_3 treatments (Tables 5,7 and 10). The same results were also reported by Heit and Slate (1950).

4. Scarification followed by chemical treatment.

With acid scarification of R. laciniatus seeds, the time seeds were soaked in concentrated sulphuric acid was found to be of the utmost importance. From Tables 6,9 and 12, it can be seen that one hour of acid treatment was the optimal. For the NaOCl trial, acid scarification for ½,1 and 1½ hours resulted in germination of 20,32 and 10% respectively (Fig. 1). This result was consistent with those reported by Heit and Slate (1950) where 1½ hours of acid scarification caused seed injury and greatly reduced the germination percentage.

Table 5. Mean percentage germination of R. laciniatus at varying lengths of acid scarification and cold stratification: For NaOCl Trial.

Scarification with H_2SO_4	Cold Stratification 5°C (month)				
	0	1	2	3	4
0 hour	0	0	0	0	0
½ hour	24.8	21.8	43.2	44.2	38.4
1 hour	39.2	32.2	48.2	46.8	44.4
1½ hour	12.4	20.2	19.6	17.2	13.6

S.E. : 0.78 (300 Error d.f.)

L.S.D. (1%) : 2.9

Table 6. Mean percentage germination of R. laciniatus at varying lengths of acid scarification and different NaOCl concentrations.

Scarification with H_2SO_4	NaOCl (%)				
	0	1	2	3	4
0 hour	0	0	0	0	0
½ hour	27.8	30.6	36.4	38.0	39.6
1 hour	35.8	39.4	42.8	45.6	47.2
1½ hour	15.0	16.4	16.4	17.4	17.8

S.E. : 0.78 (300 Error d.f)

L.S.D. (1%) : 2.9

Table 7. Mean percentage germination of R. laciniatus at varying length of acid scarification and cold stratification:- H_2CN_2

Scarification with H_2SO_4	Cold Stratification 5°C (month)				
	0	1	2	3	4
0 hour	0	0	0	0	0
½ hour	18.4	23.4	37.8	33.6	34.2
1 hour	33.5	32.0	44.0	42.4	38.6
1½ hour	10.2	21.4	19.8	16.2	13.4

S.E. : 0.74 (300 Error d.f.)

L.S.D. (1%) : 2.7

Table 8. Mean percentage germination of R. laciniatus at varying length of cold stratification and different H_2CN_2 concentrations.

H_2CN_2 mg/l	Cold Stratification 5°C (month)				
	0	1	2	3	4
0	15.5	16.8	23.3	22.3	20.8
100	17.3	17.8	25.8	24.8	24.0
200	16.1	19.3	25.3	24.5	20.8
300	14.8	20.5	28.3	21.8	21.5
400	14.0	21.8	24.5	22.0	20.8

S.E. : 0.83 (300 Error d.f.)

L.S.D. (1%) : 3.0

Table 9. Mean percentage germination of R. laciniatus at varying length of acid scarification and different H_2CN_2 concentrations.

Scarification with H_2SO_4	H_2CN_2 (mg/l)				
	0	100	200	300	400
0 hour	0	0	0	0	0
½ hour	27.8	30.2	29.8	29.8	29.8
1 hour	36.0	41.6	38.5	38.4	36.0
1½ hour	15.0	15.8	16.4	17.2	16.6

S.E. : 0.74 (300 Error d.f.)

L.S.D. (1%) : 2.7

Table 10. Mean percentage germination of R. laciniatus at varying lengths of acid scarification and cold stratification :- GA_3 Trial.

Scarification With H_2SO_4	Cold Stratification 5°C (month)				
	0	1	2	3	4
0 hour	0	0	0	0	0
½ hour	19.0	22.3	37.6	32.0	32.4
1 hour	31.0	30.2	41.6	40.8	38.0
1½ hour	11.3	18.6	23.3	18.4	15.6

S.E. : 0.64 (300 Error d.f.)
L.S.D. (1%) : 2.4

Table 11. Mean percentage germination of R. laciniatus at varying concentrations of GA_3.

GA_3 (mg/l)	0	100	200	300	400
Mean %	19.7	20.7	20.9	20.9	20.8

S.E. : 0.32 (300 Error d.d.)
L.S.D. (5%) : 0.9

Table 12. Mean percentage germination of R. laciniatus at varying lengths of acid scarification:- GA_3 Trial.

