LEAF AND STEM ANATOMY OF SEVERAL APPLE CULTIVARS, THEIR COMPACT MUTANTS, AND ALAR TREATED PLANTS

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

IN THE DEPARTMENT
of
PLANT SCIENCE

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

March, 1970

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ABSTRACT

Naturally occurring compact (spur type) apple trees (Malus sylvestris L.) were compared with standard and Alar treated trees. Stem anatomy received special attention because no comparisons had been done of the stem anatomy in standard and compact apple trees. Anatomical studies of leaves in compact mutants and in Alar treated Red Delicious are more detailed than in earlier reports.

Four cultivars were used in the studies. They were Harrold Red Delicious, a compact mutant of Red Delicious, namely Starkrimson, and standard Golden Delicious, and its compact mutant Starkspur. There were three main studies in this investigation.

In the first study, anatomical examinations were made on the four cultivars without Alar treatment. Starkspur Golden Delicious was found to have the thickest leaf and palisade parenchyma among the four cultivars studied. The compact type was found to have a thicker leaf, palisade parenchyma and greater mean palisade number when compared with the standard type.

The effect of Alar at concentrations of 0 and 1000 ppm on the same cultivars was investigated in the second study. The suppression of terminal growth by Alar varied among the cultivars. The response to Alar was greatest with Starkspur and 50 per cent inhibition of shoot growth was observed. Starkrimson was not affected by Alar treatment. Microscopic examination revealed that there were no significant differences in cell length of collenchyma, parenchyma and pith

cells or in cell diameters and tissue thickness when the samples were taken from the first internode under the growing tip.

In study three, the effect of Alar and its interaction with gibberellic acid on Red and Golden Delicious were considered. In this study, comparisons were also made with the untreated compact mutants. Alar treatments of Red Delicious were found to increase thickness of total leaf, spongy parenchyma and the length of palisade cells. The latter two accounted for the increase in total thickness of Alar-treated Red Delicious leaves.

Gibberellic acid stimulated the shoot growth of Golden Delicious and Starkspur by 29 per cent, but this stimulating effect was prevented by Alar.

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to Dr. G. W. Eaton, Associate Professor, Department of Plant Science, University of British Columbia, under whose supervision this project was undertaken, for his technical advice during the research, and for his guidance in the preparation of this thesis.

Sincere appreciation is especially expressed to Dr. N. E. Looney, Pomologist, Canada Department of Agriculture Research Station, Summerland, British Columbia, who kindly provided many of the plant materials used in this project and who also gave valuable counsel and assistance in several ways.

Also appreciation is extended to Drs. K. Beamish, C. A. Hornby, and V. C. Runeckles for their helpful suggestions during this endeavor.

This Research was supported by NRCC Operating Grant A2023 awarded to Dr. G. W. Eaton.

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INTRODUCTION

Due to the cost of labor in pruning and harvesting fruit grown on large trees, controlling the size of fruit trees has held the intermittent interest of pomologists for hundreds of years. A recent approach to attaining tree size control has involved chemical growth retardants. One showing considerable promise for use on fruit trees is succinic acid 2,2-dimethyl hydrazide commercially known and hereafter referred to as Alar. The first published report of the use of this chemical as a growth retardant on plants was made by Riddell et al. (31) in 1962.

Studies on the movement and fate of Alar in apple seedlings have been reported (25). Other studies have shown that Alar applied to apple trees caused many desirable effects without a major suppression of root growth (3). Alar suppresses terminal growth (3, 4, 14, 16), delays bloom one to three days and has also been reported to increase fruit set (14), improve fruit-keeping quality (14), reduce pre-harvest drop (14), and increase fruit color (21, 23, 33). Treatments often result in smaller and firmer fruit (5) and greener and thicker leaves (14, 16, 17). In spite of such wide-spread attention on a diversity of subjects, there is little detailed information on anatomical effects of Alar (16) or anatomical comparisons between standard and compact types.

The object of the present study was to further compare compact and standard types and the effects of alar on the morphology

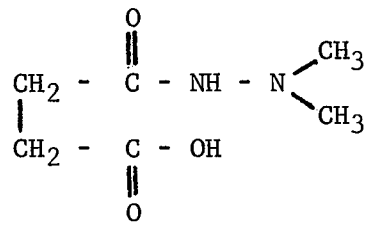
and anatomy of apple leaves and stems using standard and compact strains of Red Delicious and Golden Delicious apples.

LITERATURE REVIEW

The development of plant growth retardants has been very rapid since the report in 1949 that a new class of chemicals, the nicotiniums, reduced stem elogation of bean plants without other formative changes. The most active compound was 2,4-dichlorobenzylnicotinium. When applied in one per cent lanolin paste, the first internode of the treated plants was found to be one-quarter of the length of the control (28). One year later, Wirwile and Mitchell (39) reported that a number of quaternary ammonium carbamates retarded the growth and development of a broad variety of plant species without the development of malformed leaves, stems, roots and flowers. 4-Hydroxy-5-isopropyl-2-methylphenyl trimethyl ammonium chloride, 1-piperidine carboxylate (Amo-1618) was found to be the most active compound in this group of chemicals tested. In 1958, Preston and Link (30) found that 2,4-dichlorobenzyl-tributylphosphonium chloride (Phosfon) affected the growth of more widely different species than did Amo-1618. Then in 1960 (2-chloroethyl) trimethylammonium chloride (CCC) was found to retard the growth of a larger number of species than any of the earlier compounds (36). In 1962, Riddell *et al.* (32) reported that sprays of N-dimethylamino maleamic acid (CO11) retarded the growth of legumes, vine crops, potatoes and ornamental plants. However, whereas CO11 was found to be unstable in aqueous solution, its analogue, N-dimethylaminosuccinamic acid (B995), was stable and retarded the

growth of the same species as did CO11 (13).

B995 was the original experimental code number given by the discoverer, Uniroyal, then the Naugatuck Chemical Division of the United States Rubber Company. Later the name was shortened to B-Nine, B-9, DMAS and Alar. Originally the material was intended for use on ornamentals and sold for this purpose under the name of B-Nine. The later commercial preparation, Alar-85, is registered for use on several species of fruit crops. The chemical structure is as follows:



Among the growth retardants tested, Alar seems very promising, and has been studied by many investigators. The movement and fate of Alar in sweet cherries, apple seedlings and the short-day plant, Pharbitis nil, have been studied (33, 26). Ryugo reported residual Alar was found in new leaves of the sweet cherry, Prunus avium, in the spring following a late fall application (33). With radioactive B995, Zeevaart was able to demonstrate the mobility and persistence of this growth retardant in Pharbitis plants (40). Also, by using labeled Alar, Martin et al. (26) were able to follow the movement of Alar in apple seedlings. From chemical analysis they concluded that it was resistant to breakdown in the plant and was absorbed and translocated rapidly in the transpiration stream. Due to its rapid absorption and

high mobility, coverage should be of less importance and a more casual approach to application may be in order. This is an advantage of Alar over many other chemical sprays.

Once within the plant, Alar causes a number of effects. The effect on root growth of one year old apple trees was studied by Barden (3) who reported that the merit of Alar over some other growth retardants is that Alar causes many desirable effects on the above ground portions of an apple tree without a major supression of root growth. Other workers have studied the effect of Alar on shoots. Zeevaart reported that treatment of the short-day plant Pharbitis with B995 resulted in short, thick internodes (40). The effect of Alar on the shoot diameter of apple trees has also been noted. Halfacre et al. (16) reported that Alar treatment increased stem radius of both Golden Delicious and York Imperial apples. The increased radius in the former was due to an increase in radial thickness of the pith, phloem and cortex. For York Imperial, it was due to pith and cortex being thicker. Longitudinal sections of Alar-treated plants of both apple cultivars had fewer and shorter cells per internode. Cell division was affected more than cell expansion transversely and longitudinally.

Several workers have noted effects of Alar on flowering of fruit trees. Batjer et al. (4) reported that apple and cherry trees sprayed with Alar in the early summer of 1962 produced more flowers in 1963 than unsprayed trees. However, in the short day plant Pharbitis nil, "Violet", flower formation was inhibited by the application of Alar

via the roots for a period of 24 hours prior to one inductive long night (40). Edgerton et al. reported that flower bud formation was promoted on three year old Delicious trees sprayed with B995. The pre-bloom application of B995 on mature trees delayed bloom one to three days and resulted in higher fruit set as compared with unsprayed control when frosts occurred following the treatments (14). Several reports indicated that Alar also affected keeping quality of the fruit. Edgerton et al. found that sprays of B995 on three year old Delicious trees early in the growing season reduced fruit size at harvest (14) and pre-harvest sprays of B995 to more mature McIntosh apple trees resulted in firmer fruit than on untreated plants. Other workers have demonstrated the effects of Alar on enhancing apple quality at harvest and after storage (15).

When Alar was applied at a concentration of 2000 ppm to sweet cherry Prunus avium, early production of the anthocyanin pigments in the fruit was observed and Ryugo (33) concluded that although Alar enhanced the biosynthesis of anthocyanins, it did not measurably advance the physiological maturity of cherries. Looney, however, reports that early season application of Alar promotes several parameters of sweet cherry maturity (24).

The effect of post-bloom application of Alar on apple fruit ripening has also been studied. Looney (21) reported that the amount of chlorophyll in peel and flesh of apple was lower through the season when a spray of 4000 ppm of Alar was applied in mid-May, two weeks

after bloom but he concluded that Alar did not noticeably advance or delay maturity. He also studied the respiratory behavior of apples under storage conditions and found that a mid-July application of 2000 ppm Alar significantly reduced respiration of stored fruit at 0° C, whereas, a mid-May 2000 ppm spray, did not (21). In a later report from the same laboratory, the ripening of McIntosh apples was delayed by treatments of Alar applied two weeks after bloom, and this inhibitory effect of Alar was counteracted by 100 ppm of ethylene. Looney suggests that Alar suppressed ethylene biosynthesis within the fruit and this suppression may not be related to fruit maturity (23). One of the most noteworthy effects of Alar is its excellent control of pre-harvest drop. Batjer et al. reported that the application of Alar to Delicious and Winesap apple trees reduced pre-harvest drop and delayed the development of watercore. Treated fruits were firmer and somewhat lower in soluble solids (5).

