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ULTRASTRUCTURAL DEVELOPMENT AND OTHER CHARACTERISTICS OF STYLAR-END RUSSETING OF 'NAVEL' ORANGES IN FLORIDA

L. GENE ALBRIGO[1] AND DIANNE S. ACHOR
University of Florida
Citrus Research and Education Center
700 Experiment Station Road
Lake Alfred, FL 33850

KEN TOWNSEND
Vero Beach, FL 32962

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Abstract. **Stylar-end russeting (SER) of 'Navel' oranges often occurs in Indian River (IR) groves but is seldom seen in Central Ridge locations in Florida. Examination of the fruit peel in the summer revealed that the epidermal cell layer was ruptured, often in parallel arrays of cracks, in the stylar-end area. At this early stage of damage, there was no evidence of fungal involvement. By harvest, the injured area had a network of cracks. In the Ridge, the amount of SER was much greater in greenhouse conditions than when similar trees were outdoors. All cultivars of 'Navel' oranges examined in Indian River groves had some SER and year to year incidence was variable. Growth regulators applied during fruit growth did not affect the amount of SER, but Temik treatments in one year did reduce SER, while in the following year there was no affect of Temik on SER. Higher humidity in IR groves may favor fruit enlargement and thinner cuticles. Growth stress fracturing in relation to secondary fruit development as a mechanism for the cuticle-epidermal rupturing is discussed.**

Stylar-end russetting of 'Navel' orange fruit is a common occurrence in the more humid Indian River (IR) District along the East Coast of Central Florida, while stylar-end russetting seldom occurs in the Ridge (Central Interior Florida) District of the Florida citrus industry. Growers in the IR district commonly experience 10 to over 50% losses to the fresh fruit market due to stylar-end russetting. The disorder appears superficially to be similar to russetting caused by rust mite, except that it primarily effects the stylar-end of the fruit. On examination with a hand-lens the disorder appears to be a network of cracks in the fruit surface. The disorder is seldom reported on interior grown 'Navel' fruit. Apple russet also is reported to occur primarily in more humid conditions (Faust and Shear, 1972; Tukey, 1969).

Apple russet (Faust and Shear, 1972) and cracking of cherries (Lane et al., 2000) are perhaps similar problems for which considerable research and observations are available. A recent report on stylar-end russet of 'Navel' oranges found that fungicides significantly reduced the disorder in at least 3 of 4 year's of tests (Stover et al., 1999; Stover, pers. comm.).

The authors of this report examined 'Navel' oranges of different cultivars over a number of years in different conditions. We applied various treatments including spring sprays of growth regulators and ground application of Temik to see if they would alleviate the problem. This is a report of those research trials and a general characterization of the disorder from the ultrastructural viewpoint. The objective was to better characterize stylar-end russet of 'Navel' oranges under Florida conditions and determine if some treatments that delay senescence of citrus peel would reduce the incidence of this disorder.

Material and Methods

Characteristics and incidence of SER as influenced by plant growth regulator and/or Temik was assessed in 'Navel' cultivar blocks in the Indian River (IR) and Ridge Citrus District over several years from 1993 through 1997. In 1993 and 1994, fruit shape and time of incidence were evaluated in several IR blocks. In August 1995, fruit samples with early symptoms of stylar-end russet were collected from trees near Vero Beach, FL for microscopic examination. Surfaces near the stylar-end were examined and photographed using a dissecting microscope. Surfaces affected with this early stage of stylar-end russet also were prepared for scanning electron microscopy (SEM). Samples were taken of scarred areas over and between oil glands from affected 'Navel' fruit. These were placed in 3% glutaraldehyde in 0.1M potassium phosphate buffer pH 7.2 and fixed for 4 h at room temperature. Next the samples were washed in the buffer and postfixed in 4% osmium tetroxide in the same buffer for 4 hr at room temperature. Samples were then washed in buffer and dehydrated in an ethanol series followed by critical point drying in a Ladd critical point dryer (Ladd Industries, Burlington, VT). After mounting on stubs, the samples were sputter coated with gold/palladium (80:20) on a Ladd sputter coater (Ladd Industries, Burlington, VT) and viewed on an Hitachi S530 scanning electron microscope (Hitachi, Ltd., Tokyo, Japan).