	Scarification with H_2SO_4 (hour)			
	0	½	1	1½
Mean %	0	28.6	36.3	17.4

S.E. : 0.29 (300 Error d.f.)
L.S.D. (1%) : 1.1

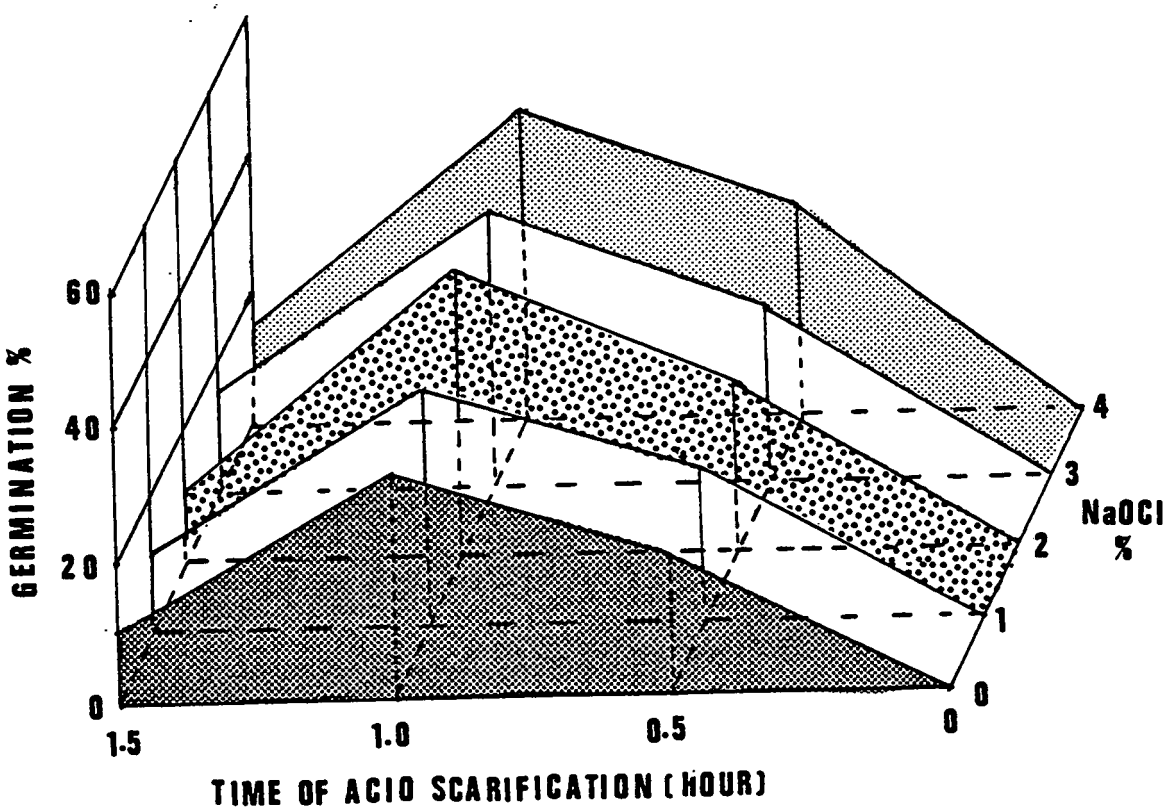

Fig.1. Effect of concentrated H_2SO_4 & NaOCl on germination of R. laciniatus :- non stratification

The chemical treatments enhanced germination only when the seeds had prior acid scarification. For non-scarified seeds, chemical treatment alone seemed to have no effect (Tables 6,9 and 11). Among the chemicals used for pre-germination treatments, NaOCl was superior to both H_2CN_2 and GA_3. A 4% concentration of NaOCl consistently yielded higher germination than treatments of lower concentration (Table 6). Following one hour of acid scarification, about 47% germination was obtained with 4% NaOCl as compared to 39% germination at 1% NaOCl. Jennings and Tulloch (1965) reported that if seeds were soaked longer than 6 days in a solution of NaOCl, a lower concentration of 0.5% was more effective than a 1% solution.

After 1 hour of acid scarification, H_2CN_2 treatment of 100 mg/l produced the best germination (Table 9). This concentration resulted in 41.6% germination compared to 36% at 400 mg/l. Compared to NaOCl, H_2CN_2 is a powerful oxidant even though it was used at much lower concentrations.

Following acid scarification, GA_3 treatments showed significant differences in germination compared to the control (Table 11). However, there was no significant difference in germination percentage with the various concentrations of GA_3. The control treatment had about 20% germination compared to 21% germination if with GA_3.

5. Scarification and chemical treatment followed by different periods of cold stratification.

Tables 5,6,7,8 and 10 show that cold stratification enhanced the germination of the acid scarified seeds followed by NaOCl or H_2CN_2 or GA_3. The stratified blackberry seeds would not germinate unless they were treated with concentrated sulphuric acid. This indicated that breaking the seed endocarp dormancy via acid scarification was more critical than cold stratification in R. laciniatus seed germination.

Following $\frac{1}{2}$ or 1 hour of acid scarification plus one of the 3 chemical treatments, cold stratification for 2 months increased average seed germination from 16.3% to 35.0% over non-stratified treatment (Table 13). Seeds which had undergone cold stratification for one month showed only a 2% germination increase over the non-stratified seeds.

The highest germination rate (54%), was obtained with 1 hour of acid treatment, followed by a 4% NaOCl treatment and 2 months of cold stratification (Fig 2). These data show that 1 month of stratification was less effective than 2,3 or 4 months.

For H_2CN_2, the highest concentration,(400 mg/l) generally provided the lowest germination percentage except at 1 month of cold stratification (Table 7). At 300 mg/l H_2CN_2, the highest germination was found to be 44% following one hour acid scarification and 2 months of cold stratification (Tables 7 and 8).

Table 13. Effect of stratification (5°C) on average germination percentage after treated with concentrated sulphuric acid, NaOCl or H_2CN_2 or GA_3 on R. laciniatus seeds.

5°C stratification	Mean germination (%)
0 month	16.3
1 month	18.5
2 months	35.0
3 months	24.3
4 months	22.4

