thesis of the B-ring substituted with hydroxyl groups in both the 3' and 4' positions, because the production of luteolinidin in light was greater than that of apigeninidin, and luteolin has been detected only after treatment with light of at least a low intensity.

Literature Cited

Changes in Calcium Levels in Cell Walls during Elongation of Oat Coleoptile Sections 1, 2

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The calcium bridge hypothesis was proposed by Bennet-Clark (1) in 1955 to explain the mode of action of IAA in controlling elongation of oat coleoptile sections. He found that the chelating agent ethylenediaminetetraacetic acid (EDTA) promotes growth of oat coleoptile sections. He assumed that the chelating agent stimulates growth by removing calcium from the cell wall and believed that IAA acted in a similar manner. Cland (4) recently tested this hypothesis by determining the effect of IAA upon the redistribution of Ca 45 within the cell wall and loss of calcium from the wall. He found that the loss of calcium from Avena coleoptile and maize mesocotyl cell walls is not enhanced by auxin and that auxin does not cause a redistribution of calcium between pectin and protopectin. On the basis of these findings he suggested that the removal of calcium cross-linkages is not brought about by auxin and that the calcium bridge hypothesis is incorrect.

If IAA has no effect on the redistribution of calcium, the calcium levels might be expected to be similar in the walls of sections incubated in inhibitory concentrations of calcium both in the presence and absence of added IAA. A search of the literature has not revealed data that either supports or refutes the assumption that the inhibition of cell elongation in the presence of supraoptimal levels of calcium is due to an increase of calcium in the cell wall. Therefore, the current study was undertaken to determine the relationship between the absolute calcium levels in

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2 This paper is based on a thesis submitted by the senior author to Dartmouth College in partial fulfillment of the requirements for the M.A. degree. This work was supported in part by a research grant from the National Science Foundation (GB705).
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Coleoptile sections were obtained from Avena sativa (var. Victory). The seeds were soaked for 3 to 5 hours in darkness in demineralized water, spread on damp paper towels in a plastic box, and covered with a transparent lid. During a 24-hour period they were irradiated with red light every 4 hours for 5 minutes to suppress elongation of the first internode and to stimulate growth of the coleoptiles. After irradiation, the seeds were sown in moist vermiculite, allowed to germinate in darkness at 25 ± 2° and 65 ± 5% relative humidity until 96 hours from initial soaking had elapsed. Coleoptiles between 27.5 and 32.5 mm in length were selected. Ten-mm sections were cut 3 mm from the tips on a double-bladed section cutter and floated on distilled water until used during the next 30 minutes. All operations were carried out under weak green light.

A series of experiments were set up using 4 different treatments: water (W); water plus 3.5 mg IAA per liter (WI); water plus 200 mg calcium per liter (WC); and water plus 3.5 mg IAA per liter plus 200 mg calcium per liter (WIC). For each treatment, 6 petri dishes containing 20 ml of media were provided. Sections were randomized and distributed 10 sections per dish. In all experiments the primary leaves were left in the coleoptile section until after analysis. It has been found that the presence of the leaves increases growth (3), reduces curvature, and has only a slight effect upon the calcium level (5). The petri dishes were placed on a rotary shaker and swirled at about 50 rotations per minute to improve growth. The sections were grown at 25 ± 2° in weak green light except during the time it took to measure the sections (approximately 20 minutes). Light seems to have no effect on the growth of sections (2). After 2, 6, and 18 hours, 2 petri dishes from each treatment were removed and shadowgraphic enlargements made of the sections. These enlargements were used in determining elongation. The sections, including those taken at zero time, were then rinsed twice in distilled water, leaves removed with a glass needle, and sections again rinsed twice. Sections from 1 dish from each treatment were transferred to tared Coors porcelain crucibles and dried at 60° for at least 8 hours. Sections in the remaining dishes were fractionated and cell walls isolated by a slight modification of the procedure of Ray (9). They were then placed in tared crucibles at 60° to dry for 8 hours. The crucibles were removed from the oven and allowed to cool in a desiccator over silica gel. Weighings were made on a Mettler M-5 balance. Reproducible weighings were made by taking readings about 60 seconds from the time the crucible was removed from the desiccator. After the dry weight was taken, the crucibles were placed in a muffle furnace at 550 to 600° for a minimum of 12 hours to ash the samples. Ash weights were determined in the same manner as the dry weights. Calcium was analyzed on a Perkin-Elmer (Norwalk, Connecticut) atomic absorption spectrophotometer, Model 303, and the procedure used to determine calcium was a modification of that suggested by Perkin-Elmer in their instruction manuals. The ash was dissolved in a stock solution containing 6 n HCl and 5% lanthanum (in the form of La203). This solution was then diluted with demineralized water so that the final concentration of lanthanum was 1%. Lanthanum protects the calcium determination from interference from as much as 200 mg per ml phosphorus in the diluted sample. Three determinations of calcium concentration were made on each sample, and the averaged values converted to absorbance. Determinations of standard solutions were interspersed with the sample readings to insure that the instrument was functioning properly. A standard curve was then plotted of absorbance vs. µg calcium per ml. Using the standard curve, the concentration of each sample was determined in µg Ca per ml. This value was multiplied by the number of ml per sample to obtain absolute amounts of calcium in the sample.

