Somaclonal Variation among ex vitro 'Thornless Evergreen' Trailing Blackberries: The Morphological Status of Selected Clones after Seven Years of Field Growth

M.A. Norton¹ and R.M. Skirvin²

University of Illinois, Department of Natural Resources and Environmental Sciences, 258 Madigan Laboratory, 1201 West Gregory Drive, Urbana, IL 61801

ADDITIONAL INDEX WORDS. *Rubus laciniatus*, fruit breeding, in vitro

ABSTRACT. Chimera 'Thornless Evergreen' (CTE), (*Rubus laciniatus* Willd.) somaclones selected in 1983 and field planted in 1985 were reexamined in 1992 for various vegetative and reproductive characteristics. Two major types of thornless (prickle-free) plants, intermediate-sized ('UI 6-6' = 'Everthornless') and dwarf ('UI 6-4'), originally selected from a chimera thornless parent plant, were compared with thorny plants. The intermediate and dwarf somaclones have maintained their distinctive habits over 7 years' growth in the field, indicating that their growth habits are stable and not a transient effect of tissue culture. Although the thornless somaclones remained thornless, the degree and type of prickly-like structures varies considerably, indicating that the thornless gene (S_{tc}) does not entirely suppress the production of prickles, but apparently alters their development. Increasing suppression was directly related to increasing dwarfism, suggesting a link between thornlessness and internode length.

'Thornless Evergreen' blackberry is a periclinal chimera in which the epidermis is composed of cells carrying a dominant (Hall et al., 1986) gene (S_{tc}) for thornlessness, while the internal tissues are composed of cells having a thorny genotype (Darrow, 1931). McPheeters and Skirvin (1983) separated the genotypes in this chimera blackberry by rapid in vitro propagation. Of 900 plants produced by this technique and evaluated based on growth habit, vigor, and presence of a unique bent petiole, 253 were evaluated in the field for 2 years (McPheeters and Skirvin, 1989). Although these plants exhibited continuous variation for size, they fell into three broad categories: normal, in which plants were vigorous, with long internodes; intermediate, in which plants had a less-vigorous growth habit than the parental type and possessed a bent petiole; and dwarf, in which plants were severely restricted in size and also possessed the bent petiole (Fig. 1). Most (410 of 421) normal plants produced thorny shoots from root cuttings, indicating that they still possessed the chimera structure of the parent plant, while 279 of 296 intermediate and dwarf plants produced thornless shoots from root cuttings. These plants had apparently arisen adventitiously from epidermal tissue and were no longer chimera.

A selection of these in vitro-derived chimera, intermediate, and dwarf 'Evergreen' plants were planted at Urbana, Ill., in 1985. To determine the stability of these phenotypes over time, examine other traits associated with the thornless versus the thorny phenotypes, and establish baseline controls for later evaluations of regenerants from these plants, we evaluated primocanes, leaves, and flowers of plants from this planting in August 1992. The plants evaluated were representative of the two classes of 'Evergreen', as previously described, as well as the thorny 'Evergreen' blackberry.

Received for publication 28 Feb. 1996. Accepted for publication 19 Sept. 1996. Funds for this research were provided in part by the Illinois Agricultural Experiment Station. Special thanks to Ken McPheeters and Alan Otterbacher for their help with this research. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Postdoctoral researcher.

²Professor of horticulture; to whom reprint requests should be addressed.

Materials and Methods

Primocane evaluations

In August 1992, primocanes of the three most fertile strains of 'Evergreen' typical of each class (thorny vigorous, dwarf growth habit, and intermediate growth habit) were measured to establish morphologies characteristic of each. The 'Evergreen' strains evaluated were in vitro-derived pure thornless intermediate 'Evergreen', designated PTE-normal ['UI 6-6' = 'Everthornless' (McPheeters and Skirvin, 1995)], in vitro-derived pure thornless dwarf 'Evergreen', designated PTE-dwarf ('UI 6-4'), and thorny 'Evergreen', designated TH. Severe winters in Urbana had killed the chimera canes from the original chimera 'Thornless Evergreen' (CTE) plantings, and none were available for evaluation. The thorny plants used in these studies were derived from root suckers of a chimera clone. We assumed these thorny suckers were typical of 'Evergreen' and used them as thorny controls. The PTE-normal clone ('UI 6-6') was a pure thornless, in vitro-derived

Fig. 1. A dwarf pure thornless 'Evergreen' (PTE) showing the bent petiole condition (left) typical of the pure thornless state, in contrast to a thorny 'Evergreen' (TH), which has straight petioles.