On May 10, 1993, three sets of six trees in each of two blocks of Bates Navels were sprayed with GA_3 (10 mg per tree) and 2, 4-D (20 mg per tree) in the IR District. Fruit were evaluated at harvest for stylar-end russet and shape. Russet was graded on a scale of 1 to 4 for none, slight, moderate, severe, respectively. Fruit with moderate or severe grades of SER were considered to be unacceptable for packing as fresh fruit. Fruit were cut longitudinally through the core axis and diameters were measured at the center and equal distance from the center to either end of the fruit.

On Mar 13, 1994 in each of two IR blocks of Navels, two plots of 0.15 or 0.42 ha were established and one plot was treated with Temik at 6 kg/ha (33 lbs/ac) and compared to a non-treated control plot. Within each Temik and control plot, 3 plot replicates of three trees each were established and treated with GA_3 (10 mg per tree) and 2, 4-D (10 mg per tree) in April and May or left as controls. Three samples of 65 to 70 fruit per plot were collected on 11 Nov., 1994 and rated for

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[1]Corresponding author.

SER on a scale of 1 to 4 as previously indicated. The Temik treatments were repeated on the same plots in 1995 with treatment and fruit evaluation dates within 2 weeks of the previous year.

In 1996, GA_3 (5 mg per tree) and 2, 4-D (2.5 mg per tree) were sprayed monthly from April through June to plots in three 'Navel' blocks in the IR District. Experimental design was a randomized complete block with three adjoining tree plots replicated four times in each grove. Russet was graded on a scale of 1 to 4 for none, slight, moderate, severe, respectively. Fruit were cut longitudinally through the core axis and diameters were measured at the center and equal distance from the center to either end of the fruit. All data were subjected to ANOVA and significant treatment means were separated by Duncan's Multiple Range Test.

A Ridge citrus nursery was identified that was growing trees of several 'Navel' cultivars under plastic greenhouse protection as well as outdoors. An IR grove was identified that had several strains of 'Navel's purchased from this nursery. Fruit at this location was evaluated for SER in the Fall of 1996.

Results and Discussion

At fruit maturity SER of 'Navel' oranges appears to be a network pattern of cracks (Fig. 1A). In late summer, the first visible signs of SER were seen as parallel cracks that radiate out from the stylar-end (Fig. 1B). Detailed examination of the cracks by SEM revealed that the cracks were breaks through the cuticle (Fig. 2 A-D, arrows) and often the epidermal cells. The inner cell areas are clearly visible in Fig. 1 F. At this time no fungal growth was evident on the ruptured surfaces.

The first appearance of SER surface cracks in 'Navel' oranges was observed in mid- to late summer in this study, which is later in fruit development than the mid June to early July dates reported for apple russet (Skene, 1982). In apples the ruptures appear to be caused by growth stresses that the epidermal cells cannot withstand (Skene, 1980) and can be largely eliminated by spring applications of GA_{4+7} (Greene, 1993; Looney et al., 1992; Taylor, 1975).

Based on parallels with apple russetting, spring spray of the growth regulators GA_3 and 2,4-D were applied in 1993 to determine whether resulting delays in peel development (Monselise, 1979) would diminish the appearance of SER, however no effect on SER was observed (data not shown). A subsequent 2 year study with two applications of the growth regulators with or without Temik again did not alter the level of SER, but the Temik did reduce the amount of SER in the first year (Table 1). Neither the PGRs or Temik affected the amount or severity of SER in the second year of these treatments (data not shown). The lack of effect from using GA_3 may be due to the GA selected or inability of GAs plus 2,4-D to promote peel expansion when internal growth pressures are extreme. The reason for the Temik effect in one year is unknown.

