The Influence of Calcium on the Development of Lettuce Tipburn[1]

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Abstract. Foliar sprays of $Ca(NO_3)_2$ or $CaCl_2$ completely controlled lettuce tipburn in the variety 'Meikoningen' when directed to susceptible immature leaves. Analysis of treated and untreated plants showed that treatment markedly increased the Ca content of tipburn susceptible leaves and revealed a 5-fold increase in Ca content as one progressed from immature heart leaves to mature basal leaves in untreated plants. Foliar sprays of organic acid salts, particularly oxalate, accelerated the development of tipburn and increased its severity. When Ca and oxalate treatments were alternated, application of Ca at the beginning of the dark period and oxalate in the morning resulted in markedly less tipburn than application of these sprays in the reverse order.

Introduction

A majority of studies dealing with lettuce tipburn have emphasized marked dependence upon environmental factors such as temperature, humidity, moisture, and more recently upon the intensity and duration of light (24). Although much of the older evidence appears contradictory it is generally agreed that tipburn is most severe when both environmental factors, and nutritional factors permit periods of very rapid leaf growth.

A detailed description of tipburn symptomology has been provided by Tibbitts, et al. (25). The visual symptoms of the disorder (marginal necrosis of inner leaves) are preceded by swelling and rupturing of laticifer cells which results in latex release. There are noteworthy similarities, however, between lettuce tipburn and some other physiological disorders, even though laticifers are not always involved. Some of these are brownheart of escarole (15), blackheart of chicory (27), blackheart of celery (8), heartrot of Chinese cabbage (10), and tipburn of cabbage (26). In each case susceptible tissues are the young leaves where rapid cellular division and enlargement occur. Their location on the plant insures a microenvironment of relatively higher humidity than that of the rest of the plant. These disorders are markedly affected by environment and appear to be correlated with rapid growth rates. When analyzed, susceptible tissues have been found to have a very low Ca content, and partial to complete control has sometimes been achieved by timely administration of this element (8, 11, 15, 16, 26, 27).

The need for Ca by young tissues is expected to be greatest during periods of rapid growth. Environmental factors such as temperature, light, humidity, and moisture not only affect growth rates, thus accentuating the requirement for Ca, but also influence the availability and translocation of this relatively immobile element within the plant (4). We therefore felt that the relationship between lettuce tipburn and calcium deserved further attention, in spite of the fact that Jenkins (13) did not influence tipburn development of field grown head lettuce by twice weekly sprays of Ca to outer leaves and that Struckmeyer and Tibbitts (22) have reported tipburn symptoms to resemble more closely those observed in lettuce grown without B than without Ca.

Material and Methods

Experiments were conducted with a small susceptible, bibb type of lettuce, *Lactuca sativa* L., cv. 'Meikoningen'. The symptomatology of the disorder referred to as tipburn was precisely as described by Tibbitts, et al. (25) in work with this same cultivar.

Experiment 1. On January 7 seedlings at the 3 leaf stage were transplanted in the greenhouse to 6-inch styro-

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foam pots filled to a level of 6 inches with a 1:1 mixture of coarse and fine washed quartz sand and watered daily with Hoagland's complete nutrient solution No. 1 containing 5 mmoles $Ca(NO_3)_2$ per liter (9). Sprays of $Ca(NO_3)_2$ (.04 *M*) or distilled H_2O were first applied at the 10-leaf stage and were continued every other day until the plants were harvested on March 28. Sprays were applied at night to runoff and directly to the heart of the plants using a small atomizer. Care was taken to prevent spray contact with the more mature leaves. Once the plants began to head it was necessary to gently open the heads to allow spray contact with heart leaves. The experiment was randomized, containing 5 replications of 4 plants each for each treatment. An attempt was made to control greenhouse temperatures at 21° C ± 2 and 15° C ± 2, day and night respectively, and periods which deviated beyond these limits are indicated. At harvest the plants were weighed and one-half the Ca-treated plants (none of which tipburned) and one-half of the check plants (all of which tipburned) were saved for mineral analysis. At this time 10 extra tipburned check plants were sprayed with $Ca(NO_3)_2$ or $CaCl_2$ at .04 *M* to see if treatment would check the development of further tipburn on newly developing leaves. The plants for analysis were separated into 7 parts. These parts were basal leaves, outer head leaves, middle leaves, inner head leaves, heart and apical leaves, stems, and roots. Tissues were dipped 10 times in each of 3 changes of distilled H_2O to insure that analyzed Ca was Ca actually absorbed rather than Ca retained on the surface of leaves. Tissues were then oven dried, weighed, and ground through a 40 mesh screen.