Fig. 2. Leaf classes of 'Evergreen' blackberry somaclones: 0 = all leaflets entire (this is typical of a broad-leaf plant); 1 = one leaflet lacinate; 3 = three leaflets lacinate; 5 = five leaflets lacinate (this is typical of a narrow-leaf plant)

clone that had been classified by McPheeters and Skirvin (1989) as intermediate in growth habit and selected for advanced study on the basis of its good fertility (about 85% drupelet set). This clone also was field tested in Oregon for several years and was recently patented as 'Everthornless' (McPheeters and Skirvin, 1995). The PTE-dwarf plant ('UI 6-4') was a pure thornless, in vitro-derived clone classified by McPheeters and Skirvin (1989) as dwarf; it was relatively sterile and <5% of its drupelets set fruit.

Five canes from each plant were evaluated. Canes were cut and leaves were removed for separate evaluation, and the following measurements were taken: total cane length, number of nodes, internode length, the number and length of secondary (lateral) branches, the number of tertiary branches, the number of prickles per node on the main cane, and the height above ground level of the last prickle on the main cane.

Leaf evaluations

The following characteristics were measured on leaves removed from primocanes: length of the entire leaf from the petiole base to the tip of the terminal leaflet, length of the petiole from its

Fig. 3. Prickle classes of 'Evergreen' blackberry somaclones: 1 = full prickle (hard, recurved; average length = 2.6 mm); 2 = soft prickle (fully formed, but not hard; average length = 0.5 mm); 3 = aborted prickle (not fully formed, average length = 0.25 mm); 4 = blister (elongated, raised area on epidermis; average length <0.25 mm); 5 = bump (small round, raised area on epidermis; average length <0.25 mm).

base to the point at which the leaflets were attached, length of the midrib of the terminal leaflet, leaf width at its widest point, number of leaflets per leaf, and number of lacinations per leaf. We defined a lacination as the fingerlike segment of leaf between two indentations that extended at least halfway to the midrib of the leaflet (Fig. 2). A leaf class number was also assigned to each leaf as follows (Fig. 2): 5 = all five leaflets were lacinate; 4 = four leaflets were lacinate; 3 = three leaflets were lacinate; 2 = two leaflets were lacinate; 1 = one leaflet was lacinate; 0 = all leaflets were entire. Prickles on each leaf were counted by leaf segment. Leaf segments consisted of the petiole, the entire midrib, the proximal half of the midrib (that is, the half of the midrib closest to the petiole), and the distal half of the midrib.

Prickle evaluations

In a separate study, ten leaves each of five 'Evergreen' strains were evaluated for prickle location and type along the petiole and the midrib of the terminal leaflet. Strains used were TH, 6-6B (a broad-leaf variant of PTE-normal), 6-6N (a narrow-leaf variant of

Fig. 4. (top) Petal classes of 'Evergreen' somaclones: 1 = petals are straplike without lobing; 2 = petals have two lobes; and 3 = petals have three lobes (typical of thorny control plants). (bottom) Sepal classes of 'Evergreen' somaclones: 1 = narrow, pointed strap that is highly twisted; 2 = narrow, pointed strap, not twisted; 3 = broad, pointed strap; and 4 = large and well-developed strap with one to three points on each sepal (this is typical of the thorny plants).

PTE-normal), 6-4B (a broad-leaf variant of PTE-dwarf), and 6-4N (a narrow-leaf variant of PTE-dwarf). Most leaflets of broad-leaf variants were entire, while most leaflets of narrow-leaf variants were lacinate.

Prickles were classified into the following types (Fig. 3): 1 = full prickles (hard, recurved); 2 = soft prickles (fully formed, but not hard, observed primarily on the terminal leaflet of 'UI 6-6'); 3 = aborted prickles (not fully formed); 4 = blister (elongated, raised area on epidermis); 5 = bump (small, round, raised area on epidermis).