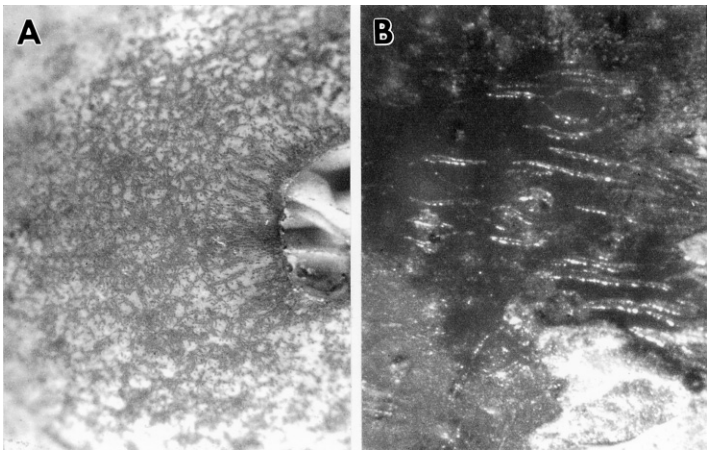

Fig. 1. A) Photograph of stylar-end russet of 'Navel' orange showing network of cracks and B) dissecting microscope view of cracks in surface near the stylar-end of a 'Navel' fruit at the early stage of SER development.

In a subsequent attempt to see if GA + 2,4-D could influence russet development on 'Navel' oranges, monthly sprays were applied from April through June, 1996, encompassing the times GA is reported to be effective against apple russet (Looney et al., 1992). Fruit diameter measurements of non-treated and treated fruit indicated a significant increase in diameter growth of the bottom (stylar-end) half of the fruit in two of three groves (Table 2), but there was no significant effect of the PGR treatment (about 20% non-useable fruit in all treatments, data not shown). The one grove width equal widths may have been another cultivar of 'Navel' or growing conditions may have influenced shape. The fruit width to height indicates that the fruit was more elongated. The greater growth of the bottom half of the 'Navel' fruit in two of the groves may relate to expansion of the secondary fruit within the 'Navel' fruit (Lima and Davies, 1984) in at least some cultivars. This expansion may contribute a growth stress on the peel that leads to cracking and should be evaluated further.

Examination of four 'Navel' cultivars in adjacent rows in an IR grove and at a Ridge citrus nursery indicated that most cultivars had some SER in the IR location. Dream 'Navel' had more SER, while Glen and Cara Cara had little and Barrington almost no SER in the IR grove. At the Ridge citrus tree nursery no cultivars growing outdoors had SER. However, 'Navel' orange trees growing in a plastic greenhouse usually had moderate to severe SER. The worst SER in the greenhouse was on SF 56-11 (a Glen strain), while Washington 'Navel' had the least SER. Work on apple russet revealed that more russet occurred under humid than dry conditions (Tukey, 1969; Faust and Shear, 1972). The air in late spring and early summer is more humid in the IR District than on the Ridge with ET values about 14% lower than for the Central Ridge (NOAA, 1994-1998). Cracking of cherries is related to moisture uptake through the cuticle, increased cell turgor and ruptuing of the epidermis (Lane et al., 2000). Reducing water uptake reduced cracking (Davenport et al., 1972).

Drawing on parallels between apple russeting and cherry cracking, we postulate that initial development of SER in the IR District is related to excessive fruit expansion particularly related to secondary fruit growth in the stylar-end of the fruit. Differences in susceptibility between cultivars may be related to overall fruit growth potential, and in particular, differences in growth of the secondary fruit. The reduced ET in the IR District may increase fruit growth by decreasing average daily water stress. Also higher humidity would decrease cuticle development leading to less resistance of the surface to rupture from internal stresses. Higher turgor of cells in cherry from surface water uptake is sufficient to lead to cracking (Glenn and Poovaiah, 1989).