Blanpied et al. thought Alar might have certain specific effects rather than a general effect on the fruit (8) because they found that the application of Alar to apple trees increased fruit firmness and delayed best harvest date for three varieties in Ireland, but did not do so for McIntosh in New York. They attribute the conflicting results to differences in the interaction of variety, season, location and the specific effects of the material.

Various effects of Alar on leaf characteristics have been reported. Edgerton and Hoffman reported that Alar applied as a foliar spray to three-year-old Delicious trees in mid-June produced leaves

normal and, in some cases, larger in size. The leaves appeared darker green and thicker in texture than the untreated leaves (14).

Halfacre and Barden investigated the leaf anatomical responses of one year old trees of Golden Delicious and York Imperial apple treated with Alar at various concentrations. They found the leaves of treated plants were thicker as a result of longer palisade cells and a looser arrangement of the spongy parenchyma cells. In Golden Delicious leaves, the lower concentrations of Alar stimulated transverse palisade cell production and expansion whereas Alar inhibited cell division and expansion at all concentrations used on York Imperial (16). Later, the same workers reported that Alar treatment decreased leaf area on York Imperial apple trees and also fresh and dry weights per unit area of leaf tissue (17). Effects of Alar on leaf anatomy should be studied in other cultivars such as Red Delicious.

All the above findings may have significance in the orchard because of the intimate relationship between the leaf structure and its maximum photosynthetic rate. McClendon studied the leaves of twenty-three different species of plant and found that the photosynthetic rate was a function of their density thickness (g/cm^2 fresh weight) (27). Beakbane concluded that the number of palisade cells per unit leaf surface was related to the photosynthetic and respiratory activity of the palisade mesophyll and also related to the growth potential of apple rootstocks (7).

The effect of a growth stimulant, gibberellic acid (GA), on stem elongation in plants has often been investigated. As there was no

evidence of cell elongation for at least 72 hours after application of gibberellin to the vegetative plants of the biennial short-day Hyoscyamus and of the long-day plant Salmolus, Sachs et al. concluded that the initial increase in stem length was due solely to an increase in cell number (34). Beakbane suggested that GA might be used as a possible expanding agent in leaf tissue subjected to shade conditions (6), because in apple leaf discs treated in a basic medium plus 0.5 ppm GA, great expansion of the epidermal cells was observed. The distribution and shape of mesophyll cells were also affected by GA.

Several workers have examined the interaction of Alar with GA in plants. Zeevaart found the inhibition of flower formation in Pharbitis nil by Alar could be completely overcome by application of gibberellin A_3 to the plumule before the long night (40). By using cucumber seedlings, Moore (29) was able to demonstrate that 1 µg of GA_3 applied to the shoot tip was sufficient to completely nullify the effect of 25 µg of Alar applied simultaneously (29). When three-year-old Delicious apple trees were sprayed with a mixture of Alar at 1000 ppm and of potassium gibberellate (KGA) at 200 ppm, the shoot growth was reduced to less than 50 per cent of that made by shoots treated with KGA alone (14). However, the real mechanism of interaction between Alar and the gibberellins still remains unknown. Further studies are needed to elucidate the relationship between Alar and GA in the apple.

The difference in growth habit, chemical content, and leaf composition between Starking Delicious and the natural compact mutant

Starkrimson have been studied extensively. In general, when compared with Starking, the mutant is reported to have shorter shoots, fewer lateral shoots, more fruiting spurs, more nodes per foot and thicker leaves with a greater depth of palisade parenchyma, more dry weight, chlorophyll, N, and Ca (1, 37, 38). Before it is possible to generalize about the anatomical differences between standard and compact types, comparisons must be made in other cultivars such as Golden Delicious and its compact mutant, Starkspur. Only Arasu (1) has considered these latter two strains and he only reports data for total leaf and palisade parenchyma thickness. Stem anatomy comparisons between compact and standard growing types have not been made in any cultivar.

Since the natural compact mutants have many merits over the standard growing strains and since Alar is reported to cause similar changes in standard apple trees, a critical comparison of the differences and similarities between these two approaches to size control is required. Intrieri reported that spur type trees and those treated with retardants showed many analogies in morphology, physiology, biochemistry and anatomy (19). This was based on an extensive literature review and not critical comparisons within any single experiment. This report therefore must be regarded as setting up a number of hypotheses which demand investigation. Likewise, Looney reported there were similarities between compact mutants and standard Delicious apple trees treated with Alar (22). He found that the net assimilation rate of Starkrimson was approximately twelve per cent higher than that of Starking Delicious and a similar difference was revealed between Starkspur and Golden Delicious (22).

According to the literature, Alar is the most promising growth retardant studied to date. While its effects on certain characteristics such as growth habit, external appearance, morphology and biochemical function have been studied, there is no information at all concerning the effect of Alar on the natural compact mutants. The horticultural importance of both growth retardants and genetic compaction as tools for size control would indicate that any possible interaction merits careful study.

Two main points arising from this literature review will be investigated for the first time in this study; effects of Alar on leaf and stem anatomy in Red Delicious and detailed anatomical comparisons of leaf and stem in standard and compact types of Red and Golden Delicious. While some anatomical effects of Alar on Golden Delicious and comparisons of leaf anatomy in compact and standard Golden and Red Delicious have already been reported, these will be included in the present study to allow more direct comparison with the results of earlier studies. Previous workers have not studied stem anatomy in any compact mutant and leaf anatomy studies to date have been rather superficial. With this information it should be possible to generalize about the anatomical effects of Alar or natural compaction upon apple varieties.

MATERIALS AND METHODS

Experiment I

Comparisons of Untreated Compact and Standard Cultivars

The leaf and shoot samples of apple trees were obtained from the orchard of the Canada Department of Agriculture Research Station at Summerland. Two varieties, Red Delicious and Golden Delicious, each with standard and compact types, i.e. Harrold Red Delicious (standard), Starkrimson (compact), Golden Delicious (standard) and Starkspur (compact), were used in this experiment. On June 11, 1968, three nine year old trees were randomly chosen from each cultivar. One shoot from the North and South side of each tree was sampled by taking two neighboring leaves from the middle part of the current year's shoot. Each leaf was sampled by taking discs from three different positions, apical, middle and basal on each side of the midrib. From the same shoot, the first internode below the shoot tip was taken. Each internode was cut transversely into two parts for both cross and longitudinal sections.

The leaf and shoot tissues were fixed in Bellings Modified Navashin Fluid (20) for which the formula is as follows:

Solution A:	Chromic acid crystals	5g
	Glacial acetic acid	500cc
	Distilled water	320cc
Solution B:	Formalin	200cc
	Distilled water	175cc
	Saponin	3g

After fixation, the samples were washed with running tap water, dehydrated in an ethanol series and embedded in paraplast according to the procedure of Johansen (20) which is shown below:

Dehydration:

1. 5% ethyl alcohol . . . . . 2 hours
2. 11% ethyl alcohol . . . . . 2 hours
3. 18% ethyl alcohol . . . . . 2 hours
4. 30% ethyl alcohol . . . . . 2 hours
5. approximate 50% alcohol . . . . . 2 hours or longer

Distilled water	5 parts
95% ethyl alcohol	4 parts
Tertiary butyl alcohol	1 part

6. approximate 70% alcohol . . . . . overnight or longer

Distilled water	3 parts
95% ethyl alcohol	5 parts
Tertiary butyl alcohol	2 parts

7. approximate 85% alcohol . . . . . at least 1 hour

Distilled water	3 parts
95% ethyl alcohol	10 parts
Tertiary butyl alcohol	7 parts

8. approximate 95% alcohol . . . . . at least 1 hour

95% ethyl alcohol	9 parts
Tertiary butyl alcohol	11 parts

9. approximate 100% alcohol. . . . . at least 1 hour

Tertiary butyl alcohol	3 parts

	100% ethyl alcohol	1 part
10.	Tertiary butyl alcohol	3 changes (one of which should remain overnight)

Infiltration:

1. mixture of equal parts of paraffin oil and Tertiary butyl alcohol — at least 1 hour
2. fill a vial three-fourth full of melted paraplast and let the paraplast solidify but not cool completely.
3. put the material on top of the solidified paraplast, just cover with the butyl alcohol-paraffin oil mixture and place the container in the oven at once.
4. about 1 hour after the material has sunk to the bottom of the vial, pour off the entire mixture of paraffin oil and what traces of alcohol remain and replace with pure melted paraplast.
5. repeat the process twice during the next 6 hours or so, discarding each change of paraplast.
6. finally replace with pure melted paraplast and the material will be ready for embedding within the next 30 minutes.

Embedding:

1. remove the vial from the oven, shake the material to get it off the bottom and quickly pour into the plastic mold.
2. add more melted paraplast from the stock container if necessary.
3. with a needle heated slightly in the flame, quickly dispose the pieces of material into an orderly arrangement.
4. as soon as the mould can be moved without disturbing the

arrangement of the pieces of material, transfer to a vessel of cold water.

5. let the mold float until the surface of the paraplast becomes sufficiently firm to permit plunging the mold slowly beneath the surface of the water.
6. leave the molds in the water for half an hour or until thoroughly cooled.

After embedding, all the leaf and stem samples were cut with a rotary microtome at ten microns thickness. Sections were affixed to slides with Haupt's adhesive (18), and stained with safranin and fast green according to the schedule described by Johansen (20) and shown below:

Staining:

1. Xylene - 10 to 15 minutes.
2. Xylene - 100% ETOH (1:1) - 5 minutes.
3. 95% ETOH - 5 minutes.
4. 70% ETOH - 5 minutes.
5. 50% ETOH - 5 minutes.
6. water - wash well.
7. Stockwell's solution - at least 24 hours, depending on material.
8. water - wash well.
9. tannic acid - 10 to 15 minutes.
10. water - wash well.
11. ferric chloride - several minutes, depending on blacking.
12. water - wash well.

13. safranin - at least overnight; usually 24 hours.

14. water - wash well.

15. 95% ETOH with 1/2% picric acid - no more than 10 seconds.

16. 95% ETOH with 4-5 drops ammonia per 100 cc - 2 minutes.

17. 100% ETOH - about 30 seconds.

18. fast green staining solution - starting with a 10 second immersion.

19. clove oil (to stop action of fast green) - a few seconds.