For the analysis of water soluble Ca, .5 g of dry ground tissue was digested on a steam bath for 15 min in a 125 ml Erlenmeyer flask containing 25 ml of distilled water and then filtered through Whatman No. 1 paper into a 100 ml volumetric flask. The residue was washed several times with hot distilled water. The liquid washings were brought to a final volume of 100 ml and comprised the soluble Ca fraction. For water insoluble Ca the residue from above was quantitatively transferred to the original Erlenmeyer flask and digested on a steam bath for one hour in 25 ml of 3.5 *N* HCl. This mixture was then filtered through Whatman No. 1, washed several times with hot distilled water, and the liquid washings brought to a final volume of 100 ml. The soluble and insoluble Ca washings were then analyzed for Ca with a Perkin-Elmer atomic absorption spectrophotometer, model 290. As recommended by Berry and Johnson (2), the filtrates were prepared to contain 500 ppm Sr in 0.1 N $HClO_4$ to prevent possible interferences from phosphate and silicate ions.

Experiment 2. Seedlings were transplanted in the greenhouse on May 3 as described above and treated with sprays of .04 *M* $Ca(NO_3)_2$ until May 23 when symptoms of tipburn first appeared on a set of indicator plants treated only with distilled water. At this time plants had an average of 25 leaves. The following sprays at the designated concentrations were then applied once every night: distilled H_2O, $Ca(NO_3)_2$ (.0125 *M*), $CaCl_2$ (.0125 *M*), $NaC_2H_3O_2$ (.025 *M*), $Na_3C_6H_5O_7$ (.008 *M*), $Na_2C_2O_4$ (.0125 *M*), and $(NH_4)_2C_2O_4$ (.0125 *M*). There were 20 plants per treatment.

Experiment 3. Plants were grown as above until the eighth true leaf emerged at which time they were moved to a controlled environment chamber with a 12 hr day and a light intensity of 2000 ft-c (cool white fluorescent supplemented with incandescent). Night and day temperatures were 15° and 18° C respectively. Heart leaves were sprayed with a .04 *M* solution of $Ca(NO_3)_2$ once every night. A set of 10 indicator plants, randomized among the Ca-treated plants, received only distilled water and when one-half of these developed tipburn, the following foliar treatments were applied to the $Ca(NO_3)_2$ treated plants:

1) H_2O(N)– Distilled water applied every night (N).
2) H_2O(D)– Distilled water applied every day (D).
3) Ca(N)– $Ca(NO_3)_2$ (.025 *M*) applied every night; heart leaves washed with distilled water in the morning.
4) Ca(D)– $Ca(NO_3)_2$ (0.25 *M*) applied every morning; heart leaves washed with distilled water at night.
5) Ox(N)– $(NH_4)_2C_2O_4$ (.025 *M*) applied every night; heart leaves washed with distilled water in the morning.
6) Ox(D)– $(NH_4)_2C_2O_4$ (.025 *M*) applied every morning; heart leaves washed with distilled H_2O at night.
7) Ca(D), Ox(N)– $Ca(NO_3)_2$ (.025 *M*) applied in the morning; heart leaves washed with distilled water the following night and then immediately sprayed with $(NH_4)_2C_2O_4$ (.025 *M*). The leaves were washed again the next morning, immediately sprayed with $Ca(NO_3)_2$ and the whole process was continually repeated until the end of the experiment.
8) Ox(D), Ca(N)– The reverse of treatment no. 7.

There were 20 plants per treatment and plants averaged 20 leaves when the spray schedule began. At this time growth chamber temperature was held to a constant 15° C ± 1 both day and night. Relative humidity was 80% ± 5 and 95% ± 5 day and night respectively. There was no indication that moisture condensed on the leaves at night. Four days after initial tipburn appeared, the temperature was raised to a constant 18° C ± 1 for the next 8 days, and finally to a constant 22° C ± 1 for the last 4 days. Tipburn development on new plants or new leaves was recorded twice a day, immediately before and immediately after the dark period.

