Fruit Varieties Journal 48(1):7-14 1994

Micropropagation of Several Lingonberry Cultivars

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Abstract

Micropropagation of lingonberry, *Vaccinium vitis-idaea* L., has become a popular method of propagation for commercial growers offering lingonberry plants; however, recommendations for basal media, plant growth regulators, and culture conditions vary substantially among publications and commercial propagators. We found that lingonberry cultivars differed in shoot growth with various concentrations of the cytokinin 2ip (N^6-[2-isopentenyl]adenine), and that the addition of the auxin IAA (indoleacetic acid) did not increase shoot quality or multiplication rates of 'Sussi,' 'Erntedank,' or 'Koralle.' In addition, tests using several concentrations of IBA (3-indolebutyric acid) in powder form did not enhance rooting of microcuttings. Woody Plant Medium containing 10 μM 2ip was an effective growing medium for the three lingonberry cultivars tested. Higher concentrations of 2ip favor increased shoot development, but the clonal fidelity of these shoots may be suspect. Stabilized cultures with consistent shoot production from 3-node stem section explants have been established for eleven lingonberry cultivars.

Introduction

Lingonberry, *Vaccinium vitis-idaea* L., is commonly propagated by division of the rhizomatous plant or by softwood cuttings. These methods, though generally successful, are inefficient especially when only one or a few stock plants are available. In addition, the failure of stem cutting-derived plants to produce a rhizomatous growth habit has been reported for *V. vitis-idaea* L. var. *minus* (2).

Micropropagation is now being used by commercial propagators to rapidly produce selected lingonberry cultivars. Hosier et al. (3) reported that plants derived from tissue culture maintain their rhizomatous habit. Research from our lab supports this finding, and we found that rhizome development occurred equally well on softwood cutting-derived plants of *V. vitis-idaea* L. (9). No direct comparison of *V. vitis-idaea* L. var. *minus* and *V. vitis-idaea* L. concerning rhizome production from cuttings has been reported.

Micropropagation protocols differ widely among published reports on lingonberry and among commercial propagators. Hosier et al. (3) were among the first to report micropropagation of lingonberry and worked with *V. vitis-idaea* L. var. *minus* seedlings. After testing four concentrations of 2ip (N^6-[2 isopentenyl]adenine), they recommended a level of 98.4 μM for multiplication. Norton and Norton (6) reported similar results with *V. vitis-idaea* L. var. *minus*, recommending 79 μM 2ip. Gebhardt and Friedrich (1) recommended a 2ip concentration of 4.9 μM for 'Erntesegen,' 'Erntedank' and 'Koralle.' Reichers and Bünemann (8) suggested a combination of 5.7 μM IAA (indoleacetic acid) and 24.6 μM 2ip in woody plant medium (WPM) (5) for micropropagation of 'Red Pearl.' Two methods of micropropagation for *V. vitis-idaea* L. were described by Sidorovich and Kutas (10): adventitious shoot production and axillary-derived shoot development. For the latter method, they used 74 μM 2ip. Reed and Abdelnour-Esquivel (7) investigated conditions conducive to initiating cultures of 22 *Vaccinium* species including *V. vitis idaea* L. Their tests with several blueberry cultivars led to a recommended initiation medium of WPM modified by doubling the Ca $(NO_3)_2$ (MWPM) and containing 18.2 μM zeatin (6-[4-hydoxy-3-methylbut-2-enylamino]purine). For multiplication and maintenance of the stock plants, MWPM + 24.6 μM 2ip was recommended. This method was successful with *V. vitis-idaea* L. and *V.*

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vitis-idaea L. var. *minus* (B. Reed, personal communication). Several different basal media are recommended in these published reports, and few mention maintaining cultures over an extended period of time. Our group has recently described micropropagation and stabilization of cultures of 'Sussi,' 'Erntedank,' and 'Koralle' on WPM + 5 μM 2ip (9).

Several commercial nurseries micropropagate lingonberry in the United States. The three surveyed are using WPM with slight variations supplemented with 2.5-8 μM 2ip or 0.5-2.5 μM zeatin. Some variable factors involved in lingonberry micropropagation as reported in scientific publications, by commercial nurseries, and by a governmental agency are outlined in Table 1.

