treated plants and the 30th day for the warm treated ones. However, at the time the plants were removed the cool treated plants had 13.4 leaves visible and the warm treated ones only 12.0. Again, the plants were past the stage when total leaf number could be influenced by temperature and the cool treated plants had only 2.8 leaves to develop to the point of visibility and the warm treated ones had 4.5. In this case the difference in the time of arrival at a specific reproductive stage was counterbalanced by the amount of vegetative development yet to take place. The date of final leaf appearance was the same, the differences in the days to tasseling and silking were 2 and 3 respectively.

Experiment 3. The only significant difference in photoperiodic response was found with the Puerto Rican variety 'Major Belle' (Table 3). A 16 hr

Table 3. Effect of photoperiod on 'Major Belle' sweet corn.[a]

Photoperiod in hr	Leaves visible at tassel initiation	Days to tassel initiation	Days to tasseling	Total leaves
10.....	9.5	26	66	19.8
13.....	9.7	27	66	19.2
16.....	17.0	50	87	25.5

[a]A nonsignificant difference between treatments is indicated by a connecting line to the right of the values.

light period compared to periods of 10 and 13 hr resulted in an increase in days to tassel initiation, days to tasseling, and total leaf number. On the average 48% of the leaves were visible at the time of tassel initiation in the 4 varieties uneffected by photoperiod. With 'Major Belle' the % visible resulting from the 10, 13, and 16 hr treatments were 48, 51, and 66 respectively. The position of the growing point at the time of tassel initiation is usually below ground level, but the growing point of 'Major Belle' was 23 inches above ground at this time.

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Some Effects of a Growth Retardant on Shoot Meristems of Apple[1]

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Abstract. Uniform, actively growing apple seedlings, 10–15 cm high, were sprayed with 1000 ppm succinamic acid 2-2 dimethyl hydrazide (Alar). The apical portion of treated and control seedlings was collected at the following intervals after treatment: 3, 27 hr; 3, 6, 8, 14 days; 5 weeks. Sections through the apex were prepared, stained and examined microscopically for mitotic figures. As compared with controls, the frequency of mitotic figures in the stem apex of treated plants progressively decreased through 3 days, thereafter the number of figures increased to 69% of that in untreated plants on the 14th day. In the rib meristem frequency of mitosis declined slightly at 3 hr, then progressively until the 3rd day, after which the number of figures increased progressively to the 14th day when it was 28% of that in untreated plants. Only a temporary decrease in mitotic activity occurred in young leaf primordia during the first 6 days. Five weeks old treated seedlings were examined for histological abnormalities associated with extreme shortening of internodes.

Introduction

ALAR (succinamic acid 2-2 dimethyl hydrazide) is one of the latest of the growth retarding chemicals to be investigated in its effects upon the growth and physiology of a number of horticultural plants including apple (1, 4). Its strong inhibition of shoot growth is of particular interest and resembles the effects of such other retardants as Amo-1618, CCC and phosfon (3). A discussion of the chemical structure and physiological effects of these compounds is given in an excellent review by Cathey (3). Cytohistological effects of Amo-1618, CCC and phosfon on *Chrysanthemum* were investigated by Sachs et al. (8, 10, 11). These growth retarding compounds were found to have a direct inhibitory effect on nuclear and cell division and cell elongation in the subapical meristem and upper internodes of the shoot. Not only was mitotic activity inhibited but the length of the subapical meristematic zone was shortened (11). Growth and leaf production at the apex were not markedly affected.

The cytohistological effects of Alar have not been investigated. The purpose of the present study, therefore, is to determine the behavior of the shoot meristems following treatment with this growth retardant on apple seedlings.

Methods and Materials

Uniform, actively growing 3 month old apple seedlings, 10–15 cm high growing in the greenhouse were sprayed with Alar at a concentration of 1000 ppm. The terminal portion of 3–4 treated and 1–4 untreated seedlings was collected at the following intervals after treatment: 3, 27 hr; 3, 6, 8 and 14 days; 5 weeks. All collections were made at noon. These were fixed in several of the "Craf" mixtures or formalin-aceto-alcohol and prepared for embedding and cutting according to the usual procedures. Longitudinal sections were cut through the apex at 10 μ and stained with iron alum hematoxylin or safranin and fast green. Sections were examined microscopically and counts made of mitotic figures in apex and rib meristem (in a defined area) and the 2 youngest leaf primordia in each collection. The slides were examined microscopically under high magnification and the position of the mitotic figures recorded on outline drawings made with the aid of a microprojector.