20. clear for one or two minutes in:

clove oil	-	50 cc
100% ETOH	-	25 cc
Xylene	-	25 cc
		100 cc

21. Xylene - at least 10 minutes.

The slides were then examined microscopically. An ocular micrometer was used for measuring the cell length and radial diameter of stem and leaf tissues.

The expected mean squares for the analysis of variance of this experiment are shown in the Appendix, Tables 1 to 4.

Experiment II

Effects of Alar on Compact and Standard Cultivars

(A) Apple trees of Golden Delicious and Starkspur on EM VII rootstocks were grown in a growth chamber at Summerland. In December 1968, Alar at 0 and 1000 ppm was applied to each cultivar. At this time Golden Delicious had an average shoot length of 14.25 cm and Starkspur had 10.6 cm. There were six trees of each treatment and cultivar. Alar

sprays and measurements of shoot length and leaf number were made at weekly intervals. The 'compact' Starkspur grew very vigorously and in fact, was not noticeably different from Golden Delicious when Alar was not applied. However, Alar appeared to reduce shoot growth more on Starkspur than on Golden Delicious.

Shoot lengths were measured from the base of the new shoot to the base of the tip leaf cluster, and leaves were counted from the base up, including half-opened ones at top of the shoot. The treatments were terminated on February 4, 1969. Samples were collected at that same time.

Sampling and preparation procedures were essentially the same as in experiment I, but the second and third fully expanded leaves below the shoot apex were chosen for leaf sampling to ensure that the leaves sampled were initiated well after the Alar treatments were begun. In each tree one or two shoots were used, and there were six trees of each cultivar randomly assigned to each of the treatments and the controls.

(B) A total of twenty-four trees of Harrold Red Delicious and Starkrimson were used in this experiment, also at Summerland. Technical grade Alar at 0 or 1000 ppm was applied to six trees of each cultivar. When first treated, the average shoot length of Harrold was 13 cm and of Starkrimson, 8 cm. Alar treatments, shoot length and leaf number determinations were done once a week for eight weeks beginning April 8, 1969 with Harrold and April 22, 1969 with Starkrimson. Sampling and preparation procedures were the same as described in

experiment I. The expected mean squares for the analysis of variance of this experiment are shown in the Appendix, Tables 5 to 13.

Experiment III (a)

Comparisons of Compact with Alar treated Red Delicious

In April, 1969, the scions of Red Delicious, and Starkrimson were grafted on EM 11 rootstocks planted in plastic pots. All lateral shoots were removed and only one shoot was allowed to develop on each tree. Twenty-one Red Delicious and seven Starkrimson trees were used in this experiment. On June 12, the Red Delicious trees had an average shoot length of 35 cm. Technical grade Alar with a small amount of Triton added, was applied at 0, 1000 and 4000 ppm. There were seven replicates within each treatment. The seven Starkrimson trees with an average shoot length of 14.3 cm were left untreated for comparison. All twenty-eight trees were randomly arranged within one block. Shoot lengths were measured at weekly intervals. The experiment was terminated five weeks after treatment.

Experiment III (b)

Effects of Alar and Gibberellic Acid on Compact and Standard Golden Delicious

Four trees each of Golden Delicious and Starkspur were randomly arranged within each of two blocks. These trees were also on EM 11 rootstocks grafted in April, 1969 and prepared as described above. On June 12, 1969, Alar at 0 and 1000 ppm, GA at 1000 ppm, and GA at 1000 ppm combined with Alar at 1000 ppm were applied individually to single trees within the same block. Shoot lengths were measured once

weekly for six consecutive weeks. Leaf and shoot samples were collected at the end of the experiment. The same collection and preparation procedures were used as in experiment I. The one exception was that only two discs along the midrib of each leaf were taken because in experiment I, no significant differences among the different positions on a leaf had been found. The expected mean squares for the analysis of variance of this experiment are shown in the Appendix, Tables 14 to 17.

RESULTS

Experiment I

Comparisons of Untreated Compact and Standard Apple Cultivars

There were no differences among Harrold Red Delicious, Starkrimson, Golden Delicious and Starkspur in the thickness of the upper and lower epidermis and spongy parenchyma. The Starkspur had a thicker palisade parenchyma than the other three cultivars (Table 1, Fig. 1). The increased thickness of the palisade parenchyma was found to be due to greater mean palisade cell layer number and length (Table 1, Fig. 2). Although the analysis of variance showed no differences in the total leaf thickness between Harrold Red Delicious and Starkrimson, discriminant function analysis showed that the cultivars (Fig. 3) differed significantly at the five per cent level. Red and Golden Delicious were compared with respect to leaf tissue thickness, pooling the compact and standard types. The thicker palisade parenchyma of Golden Delicious was probably due to both the greater mean number of layers and the length of palisade cells (Table 2). Also compact and standard types were compared pooling the two cultivars. Standard type was found to have thinner leaves, thinner palisade parenchyma and smaller mean number of palisade cell layers than the compact mutant (Table 3).

In order to investigate the cause for the thicker shoots and shorter internodes in the compact apple mutants, measurements were made of cell size in the pith and cortex, the thickness of vascular tissue as well as stem thickness. Figures 4 and 5 show longitudinal

TABLE 1

THICKNESS OF LEAF TISSUES OF FOUR CULTIVARS IN MICRONS

	Harrold Red Delicious	Starkrimson	Golden Delicious	Starkspur	Significance Level
Lower Epidermis	12a[x]	12a	12a	13a	0.1566
Spongy Parenchyma	75a	75a	75a	82a	0.1999
Palisade Parenchyma	84c	90bc	100b	112a	0.0028
Upper Epidermis	14a	14a	14a	15a	0.5218
Total	186b	191b	202b	222a	0.0047
Number of Palisade Cell Layer	3.0b	3.1b	3.0b	3.3a	0.0015
Average Palisade Cell Length	29b	29b	33ab	34a	0.0413

[x]within a row, means having a letter in common are not significantly different at the 5 per cent level by Duncan's Multiple Range Test.

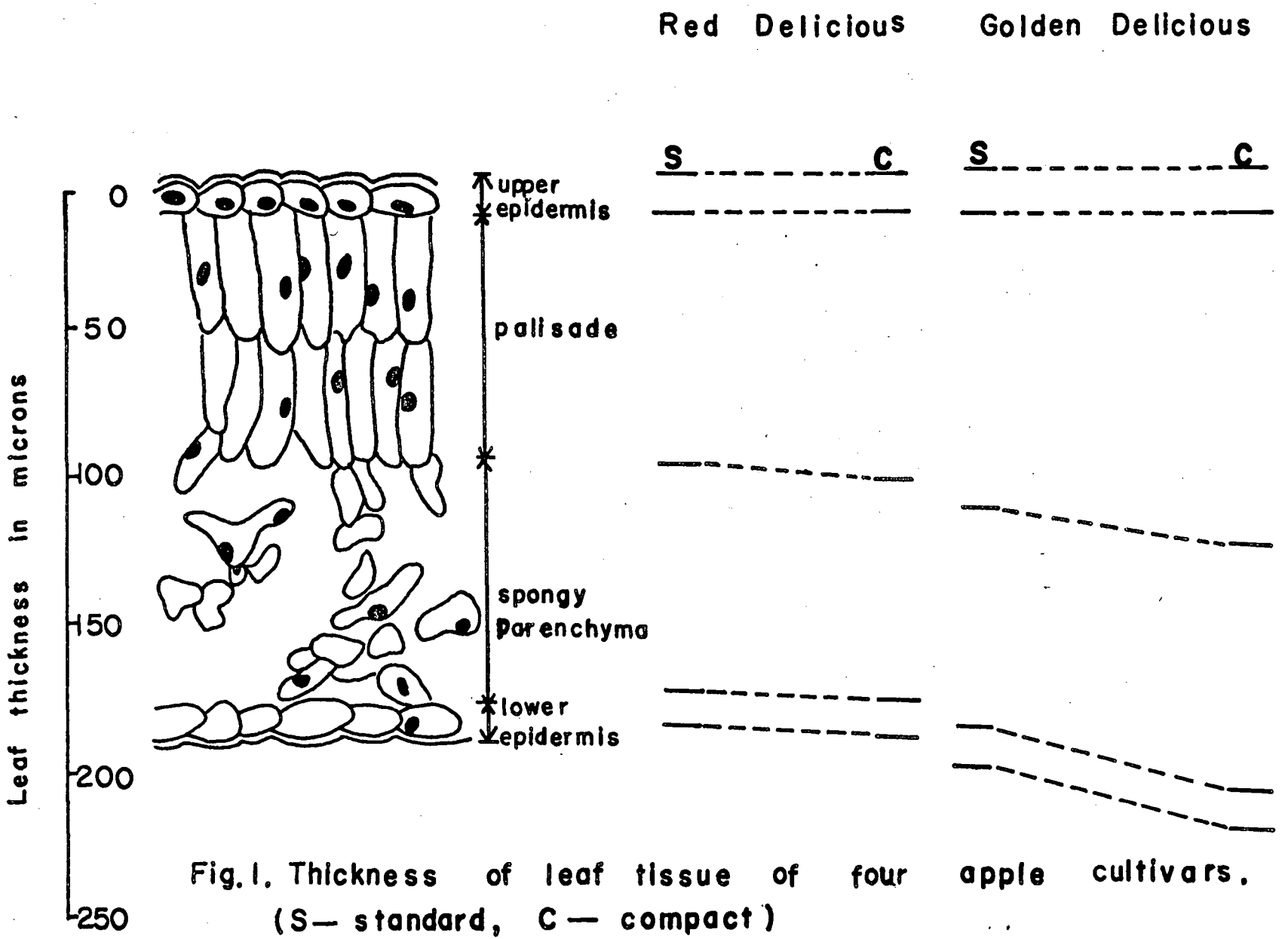


Fig. 1. Thickness of leaf tissue of four apple cultivars. (S— standard, C — compact)