Results and Discussion

Foliar sprays of $Ca(NO_3)_2$ completely controlled lettuce tipburn under conditions where plants sprayed with dis-

Table 1. Influence of .04 *M* $Ca(NO_3)_2$ sprays on tipburn occurrence and growth of lettuce.

Treatment	Per cent of plants showing symptoms at indicated days from transplanting						Average total leaves per plant	Total tipburned leaves per plant	Final fresh wt tops g
	40	45	47	51	60	80			
Distilled H_2O	30	50	60	90	100	100	104[ax]	68[a]	263.0[a]
$Ca(NO_3)_2$....	0	0	0	0	0	0	96[b]	0[b]	284.4[b]

[x]Values are means for 20 plants. Means not followed by a common letter are significantly different at 5% level.

Table 2. Rates of growth and tipburn development for water treated plants in Table 1.

Days from transplanting	Total leaves per plant >1 cm	Youngest leaf to tipburn	Rate of growth[a]	Rate of tipburn[b]
45	43.0	29	—	—
47	44.5	30	0.7	0.5
51	47.0	32	0.6	0.5
60	58.0	46	1.2	1.6
80	104.0	99	2.3	2.7

[a]Number of new leaves (1 cm or longer) per plant per day. Data is for interval from last observation.
[b]Number of new tipburned leaves per day. Data is for interval from last observation.

tilled water were severely affected (Table 1). Necrotic breakdown of the tipburned leaves probably accounts for the decreased fresh weights of the water treated plants, however, total leaf number was higher for these plants. Table 2 shows that the rate at which tipburn symptoms appeared on previously unaffected leaves closely followed the growth rate of the plants when expressed as the number of new leaves formed per day. The average maximum day temperature in the greenhouse during the latter 2 observation times was 26° C. This increase in temperature and the associated higher light intensities were likely responsible for the faster growth rate and consequently the accelerated rate of tipburn. The innermost leaves (shorter than one cm) were not affected even under very high incidence and severity of tipburn. However, as the severity of tipburn progressed a greater number of smaller inner leaves became affected.

Table 3 shows marked differences in both the soluble and insoluble Ca content of the heart leaves between

Table 3. The calcium content of various parts of the lettuce plant following foliar sprays of distilled water and .04 *M* $Ca(NO_3)_2$.

Plant parts	Foliar treatment	Per cent Ca (Dry weight)			
		Insoluble	Soluble	Total	Ratios insol:sol
heart	Ca	.34[x]	.17[a]	.51	2.1
leaves	H_2O	.24[b]	.07[b]	.31	3.4
inner	Ca	.45[a]	.21[a]	.66	2.1
leaves	H_2O	.39[b]	.12[b]	.51	3.3
middle	Ca	.62[a]	.31[a]	.93	2.0
leaves	H_2O	.54[b]	.28[a]	.82	1.9
outer	Ca	.79[a]	.51[a]	1.30	1.6
leaves	H_2O	.79[a]	.35[a]	1.14	2.3
basal	Ca	.96[a]	.75[a]	1.71	1.3
leaves	H_2O	.97[a]	.60[b]	1.57	1.6
stems	Ca	.16[a]	.06[a]	.22	2.8
	H_2O	.14[a]	.07[a]	.21	2.2
roots	Ca	.97[a]	.29[a]	1.26	3.3
	H_2O	1.04[a]	.30[a]	1.34	3.5

[x]Means of 10 plants. Columns with same letter are not significantly different at the 5% level. Analysis is for comparison of treatment effects only.

healthy (Ca-treated) and tipburned (H_2O-treated) plants. As heart leaves were the most susceptible to injury and the first to be affected the data suggest that the sprays controlled lettuce tipburn by increasing Ca in the leaf. The sprays also increased the soluble Ca of inner and middle leaves. Regardless of treatment both soluble and insoluble Ca decreased from the basal to the heart leaves, reaching the very low values of .07% Ca in heart leaves of tipburned plants. The accumulation of Ca in the older tissues and its relative immobility has been confirmed a number of times by use of ^{45}Ca (3, 4, 5, 23). The 5- to 9-fold increase of soluble Ca in the basal leaves compared to heart leaves indicates relatively slow delivery of soluble Ca to the heart tissues.