Rooting of microcuttings out of culture often takes several weeks. Under excessively low light or warm temperatures, rooting may take two months or more. All of the commercial nurseries report success with rooting and acclimation of microcuttings with the process usually taking from 8 to 12 weeks. Hosier et al. (3) noted no significant effects of IBA (3-indolebutyric acid) on rooting.

We have investigated micropropagation of three lingonberry cultivars on media with seven plant growth regulator (PGR) concentrations and report successful stable multiplication of 11 lingonberry cultivars on the medium recommended herein. In an effort to promote rapid rooting of microcuttings from several cultivars, we expanded previous investigations of IBA (3) by looking at four IBA concentrations as a powder dip.

Materials and Methods

Micropropagation

Eleven cultivars ('Red Pearl,' 'Koralle,' 'Sussi,' 'Sanna,' 'Scarlet,' 'Erntedank' 'Erntekrone,' 'Erntesegen,' 'Masovia,' 'Splendor,' and 'Regal') were grown to maturity in a greenhouse. Actively

Table 1. Published reports, and commercial and governmental nurseries dealing with micropropagation of lingonberry.

Source	Reported stable culture	Basal medium[a]	PGRs[b] (μM)	Cultivar/ clone[c]	Reported acclimation
Reference					
Gebhardt (1)	No	Kyte	4.9 2ip	Es, Ed, Ek, Ko	No
Hosier (3)	No	custom	98.4 2ip	minus seedling	Yes
Norton (6)	No	Anderson	79 2ip	minus seedling	No
Reed (7)	Yes	MWPM	24.6 2ip	unnamed selections	No
Reichers (8)	Yes	WPM	24.6 2ip + 5.7 IAA	Rp	Yes
Serres (9)	Yes	WPM	5 2ip	Ed, Su, Ko	Yes
Sidorovich (10)	Yes	Anderson	74 2ip	unnamed	No
Propagator					
Briggs[d]	Yes	WPM	0.5-2.5 zeatin or 8 2ip	Ko, unnamed selections	Yes
Hartmann's[e]	Yes	WPM	2.5 2ip	Sc, Su, Ko, Es, Rp, Sn, Sp	Yes
Knight Hollow[f]	Yes	WPM	8 2ip	Su, Sp, Re	Yes
NCGR-USDA[g]	Yes	MWPM	24.6 2ip	unnamed selections	Yes

[a]basal media names and compositions can be found in the publications cited.
[b]PGRs = plant growth regulators for maintenance (see text for explanation of abbreviations).
[c]cultivar abbreviations are Es 'Erntesegen,' Ed 'Erntedank,' Ek 'Erntekrone,' Ko 'Koralle,' Rp 'Red Pearl,' Su 'Sussi,' Sc 'Scarlet,' Sn 'Sanna,' Sp 'Splendor,' Re 'Regal.' Minus refers to *V. vitis-idaea* L. var. *minus*.
[d]Briggs Nursery. 4407 Henderson Blvd., Olympia, WA 98503.
[e]Hartmann's Plantation, Inc., 310 60th St., P.O. Box E, Grand Junction, MI 49056.
[f]Knight Hollow Nursery. 3333 Atom Ct., Middleton, WI 53562.
[g]National Clonal Germplasm Repository-USDA. 33447 Peoria Rd., Corvallis, OR 97333.

growing shoots were excised and disinfected by soaking the defoliated shoots in 10% commercial bleach for 15 minutes followed by a quick rinse in 75% ethanol and three rinses in sterile, distilled water. The disinfected stem sections were aseptically cut into two-node sections and placed on WPM supplemented with 5 μM 2ip, 20 g/L sucrose, 1.3 g/L calcium gluconate, and solidified with 3 g/L agar and 1.1 g/L gelrite. The pH was adjusted to 5.2 with KOH. In vitro shoot cultures of most of the cultivars mentioned above were stabilized and have been maintained for over one year, propagating by transferring three-node sections and shoot tips to fresh medium every eight weeks.