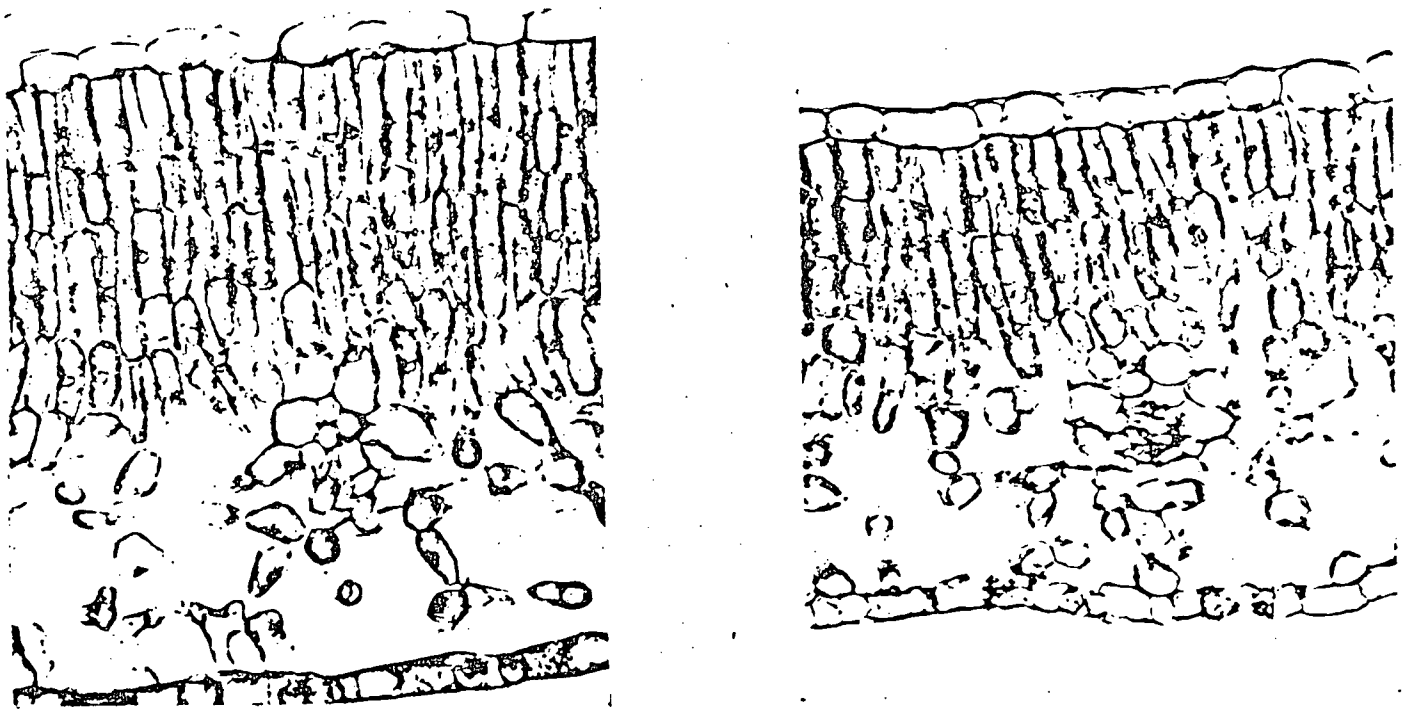


FIGURE 2

The leaf cross section of Starkspur showing longer palisade cells and greater mean palisade number (left) than in the standard Golden Delicious (right). (381X)

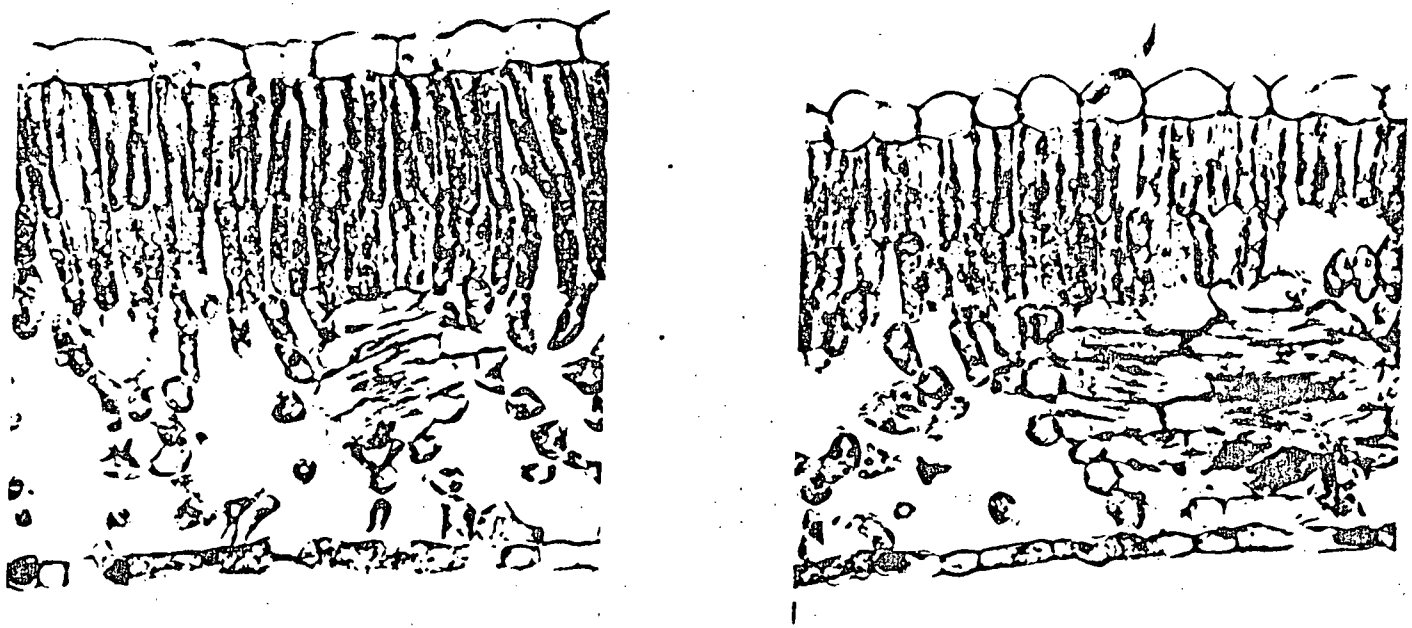


FIGURE 3

The leaf cross section of Starkrimson (left) showing greater leaf thickness than in Red Delicious (right). (300X)

TABLE 2

THICKNESS OF LEAF TISSUES OF RED DELICIOUS AND GOLDEN DELICIOUS IN MICRONS

	Red Delicious[x]	Golden Delicious[x]	Significance Level
Lower Epidermis	12	13	0.0456
Spongy Parenchyma	75	78	0.2317
Palisade Parenchyma	87	106	0.0008
Upper Epidermis	14	15	0.2107
Total	188	212	0.0018
Number of Palisade Cell Layer	3.02	3.19	0.0039
Average Palisade Cell Length	29	33	0.0078

[x]mean of standard and compact types.

TABLE 3

THICKNESS OF LEAF TISSUES OF STANDARD AND COMPACT TYPES IN MICRONS

	Standard[x]	Compact[xx]	Significance Level
Lower Epidermis	12	12	0.6533
Spongy Parenchyma	75	79	0.1923
Palisade Parenchyma	92	101	0.0350
Upper Epidermis	14	14	0.7804
Total	195	206	0.0345
Number of Palisade Cell Layer	3.0	3.2	0.0013
Average Palisade Cell Length	31	32	0.3983

[x]means of the Red Delicious and Golden Delicious.

[xx]means of the Starkrimson and Starkspur.

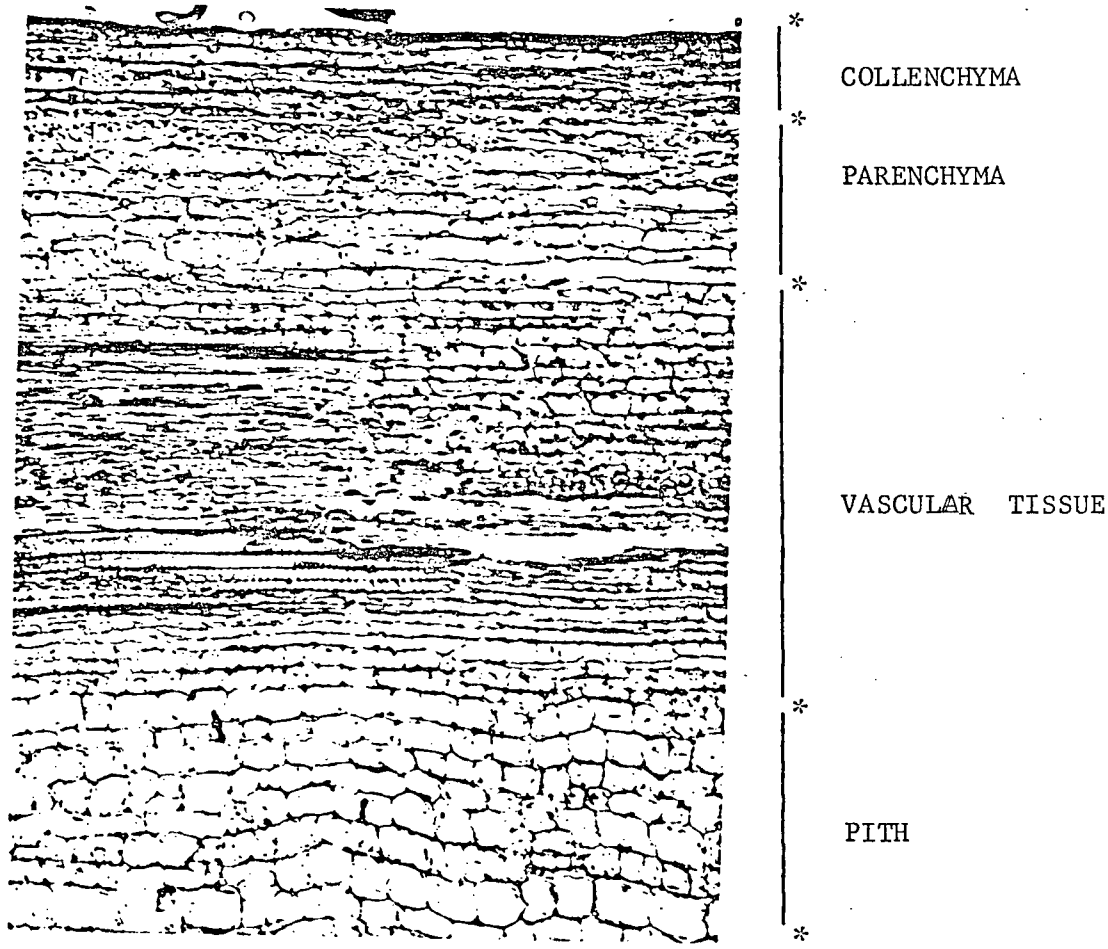


FIGURE 4

Longitudinal section of Red Delicious stem showing collenchyma, parenchyma, vascular tissue, and part of pith. (118 X)