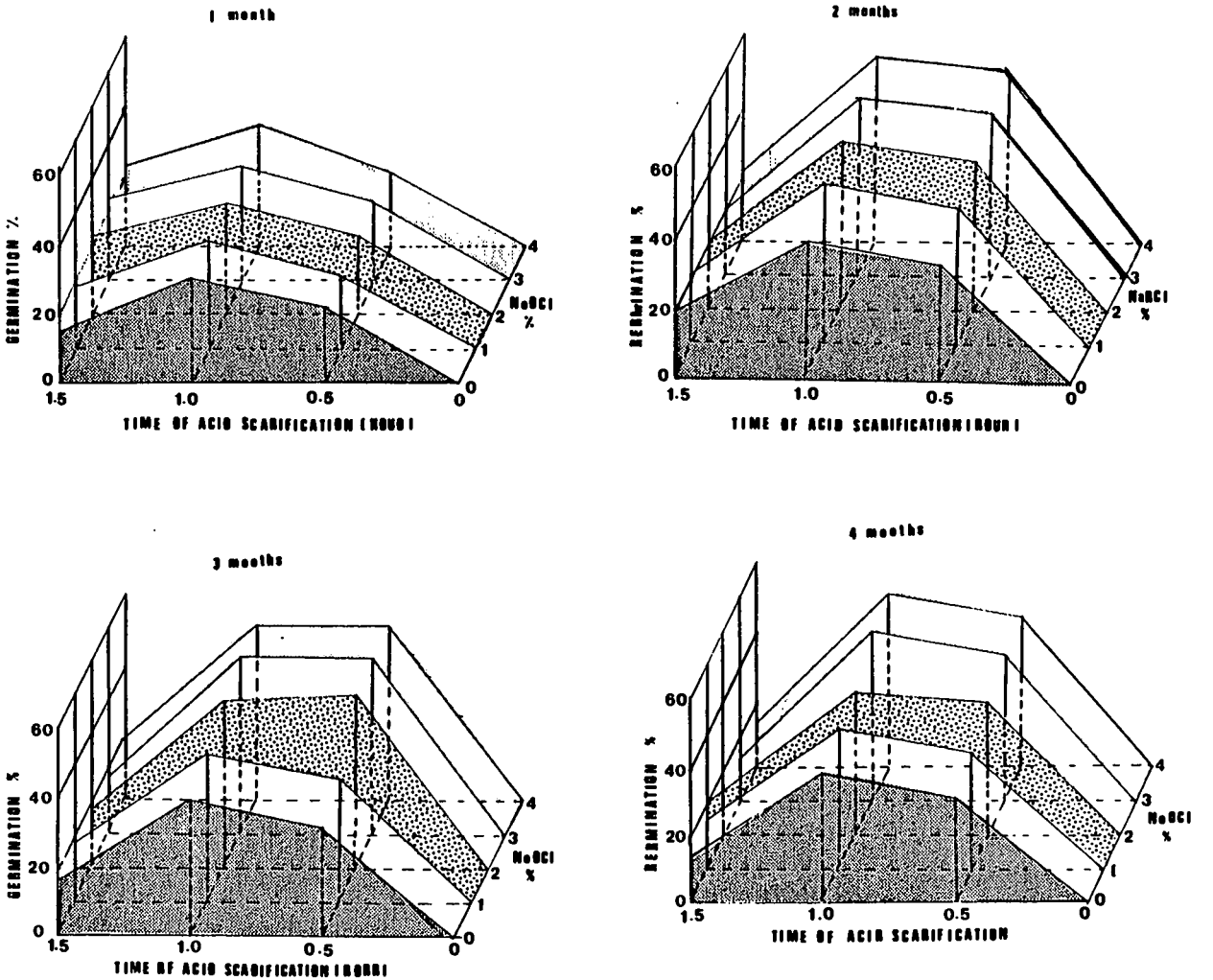

Fig. 2. The effect of stratification 5°C on the conc. sulphuric acid & sodium hypochlorite pre treated Rubus laciniatus seeds.

GA_3 at 300 mg/l produced the mean germination of 41% following 1 hour of acid scarification and 3 months cold stratification as compared to other GA_3 concentrations (Table 10).

By and large, cold stratification and acid scarification played the most important roles in germinating R. laciniatus seeds. As can be seen from Tables 2,3 and 4, the interactions between cold stratification and acid scarification consistently showed highly significant differences for each of the 3 experiments. Lasheen and Blackhurst (1956) reported that as cold stratification progressed, the percentage of starch in the seed decreased whereas the percentage of reducing sugars and sucrose increased. The initial rate of starch break down was accelerated by concentrated sulphuric acid treatment (45 minutes). They reported that the reduction of starch level coincides with the disappearance of germination inhibitors as indicated by Avena coleoptile bioassay. This disappearance was correlated with the breaking of dormancy and the ability of the seed to germinate.

B. *In Vitro* Seed Germination

For acid scarification, seeds have to be relatively dry. If the seeds are moist, then the water-acid interaction can create tremendous heat and kill the embryo. This heating can reach a temperature as high as 70°C. When the *Rubus* seeds are stored dry, it is believed that germination inhibitors from the endosperm may be moving into the embryo to induce embryo dormancy. In the study of this phenomenon, Dale and Jarvis (1983) reported that acid scarification of fresh red raspberry seeds resulted in 75% germination when the seeds were dried overnight compared with 34% germination if dried for 7 weeks.

Table 14 shows that fresh halved seeds of 'Cherokee' blackberry resulted in 77% *in vitro* germination. This was the highest percent germination obtained with only *Rubus* seed. Seeds dried at room temperature for more than 24 hours before being halved, showed significant reduction in germination rates. For the intact seeds, there was no germination at all whether they were fresh or air-dried (Table 14). These results indicate that air-drying significantly reduced germination.

The halved seed could successfully develop into a whole plant. Ke, Skirvin, McPheeters and Otterbacher (1985) reported that some of these embryos might be injured, but generally the physical action of cutting the seeds in half did not impede plant development.

Table 14. Effect of seed drying on the germination of 'Cherokee' blackberry *in vitro*.

Treatments	Mean germination (%)	
	Intact seeds	Halved seeds
Fresh	0	77
24 hours air-dried	0	54
48 hours air-dried	0	50
72 hours air-dried	0	52
L.S.D. (1%)	N.S.	9.8

The advantage of *in vitro* germination of fresh seed may be the elimination of scarification and the time consuming stratification process. Ke, Skivin, McPheeters and Otterbacher (1985) reported that *in vitro* germination bypassed the drying and cold stratification treatments. The fresh halved seeds gave them 81% germination within 8 to 12 days. They also indicated that this system may provide an alternative propagation method for breeders who deal with a limited number of seeds.