To investigate various levels of 2ip and IAA, six-week old in vitro-grown shoots were excised from their callused bases and cut into three-node sections. The tips were discarded. The sections were placed vertically in 8-dram shell vials containing 10 ml of medium, assuring that at least one node was submerged and one node was above the surface of the medium. The medium was supplemented WPM, as described above, but containing one of seven plant growth regulator combinations: 1, 5, 10, 25, 50 μM 2ip, 5 μM 2ip + 6 μM IAA, or 5 μM 2ip + 10 μM IAA. IAA was filter sterilized and added to the medium after autoclaving. The cultivars 'Erntedank,' 'Sussi,' and 'Koralle' were used. Ten vials per medium and cultivar were set up. All vials were randomized and incubated at 25 $\pm$ 3°C under continuous lighting at 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Ten weeks after subculture, the number of shoots > 5mm in length, total shoot length and presence of adventitious buds was recorded. The experiment was repeated twice for the 5, 10, and 25 μM 2ip treatments.

Rooting and acclimation

'Erntedank,' 'Sussi,' 'Koralle,' and 'Regal' stock cultures grown for eight weeks on WPM as described above but containing 10 μM 2ip + 6 μM IAA were harvested for rooting and acclimation. The callused bases and lower leaves were removed leaving a shoot approximately 5 cm in length. Randomly selected shoots were dipped in one of five IBA preparations and placed 1-2 cm deep in a soilless medium for rooting. The IBA preparations were Hormex powders (Brooker Chemical, N. Hollywood, CA) containing 0.1, 0.3, 0.8, or 3% IBA in talcum powder. We included a treatment with no IBA in talcum powder. The arrangement for the experiment was a split-plot design in which lingonberry cultivars were put into whole plots with 2 replicates, and IBA concentrations were put into subplots with 10 replicates. Treated shoots were placed in 2 peat:1 perlite medium in standard (28 x 56 cm) planting flats. The flats were covered with clear plastic lids and placed under cool white fluorescent lights to give a continuous light intensity of 75 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a temperature of 28 $\pm$ 1°C. High humidity was maintained by misting with distilled water every two days. After four weeks, the number of roots > 2mm in length per shoot was recorded. After six weeks, all plants were potted in peat in 10 cm diameter pots and grown in the greenhouse with the same experimental arrangement as previously described. After five months in the greenhouse, survival, rhizome number, shoot number, root length, and shoot length were measured on three randomly selected plants for each treatment.

Results and Discussion

Micropropagation

Increasing 2ip concentrations produced more shoots and a generally decreased average shoot length for all three cultivars (Figure 1). 'Erntedank' and 'Sussi' produced nearly the same number of shoots at 10 μM 2ip as 25 μM but produced a greater number of shoots at 50 μM 2ip. For both of these cultivars, average shoot length was optimal at 5 or 10 μM 2ip. 'Koralle'

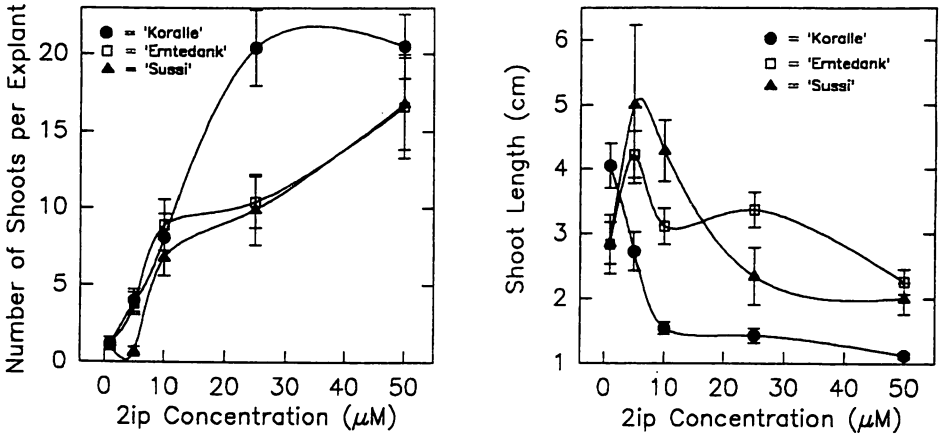

Figure 1. ***In vitro*** **shoot growth of three lingonberry cultivars after 10 weeks as influenced by 2ip concentration—number of shoots (left) and average shoot length (right). Vertical bars represent the average ± one SE for 10 explants.**

produced significantly more shoots at 25 μM 2ip than 10 μM, but the number of shoots at 25 μM and 50 μM 2ip did not differ. Average shoot length for 'Koralle' decreased with increasing 2ip concentration at all levels. A typical response to increasing 2ip concentrations is shown in Figure 2 with 'Erntedank.'