The reported effect of the fungicide Ferbam in decreasing incidence of SER 3 of 4 yr (Stover et al., 1999; Stover, pers. comm.) may be from decreasing secondary development of

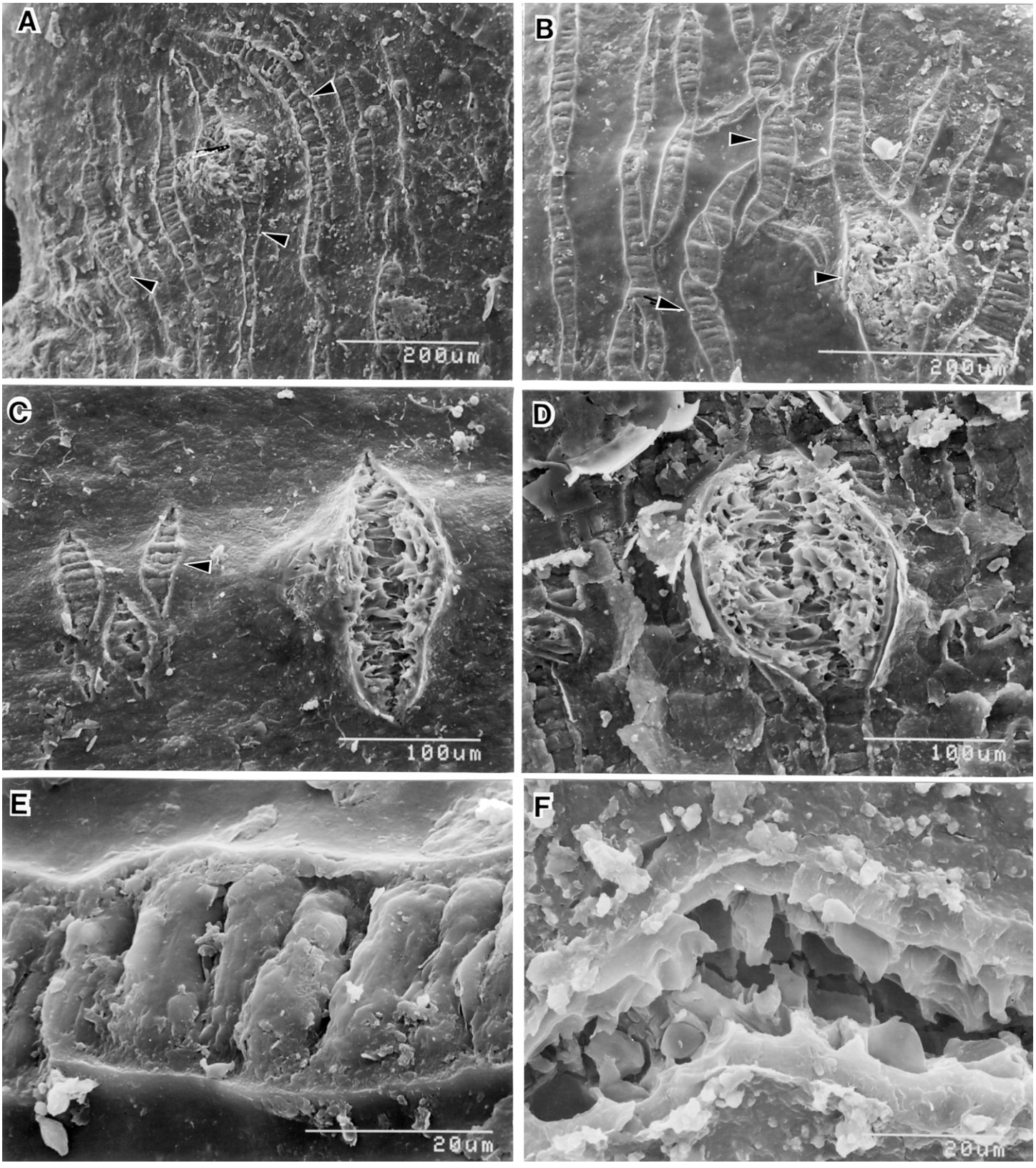

Fig. 2. Various views (A-F) of cracks in surface of 'Navel' fruit as seen by SEM. SER at same stage as shown in Fig. 1B. Note parallel cracks at earliest stage (A and B) and exposed (E) or ruptured epidermal cells (F) at higher magnification.

the disorder. Fungal invasion of the initial cracks may lead to further cracking of the surface. This might be the case particularly if wound periderm formation is an important part of the final appearance of the disorder. Wound periderm formation is an important aspect of apple russet development (Skene, 1981) and citrus fruit readily form wound periderms under

Table 1. Effect of Temik and the growth regulators GA_3 and 2, 4-D on SER development in 'Navel' oranges in 1994.

Treatments	Average rating of SER[z]
Control	3.0 a
GA_3 + 2, 4-D	3.3 a
Temik	2.4 b
Temik and GA_3 + 2, 4-D	2.3 b

[z]Ratings were 1, 2, 3 and 4 for none, slight, moderate and severe. Treatment ratings followed by different letters are significantly different at the 1% level.

fruit surface injuries (Albrigo and McCoy, 1974). The fungicide applications could directly reduce further cracking by somehow reducing wound periderm formation. Yeast fungal species have been associated with apple russet (Matteson Heidenreich et al., 1997). They postulated that the yeast may weaken the cuticle (cutinase etching), alter IAA balance (effect cell wall elasticity) or produce phytotoxins that kill epidermal cells. No matter what the mechanism, fungicides reduce apple russet (Matteson Heidenreich et al., 1997; Reuveni et al., 2001). Whether fungal growth on the surface contributes to weakening