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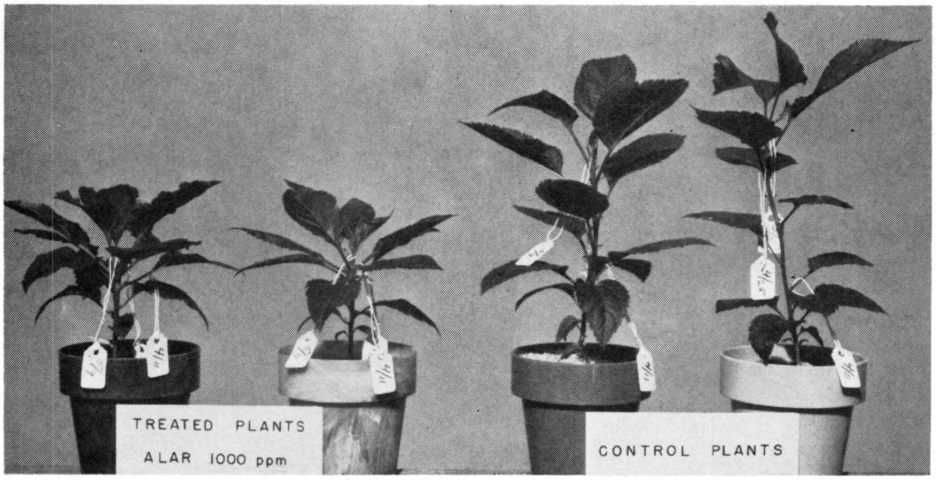

Fig. 1. Apple seedlings showing reduction of internodes on Alar treated plants (left) and normal growth of control plants (right).

An analysis of the position and number of leaf primordia was made also from the outline drawings.

As an aid in studying the growth pattern of the seedlings, the outermost unrolling leaf separating from the terminal bud was tagged every 2 weeks from 4/11/67, the spraying date, for a month and a half.

Results

The most conspicuous external effect of Alar treatment on apple seedlings is the reduction of internodal growth. Over a period of 5 weeks following treatment the average total growth of 8 treated seedlings was 14.2 mm while that of 8 untreated plants averaged 77.8 mm (Fig. 1). The present study indicates that this reduction is clearly caused by an interruption in the normal rate of mitotic division in both stem apex and rib meristem.

The apical meristem was considered, for convenience, to be the outer 8 cell layers in longitudinal section. The frequency of mitotic figures present in 7 untreated apices as observed in 10 sections, each 10 microns in thickness, was found to average 18 (Fig. 2). Mitotic divisions in early initiation of new leaf primordia at the apex were included when present. Three hours after treatment with Alar the average number of mitotic figures found in the apical meristem of apple seedlings had dropped to 9 and continued to drop over a period of 3 days to an average low number of 3 (Fig. 2). Samples collected 6, 8 and 14 days respectively after treatment showed a progressive rise in the frequency of mitotic activity with an average of 12 mitotic figures per apical meristem on the 14th day (Fig. 2).

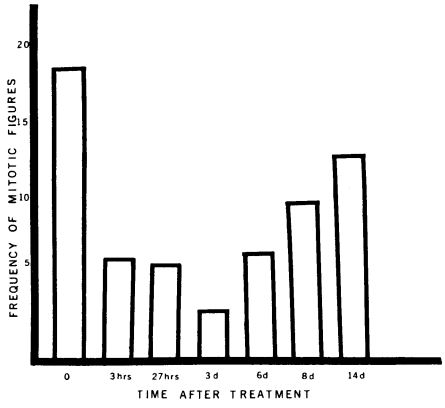

Fig. 2. Effect of Alar treatment on the number of mitotic figures in the apical meristem of apple seedlings at various collection times as compared with untreated seedlings; d – days.