The addition of 6 μM or 10 μM IAA to the medium had no significant effect on the number of shoots produced by 'Erntedank' nor 'Koralle'; however, both IAA concentrations significantly reduced the number of shoots produced by 'Sussi' (Figure 3).

Shoots growing on 2ip concentrations of 25 and 50 μM occasionally produced adventitious bud masses which appeared to arise from dense callus growing at the base of the shoots in the medium (Figure 4). Buds pro-

Table 2. Growth of greenhouse lingonberry plants derived from microcuttings treated with various concentrations of IBA.

Variable	Plant survival (%)	Avg. root length (cm)	Avg. shoot length (cm)	Avg. # rhizomes/ plant	Avg. # shoots/ plant
Lingonberry Cultivar					
'Erntedank'	100	10.5	11.8[a]	0.7	5.6
'Sussi'	91.7	10.3	9.1	0.3	3.5
'Koralle'	83.3	10.6	11.2	1.1	5.5
'Regal'	96.7	12.6	9.2	0.4	4.1
IBA Concentration					
0.0%	78.3	10.9	10.4	0.5	4.1
0.1%	85.0	11.3	11.3	1.0	5.0
0.3%	83.3	11.0	10.4	0.6	4.6
0.8%	73.3	11.4	11.7	0.6	4.4
3.0%	68.3	10.4	10.3	0.1	4.2

[a]All means within columns for each variable are not significantly different except for the average shoot length where 'Erntedank' and 'Koralle' are significantly different (P = 0.05) from 'Sussi' and 'Regal.'

Figure 2. 'Erntedank' *in vitro* shoot development after 10 weeks on 1, 5, 10, 25, and 50 μM 2ip (left to right).

duced adventitiously have been known to produce somaclonal variants of "off-types" in other plants (for review see ref. 4) and thus 2ip concentrations of 25 μM or greater should be avoided. We have maintained and propagated 11 lingonberry cultivars *in vitro*, some for three years, without obvious morphological variation arising. Shoots from five of the 11 cultivars have been taken out of culture and grown in a greenhouse, and, once again, no aberrant morphological characters were apparent.

Rooting and acclimation

Applications of IBA influenced the number of adventitious roots per cutting; however, IBA application did not significantly influence survival and development of the plant. IBA at 0.8% significantly increased the number of roots per cutting for all four lingonberry cultivars (Figure 5). At 3.0% IBA, however, toxicity effects (dead tissue

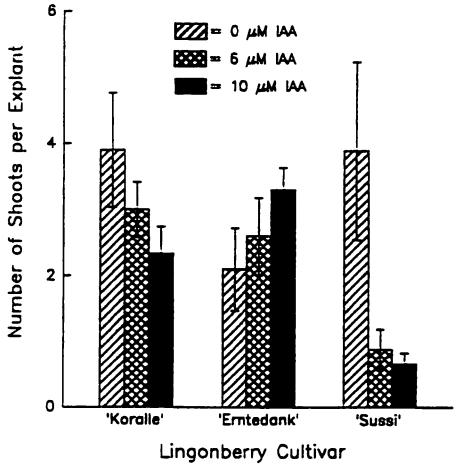

Figure 3. Effect of IAA on *in vitro* shoot growth of three lingonberry cultivars after 10 weeks. Vertical bars represent the average ± one SE for 10 explants.

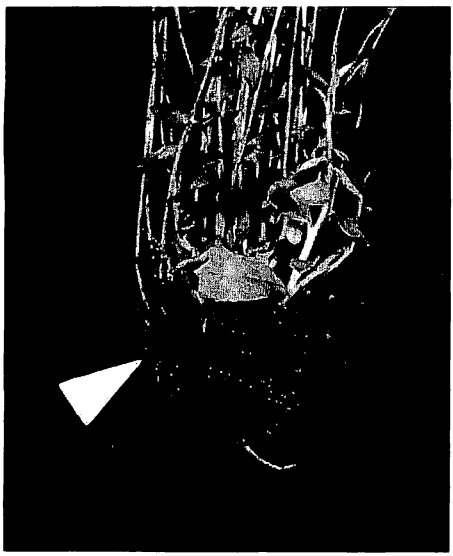

Figure 4. Adventitious bud mass (arrow) developing on basal callus of 'Erntedank' growing on 50 μM 2ip.