The location of each type of prickles, measured from the base of the petiole, was determined. In addition, the prickles locations were classified as to segment, with Segment 1 being the basal half of the petiole, Segment 2 being the upper half of the petiole, Segment 3 being the lower half of the midrib of the terminal leaflet, and Segment 4 being the tip half of the midrib of the terminal leaflet.

Flower evaluation

In August 1992, flowers of all field plantings of the in vitro-

derived 'Evergreen' clones were evaluated. Twenty flowers of each of the following clones were measured: 'UI 1-11' (thorny root sucker, fully fertile), 'UI 3-7' (intermediate, poor fertility); 'UI 4-1' (intermediate, poor fertility); 'UI 4-3' (intermediate, poor fertility), 'UI 5-24' (intermediate, poor fertility); 'UI 6-6' (intermediate, fully fertile), 'UI 7-9' (intermediate, poor fertility), 'UI 5-5' (dwarf, poor fertility), 'UI 6-4' (dwarf, poor fertility), and 'UI 8-1' (dwarf, poor fertility).

Petals were counted and their shape given a numerical value to reflect their degree of normalcy (Fig. 4). Thorny (TH) flowers were used as a control. Numerical values given were 1 = narrow, pointed, and straplike; 2 = broad strap with a single notch at the tip; and 3 = broad and rounded, with three regular lobes at the tip (this was typical of thorny plants). Sepals were also counted and their shape was given a numerical value to reflect their degree of normalcy (Fig. 4). Numerical values given were 1 = narrow, pointed strap that is highly twisted; 2 = narrow, pointed strap, not twisted; 3 = broad pointed strap; and 4 = large and well-developed strap with one to three points on each sepal (this was typical of thorny plants). In addition, stamens and pistils were counted for each flower, and pollen were stained with acetocarmine dye and their diameter measured on the screen of a Reichert projection microscope.

Results

Primocane evaluations

Significant differences were apparent among primocanes of the three 'Evergreen' strains tested for most of the characteristics measured (Table 1).

There was no significant difference in the number of internodes per cane for the three clones analyzed. However, each class of 'Evergreen' differed from the others in the length of its internodes. TH internodes were the longest, followed by PTE-normal. PTE-dwarf internodes were significantly shorter than either TH or PTE-normal internodes.

The thorny clone had 13 times more prickles per cane than the PTE-normal thornless clone, and these prickles were present on the entire length of the cane, from ground level to the unexpanded leaves at the growing point. However, PTE-normal plants, even though they were thornless, possessed some prickles, which appeared only near the base of the canes, from ground level to a mean of 19 cm from ground level. Above this level, the canes were smooth. PTE-dwarf plants had no prickles on their canes. PTE-normal canes also had significantly greater amounts of secondary branching than TH or PTE-dwarf plants.

Leaf evaluations

LEAF SIZE. There were no significant differences for total leaf

Table 1. A comparison of primocanes from three strains of 'Evergreen' blackberry (n = 5).

Evergreen clone ^a	Cane length (cm)	Internodes		Prickles/cane (no.)	Ht above ground of last prickle (cm)	Axillary branching	
		No.	Length (cm)			Secondary (no.)	Tertiary (no.)
TH	239 a	39 a	5.9 a ^b	505 a	239 a	4 b	0 a
PTE-normal	203 a	43 a	4.3 b	39 b	19 b	14 a	3 a
PTE-dwarf	177 a	39 a	3.8 c	0 b	0 b	4 b	1 a

^aTH = thorny; PTE = pure thornless (in vitro-derived) 'Evergreen'.

^bStudent-Newman-Keuls multiple comparisons. Numbers within columns followed by different letters are significantly different at *P* = 0.05.

Table 2. A comparison of mean leaf morphology and prickles distribution of three strains of 'Evergreen' blackberry.