Results

Fresh weight, dry weight, and calcium levels of oat coleoptile sections were determined immediately after harvesting on 11 separate occasions. These data, presented in table 1, show that the dry weight of sections represents 7.2% of the fresh weight, compared to 7.5% found by Ray (9), and the calcium content is 0.18% of the dry weight. A search of the literature has not revealed any data on absolute calcium levels in oat coleoptile sections. However, Neales and Kinde (8), using flame photometry and atomic absorption spectrophotometry, found that the level of calcium in bean roots was 0.05% of the dry weight before they were subjected to calcium in the external medium and Vivilaan et al. (11) give a value of 0.06% calcium on a dry weight basis for maize coleoptile walls.

No studies have been made of changes in absolute calcium levels in the cell wall during growth. Therefore, it was of interest to determine whether the calcium level of coleoptiles, and more specifically of cell walls and cell elongation in the presence and absence of IAA.

Materials and Methods

Table I. Average Fresh Weight, Dry Weight, and Calcium Content of 10-mm Avena Coleoptile Sections and Cell Walls (with se, N = 11)

<table>
<thead>
<tr>
<th>Avena coleoptile section</th>
<th>Weight and calcium content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fr wt/section</td>
<td>7.93 ± 0.13 mg</td>
</tr>
<tr>
<td>Dry wt/section</td>
<td>0.57 ± 0.01 mg</td>
</tr>
<tr>
<td>% dry wt of the fr wt</td>
<td>7.15 ± 0.06 %</td>
</tr>
<tr>
<td>Fr wt: dry wt</td>
<td>14:1</td>
</tr>
<tr>
<td>Calcium content/section</td>
<td>1.01 ± 0.09 µg</td>
</tr>
<tr>
<td>% calcium of the dry wt</td>
<td>0.18 ± 0.05 %</td>
</tr>
<tr>
<td>Dry wt of cell wall/section</td>
<td>1.81 ± 0.03 mg</td>
</tr>
<tr>
<td>Wt of calcium in cell wall/section</td>
<td>4.09 ± 0.17 µg</td>
</tr>
<tr>
<td>% calcium in cell wall</td>
<td>0.23 %</td>
</tr>
</tbody>
</table>

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walls, actually does increase as growth proceeds. Sections subjected to calcium in the presence or absence of IAA accumulated calcium at constant rates over the 18-hour period, and these rates were very nearly equal (fig 1A). In all 4 treatments the dry weight of intact sections decreased slightly during the 18-hour period. The wall fraction in all treatments showed no significant change in dry weight and thus any weight change of the intact sections must be accounted for by a decrease in the dry weight of the protoplast. Protoplast is used here to refer to the nonwall portion of the cell. The average ash weight of intact sections at time zero was 4.4% of the dry weight and 0.3% of the calculated fresh weight. Using the ratio of fresh weight to dry weight (14:1) obtained in table I, the fresh weight of the sections can be estimated. Assuming that the specific gravity of the tissue is approximately 1.0, the volume of the 10 sections subjected to calcium for 18 hours can be estimated at 0.076 ml. Since 1 ml of incubation medium contains 200 μg calcium, the 0.076 ml of coleoptiles would contain 15.2 μg of calcium if equilibrium were reached with the external medium. However, at the end of 18 hours these sections had accumulated 46 μg of calcium. Since the endogenous level was 9 μg, the final level in the sections was 5 times the original, and 3 times greater than the level in the external medium. In the absence of calcium in the medium (W and WI), the intact sections maintained the endogenous level of calcium throughout the 18-hour period (fig 1A).

Figure 1B shows that the percentage of calcium in the walls in both WC and WIC increased 2-fold during the treatment period. The wall calcium in the W and WI treatments did not change significantly. The accumulation of calcium in both WC and WIC was twice as great in intact sections as the accumulation by the cell walls alone.