at the treated end) were evident on 'Koralle,' 'Sussi,' 'Regal,' and 'Erntedank,' but 'Erntedank' continued to respond positively in root number (Figure 6). 'Erntedank' responded more dramatically to all IBA concentrations than the other three lingonberry cultivars (Figure 6). In the greenhouse, most plants produced branch shoots and rhizomes. Survival of the plants after five months in the greenhouse ranged from 83 to 100% with no significant difference among IBA treatments or cultivars (Table 2). IBA treatments did not differ significantly in regards to root length, shoot height, rhizome number, or shoot number. Among lingonberry cultivars, root length, shoot number and rhizome number did not differ, but 'Erntedank' and 'Koralle' produced significantly longer shoots than 'Sussi' and 'Regal.'

Conclusions

Lingonberry cultivars respond differentially to various 2ip concentrations in the tissue culture medium; however, we have used 10 μM 2ip in Woody Plant Medium to successfully micropropagate eleven lingonberry cultivars. 2ip concentrations of 25 μM and 50 μM resulted in the production of adventious buds from stem explants. The addition of IAA to the basal medium did not significantly enhance shoot growth and, in 'Sussi,' significantly decreased shoot number. Microcuttings rooted *ex vitro* and acclimated easily. Applications of IBA, especially at 0.8%, significantly increased the number of adventitious roots per cutting, but this increase did not affect ultimate plant production or quality.

Acknowledgments

We thank Knight Hollow Nursery, Hartmann's Plantations, Briggs Nursery, and The National Clonal Germplasm Repository—USDA for sharing their micropropagation protocols. We also thank John Klueh for greenhouse plant maintenance and Heidi Hoel for tissue culture maintenance. This research was supported by the Agricultural Research Station, College of Agriculture and Life Sciences, Univ. of Wisconsin-Madison, Hatch project no. 3215.

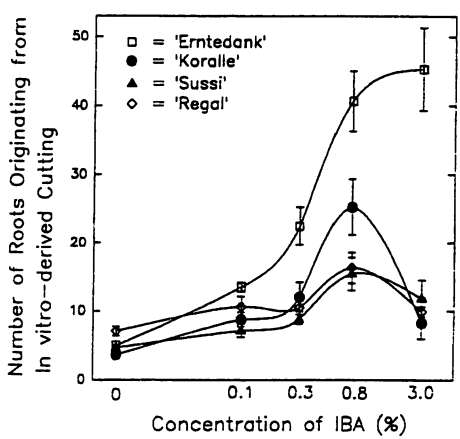

Figure 5. Effect of IBA concentration on root initiation from microcuttings of four lingonberry cultivars. Vertical bars represent the average ± one SE for 20 shoots.