Evergreen clone ^z	Leaf length			Leaf width (cm)	Leaflets/ Laciniations/ leaf		Leaf class		No. prickles		
	Total leaf	Petiole	Midrib		(no.)	(no.)			Midrib half		
							Mean	Range	Petioles	Proximal	Distal
TH _(n = 151)	15.3 a ^y	5.6 b	9.6 b	13.8 b	4.7 b	44 a	4.3 a	0–5	20 a	19a	7a
PTE-normal _(n = 133)	15.9 a	5.7 b	10.0 a	14.9 a	5.0 a	34 b	3.4 b	1–5	3 b	5b	7a
PTE-dwarf _(n = 110)	16.1 a	6.9 a	9.0 c	14.0 b	4.9 a	30 b	3.5 b	0–5	1 c	1c	1b

²TH = thorny; PTE = pure thornless (in vitro-derived) 'Evergreen'.

^yStudent-Newman-Keuls multiple comparisons. Numbers within columns followed by different letters are significantly different at $P = 0.05$.

lengths among TH, PTE-normal, and PTE-dwarf plants (Table 2). However, when the total leaf length was divided into two components—petiole and midrib—differences among the clones became apparent. Petioles of PTE-dwarf plants were significantly longer than TH or PTE-normal plants, while midribs of PTE-dwarf plants were significantly shorter. PTE-normal plants had the longest midribs, while TH plants were intermediate. PTE-normal plants had the widest leaves, while leaves of TH and PTE-dwarf plants were not significantly different from each other.

LEAFLET NUMBER AND MORPHOLOGY. 'Evergreen' clones also differed for the number of leaflets present. PTE-normal and PTE-dwarf approached a mean of 5.0 leaflets per leaf (the typical 'Evergreen' leaf has five leaflets in a palmate arrangement), while TH leaves had significantly fewer leaflets per leaf (4.7). In addition, leaves among clones varied widely in the number of laciniations. Thorny leaves were more lacinate (44 laciniations per leaf) than leaves of either PTE-normal (34 per leaf) or PTE-dwarf (30 per leaf).

The three 'Evergreen' clones also differed in their leaf class. Leaf samples of TH and PTE-dwarf contained leaves representative of each class, while samples of PTE-normal had no completely broadleaf (class 0) samples. TH leaves had a significantly higher mean leaf class than either PTE-normal or PTE-dwarf leaves. That is, TH leaves tended to have more leaflets of the lacinate type than did PTE-normal or PTE-dwarf.

PRICKLES. There were significant differences in the number of leaf prickles among all three clones. TH leaves had the greatest number of prickles, but PTE-normal and PTE-dwarf leaves still formed some prickles. Among the thornless clones, PTE-normal leaves had more prickles than PTE-dwarf leaves.

Midribs of TH leaves had fewer prickles toward the tip (distal) of the leaflet than toward the leaf-petiole junction (proximal). However, prickles of PTE-normal and PTE-dwarf types were evenly distributed between the proximal and distal regions of the

midrib. There was no significant difference between the number of prickles at the distal end of PTE-normal leaves and TH leaves.

Prickle evaluations

A comparison of prickle characteristics among TH and broad- and narrow-leaf variants of PTE-normal and PTE-dwarf plants showed significant differences for number of prickles per leaf and the prickle type (Table 3). TH leaves had significantly more prickles than any of the thornless clones, but among the thornless plants, broad- and narrow-leaf clones of PTE-normal had significantly more prickles than broad- and narrow-leaf PTE-dwarfs. Narrow-leaf PTE-dwarfs had the fewest prickles per leaf.

TH had a mean prickle value of 1.0, as would be expected for a clone which was completely thorny. However, broad- and narrow-leaf variants of PTE-normal and narrow-leaf PTE-dwarfs had mean prickle types between 3 and 4, indicating that the prickles present on these leaves were not as well developed. Mean prickle type for broad-leaf PTE-dwarfs was significantly higher than other thornless clones, indicating very little prickle development.

TH had significantly more prickles than any of the thornless clones in Segment 1, the basal half of the petiole. The broad- and narrow-leaf variants of PTE-dwarf had the fewest prickles in this segment. Broad- and narrow-leaf variants of PTE-normal had more prickles than the other thornless clones, but far fewer than TH.

In Segment 2, the distal half of the petiole, TH again had significantly more prickles than any other clone. Among the thornless clones, broad- and narrow-leaf variants of PTE-normal had the most prickles in Segment 2. Narrow-leaf PTE-dwarf leaves had the fewest prickles in this segment.