of the cuticle-epidermal barrier was not determined nor tested, but might be a logical next step, since the broad spectrum fungicide, Ferbam, did appear to reduce SER. If any of these proposed fungal mechanisms are involved, a variety of general fungicides applied in late spring to early summer should reduce the incidence of SER as Ferbam did.

The early development of SER in mid- to late August was evidenced by surface cracks around the stylar-end of the 'Navel' fruit. This is likely related to expansion of the stylar-end of the fruit creating a stress that the epidermal layer could not expand to meet. Growth regulators that could delay peel senescence did not increase epidermal elasticity sufficiently, if at all, so that the peel could expand sufficiently to avoid epidermal splitting. Testing GA_{4+7}, which worked to reduce russet in apples, may be warranted, but GA_3 is effective in enhancing early fruit growth (Guardiola et al., 1993) and in delaying peel senescence of citrus indicating its general effectiveness in citrus (Monselise, 1979).

Table 2. Measurements of diameters at the center and equal distances to the button and stylar-end of 'Navel' oranges treated or not treated with GA_3 and 2, 4-D in 3 Indian River groves.

Treatment	Measurement	Grove 1	Grove 2	Grove 3
Control	Top width	49.7 mm	53.8 mm	59.2 mm
	Equatorial W	58.2	64.3	68.7
	Bottom W	52.8	55.4	59.4
	Height	58.7	64.4	60.7
	B/T Ratio[z]	1.06	1.06	1.00
Treated	Top width	49.7	54.3	60.1
	Equatorial W	59.5	65.1	68.7
	Bottom W	52.7	57.6	59.8
	Height	56.9	64.4	62.8
	B/T Ratio	1.06	1.06	1.00

[z]B/T ratio is the bottom to top ratio of fruit width. There were significant differences in this ratio for groves 1 and 2 when compared to grove 3 but not between treated and non-treated fruit.

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