Figure 2 shows the growth of coleoptile sections for all 4 treatments for the 18-hour period. As expected, maximum growth occurred in sections subjected to IAA alone (WI), but some growth occurred in the absence of an exogenous supply of IAA (W). Addition of calcium to the medium inhibited growth under both conditions (WIC and WC).

Discussion

Thimann and Schneider (10) showed that calcium has an inhibitory effect on auxin-induced elongation of oat coleoptiles. In 1955, Bennet-Clark (1) proposed that IAA exerts its effect on growth by removing calcium from the cell wall. Tagawa and Bonner (10) showed that inhibitory levels of cal-

![Figure 1](image1.png)

![Figure 2](image2.png)

**Fig. 1.** Calcium content of oat coleoptile sections incubated in distilled water (W), 3.5 mg/liter IAA (WI), 200 mg/liter calcium (WC), and IAA plus calcium (WIC). A, intact sections; B, cell walls.

**Fig. 2.** Increase in length of oat coleoptile sections in water (W), 3.5 mg/liter IAA (WI), 200 mg/liter calcium (WC), and IAA plus calcium (WIC). The initial section length was 10 mm.
calcium actually did increase the rigidity of the cell wall. These results are consistent with the calcium bridge hypothesis of Bennet-Clark. Cleland (4), however, demonstrated that IAA in the absence of added calcium caused no redistribution of Ca\(^{45}\) in the cell wall nor loss of it from the cell wall. We have shown that calcium is accumulated by coleoptile sections, and that this calcium uptake is independent of exogenous IAA. There was no detectable change in rate of calcium uptake by intact coleoptile sections over an 18-hour period in either the presence or absence of IAA when calcium was present in the medium. Cool and Bonner (5), however, found an initially rapid (30–60 minutes) uptake of Ca\(^{45}\), attributed to adsorption and diffusion, followed by a slower continuing accumulation, attributed to metabolic uptake. But, Machett and Nance (7) have found that pretreatment of pea stem slices with growth-promoting levels of IAA results in an increased capacity for rapid uptake of calcium ions. Furthermore, they found that loss of calcium from these sections was greatly enhanced by the presence of IAA. Both Cleland and Machett and Nance followed changes in calcium levels with Ca\(^{45}\). Our determinations of changes in absolute levels of calcium in intact sections and in cell walls subjected to supraoptimal levels of calcium in the presence and absence of IAA are consistent with those of Cleland. Since the values obtained by Machett and Nance were for pea stem sections, as opposed to *Avena* coleoptile cell walls, it is difficult to relate their work to ours.

Jansen et al. (6) determined by means of flame photometry the calcium level in an aqueous suspension of oat coleoptile cell walls. They found that the cell wall contained 0.6 \(\mu\)g Ca per mg dry weight. They were also able to calculate the cation exchange capacity of cell walls (3.30 \(\mu\)g Ca/mg dry weight) from their determinations of pectin concentrations and the estimated number of free pectic carboxyl groups per pectin molecule. Using the cell wall preparation and a range of calcium concentrations, they were able to determine that the maximum cation exchange capacity of the cell wall was 3.60 \(\mu\)g Ca/mg dry weight. The present study shows that the initial level of calcium in the cell walls is 2.24 \(\mu\)g Ca/mg dry weight, a value higher than those obtained by Jansen and by Vivilaan et al. (12) for maize coleoptile cell walls. The calcium content of cell walls doubled within 18 hours in a medium containing calcium, but remained essentially unchanged in a calcium-free medium. We have confirmed the finding that calcium inhibits elongation of oat coleoptiles in the presence of added IAA (5, 10), and have further shown that calcium inhibits elongation in the absence of added IAA.

IAA does not cause a redistribution of Ca\(^{45}\) within the cell wall or loss from the cell wall in the absence of added calcium (4), and we have shown that IAA has no effect on calcium uptake in the cell wall in the presence of added calcium. Since calcium inhibits growth in either the presence or absence of IAA and since IAA does not alter absolute calcium levels in cell walls in the presence or absence of added calcium, it is suggested that the 2 phenomena are independent of one another.

**Summary**

Calcium levels in oat coleoptile sections were determined on intact sections and cell wall fractions by atomic absorption spectrophotometry. The measurements were made over an 18-hour period in both the presence and absence of 3.5 mg per liter of indoleacetic acid and in both the presence and absence of 200 mg per liter calcium. Calcium actively accumulates in the intact sections and shows a 2-fold increase in the walls over the 18-hour period if calcium is present in the medium. Indoleacetic acid has no effect on this accumulation. These results, based on determinations of calcium rather than Ca\(^{45}\), provide additional evidence that the mode of action of auxin is not through effects upon calcium levels in cell walls.

**Literature Cited**