II. Asexual Propagation

A. Leaf Wedge Cuttings -Screening Trial

Propagation of *Rubus* via leaf cuttings has not yet been documented. Twenty-five *Rubus* cultivars were screened for their ability to regenerate from leaf cuttings. Of these, there were 12 clones which produced roots. The effect of the rooting hormones, Hormodin #1 and #2 on rooting varied among the clones (Table 15). There was no significant effect on rooting with Hormodin #1 or #2 in *R. shankii*, *R. rigidus*, *R. calycinoides*, *R. lambertianus*, *R. moluccanus*, *R. biflorus*, *R.* spp.(Chehalem X Cascade), *R.* spp.(OSC 884 X OSC 743) and *R. ellipticus*. Hormodin #1 and #2 produced statistically significant rooting in *R. illecebrosus*, *R.* spp. (OSC 743 X Chehalem), and *R. simplex*.

Of the 12 clones that rooted, only 4 species were found to have the ability to produce adventitious buds and

Table 15. Effect of Hormodin* on the rooting of Rubus leaf wedge cuttings.

Species	Mean % of cuttings rooted on various Rubus				
	Control	Hormodin #1	Hormodin #2	Mean	LSD (1%)
R. illecebrosus	58.5	79.0	95.8	77.8	20.2
R. shankii	58.3	87.5	66.8	70.8	N.S.
R. rigidus	75.0	70.8	91.8	79.2	N.S.
R. calycinoides	62.8	79.0	75.0	72.2	N.S.
R. lamberitianus	62.5	71.0	66.8	66.8	N.S.
R. biflorus	66.8	75.0	83.3	75.0	N.S.
R. moluccanus	62.5	75.0	71.0	69.5	N.S.
R. spp. (#346)	8.5	54.0	87.0	50.0	37.2
R. spp. (#360)	58.3	83.3	75.0	72.2	13.6
R. spp. (#368)	37.5	45.8	62.5	48.9	N.S.
R. ellipticus	66.8	70.8	70.8	69.4	N.S.
R. simplex	25.3	62.5	79.3	55.7	36.8

* Hormodin #1 contains 0.1% IBA

Hormodin #2 contains 0.3% IBA

shoots within 6 months of planting: R. illecebrosus, R. spp. (OSC 884 X OSC 743), R. rigidus and R. simplex. The latter was superior to the other clones in shoot regeneration, i.e., 25% as compared to 3%, 6% or 10% (Table 16, Plate 3). R. simplex shoots developed within 3 months of planting, much earlier than the other clones.

B. Leaf Disk Propagation

Rubus simplex was able to regenerate from leaf disks as well as leaf wedges. Both IBA and BA were found to be independent of each other and there was no interaction between IBA and BA (Table 17). Table 18 shows that the highest number of leaf disks regenerated was only 13 out of 128 (about 10%) using 25 mg/l IBA. The same success rate was also obtained at 5.0 mg/l BA. With the highest concentration of IBA (100 mg/l), the root and shoot regeneration of the leaf disks was greatly reduced. With BA treatments, the number of shoots regenerated was not consistent.

Where possible, propagation by leaf disks represents an economical use of the parent plant material, i.e., a single leaf has the potential to yield a large number of plants. However, leaf disk propagation does not appear to be a practical method for Rubus propagation as the rate of regeneration was very low.

Table 16. Percentage of plantlets regenerated via leaf wedge cuttings of four Rubus species.

Species	Total # leaf wedges regenerated/ 72 cuttings	% regeneration
R. illecebrosus	4	5.6
R. rigidus	7	9.8
R. spp.(OSC 884 X)SC 743)	2	2.8
R. simplex	18	25.0

Plate 3. Rooted leaf wedge cutting of Rubus simplex with emerging shoot.

Table 17. ANOVA of the effect of IBA and BA on the plantlets regeneration via leaf disks of R. simplex.

Source	df	SS	MS	F
Total	63	17.61		
IBA	3	2.05	0.68	3.24*
BA	3	2.05	0.68	3.24*
IBA X BA	9	3.26	0.36	1.71
Error	48	10.25	0.21	

Table 18. Effect of IBA and BA on the plantlets regeneration via leaf disks of R. simplex.

IBA (mg/l)	BA (mg/l)				Total # plantlets regenerated/128 leaf disks
	0	2.5	5.0	10.0	
0	2	0	4	3	9
25	2	3	5	3	13
50	3	1	4	2	10
100	2	1	0	2	5
Total	9	5	13	10	

C. *In Vitro* Propagation

In vitro propagation of *R. leucodermis* showed that both shoot and root proliferation could be obtained in shoot multiplication medium. Axillary bud explants rooted well and this may eliminate the process of transferring the proliferated shoots onto a rooting medium.

Table 19 shows that there was no interaction between IBA and BA for shoot development at 4, 8 and 12 week evaluations. However, BA treatments consistently showed statistically significant difference throughout the 12 week evaluations. An increase in shoot proliferation occurred in the treatments with increasing BA concentration (Table 20, Fig. 3 and Plate 4). At 3.0 mg/l, the highest concentration of BA, the mean shoot numbers were 1.6; 4.4 and 5.8 at 4, 8 and 12 weeks respectively. The mean number of shoots proliferated showed no significant difference between the control treatment and 1.0 mg/l BA at 4 week evaluation.