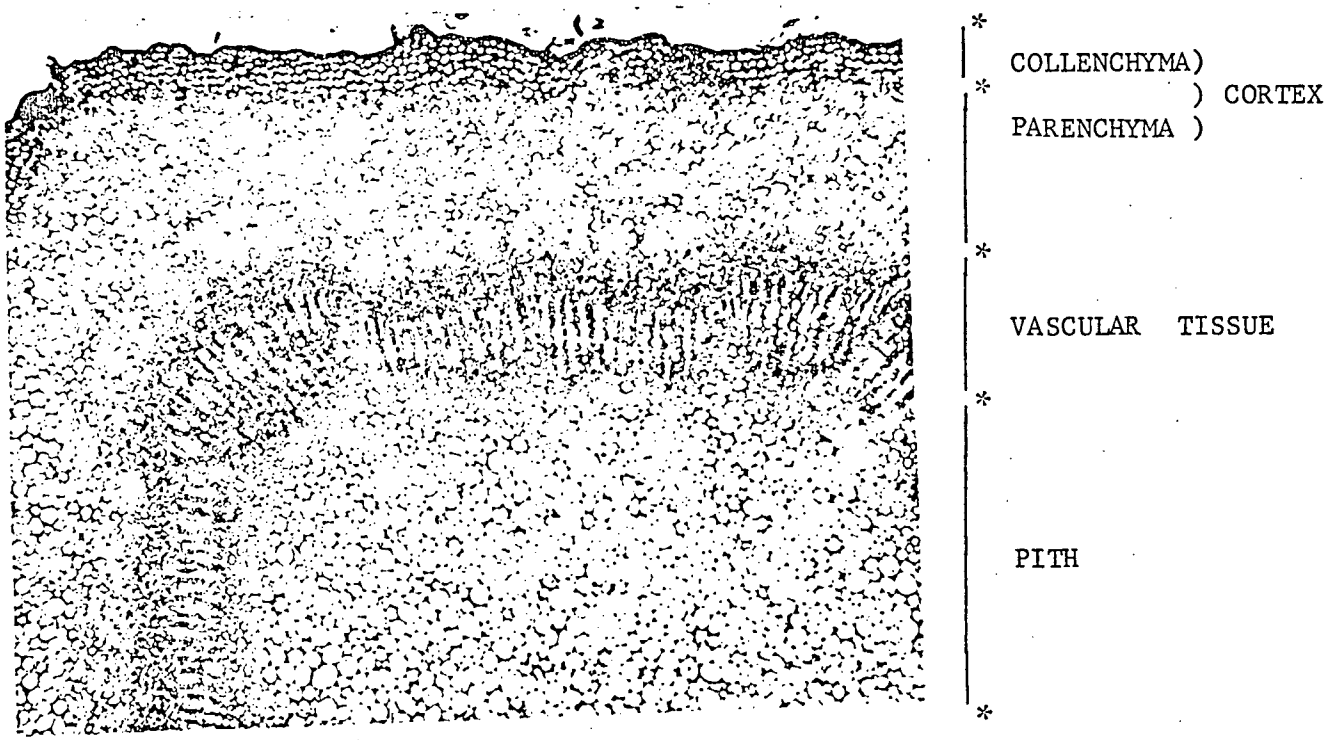


FIGURE 5

Cross section of Red Delicious stem showing part of collenchyma, parenchyma, vascular tissue and pith. (60 X)

TABLE 4

CELL LENGTHS OF STEM COLLENCHYMA, PARENCHYMA AND PITH OF FOUR APPLE CULTIVARS IN MICRONS

Cultivar	Collenchyma	Parenchyma	Pith
Harrold	48a[x]	61a	41a
Starkrimson	44a	67a	38a
Golden	45a	57a	37a
Starkspur	41a	51a	38a

[x]within a column, means having a letter in common are not significantly different at the 5 per cent level by Duncan's Multiple Range Test.

and cross sections respectively of Red Delicious shoot tissue. There were no significant differences in cell length of collenchyma, parenchyma or pith cells among the four cultivars (Table 4). In stem cross sections, Starkrimson had a thicker vascular tissue than the other cultivars (Table 5), but the thickness of the cortex, pith and total stem were not significantly different in the cultivars. The cell diameter of the collenchyma of Red Delicious and Starkrimson Red Delicious was greater than that of Golden Delicious and Starkspur. Starkspur had a smaller pith cell diameter than the other three cultivars, but the mean diameter of the parenchyma cells did not differ among cultivars (Table 6). The data presented in Table 5 were measured as though each tissue were circular.

Experiment 11

Effects of Alar on Compact and Standard Apple Cultivars

The shoot growth of Red Delicious was not significantly affected by Alar until six weeks after treatment, but growth rate suppression was first suspected four weeks after treatment (Table 7). Alar at 1000 ppm did not show any inhibiting effect on the shoot growth of Starkrimson (Table 7).

Alar did not significantly affect leaf number (Table 8), leaf length or width in either Red Delicious or Starkrimson (Table 9). However, in Golden Delicious, significant inhibition on shoot length was observed from the second week after Alar treatment. The difference due to treatment increased gradually till the termination of the experiment (Table 10). The effect of Alar on Starkspur shoot growth was

TABLE 5

THICKNESS OF STEM CORTEX, VASCULAR TISSUE, DIAMETERS OF PITH, AND STEM OF FOUR APPLE CULTIVARS IN MICRONS

Cultivar	Cortex	Vascular Tissue	Pith Diameter	Shoot Diameter
Harrold Red Delicious	256a[x]	248b	1073a	2160a
Starkrimson	263a	414a	973a	2273a
Golden Delicious	233a	327ab	908a	2006a
Starkspur	288a	279b	939a	1950a

[x]within a column, means sharing the same letter are not significantly different at the 5 per cent level by Duncan's Multiple Range Test.

TABLE 6

CELL DIAMETERS OF STEM COLLENCHYMA, PARENCHYMA AND PITH OF FOUR APPLE CULTIVARS IN MICRONS

Cultivar	Collenchyma	Parenchyma	Pith
Harrold Red Delicious	20a[x]	29a	35a
Starkrimson	21a	27a	33a
Golden Delicious	18b	25a	30a
Starkspur	18b	25a	26b

[x]within a column, means sharing the same letter are not significantly different at the 5 per cent level by Duncan's Multiple Range Test.

TABLE 7

EFFECT OF ALAR ON SHOOT GROWTH OF RED DELICIOUS AND STARKRIMSON (CM)

Cultivar	Alar Concentration (ppm)	Weeks After Treatment							
		1	2	3	4	5	6	7	8
Red Delicious	0	8h[x]	15g	23f	29e	35d	43b	50a	55a
	1000	8h	15g	21f	28e	32de	37cd	41bc	44b
Significance Level	0.0135								
Starkrimson	0	12a	18a	26a	33a	40a	45a	45a	
	1000	14a	24a	30a	38a	42a	45a	47a	
Significance Level	0.9389								

[x]within each cultivar, means having a letter in common are not significantly different at the 5 per cent level by Duncan's Multiple Range Test.

TABLE 8

EFFECT OF ALAR ON LEAF NUMBER OF RED DELICIOUS AND STARKRIMSON

Cultivar	Alar Concentration (ppm)	Weeks After Treatment 1	2	3	4	5	6	7	8
Red Delicious	0	6	8	11	14	16	18	21	23
	1000	6	8	11	14	16	18	20	22
Significance Level	0.5581								
Starkrimson	0	8	12	15	18	20	22	23	
	1000	10	13	17	19	20	23	24	
Significance Level	0.7543								

TABLE 9

EFFECT OF ALAR ON AVERAGE LEAF LENGTH AND WIDTH OF RED DELICIOUS AND STARKRIMSON (CM)

Cultivar	Alar Concentration (ppm)	Leaf Length	Leaf Width
Red Delicious	0	10.1	5.3
	1000	10.0	4.9
Significance Level		0.8042	0.2430
Starkrimson	0	12.4	6.1
	1000	12.0	5.7
Significance Level		0.2673	0.1551

TABLE 10

INFLUENCE OF ALAR ON SHOOT GROWTH OF STARKSPUR AND GOLDEN DELICIOUS (CM)

Cultivar	Alar Concentration (ppm)	Weeks After Treatment 1	2	3	4	5	6	7
Golden Delicious	0	16i[x]	20h	26f	33d	40c	46b	51a
	1000	14i	16i	19h	23g	27f	30e	33d
Significance Level	0.0000							
Starkspur	0	13hij	16gh	22ef	31d	41c	47b	52a
	1000	9i	11ij	14hi	16hi	21fg	24ef	26de
Significance Level	0.0000							

[x]within each cultivar, means having a letter in common are not significantly different at the 5 per cent level by Duncan's Multiple Range Test.

TABLE 11

EFFECT OF ALAR ON LEAF NUMBER OF GOLDEN DELICIOUS

Cultivar	Alar Concentration (ppm)	Weeks After Treatment 1	2	3	4
Golden Delicious	0	14f[x]	16.6d	19b	22a
	1000	12g	15e	17cd	17.6b
Significance Level	0.0007				

[x]means having a letter in common are not significantly different at the 5 per cent level by Duncan's Multiple Range Test.

quite different from expectation. The retardation effect was noted during the second week after treatment, but less retardation was found during the fourth week. However, six weeks after treatment, Alar inhibited shoot growth by 50% (Table 10). Four weeks after treatment, Alar 1000 ppm significantly reduced Golden Delicious leaf number by 25% (Table 11). Alar did not show any significant effect on the leaf number of Starkspur.

Microscopic examination of Harrold and Starkrimson leaf lamella sections revealed no significant differences between treatments in any one of the seven variables measured. In Golden Delicious, the treated plants had thinner lower epidermis and fewer palisade cells than the control. Treated Starkspur had thinner spongy parenchyma, fewer palisade cells and less total leaf thickness (Table 12).