Table 3 shows that ratios of insoluble to soluble Ca always exceeded unity and generally increased from the basal to the heart leaves. The high ratios in heart leaves suggests that soluble Ca is rapidly tied up and immobilized in the formation of new tissues. Consequently, it would be expected that environmental factors causing rapid leaf growth would not only increase demands for soluble Ca but at the same time might accelerate immobilization of Ca already available, possibly through increased production of respiratory organic acids such as oxalate and citrate during dark periods (6, 18). A number of reports (1, 7, 12, 19) indicate that immature leaves have the highest respiratory rates, and in lettuce these are the leaves most susceptible to tipburn.

At the end of the experiment $Ca(NO_3)_2$ and $CaCl_2$ sprays were applied to the heart of 10 extra tipburned check plants in an attempt to correct the disorder. Two days later new healthy leaves appeared. One week later, 8 to 12 new leaves had developed completely free of tipburn and were growing vigorously. Previously sprayed healthy plants whose treatment was discontinued developed tipburn within 3 to 4 days during this same period. Differences in effectiveness between $CaCl_2$ and $Ca(NO_3)_2$ were not evident. After 20 new leaves had developed, recovered plants were harvested and analyzed and it was found that treatment had increased soluble and insoluble Ca to levels similar to those of treated plants that never tipburned. Data are not shown. Attempts to correct tipburn by applying Ca sprays to basal or outer head leaves failed. These results suggesting a slow translocation of Ca in the lettuce plant, and the well documented immobility of Ca in many plant species logically explain why twice weekly sprays of Ca applied to head lettuce in the field failed to influence tipburn (13).

It appears that lettuce tipburn is not associated with Ca-deficiency in the usual sense of the term but rather a temporary localized shortage of soluble Ca during a period of great need. Tissue analysis may indicate relatively high levels of Ca and the root media may be well supplied. However, when environmental conditions are such as to produce rapid leaf growth the delivery of soluble Ca to a new site may be too slow and immobilization, possibly through respiratory products, too great to meet the need. It is felt that if this condition exists for even a short period (1–2 hr) tipburn may result.

The role of Ca in maintaining the stability and functional integrity of cell walls and membranes is well documented (14, 20, 21). Olson and Tibbitts (17) have shown that only in young developing leaves during laticifer differentiation do laticifer cells bulge, unite with parenchyma cells, and rupture. These events precede visible symptoms and result in latex release. Such aberrations are thought to be caused by excessive pressures occurring within the differentiating laticifers, possibly due to increased accumulation of osmotically active solutes within the laticifers (17). Regardless of the cause of the pressure it is reasonable that greater pressures would be required to rupture membranes and walls developing with adequate Ca than those developing under conditions of Ca stress. Therefore, it is postulated that Ca sprays controlled tipburn by stabilizing cell wall components susceptible to rupture.

It was expected that factors reducing soluble Ca would accelerate tipburn development. Table 4 shows the results of an experiment where plants were sprayed with various organic acid salts as well as with $Ca(NO_3)_2$ and $CaCl_2$.

Table 4. Influence of Ca sprays and sprays of organic acid salts on the incidence and development of lettuce tipburn.

Foliar treatment	Molar concentration	Per cent of plants showing synptoms at the indicated days from transplanting[a]							
		21	22		23		24		25
		D[b]	D	N	D	N	D	N	D
$Ca(NO_3)_2$	.0125	0	0	0	0	0	0	0	0
$CaCl_2$	.0125	0	0	0	0	0	0	0	0
H_2O	—	0	0	0	0	0	50	65	100
$NaC_2H_3O_2$	.025	0	0	0	40	40	75	80	100
$Na_3C_6H_5O_7$	.008	0	0	25	75	80	100	100	100
$Na_2C_2O_4$	.0125	0	20	40	100	100	100	100	100
$(NH_4)_2C_2O_4$	.0125	0	25	40	100	100	100	100	100

[a]All plants received .04 *M* $Ca(NO_3)_2$ until the 20th day after transplanting when H_2O treated indicator plants showed tipburn. At this time the above treatments were started. There were 20 plants pretreatment.

[b]Tipburn is indicated when data is taken in the morning (D) and at the beginning of the dark period (N).