In root development, IBA and BA did not show any interaction (Table 21). There was also no significant difference in the root index for 4 and 8 weeks at various concentrations of IBA and BA (Table 21). However, evaluation at 12 weeks showed that higher IBA concentrations produced slightly more roots (Table 22, Fig. 4). At 0.3 mg/l IBA, the root index was 2.8 compared to 2.4 in the control (Table 22). The high concentration of BA (3.0 mg/l) did not inhibit root growth (Fig. 4). Slivinski, Preece and Myer (1984)

Table 19. Effect of IBA and BA on the shoot proliferation of *R. leucodermis in vitro*: ANOVA

After 4 weeks

Source	df	SS	MS	F
Total	63	12.47		
IBA	3	0.57	0.19	1.15
BA	3	2.62	0.87	5.28**
IBA X BA	9	1.33	0.15	0.90
Error	48	7.90	0.17	

After 8 weeks

Source	df	SS	MS	F
Total	63	114.96		
IBA	3	4.07	1.36	2.28
BA	3	77.13	25.70	43.31**
IBA X BA	9	5.27	0.59	1.0
Error	48	28.49	0.59	

After 12 weeks

Source	df	SS	MS	F
Total	63	266.20		
IBA	3	10.73	3.58	2.78
BA	3	182.60	60.87	47.31**
IBA X BA	9	11.12	1.24	0.96
Error	48	61.75	1.29	

Table 20. Effect of BA on shoot proliferation of *R. leucodermis in vitro*.

Mean # of shoots	BA (mg/l)				Standard Error*	L.S.D. (1%)
	0	1.0	2.0	3.0		
After 4 weeks	1.1	1.4	1.5	1.6	0.1	0.4
After 8 weeks	1.1	2.1	2.7	4.1	0.2	0.7
After 12 weeks	1.2	2.4	3.4	5.8	0.3	1.0

* 48 Error d.f.

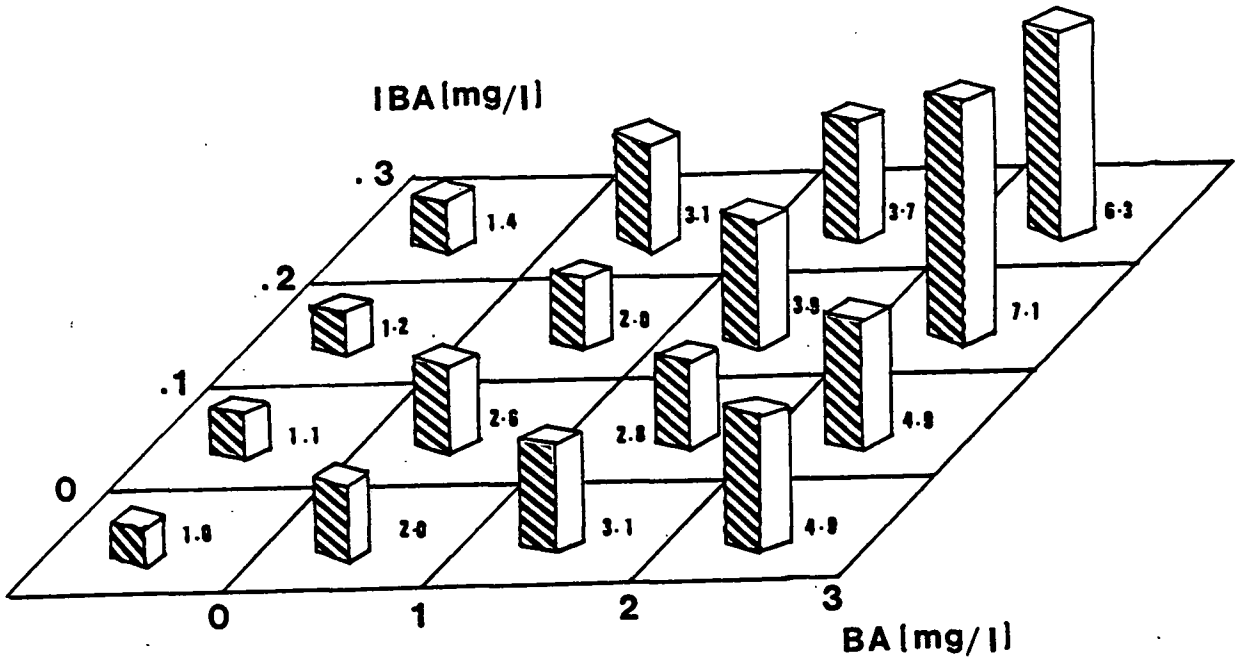

Fig.3. Effect of IBA & BA on the number of shoots proliferated from R. leucodermis in vitro (After 12 weeks).

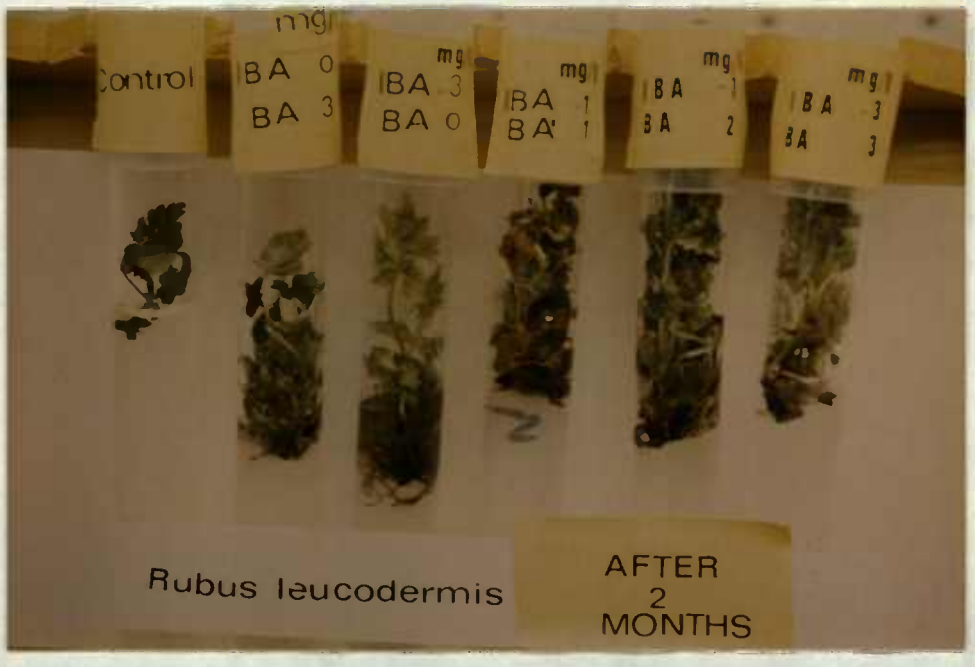

Plate 4. In vitro propagation of Rubus leucodermis (after 2 months).