In Segment 3, the proximal half of the midrib, TH had significantly more prickles than any of the thornless clones. Among the thornless clones, narrow-leaf variants of PTE-normal plants had the most prickles at this site along the midrib. The broad-leaf

Table 3. A comparison of prickle type and location among five strains of 'Evergreen' blackberry (n = 10).

Evergreen' clone ^y	Prickle type ^x	Prickles/ leaf	Prickles/segment ^z			
			S1	S2	S3	S4
TH	1.0 a ^w	49.4 a	11.7 a	16.9 a	16.0 a	4.8 a
PTE-normal broad	3.3 b	15.9 b	3.1 b	3.5 b	5.8 c	3.5 b
PTE-normal narrow	3.3 b	19.5 b	2.8 b	3.2 b	9.0 b	4.5 a
PTE-dwarf broad	4.4 c	7.6 c	0.9 c	1.2 bc	4.0 c	1.5 c
PTE-dwarf narrow	3.9 b	3.0 d	0 c	0.3 c	1.6 d	1.1 c

^zS1 = basal half of petiole; S2 = upper half of petiole; S3 = lower half of midrib of terminal leaflet; S4 = tip half of midrib of terminal leaflet.

^yTH = thorny; PTE = pure thornless (in vitro-derived) 'Evergreen'.

^x1 = full prickle (hard, recurved; average length = 2.6 mm); 2 = soft prickle (fully formed, but not hard; average length = 0.5 mm); 3 = aborted prickle (not fully formed, average length = 0.25 mm); 4 = blister (elongated, raised area on epidermis; average length <0.25 mm); 5 = bump (small, round, raised area on epidermis; average length <0.25 mm).

^wStudent-Newman-Keuls multiple comparisons. Numbers within columns followed by different letters are significantly different at $P = 0.05$.

Table 4. A comparison of prickly type distribution on leaves of five strains of 'Evergreen' blackberry (n = 10).

Prickle type ^y	Mean number of prickles of each type per leaf segment ^z									
	S1 (petiole proximal half)					S2 (petiole distal half)				
	1	2	3	4	5	1	2	3	4	5
TH	7.9 a ^x	0.0	0 b	0	0 b	11.9 a	0	0 b	0	0 b
PTE-normal broad	0 b	0.0	0.9 a	0	0.7 ab	0 b	0	2.3 a	0	0.3 ab
PTE-normal narrow	0 b	0.0	0.7 ab	0	1.0 a	0 b	0	1.7 a	0	0.4 ab
PTE-dwarf broad	0 b	0.0	0 b	0	0.4 ab	0 b	0	0 b	0	0.8 a
PTE-dwarf narrow	0 b	0.0	0 b	0	0 b	0 b	0	0 b	0	0 b

Prickle type	S3 (midrib proximal half)					S4 (midrib distal half)				
	1	2	3	4	5	1	2	3	4	5
	1	2	3	4	5	1	2	3	4	5
TH	20.6 a	0 a	0 b	0 b	0 b	9.0 a	0 b	0 c	0 b	0 a
PTE-normal broad	0 b	0 a	5.5 a	0.2 b	1.1 b	0.1 b	0.4 b	3.2 b	1.0 b	0.2 a
PTE-normal narrow	0 b	0.3 a	6.7 a	0.1 b	1.4 b	0 b	1.2 a	5.4 a	0.2 b	0.4 a
PTE-dwarf broad	0 b	0 a	0.1 b	1.1 a	2.6 a	0 b	0.2 b	0 c	2.4 a	0 a
PTE-dwarf narrow	0 b	0 a	0.4 b	0.2 b	0.9 b	0 b	0 b	0.9 c	0.6 b	0 a

^zS1 = basal half of petiole; S2 = upper half of petiole; S3 = lower half of midrib of terminal leaflet; S4 = tip half of midrib of terminal leaflet.

^yTH = thorny; PTE = pure thornless (in vitro-derived) 'Evergreen'.

^xStudent-Newman-Keuls multiple comparisons. Numbers within columns followed by different letters are significantly different at $P = 0.05$.

variants of PTE-normal and PTE-dwarf plants had significantly fewer prickles than narrow-leaf PTE-normal plants. Narrow-leaf PTE-dwarfs had the fewest Segment 3 prickles.