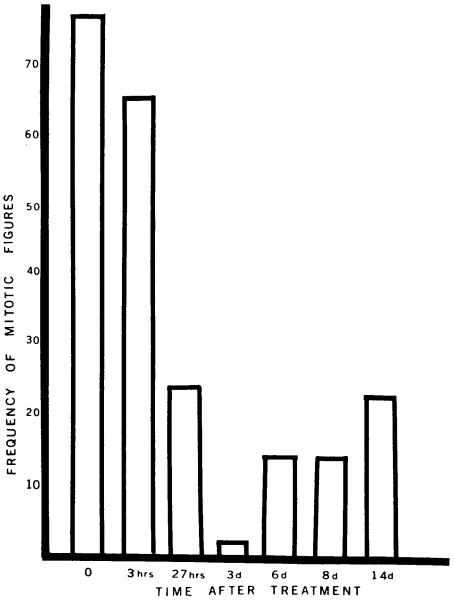

Fig. 3. Effect of Alar treatment on the number of mitotic figures in the rib meristem of apple seedlings at various collection times as compared with untreated seedlings; d – days.

In all seedlings collected at 3 and 27 hr following treatment, evidence of new leaf initiation at the apex was completely lacking, while in untreated apices and apices collected 3, 6, 8 and 14 days following treatment various stages of initiation of a new leaf primordium were present, that is, evidence of periclinal divisions having occurred in 1 to several apical layers.

The rib meristem consists of enlarging, but still meristematic, potential pith cells under the 8 apical cell layers. Mitotic figures in this area were counted in 3 consecutive longitudinal fields below the apex (750 microns) in 12 sections from each seedling. In 7 untreated seedlings an average frequency of 78 mitotic figures was found in this prescribed area of the rib meristem (Fig. 3).

At 3 hr following treatment with Alar the average frequency of mitotic figures in this area of the rib meristem had fallen to 67 (Fig. 3), a drop less conspicuous than that in the apical meristem. This decrease in the frequency of mitotic division continued, as in the apex, until the 3rd day, when it reached an average low number of 2 in the prescribed area. Samples taken on the 6th and 8th days showed a marked increase in mitotic activity in the rib meristem. On the 14th day the average number of mitotic figures had risen to 22 in this area (Fig. 3), still considerably below that found in untreated seedlings.

The typical arrangement of rib meristem cells in normal seedlings is in rather regular longitudinal rows (Fig. 4). These cells are formed by repeated nuclear and cell divisions oriented longitudinally. Few transversely oriented divisions occur in the rib meristem at this stage in the growth of normal untreated seedlings. In treated seedlings, collected 6, 8 and 14 days after treatment, however, mitotic figures oriented transversely are much more frequent than in untreated seedlings. Similar transversely oriented divisions have been found also in *Chrysanthemum morifolium* treated with other growth retardants (10, 11). In seedlings collected 8 and 14 days respectively after Alar treatment, an irregular arrangement of rib meristem cells is apparent (Fig. 5, 9); the regular longitudinal cell tiers characteristic of normal seedlings are interrupted by groups of cells formed in a transverse plane or sometimes obliquely. This frequency of transverse division produces a broader than normal pith composed of many more cells along the radial diameter, quite obvious in Fig. 5 when compared with

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the untreated seedling in Fig. 4. Although cell size in the rib meristem of treated seedlings appears to be little affected, cell shape is changed. Normal rib meristem cells in longitudinal section are rectangular, in this region usually broader than long, compactly arranged without intercellular spaces and regularly tiered (Fig. 4). The longitudinal walls are slightly thicker than the transverse walls. In seedlings collected 14 days after treatment subapical meristem cells are generally nearly isodiametric, irregularly arranged and, because of a loss of angularity, show intercellular spaces. Wall thickening seems to have begun in irregular patches in no consistent position (Fig. 9).

Frequencies of mitotic division in leaf primordia are difficult to compare because of the great variation in size of primordia and variation among seedlings. Compared with untreated apices (Fig. 6) mitotic division was definitely inhibited in Alar treated leaf primordia (Fig. 7) in all collections except on the 14th day (Fig. 8). By comparing the counts of mitotic figures in leaf primordia of approximately the same ranges in size in treated and untreated seedlings (Table 1) a definite trend from initial inhibition back to renewed activity is obvious, although there is little difference between the first 4 collections of treated apices through 6 days (Table 1). In some seedlings a period of lower division rate seemed to occur at 3 hr, in other seedlings a lower rate was found at 3 days. Thereafter figure counts in treated seedlings rose (Table 1) and by the 14th day after treatment the frequency of mitotic figures in all leaf primordia of treated plants appeared to have returned to normal (Fig. 8).