Table 21. Effect of IBA and BA on the root index of *R. leucodermis in vitro*: ANOVA

After 4 weeks

Source	df	SS	MS	F
Total	63	2.5000	0.	
IBA	3	0.0869	0.0290	0.88
BA	3	0.1494	0.0498	1.52
IBA X BA	9	0.6837	0.0760	2.30
Error	48	1.5800	0.0329	

After 8 weeks

Source	df	SS	MS	F
Total	63	11.4300		
IBA	3	0.7469	0.2489	1.37
BA	3	0.8456	0.2818	1.55
IBA X BA	9	1.0975	0.1219	0.67
Error	48	8.7400	0.1821	

After 12 weeks

Source	df	SS	MS	F
Total	63	8.9900		
IBA	3	1.4381	0.4794	4.00*
BA	3	0.5969	0.1989	1.70
IBA X BA	9	1.1950	0.1328	1.11
Error	48	5.7500	0.1198	

Table 22. Effect of IBA on the root index of *R. leucodermis* *in vitro*.

Mean root index	IBA (mg/l)				Standard Error*	L.S.D (5%)
	0	0.1	0.2	0.3		
After 4 weeks	1.2	1.1	1.1	1.2	0.05	
After 8 weeks	1.5	1.6	1.7	1.7	0.11	
After 12 weeks	2.4	2.6	2.6	2.8	0.09	0.3

* 48 Error d.f.

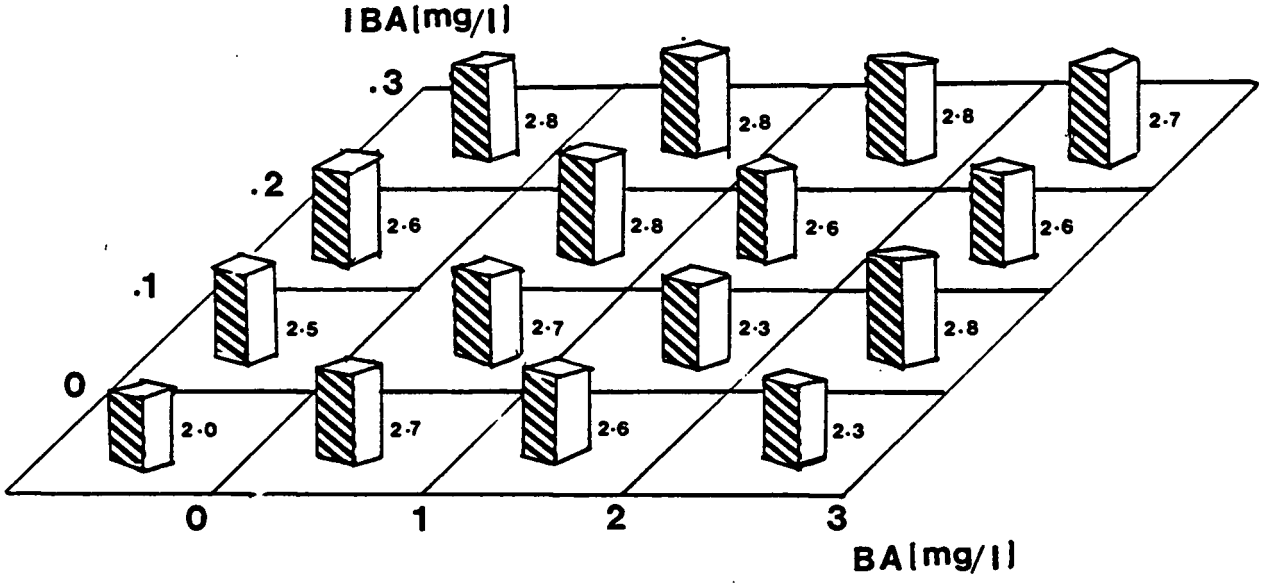

Fig. 4. Effect of IBA & BA on roots growth of

R. leucodermis in vitro - root index
(After 12 weeks).

indicated that there was no significant difference due to BA at 1, 2 or 5 mg/l in the in vitro propagation of thornless blackberry.

Rubus leucodermis explants rooted well even in the shoot multiplication media and without added charcoal. The roots seemed to be vigorous and functional. The ability of the explant to root in the shoot multiplcation medium without charcoal does not agree with the results of Snir (1981). He reported that if charcoal was omitted, the red raspberry explant did not root.

Fifty each of rooted and non-rooted shoots were excised from our cultures and planted in a mixture of soil: sand:perlite (1:1:1) in an enclosed mist chamber. The survival rates of these cuttings were 97% and 94% respectively.

CONCLUSIONS

Supposely sound Rubus laciniatus seeds were found to contain up to 16% defective seeds. The embryo was enclosed by a thick endocarp and acid scarification was necessary to break down some of this thick endocarp to permit germination. The optimal time of acid scarification was 1 hour and scarification in excess of 1 hour was detrimental to germination.

Sodium hypochlorite or hydrogen cyanamide or gibberellic acid treatments enhanced germination in a few cases and then only when the seeds had prior acid scarification. Of the three chemicals, sodium hypochlorite was best.