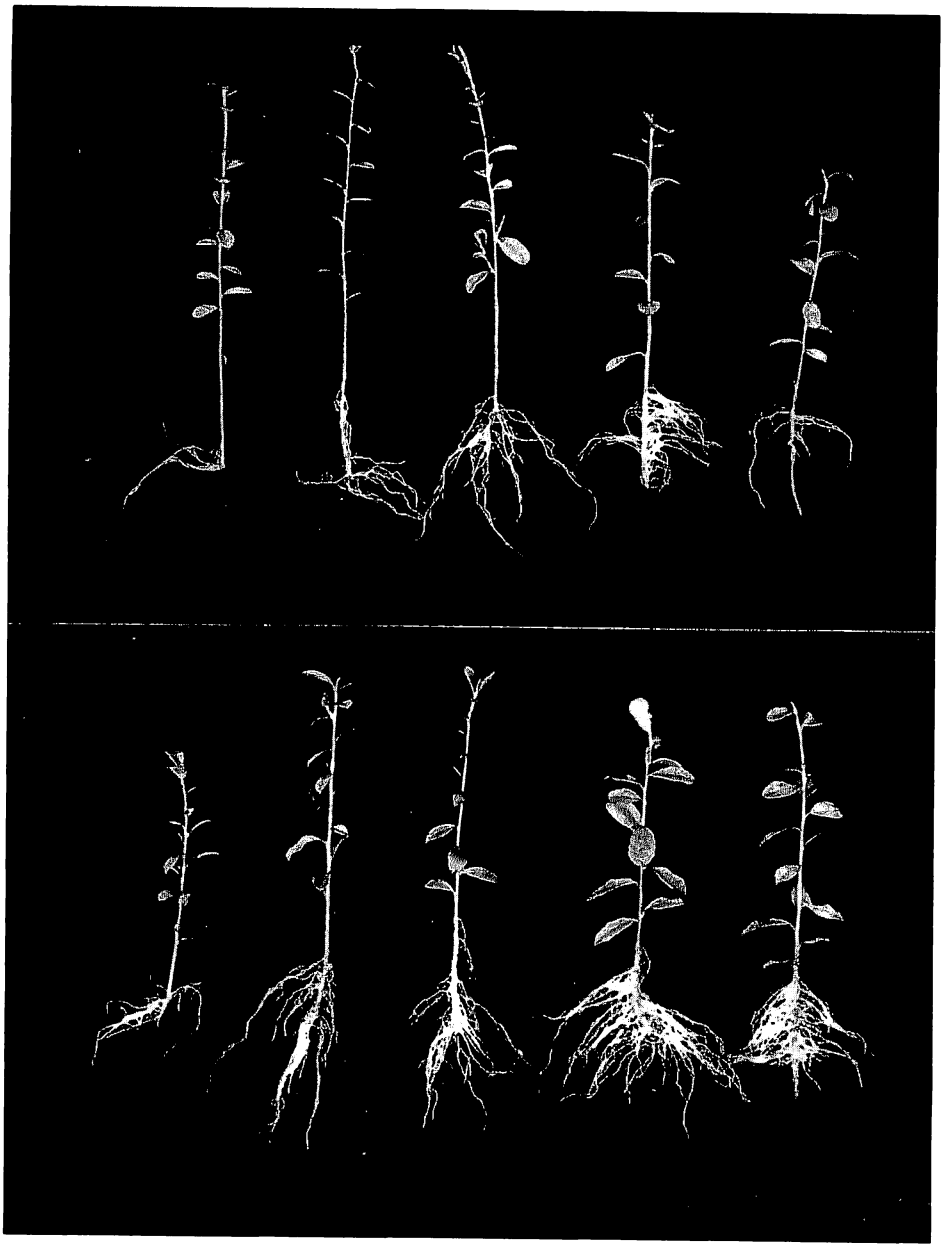

Figure 6. Adventitious root development on 'Koralle' (top) and 'Erntedank' (bottom) microshoots four weeks after treatment with 0, 0.1%, 0.3%, 0.8% and 3% IBA rooting hormone (left to right). Note toxic effect of 3% IBA on treated end of cuttings (no roots) from both cultivars.

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Fruit Varieties Journal 48(1):14-19 1994

Comparison of Sensory, Chemical and Color Attributes of Disease-Resistant Apple Cultivars[1]

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Abstract

In 1989 and 1990, the sensory preference, chemical and color attributes of three disease resistant apple cultivars (DRC), 'Liberty,' 'Nova Easygro,' and 'Jonafree,' were compared to 'McIntosh' at harvest and following three months storage at 2°C. Throughout the testing period panelists equally preferred the flavor of 'Liberty' and 'McIntosh.' Liberty was significantly ($P \leq 0.05$) preferred for texture during the four sampling periods. 'Jonafree' was significantly ($P \leq 0.05$) less preferred when compared to 'McIntosh.' The color of 'McIntosh' was preferred overall, followed by 'Liberty.' 'Jonafree' was least preferred for color. The % soluble solids, titratable acidity, fructose, and sucrose concentrations decreased over time. Glucose and the sugar:acid ratio increased with time. Significant differences in chemical and color evaluations were found from year to year.

Introduction

Newly introduced apple cultivars with resistance to apple scab, as well as some other diseases provide growers an opportunity to reduce disease control costs, lessen the risk of environmental contamination associated with fungicide applications, and meet consumer demands for reduced pesticide residue on produce. Despite these obvious advantages, growers have been slow to plant disease-resistant cultivars (DRC).

A recent survey of the New England Apple Industry indicated that DRC comprised less than 1% of the respondents' acreage in 1989 and would account for 10% of the acreage planted in 1990-1994 (2). Growers have been reluctant to plant DRC because of the uncertainty of consumer acceptance. Appearance and flavor are key attributes determining whether consumers accept a new cultivar (15). Little information is available on fruit quality and consumer preferences for DRC.

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