In Segment 4, the tip half of the midrib, the distribution of prickles changed markedly. Although all clones had fewer prickles in this segment than in any other segment, differences between TH and thornless clones became less pronounced. Narrow-leaf PTE-normal plants had about the same number of prickles at the leaf tip as TH plants. Broad-leaf PTE-normal plants had only slightly fewer prickles at the leaf tips compared to TH. PTE-dwarf variants had the fewest prickles in Segment 4.

TH plants had only Type 1 prickles; most of these were present in Segment 3 (Table 4). Among the thornless clones, the least suppressed types of prickles (Types 1 and 2) were expressed in greater numbers in Segment 4, the leaf tip. The more suppressed types of prickles accounted for a greater percentage of the prickles present on thornless leaves. The greatest numbers of these suppressed-type of prickles appeared in Segment 3 for the PTE-normal clones and in Segments 3 and 4 for PTE-dwarf clones.

Percentage prickly suppression in each segment was calculated for all clones, based on the number of prickles of all types expressed (Table 5). Using the number of prickles expressed by TH in each segment as the expected number and the number of prickles expressed by thornless clones as the observed number, we calculated percentage prickly suppression with the formula percentage suppression = $100 - [(observed/expected) \times 100]$. Among

the segments, Segment 1 and Segment 2 consistently had the highest levels of prickly suppression, and Segment 4 had the least amount of prickly suppression.

The total numbers of each prickly type differed markedly among the clones. All prickles of TH plants were Type 1, while most of the prickles of PTE-normal were Type 3. PTE-dwarf plants had mostly Types 4 and 5 prickles.

Among the clones, PTE-normal (both variants) had the least amount of prickly suppression over all segments. Narrow-leaf PTE-dwarfs consistently had the most prickly suppression of all cultivars.

Flower evaluations

A comparison of flower morphology among ten in vitro-derived 'Evergreen' somaclones showed significant differences among individuals (Table 6). A summary of the ten clones by plant class indicated that TH, PTE-normal, and PTE-dwarf classes differed for all traits except number of pistils.

PETALS. TH had significantly fewer petals than either thornless class. Of the thornless classes, PTE-dwarf had significantly more petals than PTE-normal clones. A comparison of the three classes of somaclones showed PTE-dwarfs to have a significantly greater number of abnormal petals than PTE-normal.

SEPALs. TH had significantly fewer sepals than either thornless class. Of the thornless classes, PTE-dwarf had significantly more sepals than PTE-normal clones. A comparison of the three classes

Table 5. Percentage prickly suppression per leaf segment for five strains of 'Evergreen' blackberry.

'Evergreen' clone	Percent prickly suppression ^z			
	S1	S2	S3	S4
	(Petiole proximal)	(Petiole distal)	(Midrib proximal)	(Midrib distal)
TH ^y	0	0	0	0
PTE-normal broad	80	78	67	46
PTE-normal narrow	79	82	59	20
PTE-dwarf broad	95	93	82	71
PTE-dwarf narrow	100	100	93	83

^z $100 - [(observed/expected) \times 100]$.

^yTH = thorny; PTE = pure thornless (in vitro-derived) 'Evergreen'.

Table 6. A comparison of flower morphology among three classes of somaclones from 'Thornless Evergreen' blackberry.

Evergreen clone	No. petals	Petal shape	No. sepals	Sepal shape	No. stamens	No. pistils	Pollen diam (μm)
TH ^a	5.15 a ^y	3.00 a	5.10 a	4.00 a	164.85 a	63.65 a	19 a
PTE-normal (n = 120)	6.30 b	1.51 b	6.66 b	2.64 b	185.31 b	77.42 a	15 b
PTE-dwarf (n = 60)	6.90 c	1.28 c	6.97 c	2.37 c	188.98 b	92.62 a	16 b

^aTH = thorny; PTE = in vitro-derived pure thornless.

^yStudent-Newman-Keuls multiple comparisons. Numbers within columns followed by different letters are significantly different at $P = 0.05$.

of somaclones showed PTE-dwarfs to have the most abnormal sepals. This followed the same trend noted for petals.