Relative to water these salts increased the incidence and severity of tipburn in the order oxalate > citrate > acetate. On the oxalate treated plants some of the youngest leaves (barely 1 cm in length) were affected. These plants were also the first to tipburn followed closely by citrate and finally by acetate and water. As before, sprays of $Ca(NO_3)_2$ or $CaCl_2$ resulted in complete control. The data suggests that rapid growth and ensuing high respiratory rates promote tipburn by temporarily immobilizing soluble Ca as salts of organic acids, particularly oxalate. Note that organic acids closest to the end of the oxalate pathway (18) initiated the earliest tipburn. Therefore, possibly the differences in time of symptom appearance may be due to time needed to convert the treatments to oxalate in the tissue. One cannot be sure however, because the occurrence of symptoms also seemed to follow the order of solubility of the Ca salts of the compounds applied, e.g. Ca-acetate (.374g/100 ml), Ca-citrate (.085g/100 ml), and Ca-oxalate (.00067g/100 ml).

The results of a third experiment showed that Ca sprays were equally effective in controlling tipburn whether applied at night or in the morning. (Table 5).

Table 5. Influence of $(NH_4)_2C_2O_4$, $Ca(NO_3)_2$, and H_2O foliar sprays supplied at the beginning of the dark period (N) or in the morning (D) on the development of lettuce tipburn.

Foliar treatment	Observation period[a]					
	I		II		III	
	Per cent[b] tipburn	No. of leaves[c] affected	Per cent tipburn	No. of leaves affected	Per cent tipburn	No. of leaves affected
H_2O(N)	5	1.0	50	3.8	70	5.5
H_2O(D)	5	2.0	50	4.2	70	6.0
Ca(N), H_2O(D)	0	0	0	0	0	0
Ca(D), H_2O(N)	0	0	0	0	0	0
Ca(N), Ox(D)	0	0	5	1.0	20	1.2
Ca(D), Ox(N)	20	1.5	40	1.7	55	3.6
Ox(N), H_2O(D)	75	1.7	100	10.0	100	15.7
Ox(D), H_2O(N)	60	1.7	100	10.0	100	15.5

[a]I = 1st observation; temperature to this time = 15°C. II = 2nd observation after 4 days at 18°C. III = 3rd observation after 8 days at 22°C. R.H. = 80 ± 5 and 95 ± 5 for day and night respectively for I, II, and III.
[b]Per cent of plants showing new tipburn symptoms since time of last observations. Data is not accumulative. Twenty plants per treatment.
[c]Average number of tipburned leaves per plant. Data is accumulatve.

As in the second experiment oxalate sprays greatly accelerated tipburn development relative to water sprayed plants. When Ca sprays were alternated with oxalate sprays tipburn was less than on water sprayed plants, however, Ca sprays did not result in complete control when alternated with oxalate. Of particular interest is the fact that Ca applied at night and oxalate applied during the day resulted in markedly less tipburn than when sprays were applied in the reverse order. With the alternating Ca and oxalate treatments practically all of the tipburn became visible in the period immediately following oxalate sprays and practically none appeared following Ca sprays regardless of which treatment was applied in the morning or at night. Most of the tipburn on H_2O treated plants first become visible during the night. This was true with alternating H_2O and oxalate treatments when the oxalate was applied at night. Approximately 50% of the symptoms appeared during the day when oxalate was applied in the morning. It is recognized that these observations, although highly indicative, do not conclusively fix the time of tipburn initiation because it is not known by how many hours the actual laticifer rupture precedes visible symptoms under the conditions of the experiment.

Nevertheless, the influence of treatment timing on tipburn development suggests that immobilization of Ca by products which accumulated during darkness may cause tipburn. The capacity to accumulate these Ca-binding products in a short period of time would in large part depend on the amounts of photosynthate produced during previous light periods. The fact that tipburn does not occur under conditions of low light intensity or extended periods of darkness does not contradict the above reasoning. Firstly, growth rates and thus the demand for soluble Ca would be less under these conditions and secondly, the amount of photosynthate produced might be too low to allow substantial production and accumulation of respiratory Ca-binding acids.

It is suggested that any factor or combination of factors, environmental or otherwise, limiting the supply of available Ca to heart leaves below the minimum dictated by their growth rate at a particular time will promote tipburn. The effect may be exerted by 1) depressing Ca absorption or translocation, 2) by increasing growth rates to the point where Ca requirements exceed what might otherwise be a sufficient supply, 3) by immobilizing Ca before it can be utilized by young developing tissues, and 4) by combinations of the above.