Overcoming endocarp dormancy via acid scarification was more critical than cold stratification for R. laciniatus seed germination. Seeds which had 4 months of cold-moist stratification did not germinate unless they had been acid scarified. One month of cold stratification was less effective than 2, 3, or 4 months cold stratification.

In vitro seed germination resulted in 77% germination when fresh seeds with cut endocarp were used. Air drying of seeds for 24 hours or more before they were halved, reduced germination greatly. This method of germination could be used to bypass the need for acid scarification or the time consuming stratification process.

Asexual propagation via leaf wedge cuttings was found to be possible with Rubus simplex, R. illecebrosus, R. rigidus and R. spp. (OSC 884 X OSC 743). Among these four clones, R. simplex was found to be superior to the others in terms of ability to regenerate. Plant regeneration via leaf disks was also found to be successful with R. simplex. However, this leaf propagation does not appear to be a practical method as the regeneration rate was very low and very slow.

Propagation of R. leucodermis in vitro resulted in both shoot and root proliferation in shoot multiplication medium. This finding could eliminate the process of transferring the proliferated shoots onto a special rooting medium.

BIBLIOGRAPHY

1. Anderson, W.C. 1980. Tissue culture propagation of red and black raspberry, Rubus idaeus and R. occidentalis. Acta Hort. 112: 13-20.

2. Angelo, E. 1937. Experiences in propagating Rubus and other species by leaf-bud cuttings. Proc. Amer. Soc. Hort. Sci. 35: 448-50.

3. Bailey, L.H. 1941. The standard cyclopedia of horticulture. Vol. III: 3021-3033.

4. Becking, J.H. 1979. Nitrogen fixation by Rubus ellipticus J.E. Smith. Plant and Soil 53(4): 541-545.

5. Boe, A.A.; R.B. Stewart and T.J. Banko. 1972. Effects of growth regulators on root and shoot development of Sedum leaf cuttings. HortSci, 7(4): 404-405.

6. Butterfield, H.M. 1947. Bushberry culture in California. Calif. Agric. Ext. Serv. Circ. 80.

7. Carrillo, C.G. and J.L. Mendoza. 1979. Effect of natural products on in vitro development of Rubus sp. buds. Turrialba 29(4): 299-303.

8. Fejer, S.O. and L.P.S. Spangelo. 1971. Seed germination in the red raspberry. Fruit Varieties and Horticultural Digest 25(4): 75-76.

9. Hammerschlag, F.A. 1978. Influence of light intensity and date of explantation on growth of Geranium callus. HortSci. 13(2): 153-4.

10. Harris, G.P. and M.H. Hart Ennid. 1964. Regeneration from leaf squares of Peperomia sandersii A.D.C.: A relationship between rooting and budding. Ann. Bot. 28 (111): 509-527.

11. Heide, O.M. 1964. Effects of light and temperature on the regeneration ability of Begonia leaf cuttings. Physiol. Plant. 17: 789-804.

12. _____. 1965 a. Interaction of temperature, auxins and kinins in the regeneration ability of Begonia leaf cuttings. Physiol. Plant. 18: 891-920.

13. _____. 1965. Photoperiodic effects on the regeneration ability of Begonia leaf cuttings. Physiol. Plant. 18: 185-190.

14. Heit, C.E. 1967. Propagation from seed. Part 7: Successful propagation of 6 hard seeded group species. Amer. Nurseryman 125 (12): 10-12, 37-41, 44-45.

15. _____. and G.L. Slate. 1950. Treatment of blackberry seed to secure first year germination. Proc. Amer. Sco. Hort. Sci. 55: 297.

16. Henny, R.J. 1978. In vitro propagation of Peperomia 'Red Ripple' from leaf discs. HortSci. 13(2): 150-51.

17. Hudson, J.P. 1954. Propagation of plants by root cuttings I. Regeneration of raspberry root cuttings. J. Hort. Sci. 29: 27-43.

18. _____. 1955. Propagation of plants by root cuttings II. Seasonal fluctuation of capacity to regenerate from roots. J. Hort. Sci. 30: 242-251.

19. James, D.J. 1979. The role of auxins and phloroglucinol in adventitious root formation in Rubus and Fragaria grown in vitro. J. Hort. Sci. 54(4): 273-77.

20. James, D.J.; V.H. Knight and I.J. Thurbon. 1980. Micropropagation of red raspberry and the influence of phoroglucinol. Scientia Hort. 12(4): 313-319.

21. Jennings, D.L. and B.M.M. Tulloch. 1965. Studies on factors which promote germination of raspberry seeds. J. Exp. Bot. 16(47): 329-40.

22. Joley, L.E. and A.W. Close. 1940. The rooting response of various species of Rubus to conventional methods of propagation. Proc. Amer. Soc. Hort. Sci. 38: 420-423.

23. Kains, M.G. and L.M. McQuesten. 1956. Propagation of plants. Orange Judd Publ. Co. Inc. 639 pp.

24. Ke, S.Q.; R.M. Skirvin; K.D. McPheeters and A.G. Otterbacher. 1985. In vitro germination and growth of Rubus seeds and embryos. HortSci. 20(6): 1047-1049.

25. Kerr, E.A. 1954. Seed development in blackberries. Can. J. Bot. 32: 654-672.

26. Lagerstedt, H.B. 1967. Propagation of Begonia from leaf discs. HortSci. 2(1): 20-22.

27. _____. 1968. Leaf disc propagation technique. Begonia 35(3): 44-46.

28. Lasheen, A.M. and H.T. Blackhurst. 1956. Biochemical changes associated with dormancy and after-ripening of blackberry seeds. Proc. Amer. Soc. Hort. Sci. 67: 331-340.

29. Lundergan, C.A. and J.A. Carlisi. 1984. Acceleration of the reproductive cycle of the cultivated blackberry. HortSci. 19(1): 102-103.