A study of the growth patterns in treated and untreated seedlings revealed only slight deviations caused by Alar treatment other than the conspicuous inhibition of internodal growth. In order to orient later that portion in the bud which was exposed to the spray treatment, the outermost young leaf separating from the bud was tagged on the spraying date and thereafter at fortnightly intervals (Fig. 1). This leaf blade was still rolled or conduplicate but with the short petiole sufficiently free of the bud to slip a tag over it. The same procedure was followed with untreated seedlings. From longitudinal sections through the growing tip it was determined that this first tagged leaf (4/11/67) was most frequently the 7th from the apex. At the end of the first 14 days the

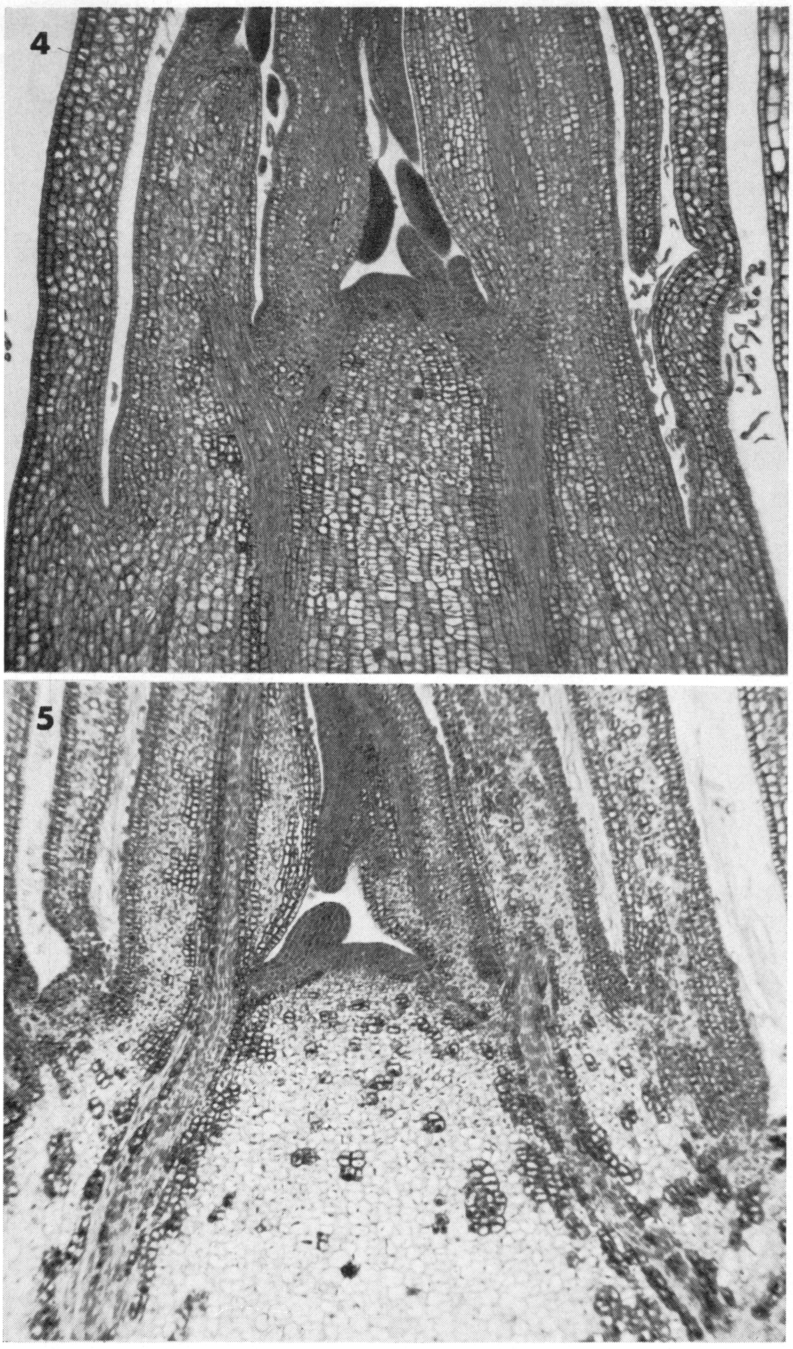

Fig. 4–5. Median longitudinal sections of the apical portion of apple seedlings (×90). *Fig. 4.* Untreated seedling. *Fig. 5.* Seedling taken 14 days after Alar treatment.