The data for cell length in the stem longitudinal sections of four cultivars, each with two different treatments is shown in Table 13. Apparently the Alar treated Starkrimson plants had significantly shorter collenchyma cells but the length of parenchyma cell in cortex and pith were not significantly shorter than the untreated ones. Starkspur Golden Delicious plants showed longer parenchyma cell in cortex as compared with the treated plants. The cell lengths in treated Harrold and Golden Delicious were not significantly different from the untreated plants.

Experiment III (a)

Comparisons of Compact with Alar treated Red Delicious

Alar was found to increase the thickness of total leaf, spongy

TABLE 12

INFLUENCE OF ALAR ON THICKNESS OF LEAF TISSUES OF FOUR APPLE CULTIVARS (MICRONS)

Cultivar	Alar Concentration (ppm)	Lower Epidermis	Spongy Parenchyma	Palisade Parenchyma	Upper Epidermis	Total	Palisade Number	Average Palisade
Red Delicious	0	12	76	124	16	226	3	41
	1000	11	73	114	15	213	3	39
Starkrimson	0	11	81	100	16	208	3	33
	1000	11	90	113	15	229	3	36
Golden Delicious	0	11	77	96	14	198	3	32
	1000	10**	81	90	14	195	2.8*	33
Starkspur	0	11	80	97	14	203	3	33
	1000	11	71*	89	14	184*	2.9*	31

*significant at 5 per cent level.

**significant at 1 per cent level.

TABLE 13

INFLUENCE OF ALAR ON THE CELL LENGTHS OF COLLENCHYMA, PARENCHYMA AND PITH OF FOUR APPLE CULTIVARS (MICRONS)

	Alar Concentration (ppm)	Red Delicious	Starkrimson	Golden Delicious	Starkspur
Collenchyma	0	33	46	38	42
	1000	35	31**	43	33
Parenchyma	0	59	54	50	56
	1000	53	50	54	42*
Pith	0	35	37	37	41
	1000	38	34	39	32

*significant at 5 per cent level.

**significant at 1 per cent level.

TABLE 14

INFLUENCE OF ALAR ON THE THICKNESS OF LEAF TISSUES OF RED DELICIOUS IN MICRONS

Cultivar	Alar Concentration (ppm)	Lower Epidermis	Spongy Parenchyma	Palisade Parenchyma	Upper Epidermis	Total	Palisade Number	Average Palisade
Red Delicious	0	11a[x]	63ab	78b	16a	168b	3a	26b
Red Delicious	1000	10a	71a	87a	16a	184a	3a	30a
Red Delicious	4000	10a	69a	90a	16a	186a	3a	30a
Starkrimson	0	10a	59b	75b	16a	161b	3a	27b
Significance Level		0.8043	0.0342	0.0039	0.7688	0.0062	0.0907	0.0085

[x]within each column, means having a letter in common are not significantly different at the 5 per cent level by Duncan's Multiple Range Test.

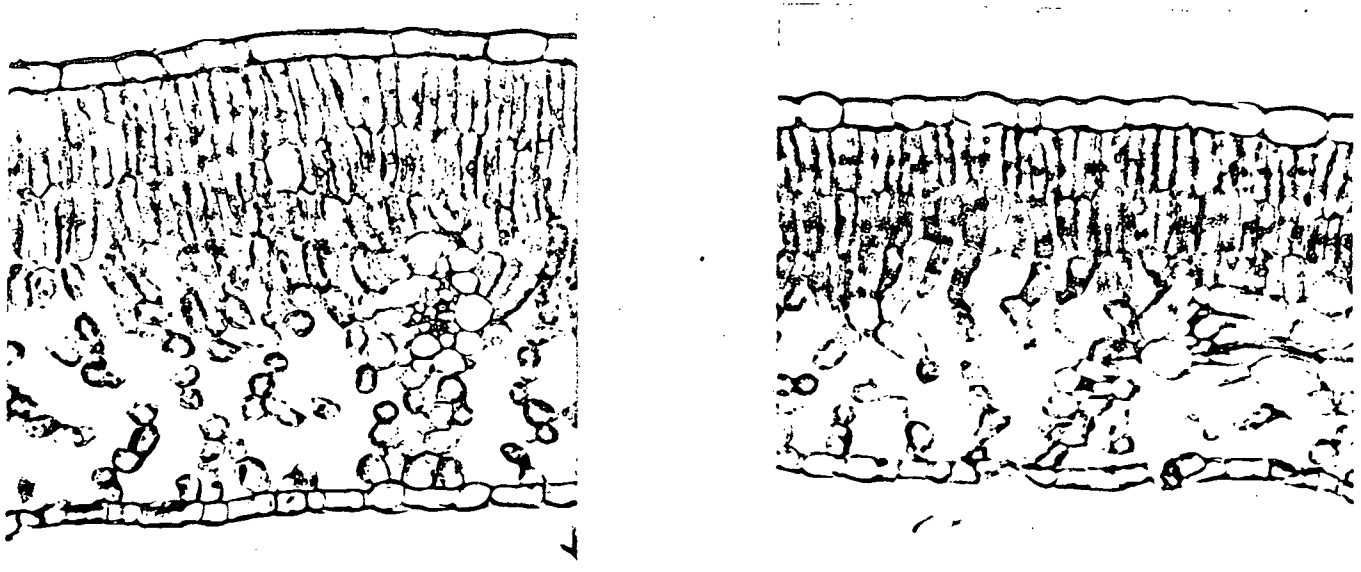


FIGURE 6

Red Delicious leaves treated with Alar at 4000 ppm (left) were thicker than untreated leaves. (381 X)

parenchyma and length of palisade cell in Harrold Red Delicious (Table 14). The results from two concentrations i.e. 1000 and 4000 ppm, were not different from each other. The total leaf thickness, palisade parenchyma and average palisade parenchyma of Starkrimson were not different from untreated Harrold Red Delicious, although slightly thicker spongy parenchyma was found in the latter. The upper and lower epidermis and palisade number were not different from treatment to treatment, hence the thicker leaf of Alar treated Red Delicious resulted from an increase in the thickness of spongy parenchyma and palisade parenchyma (Fig. 6).

Alar inhibited shoot growth of Red Delicious apple trees and the inhibiting effect was greater at the higher concentration, i.e. at 4000 ppm (Table 15). The treatment effect was noticeable during the second week's growth and was still present at the termination of the experiment. After two weeks, the growth rate of Alar-treated Red Delicious was less than that of Starkrimson Red Delicious although Starkrimson was still smaller in total size.

Experiment III (b)

Effects of Alar and Gibberellic Acid on Compact and Standard Golden Delicious

There were no significant differences between means for the thickness of Golden Delicious leaf tissues as a result of treatment with Alar or GA (Table 16). However, GA increased mean shoot growth of Golden Delicious and Starkspur Golden Delicious by 29 per cent by the end of the experiment (Table 17). Alar at 1000 ppm or the Alar and GA

combination did not affect the shoot growth of Golden Delicious and Starkspur Golden Delicious (Table 17). Apparently Alar nullified the stimulating effect of GA.

TABLE 15

EFFECT OF ALAR ON SHOOT GROWTH OF STARKRIMSON AND RED DELICIOUS (CM)

Cultivar	Alar Concentration (ppm)	Weeks After Treatment 1	2	3	4	5	6
Red Delicious	0	36h[x]	47ef	54c	59b	63a	66a
Red Delicious	1000	36h	45gh	48ef	50de	52cd	54c
Red Delicious	4000	34i	42gh	45fg	46f	47ef	48ef
Starkrimson	0	14m	211	25kl	28jk	31j	33i
Significance Level for Interaction				0.0000			

[x]means having a letter in common are not significantly different at the 5 per cent level by Duncan's Multiple Range Test.

TABLE 16

INFLUENCE OF ALAR AND GIBBERELLIC ACID ON THICKNESS OF LEAF TISSUES OF STARKSPUR AND GOLDEN DELICIOUS[x]

Cultivar	Treatment (ppm)	Lower Epidermis	Spongy Parenchyma	Palisade Parenchyma	Upper Epidermis	Total	Number of Palisade Layer	Average Palisade Cell Length
Golden Delicious	control	11	70	93	15	189	3	32
	A - 1000	11	65	89	15	180	3	33
	GA - 1000	11	54	85	16	166	3	28
	A - 1000, GA - 1000	10	58	86	15	169	3	31
Starkspur	control	11	66	90	14	182	3	31
	A - 1000	11	60	88	15	174	3	31
	GA - 1000	12	61	87	15	175	3	29
	A - 1000, GA - 1000	12	72	94	14	191	3	34
Significance Level		0.4403	0.2187	0.6475	0.8584	0.1668	0.9695	0.4339

[x]There was no significant effect of any of these treatments on leaf tissues thickness of either cultivar.

TABLE 17

INFLUENCE OF ALAR AND GIBBERELLIC ACID ON MEAN SHOOT GROWTH OF STARKSPUR AND GOLDEN DELICIOUS (CM)

Treatment (ppm)	Weeks After Treatment 1	2	3	4	5	6
Control	23h[x]	31g	33fg	35cdefg	38cdef	40cd
Alar - 1000	24h	34efg	38cdef	39cdef	39cdef	41c
GA - 1000	23h	34efg	41c	48b	56a	58a
Alar - 1000, GA - 1000	19h	32g	35defg	38cdef	40cd	41c
Significance Level of Interaction			0.0001			

[x]means having a letter in common are not significantly different at the 5 per cent level by Duncan's Multiple Range Test.

DISCUSSION

Starkspur was found to have a thicker leaf, palisade parenchyma and a greater mean palisade number and size than the other three cultivars. These results agree with the conclusions of Westwood (37), Arasu (1) and Westwood and Zielinski (38) that the spurtype mutants have thicker leaves and palisade tissue. They also found the mutant to have shorter internodes, greater leaf surface per foot of shoot, fewer side branches but more spurs, and greater chlorophyll content per cm^2 of leaf. These attributes of spurtypes favor them with regard to light distribution, bearing surface, photosynthetic efficiency and fruit-bearing potential. The present study has demonstrated the thicker leaf and palisade tissues of the spurtype mutants by careful examination of the leaf anatomy of the spurtype mutants. The increase in thickness of the palisade parenchyma was found due both to longer palisade cells and a greater mean palisade layer of them. The Starkrimson leaves were only slightly thicker than those of Red Delicious and this difference was attributed to thicker palisade parenchyma.