30. Moore, J.N.; G.R. Brown and C. Lundergan. 1974. Effect of duration of acid scarification on endocarp thickness and seedling emergence of blackberry. HortSci. 9(3): 204-225.

31. Plummer, T.H. and A.C. Leopold. 1957. Chemical treatment for bud formation in Saintpaulia. J. Amer. Soc. Hort. Sci. 70: 442-444.

32. Pyott, J.L.; R.H. Converse. 1981. In vitro propagation of heat-treated red raspberry clones. HortSci. 16(3): 308-309.

33. Rosati,P. and W. Faedi. 1978. The rooting of thornless blackberry cuttings. Bologna 96: 63-66.

34. Schimmelpfeng, H. 1974. Methods of propagating thornless blackberry. Erwerbsobstbau 16(4): 62-64.

35. Schopmeyer, C.S. 1974. Seeds of woody plants in the United States. Agric. Handbook No. 450: 738-742.

36. Schraudolf, H. and J. Reinert. 1959. Interaction of plant growth regulators in regeneration processes. Nature 184: 465-466.

37. Scott, D.H. and D.P. Ink. 1957. Treatment of Rubus seeds prior to after-ripening to improve germination. Amer. Soc. Hort. Sci. 69: 261-267.

38. _____. and A.O. Draper. 1967. Light in relation to seed germination of blueberry, strawberry and Rubus. HortSci. 2: 107-108.

39. Skirvin, R.M.; M.C. Chu and E. Gomez. 1981. In vitro propagation of thornless trailing blackberries. HortSci. 16(3): 310-2.

40. Slivinski, J.A.; J.E. Preece and O. Myers. 1983. In vitro micropropagation of thornless blackberries utilizing single node explants. Plant Propagator 30(1): 4-5.

41. Snir, I. 1981. Micropropagation of red raspberry. Scientia Hort. 14: 139-143.

42. Sokolova, V.A. and V.V. Kichira. 1971. Increasing the germination rate of raspberry seeds. Vestnik Sel 'skokhozyaistvennol Naul 16(10): 87-90.

43. Spriovska, R. 1982. Rooting hardwood and softwood cuttings of Chinese Gooseberry and blackberry using mist and bottom heat. Jugoslovensko Vocarstvo 16(61/62): 61-65.

44. Stoutemyer, V.T.; T.J. Maney and B.S. Pickett. 1933. A rapid method of propagation raspberries and blackberries by leaf-bud cuttings. Proc. Amer. Soc. Hort. Sci. 30: 278-282.

45. Swartz, H.J.; G.J. Galletta and R.H. Zimmerman. 1981. Phenotypic stability and field performance tissue culture propagated thornless blackberry. HortSci. 16(3): 418.

46. Thomas, C.C. 1935. Leaf-bud propagation of several introduced species of Rubus. Proc. Amer. Soc. Hort. Sci. 33: 379-9.

47. Tumanova, R.N. 1971. The effect of potassium iodide on raspberry seed germination and on physiological process in the seedlings. Obrazovania 42: 169-171.

48. Wenzel, W.G. and C.W.J. Smith. 1975. Germination tests with blackberry seeds. Angenandte Botanik 49(1/2): 11-14.

49. Wildern, J.A. and R.A. Criley. 1975. Cytokinins increase shoot production from leaf cuttings of Begonia. Plant Propagator 20(4): 7-9.

50. Zimmerman, R.H.; G.J. Galletta and O.C. Broome. 1980. Propagation of thornless blackberries by one-node cuttings. J. Amer. Soc. Hort. Sci. 105(3): 405-407.

51. Zych, C.C. 1965. Rooting of leaf-bud cuttings of Rubus neglectus inhibition by Gibberellin. Proc. Amer. Soc. Hort. Sci. 87: 238-239.

APPENDICES

Appendix 1. Mean % germination of R. laciniatus at varying length of acid scarification and cold stratification and at different NaOCl concentrations.

Stratification (month)	Scarification (hour)	NaOCl (%)				
		0	1	2	3	4
0	0	0	0	0	0	0
	½	20	22	25	26	31
	1	32	35	42	41	46
	1½	10	12	10	15	15
1	0	0	0	0	0	0
	½	22	21	23	22	21
	1	30	31	32	33	35
	1½	15	18	22	23	23
2	0	0	0	0	0	0
	½	34	40	44	48	50
	1	40	47	49	52	53
	1½	19	20	20	19	20
3	0	0	0	0	0	0
	½	32	36	51	51	51
	1	44	38	49	52	51
	1½	17	17	17	17	18
4	0	0	0	0	0	0
	½	31	34	44	43	45
	1	38	41	42	50	51
	1½	14	15	13	13	13

S.E. : 1.74 (300 Error d.f.)

Appendix 2. Mean % germination of R. laciniatus at varying length of acid scarification and cold stratification and at different H_2CN_2 concentrations.

Stratification (month)	Scarification (hour)	H_2CN_2 (mg/l) 0	100	200	300	400
0	0	0	0	0	0	0
	½	20	17	20	18	17
	1	32	42	35	50	19
	1½	10	10	10	11	10
1	0	0	0	0	0	0
	½	22	20	24	23	28
	1	30	32	30	33	35
	1½	15	19	23	26	24
2	0	0	0	0	0	0
	½	34	40	37	40	38
	1	40	38	45	54	34
	1½	14	20	14	20	21
3	0	0	0	0	0	0
	½	32	37	35	33	31
	1	40	47	46	38	41
	1½	17	15	17	16	16
4	0	0	0	0	0	0
	½	31	37	33	35	35
	1	38	44	37	38	36
	1½	14	15	13	13	12

S.E. : 1.65 (300 Error d.f.)