4/11 tagged leaf was the 4th expanded leaf in nearly all 15 control plants, it was the 3rd expanded leaf in nearly all of 15 treated plants. After 6 weeks of seedling growth most treated plants still lagged by 1 node in the treated region, further evidence that leaf production is only briefly interrupted. The slowing down of growth in the treated plants was in some respects like the normal slowing down of growth previous to bud formation but leaf primordia continued to be formed, and to grow and unfold.

In both treated and untreated seedlings the first lateral bud was found most frequently in the axil of the 7th or 8th young leaf. The first evidence of mature protoxylem was consistently in the 4th leaf primordium in untreated plants, but 8 and 14 days after

Table 1. Mitotic figure counts in apple leaf primordia.

Time after Alar treatment	Height-microns		Number figures	
	Average	Range	Average	Range
3 hours.....	32.9	23.0–41.4	6	2–12
27 hours.....	36.8	25.3–48.3	8	8–9
3 days.....	51.7	46.0–57.5	6	4–9
6 days.....	43.7	27.6–59.8	6	5–7
8 days.....	55.2	—	19	—
14 days.....	48.3	29.9–66.7	24	15–33
Control.....	55.2	48.3–62.1	24	18–31

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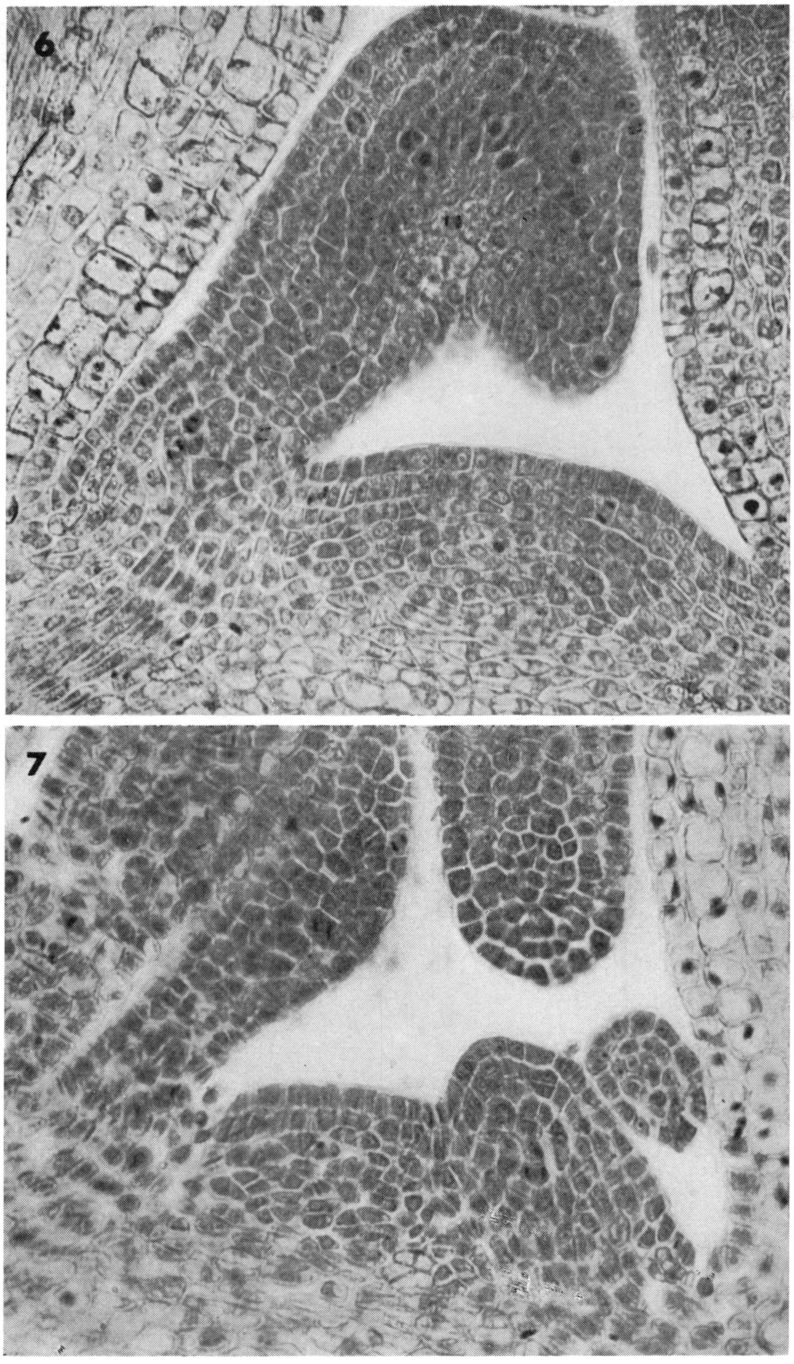