By comparing the thickness of leaf tissues of Red Delicious with Golden Delicious (Table 2), it is evident that the latter was 11.3 per cent thicker in terms of total leaf thickness. Apparently there were no significant differences in thickness of lower epidermis, upper epidermis and spongy parenchyma between these two varieties. The difference in total leaf thickness is attributable to differences in palisade parenchyma which was 18 per cent thicker in Golden Delicious than

in Red Delicious. Looney reported that Golden Delicious had an 18 per cent higher net assimilation rate than Red Delicious (22).

Compact mutants have been reported to have a thicker stem and shorter internodes than standard varieties (37, 38). However, in this study it was found that there were no significant differences in cell length of collenchyma, parenchyma and pith cells among the four cultivars studied (Table 4). In stem cross sections, the thickness of cortex, pith and total stem of mutants were also not different from the standard (Table 5). Furthermore, in cell diameter of different tissues (Table 6), standard types were not different from the spur types. These results represent the first comparisons between compact and standard types with respect to stem anatomy. Since the shoot material used in this experiment was taken from the first internode under the growing tip, manifestation of the reasons for the reported thicker and shorter shoots may not occur in this region of the shoot. Future work should consider mature tissues. It is quite possible that the effects of Alar treatment have not shown up completely on this premature tissue.

In experiment II, Red Delicious treated with Alar at 1000 ppm did not show any significant difference in leaf thickness when compared with the control (Table 12). However, in experiment III, Alar at 1000 ppm was found to increase the thickness of total leaf, spongy parenchyma and length of palisade cells (Table 15). These conflicting results might be due to the differences of season, location and the specific effect of the material used in the experiment. These are the

first results reported on the effects of Alar on the anatomy of either compact or standard Red Delicious.

In Golden Delicious (Table 12), the leaves of treated plants had thinner lower epidermis and fewer palisade cells and the treated Starkspur Golden Delicious had thinner spongy parenchyma, a smaller total leaf thickness, and fewer palisade cells. Thus cultivar might influence the effect of Alar treatment. Halfacre (17) also reported that the Golden Delicious and York Imperial responded to Alar differently when treated at the same concentration.

The present study also revealed that suppression of terminal growth by Alar varied among the cultivars used. It was found that of the four cultivars used in experiment II, Starkrimson trees were not affected by Alar treatment (Table 7, Fig. 7) and Starkspur was the most susceptible to the Alar treatment (Table 10). Besides differences among cultivars, the above results could be explained if incorrectly labeled material had been used in the experiment. However, even though the Starkspur trees sampled grew very vigorously before the application of Alar, they responded to the treatment much differently than the Golden Delicious trees.

Neither leaf number, length or width of Red Delicious and Starkrimson were affected by Alar treatment. These findings do not agree with those of Halfacre (16), and the findings need verification. In Golden Delicious Alar at 1000 ppm was found to reduce the leaf number by 19 per cent (Table 11), but no effect was observed on Starkspur. GA stimulated shoot growth of Golden Delicious and Starkspur but when GA was applied in combination with Alar, the stimulating effect was

cancelled completely by Alar. Edgerton and Hoffman (14) also found that the stimulating effect of GA on Red Delicious apple trees could be cancelled by Alar.

Since the natural compact mutants have many merits over the standard growing strains, it may be desirable to induce similar changes in standard apple trees or intensify the desirable traits of the compacts by means of treatment with growth regulators such as Alar. However, there are only a few workers studying the relationships among the natural compact mutants and the chemically induced compact habit. One worker, Intrieri (19), reported, upon reviewing the literature, that spur type trees and standard trees treated with Alar showed many similarities in morphology and anatomy.

Alar treatment increased leaf thickness of Harrold Red Delicious by 9.5 per cent. Apparently the increase in total thickness of leaf tissue after Alar treatment was due to an increase in thickness of the palisade parenchyma. Hence it seems that this tissue is the primary active site within the leaf tissue which responds to the growth retardant. After the treatment with Alar 4000 ppm, a 16 per cent increase in thickness of palisade parenchyma was noted. These anatomical findings help explain the report of Edgerton and Hoffman (14) that Alar treated apple trees of several cultivars had thicker leaves.

Alar inhibited the growth rate of Red Delicious apple shoots. This inhibitory effect was immediate and lasted for more than six weeks after the application of Alar. When Alar was applied at concentrations of 1000 ppm and 4000 ppm, the inhibitions of the shoot growth of Harrold Red Delicious were found to be 18 per cent and 27 per cent

respectively. This result concurs with the findings of other workers (3, 4, 14, 15, 16, 17) that Alar inhibits the terminal growth of apples. Interestingly, Alar did not appear to influence the length of any of the stem cell types examined in the current study. As discussed earlier, this may have been due to the sampling procedure but it also may support the findings of Martin et al. (24) who found that Alar had a greater effect on cell division in apple fruits than on cell size.

Untreated Harrold Red Delicious shoots have a tendency to grow continuously. This would result in a taller tree. However, in the Starkrimson and Alar treated Harrold Red Delicious the shoot growth curves are quite similar and become level at about the fourth week. These results are consistent with the review of Intrieri (19) who concluded that standard apple trees treated with Alar and spur type trees are quite similar in morphology and anatomy.

This present study has contributed to the understanding of the effects of Alar on apple trees. Apparently both the nature of the cultivar and the concentration of Alar are important factors when considering the use of this growth retardant.

SUMMARY

A two year study was conducted to investigate the morphological and anatomical changes in apple leaf and stem tissues. Factors analyzed were leaf anatomy, leaf number, leaf length and width, shoot anatomy and shoot length.

There is no previous anatomical comparisons of stems between standard and compact apple trees or between Alar treated and untreated trees of Red Delicious. Detailed measurements of spongy parenchyma thickness and numbers of palisade layers in compact mutants or in Alar treated Red Delicious are the first reported.

In 1968, two varieties, Red Delicious and Golden Delicious, each with standard and compact types, were studied without Alar treatments. In the second phase of the experiment Alar at the concentrations of 0 and 1000 ppm was applied to additional trees of the same cultivars. From April to June 1969, concentrations of 0, 1000 and 4000 ppm of Alar were applied to cultivars which had been grafted on EM II rootstocks.

Without Alar treatment, it was found that Starkspur had a thicker leaf and thicker palisade parenchyma than the other three cultivars studied and Red Delicious was found to have less total leaf thickness than Golden Delicious. Results also indicated that compact apple mutants had on average thicker leaves and palisade parenchyma and greater mean palisade number as compared with standard types.

The compact mutants have been reported to have thicker stems and shorter internode. However, microscopic examination of samples taken

from the first internode under the growing tip revealed no significant differences in cell length of collenchyma, parenchyma, pith cells, cell diameter or thickness in the same tissues.

The suppression of terminal growth by Alar varied among cultivars. The response to Alar was greatest with Starkspur where an inhibition of shoot growth by 50 per cent was observed. Starkrimson was not affected by Alar treatment in the same experiment.

In the third phase of the experiment, Alar treated leaf blades of plants were found to increase in total thickness, in thickness of spongy parenchyma and in the length of palisade cells. The results from two concentration of Alar, i.e. 1000 and 4000 ppm, were not found to differ from each other. These data indicate that the site which is most affected by Alar is in the palisade parenchyma cells of the leaf tissue.

GA stimulated the shoot growth of Golden Delicious and Starkspur by 29 per cent, but this stimulating effect was prevented by Alar.

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APPENDIX

Table 1 Analysis of Variance Models (Table numbers correspond to those in text)

Line Number	Source of Variation	Degrees of freedom	Line number of F denominator	Expected mean squares
1	Clone	(c-1) = 3	2	$\sigma^2_E+2\sigma^2_{D(TCSLP)}+6\sigma^2_{L(TCS)}+12\sigma^2_{S(TC)}+24\sigma^2_{T(C)}+72\theta^2_C$
2	Trees within Clones	c(t-1) = 8	3	$\sigma^2_E+2\sigma^2_{D(TCSLP)}+6\sigma^2_{L(TCS)}+12\sigma^2_{S(TC)}+24\sigma^2_{T(C)}$
3	Shoots within Trees	ct(s-1) = 12	4	$\sigma^2_E+2\sigma^2_{D(TCSLP)}+6\sigma^2_{L(TCS)}+12\sigma^2_{S(TC)}$
4	Leaves within Shoots	cts(e-1) = 24	10	$\sigma^2_E+2\sigma^2_{D(TCSLP)}+6\sigma^2_{L(TCS)}$
5	Position	p-1 = 2	7	$\sigma^2_E+2\sigma^2_{D(TCSLP)}+2\sigma^2_{PL(TCS)}+4\sigma^2_{PS(TC)}+8\sigma^2_{TP(C)}+96\theta^2_P$
6	Position x Clone	(p-1)(c-1) = 6	7	$\sigma^2_E+2\sigma^2_{D(TCSLP)}+2\sigma^2_{PL(TCS)}+4\sigma^2_{PS(TC)}+8\sigma^2_{TP(C)}+24\sigma^2_{PC}$
7	Position x Trees within Clones	c(p-1)(t-1) = 16	8	$\sigma^2_E+2\sigma^2_{D(TCSLP)}+2\sigma^2_{PL(TCS)}+4\sigma^2_{PS(TC)}+8\sigma^2_{TP(C)}$
8	Position x Shoots within Trees	ct(p-1)(s-1) = 24	9	$\sigma^2_E+2\sigma^2_{D(TCSLP)}+2\sigma^2_{PL(TCS)}+4\sigma^2_{PS(TC)}$
9	Position x Leaves within Shoots	cts(p-1)(l-1)= 48	10	$\sigma^2_E+2\sigma^2_{D(TCSLP)}+2\sigma^2_{PCL(TCS)}$
10	Disc	ctspl(d-1) = 144	E	$\sigma^2_E+2\ ^2_{D(TCSLP)}$
E	Measurements within Discs	ctspld(m-1) = 864		σ^2_E
	Total	ctspldm-1 =1151		