STAMENS AND PISTILS. TH had significantly fewer stamens than PTE-normal or PTE-dwarf clones. Pistil numbers were similar for all three classes.

POLLEN DIAMETER. TH had significantly larger pollen grains than either PTE-normal or dwarf clones.

Discussion

TH, PTE-normal, and PTE-dwarf classes of 'Evergreen' distinctly differed from each other in primocane structure and leaf, prickle, and flower morphology. These differences were first apparent shortly after the plantings were made (McPheeters and Skirvin, 1989) and were still present after 7 years' growth in the field. The PTE-normal plants, originally classified as intermediate based on their relative size (McPheeters and Skirvin, 1989), retained their more vigorous habit, while the PTE-dwarf plants remained smaller and more abnormal.

The trend among the classes was one of increasing abnormality in comparison to the thorny 'Evergreen' clone after the plants lost their chimeral nature and became pure thornless. TH was the most vigorous of the plant classes evaluated. It had vigorous, long canes with long internodes, and these canes were heavily armed with fully formed prickles from ground level to cane tip. PTE-normal plants had shorter canes with shorter internodes. Although PTE-normal plants were thornless and no longer chimeral (i.e., we presumed them to be completely thornless in all histogenic layers), they possessed small prickles at the base of their canes. Leaves possessed few prickles, and these prickles were only partially formed. PTE-dwarf plants, which had the shortest canes and the shortest internodes, showed the greatest prickle suppression. Canes were completely without prickles. Fewer prickles were present on the leaves compared to PTE-normal plants, and these prickles were malformed and were present on the leaf tips only. Malformation of flowers followed the same trend of increasing abnormality with increasing dwarfness of the plant. Dwarf plants had more petals and sepals than other 'Evergreen' classes, and these petals and sepals were smaller and often twisted. This suggests that dwarfism, with its accompanying effects on plant morphology, is linked with prickle suppression.

The differences in prickle development among 'Evergreen' strains may be explained in terms of a suppression factor that interferes with prickle growth. For instance, the TH strain had an average prickle value of 1, that is, they possessed the hard, recurved fishhook type of prickle characteristic of wild-type thorny 'Evergreen' (Fig. 3). The thornless strains received much higher average prickle ratings, indicating their reduced prickle number and suggesting that prickle development had been suppressed by an unknown factor.

To summarize, the intermediate and dwarf somaclones of 'Evergreen' derived by McPheeters and Skirvin (1989) from epidermal tissue of the CTE periclinal chimera have maintained their distinctive habits over seven years' growth in the field. This suggests that these growth habits are not transient effects of tissue culture. The thornless gene does not entirely stop prickles from being produced, but apparently represses them at some stage of their development. There appears to be a link between the degree of thornlessness and the length of the internodes in PTE plants: increasing prickle suppression is directly related to increasing dwarfism. Although there are quantitative variations among chimeral and pure thornless types, the pure thornless plants continue to produce thornless root suckers (indicating they are no longer chimeral and are free of thorny tissues). The stability exhibited by our somaclones after 7 years of field growth is encouraging news for scientists planning to use somaclonal variation as a means to improve particular cultivars (Skirvin et al., 1994).

Literature Cited

- Darrow, G.M. 1931. A productive thornless sport of the Evergreen Blackberry. *J. Hered.* 22:405-406.
- Hall, H.K., D. Cohen, and R.M. Skirvin. 1986. The inheritance of thornlessness from tissue culture-derived 'Thornless Evergreen' blackberry. *Euphytica* 35:891-898.
- McPheeters, K.D. and R.M. Skirvin. 1983. Histogenic layer manipulation in chimeral 'Thornless Evergreen' trailing blackberry. *Euphytica* 32:351-360.
- McPheeters, K.D. and R.M. Skirvin. 1989. Somaclonal variation among *ex vitro* 'Thornless Evergreen' trailing blackberry. *Euphytica* 42:155-162.
- McPheeters, K.D. and R.M. Skirvin. 1995. 'Everthornless Blackberry'. U.S. Plant Patent No. 9407.
- Skirvin, R.M., K.D. McPheeters, and M.A. Norton. 1994. Sources and frequency of somaclonal variation. *HortScience* 29:1232-1237.