Fig. 6–7. Median longitudinal sections of apices of apple seedlings showing distribution of mitotic figures. ×440. *Fig. 6.* Untreated apex. *Fig. 7.* Apex, 27 hr after Alar treatment.

treatment mature protoxylem elements first appeared in the 4th or 5th leaf primordium of treated plants.

Discussion

The important part played by a subapical zone of cell division in the elongation of stems was pointed out in 1942 by Bindloss (2) and more recently emphasized by Sachs et al. (9) in their study of the effect of gibberellin on stem elongation in 2 rosette plants. Gibberellin has a strong stimulating effect on nuclear and cell division and has been found to counteract the inhibiting effect of Amo-1618, CCC and phosfon when the retardants were used in combination with gibberellin on *Chrysanthemum morifolium* (8, 10, 11). From these studies it was concluded that a function of the native gibberellins is "to regulate the activity of the subapical meristem" (10), and the growth retardants are, in some way not yet clearly understood, often antagonistic to gibberellin or its formation.

Results in the present investigation show that inhibition of mitotic activity by Alar in the rib meristem of apple seedlings is essentially similar to the effects produced by Amo-1618, CCC and phosfon on *Chrysanthemum* (8, 10, 11). In the present study, however, gradual changes in mitotic frequency for one diurnal period (noon) were observed over a span of 14 days, showing that an initial decline in mitotic activity after treatment is followed by a gradual rise. Although mitotic figure counts were not made beyond 14 days it may be reasonably concluded from the continued occurrence of extremely shortened internodes on those plants allowed to grow beyond this date that mitotic rate stabilized for a while at least, somewhere near the figure found on the 14th day, certainly still far below that of untreated plants.

Alar is a water soluble compound and has been reported to move very rapidly through plants. Thirty minutes after the stem of an excised seedling was dipped in a ^{14}C Alar solution, distribution of the compound throughout the seedling was evident on an autoradiograph (7). Alar sprayed on young fruits was found in the flesh in traces after one minute and to a considerable degree after 4 hr (5). It is not surprising therefore to find an effect on leaf primordia, apical and rib meristem 3 hr after spraying.

An interruption in the initiation of new leaf primordia in all apices taken at 3 and 27 hr suggests that there may be a diurnal rhythm in leaf initiation in apple as was found by Jacobs and Marrow in *Coleus* apices (6). Most leaf initiation in *Coleus* occurred during the middle of the dark period when the apex had increased to its greatest size and therefore showed its highest mitotic figure count. Since the Alar application was made at 9 AM and the first collection taken at noon it might be supposed that leaf initiation normally occurs some time during this period. Too few seedlings were examined, however, to draw any certain conclusions. The youngest leaf primordia on the 3 and 27 hr treated seedlings varied considerably in size but all were smaller than the 1st leaf primordium and larger than newly initiated primordia on untreated plants. Normal leaf primordia were much more uniform in height.