Practical control of tipburn for field grown head lettuce by use of Ca sprays seems remote due to the relative immobility of Ca and the inaccessibility of the susceptible immature leaves. However, we have found Ca sprays to be of some use in checking tipburn where lettuce is grown for seed in the greenhouse. In this case, to encourage bolting, heading is discouraged by periodically unfolding the inner leaves. Therefore, it is possible to reach the susceptible parts at the frequency necessary to check tipburn.

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Tissue Response of Young Developing Apple Fruits to Freeze Injury[1]

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Abstract. Morphological and anatomical development of young fruit were studied in relation to the effects of a late spring freeze. 'Lodi' and 'Duchess' apples were frozen 16 days after full bloom. Samples were collected for anatomical study on 4 subsequent dates following the freeze. The secondary vascular tissues in the outer cortex were affected, resulting in lesions in those tissues where adjacent parenchyma cells had collapsed. The main vascular strands in the outer cortex were killed, the injury extending from fruit base to apex. Separation of the hypodermis from the outer cortex occurred in the fruit apex where tissues were injured most severely. Also injuries were evident contiguous to the core line. Internal phellogen, phellem, and phelloderm in the parenchyma had formed within 18 days after injury. In some cases, cell proliferation and dedifferentiation of cortical parenchyma was noted 46 days after injury.

Introduction

Young developing apples are often subjected in many areas to freezing temperatures late in the spring season. Tissues of young fruits have been studied in their responses to injury and their ability to recover.

Groves (6) reported freeze damage to young apples that were a half-inch in diameter. A large number of frozen fruit dropped from the tree, but many remained and developed to maturity. The regions of killed cells, apparent as light brown areas, were rendered obscure by subsequent growth of uninjured cells. Fruits were often deformed as a result of the internal injury, although there was no frost russet on the fruit. Gardner (4) states that frost occurring after the time of fruit set may occasionally arrest the further development of seeds and still permit the fleshy tissues to develop and mature, giving rise to fruits abnormal in size and shape.

Rogers (8) has found that the tissues commonly supercool to about 28 or 29°F before any ice formation takes place. If supercooling persists and no ice has formed, the plant is endangered, even by severe frost. At full bloom in the 'Cox's Orange Pippin', a temperature of 28° resulted in formation of a layer of ice beneath the skin (including the epidermis and hypodermis) which was thereby lifted from the cortex. This damage heals readily. At 27°, damage occurs at the base of the style. This damage appears as brown discoloration after thawing, may spread to the placenta and ovules, and is often fatal. If the temperature falls to 25 or 26°, the damage becomes widespread and the crop is reduced. Rogers also found that most apples are more susceptible to frost in the fruitlet stage than at full bloom.

Freezing of the fruit has many implications for its development to maturity and for its susceptibility to storage disorders. In instances where the injury is not severe, growth will continue. This may be one source of the physiological disorders found in apples such as watercore and cork spot. Abnormal development of mature apples injured by a late spring freeze has been discussed in a previous report (12). Another type of freeze damage already reported is the occurrence of superficial injuries, i.e. frost rings and the sequential development of injured tissues (9, 11).

This study recorded the effects of a late spring freeze on the morphological and anatomical development of very young fruit in its early stages of development.

Materials and Methods

A freeze in the early morning of April 24, 1967 in western Illinois, 16 days after full bloom for 'Lodi' and 'Duchess' apples, caused severe damage to fruit tissues. The lowest temperature for the area was 27°F.

In each sampling date, 12 representative injured fruits were collected for anatomical study on April 28, May 12, May 23, and June 9. Additional samples were made for the X-Sections of the fruit base and apex. Approximately 100 fruits were observed in obtaining each morphological observation for the 2 cultivars. These morphological observations were made 4 and 46 days respectively for 'Lodi' and 29 days after injury for 'Duchess'. These observations were recorded as growth anomalies appeared macroscopically.

Anatomical studies were aimed toward determination of specific tissue responses to be found within the various parts of the apple. Specimens of injured tissue were preserved immediately in a standard formalin-acetic acid-alcohol killing and fixing solution. After a brief aspiration they were carried through an alcohol-xylol dehydration series and then infiltrated and embedded in par-

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