Table 2, 3

Line Number	Source of Variation	Degrees of freedom		Line number of F denominator	Expected mean squares
1	Variety	(v-1)	= 1	E	$\sigma^2_E+96\sigma^2_{R(VT)}+576\theta^2_V$
2	Type	(t-1)	= 1	E	$\sigma^2_E+96\sigma^2_{R(VT)}+576\theta^2_T$
3	Variety x Type	(v-1)(t-1)	= 1	4	$\sigma^2_E+96\sigma^2_{R(VT)}+288\sigma^2_{VT}$
4	Tree with Variety and Type	vt(r-1)	= 8	E	$\sigma^2_E+96\sigma^2_{R(VT)}$
E	Measurements with Variety, Type	vtr(m-1)	=1140		σ^2_E
	Total	vtrm-1	=1151		

Table 4, 6

Line Number	Source of Variation	Degrees of freedom		Line number of F denominator	Expected mean squares
1	Variety	(v-1)	= 3	2	$\sigma^2_E+5\sigma^2_{C(VTS)}+10\sigma^2_{S(VT)}+20\sigma^2_{T(V)}+60\theta^2_V$
2	Tree within Variety	v(t-1)	= 8	3	$\sigma^2_E+5\sigma^2_{C(VTS)}+10\sigma^2_{S(VT)}+20\sigma^2_{T(V)}$
3	Shoot within Tree	vt(s-1)	= 12	4	$\sigma^2_E+5\sigma^2_{C(VTS)}+10\sigma^2_{S(VT)}$
4	Section within Shoot	vts(c-1)	= 24	E	$\sigma^2_E+5\sigma^2_{C(VTS)}$
E	Measurements within Shoot	vtsc(m-1)	= 192		σ^2_E
	Total	vtscm-1	= 239		

Table 5, 9

Line Number	Source of Variation	Degrees of freedom			Line number of F denominator	Expected mean squares
1	Variety	(v-1)	=	3	2	$\sigma^2_E+3\sigma^2_{S(VT)}+6\sigma^2_{T(V)}+18\theta^2_V$
2	Tree within Variety	v(t-1)	=	8	3	$\sigma^2_E+3\sigma^2_{S(VT)}+6\sigma^2_{T(V)}$
3	Shoot within Tree	vt(s-1)	=	12	E	$\sigma^2_E+3\sigma^2_{S(VT)}$
E	Measurements within Shoot	vts(m-1)	=	48		σ^2_E
	Total	vtsm-1	=	71		

Table 7, 8, 10, 11

Line Number	Source of Variation	Degrees of freedom		Line number of F denominator	Expected mean squares
1	Treatment	(t-1)	= 1	2	$\sigma^2_E + 8\sigma^2_{S(T)} + 48\theta^2_T$
2	Shoot within Treatment	t(s-1)	= 10	E	$\sigma^2_E + 8\sigma^2_{S(T)}$
3	Date	(d-1)	= 7	E	$\sigma^2_E + 12\theta^2_D$
4	Date x Treatment	(d-1)(t-1)	= 7	E	$\sigma^2_E + 12\sigma^2_{DT}$
E	Date x Shoot	t(d-1)(s-1)	= 70		σ^2_E
	Total	tds-1	= 95		

Table 12

Line Number	Source of Variation	Degrees of freedom		Line number of F denominator	Expected mean squares
1	Treatment	(t-1)	= 1	2	$\sigma^2_E+2\sigma^2_{C(TSLD)}+4\sigma^2_{D(TSL)}+24\sigma^2_{L(TS)}+48\sigma^2_{S(T)}+240^2_T$
2	Shoot within Treatment	t(s-1)	= 8	3	$\sigma^2_E+2\sigma^2_{C(TSLD)}+4\sigma^2_{D(TSL)}+24\sigma^2_{L(TS)}+48\sigma^2_{S(T)}$
3	Leaf within Shoot	ts(l-1)	= 10	4	$\sigma^2_E+2\sigma^2_{C(TSLD)}+4\sigma^2_{D(TSL)}+24\sigma^2_{L(TS)}$
4	Disc within Leaf	tsl(d-1)	=100	5	$\sigma^2_E+2\sigma^2_{C(TSLD)}+4\sigma^2_{D(TSL)}$
5	Section within Disc	tsld(c-1)	=120	E	$\sigma^2_E+2\sigma^2_{C(TSLD)}$
E	Measurements within Section	tsldc(m-1)	=240		σ^2_E
	Total	tsldcm-1	=479		

Table 13

Line Number	Source of Variation	Degrees of freedom			Line number of F denominator	Expected mean squares
1	Treatment	(t-1)	=	1	2	$\sigma^2_E+5\sigma^2_{C(TS)}+10\sigma^2_{S(T)}+50\theta^2_T$
2	Shoot within Treatment	s(t-1)	=	8	3	$\sigma^2_E+5\sigma^2_{C(TS)}+10\sigma^2_{S(T)}$
3	Section within Shoot	st(c-1)	=	10	E	$\sigma^2_E+5\sigma^2_{C(TS)}$
E	Measurements within Section	stc(m-1)	=	80		σ^2_E
	Total	stcm-1	=	99		

Table 14

Line Number	Source of Variation	Degrees of freedom		Line number of F denominator	Expected mean squares
1	Treatments	(t-1)	= 3	2	$\sigma^2_E+2\sigma^2_{S(TRLD)}+4\sigma^2_{D(TRL)}+8\sigma^2_{L(TR)}+16\sigma^2_{R(T)}+80\theta^2_T$
2	Tree within Treatment	t(r-1)	= 16	3	$\sigma^2_E+2\sigma^2_{S(TRLD)}+4\sigma^2_{D(TRL)}+8\sigma^2_{L(TR)}+16\sigma^2_{R(T)}$
3	Leaf within Tree	tr(l-1)	= 20	4	$\sigma^2_E+2\sigma^2_{S(TRLD)}+4\sigma^2_{D(TRL)}+8\sigma^2_{L(TR)}$
4	Disc within Leaf	trl(d-1)	= 40	5	$\sigma^2_E+2\sigma^2_{S(TRLD)}+4\sigma^2_{D(TRL)}$
5	Section within Disc	trld(c-1)	= 80	E	$\sigma^2_E+2\sigma^2_{S(TRLD)}$
E	Measurement within Section	trldc(m-1)	= 160		σ^2_E
	Total	trldcm-1	= 319		

Table 15

Line Number	Source of Variation	Degrees of freedom		Line number of F denominator	Expected mean squares
1	Treatment	(t-1)	= 3	2	$\sigma^2_E+6\sigma^2_{R(T)}+42\theta^2_T$
2	Tree within Treatment	t(r-1)	= 24	E	$\sigma^2_E+6\sigma^2_{R(T)}$
3	Date	d-1	= 5	E	$\sigma^2_E+28\sigma^2_D$
4	Date x Treatment	(d-1)(t-1)	= 15	E	$\sigma^2_E+7\sigma^2_{DT}$
E	Date x Tree within Treatment	t(d-1)(r-1)	= 120		σ^2_E
	Total	tdr-1	= 167		

Table 16

Line Number	Source of Variation	Degrees of freedom			Line number of F denominator	Expected mean squares
1	Block	(r-1)	=	1	6	$\sigma^2_E+2\sigma^2_{S(VTRLP)}+4\sigma^2_{P(VTRL)}+8\sigma^2_{L(VTR)}+128\sigma^2_R$
2	Variety	(v-1)	=	1	6	$\sigma^2_E+2\sigma^2_{S(VTRLP)}+4\sigma^2_{P(VTRL)}+8\sigma^2_{L(VTR)}+128\theta^2_V$
3	Treatment	(t-1)	=	3	6	$\sigma^2_E+2\sigma^2_{S(VTRLP)}+4\sigma^2_{P(VTRL)}+8\sigma^2_{L(VTR)}+64\theta^2_T$
4	Variety x Treatment	(v-1)(t-1)	=	3	5	$\sigma^2_E+2\sigma^2_{S(VTRLP)}+4\sigma^2_{P(VTRL)}+8\sigma^2_{L(VTR)}+16\sigma^2_{RVT}+32\sigma^2_{VT}$
5	Black x Variety and Treatment	(r-1)(vt-1)	=	7	6	$\sigma^2_E+2\sigma^2_{S(VTRLP)}+4\sigma^2_{P(VTRL)}+8\sigma^2_{L(VTR)}+16\sigma^2_{RVT}$
6	Leaf within Treatment	vtr(l-1)	=	16	7	$\sigma^2_E+2\sigma^2_{S(VTRLP)}+4\sigma^2_{P(VTRL)}+8\sigma^2_{L(VTR)}$
7	Position within Leaf	vtrl(p-1)	=	32	8	$\sigma^2_E+2\sigma^2_{S(VTRLP)}+4\sigma^2_{P(VTRL)}$
8	Section within Position	vtrlp(s-1)	=	64	E	$\sigma^2_E+2\sigma^2_{S(VTRLP)}$
E	Measurement within Section	vtrlps(m-1)	=	128		σ^2_E
	Total	vtrlpsm-1	=	255		

Table 17

Line Number	Source of Variation	Degrees of freedom		Line number of F denominator	Expected mean squares
1	Block	(b-1)	= 1	E	$\sigma^2_E+48\sigma^2_B$
2	Variety	(v-1)	= 1	E	$\sigma^2_E+48\theta^2_V$
3	Treatment	(t-1)	= 3	E	$\sigma^2_E+24\theta^2_T$
4	Variety x Treatment	(t-1)(v-1)	= 3	5	$\sigma^2_E+6\sigma^2_{TBV}+12\sigma^2_{TV}$
5	Block x Treatment, Variety	(b-1)(tv-1)	= 7	E	$\sigma^2_E+6\sigma^2_{TBV}$
6	Date	(d-1)	= 5	E	$\sigma^2_E+16\theta^2_D$
7	Date x Variety	(d-1)(v-1)	= 15	E	$\sigma^2_E+8\sigma^2_{DV}$
8	Date x Treatment	(d-1)(t-1)	= 15	E	$\sigma^2_E+4\sigma^2_{DT}$
9	Date x Treatment x Variety	(d-1)(t-1)(v-1)	= 15	E	$\sigma^2_E+2\sigma^2_{DTV}$
E	Date x Block within Treatment	vt(d-1)(b-1)	= 40		σ^2_E
	Total	vtdb-1	= 95		