Although Sachs et al. (10) have concluded that the apical meristem continues to function almost normally in Amo-1618 treated *Chrysanthemum*, Alar treatment of apple seedlings has been found to have a very definite

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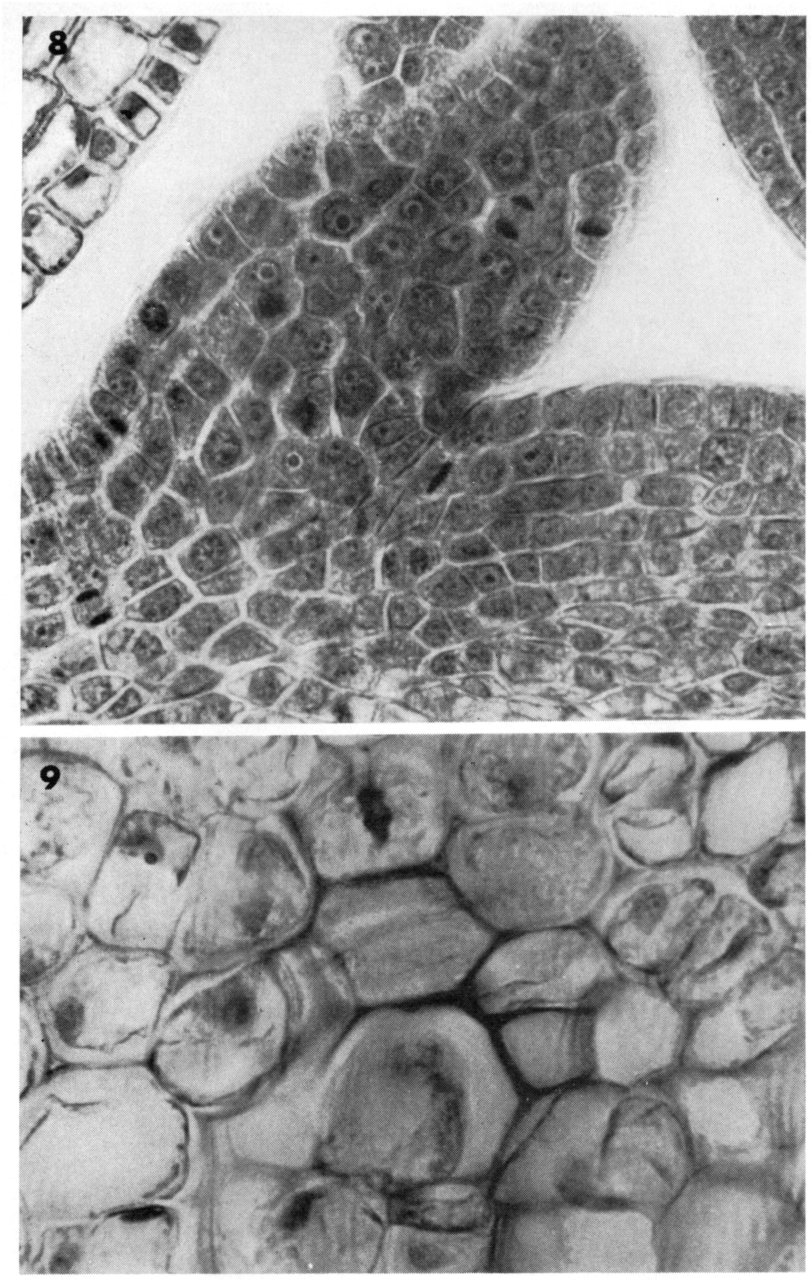

Fig. 8–9. Median longitudinal sections of portions of apple seedling apices 14 days after Alar treatment. ×870. *Fig. 8.* Mitotic figures in young leaf primordium. *Fig. 9.* Irregular wall thickening and atypical arrangement of rib meristem cells.

effect on cell division in the apical meristem. Three hours after Alar spraying the reduction in mitotic figures in the apical meristem fell 66% while the decrease in the rib meristem was only 14%. Over the next 24 hr mitotic figures in the apex decreased only slightly more, but in the rib meristem a further decrease of 54% now occurred, suggesting that Alar was moving first into the leaves and apex then into the rib meristem where it was accumulating. By the 3rd day the number of figures in the apex of treated seedlings had fallen to 16% while those in the rib meristem decreased to 3% of the number of figures in untreated plants. After 14 days the figure count in the apical meristem had risen to 69% of that in untreated plants, but in the rib meristem recovery did not rise above 28%. Unfortunately, mitotic figure counts were not made after the 14th day.

Certainly Alar treatment produces the most severe effect on the rib meristem of apple seedlings, but there is a similar, initial though temporary inhibition of mitotic activity in both leaf primordia and apical meristem.

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