

shortest juvenile periods, those in progenies of *M. Sieboldii* still longer. The mean juvenile period varied considerably depending on the particular parentage of the progeny. In all cases, the juvenile periods for *M. hupehensis* and *M. Sargentii* were longer than those which I found. However, Schmidt stated that no attempt was made to shorten the juvenile period of the seedlings.

Saure (4) reported that when apple progenies with the same parentage were planted in successive years, the mean juvenile period of each succeeding progeny was shorter. This was because changes in cultural practices increased the growth of each succeeding progeny. A similar response occurred with the apomictic crabapples grown on 2 different sites in the field since the most vigorously growing seedlings had the shortest juvenile period. This also agrees

with other reports on the effect of growth on the length of the juvenile period in tree seedlings (9).

The juvenile period could be shortened by accelerating the growth of the seedlings in the greenhouse before field planting only with *M. toringoides*. However, these seedlings grew so tall in the greenhouse (up to 5 m in 15 months) that they were unwieldy to handle. The results presented here together with those published earlier (8) clearly show that *M. hupehensis* is the most suitable for physiological studies of those species that I tested.

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## A Method for Feeding Chemicals into Young Apple Trees<sup>1</sup>

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**Abstract.** A method is described for introducing small quantities of chemicals by gravity flow into terminal shoots of young apple trees. The destruction of acid fuchsin and <sup>14</sup>C labelled growth regulators by this method was down the main stem, out to the lateral branches, and into the leaves.

When one obtains negative data with growth regulators it is not known whether the compounds are ineffective or are not taken up by the tree. Direct introduction of the growth regulator into the tree may circumvent the absorption problem, but the distribution throughout the tree may not be uniform. A method by which growth regulators can be administered to the tree with good distribution would be desirable. Various methods have been used to inject chemicals to fruit trees including pressure (3, 6) and gravity flow (2, 4).

In this report we describe a method to introduce growth regulators and dye through the terminals of young shoots. In our method the terminal shoot of a main limb is cut off with sharp pruning shears or a knife so as not to crush the conductive tissue. A rubber stopper

with a single hole is placed over the cut terminal shoot with the tapered end pointing upward. Either a test tube with the end removed, or a section of glass tubing is placed tightly over the stopper (Fig. 1). The test chemical is poured into the tube and at least 2 volumes of water added after uptake of the solution to aid distribution in the tree and prevent desiccation.

This method was used on 3-year field grown 'Delicious' apple trees to determine the path of movement of a dye using 60 ml of an aqueous 0.1% acid fuchsin solution per tube. The trees were grown under irrigation which allowed normal uptake and movement of water. After 3 days of treatment the trees were dug and dissected. The dye apparently moved into the laterals in the transpiration flow. The acid fuchsin dye moved readily downward through the xylem until intercepted by a lateral limb, then moved out into the upper side of the lateral (Fig. 2). When not intercepted by a lateral branch, the dye continued to move downward in the xylem of the main stem. After numerous lateral interceptions, little dye remained. The 60 ml of dye moved down the limb approximately 1.2 m. Our results support those of Dixon (1) who found that dye moved downward readily through the xylem when introduced into terminal shoots. Thomas (7) injected dogwood trees with 0.5% acid fuchsin and was successful in identifying the transpiration stream and concluded the dye moved basipetally in



Fig. 1. Installation of feeding apparatus on terminal of apple tree.

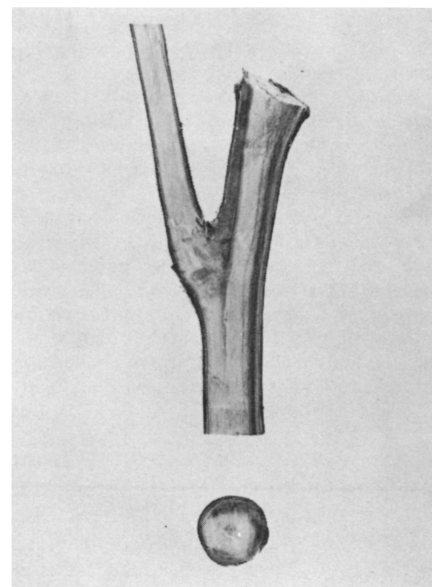


Fig. 2. Interception of acid fuchsin dye in xylem by lateral limb.

the same pattern as it moved acropetally. However, we found a concn of 0.5% too high in apple trees.

To further determine the distribution pattern of chemicals applied by this method <sup>14</sup>C labelled materials were fed into 4 six-month old 'Delicious' apple

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trees growing in the greenhouse. Two trees were fed with 50 ml 5 ppm 2,4-dichlorophenoxyacetic acid (2,4-D) and 2 trees were fed with 50 ml 1000 ppm succinic-2,2-dimethylhydrazide (SADH). The 2,4-D contained 1%  $^{14}\text{C}$  on the carboxyl group, and the SADH 1%  $^{14}\text{C}$  on the dimethylhydrazine group. After 3 days, the trees were cut into sections and the sections were divided into leaves, bark and wood (Table 1). The tissues were extracted with 80% ethanol in a blender and the extracts were filtered through Whatman No. 1 filter paper. Radioactivity of the filtrate was evaluated with a Packard Model No. 3320 liquid scintillation spectrometer.

In the ethanol extract, 42% of the 2,4-D and 46% of the SADH were recovered. Both 2,4-D and SADH were distributed throughout the tree (Table 1) with leaves containing the highest quantity. In the main trunk, the wood contained approx 3 times the quantity in the bark, an indication of xylem transport. It must be recognized, however, that this method provides for a xylem transport; whereas some substances may translocate through the phloem. We have no way to assess the similarity between xylem feeding and natural transport of these substances, although the results are similar to those of Schonherr and Bukovac (5) from

Table 1. The distribution of injected 2,4-D ( $^{14}\text{C}$ ) and SADH ( $^{14}\text{C}$ ) in 6 month old apple trees grown in pots in the greenhouse, 3 days after injection (cpm  $\times 10^{-3}$ ).

		Distribution (1000 cpm) ± SE	
		2,4-D	SADH
Main Trunk <sup>2</sup>			
( 0-3 cm)	Bark	95± 18	18± 4
	Wood	207± 37	17± 1
( 4-10 cm)	Bark	61± 9	14± 6
	Wood	165± 10	15± 3
(11-25 cm)	Bark	377± 14	48± 10
	Wood	969±159	112± 41
(26-50 cm)	Bark	255± 1	39± 5
	Wood	1009±164	96± 38
Buds		28± 24	3± 2
Laterals <sup>y</sup>			
( 0-20 cm)	Bark	329± 43	50± 4
	Wood	419± 69	52± 10
(21-40 cm)	Leaves	3687±306	383±146
	Bark	332± 27	41± 11
(41-60 cm)	Wood	460± 26	49± 18
	Leaves	4212± 33	415± 56
	Bark	268± 55	30± 6
	Wood	278± 26	28± 9
Crown		268± 73	88± 24
Roots		652± 27	181± 62
Total Recovered		14,216± 37	1680±410
Injected		33,899± 22	3652±911

<sup>2</sup>Measurement from apex to ground.

<sup>3</sup>Measurement from main trunk to apex.

dipping the terminal 8 leaves in  $^{14}\text{C}$  SADH.

Although all substances may not behave in the same manner as acid fuchsin, 2,4-D and SADH, the dye and

isotope studies show the potential distribution of substances fed by this method. It was evident from appearance of red color and high radioactivity in the leaves that chemicals fed through the terminal shoot are distributed throughout the tree. The method is easy to use and appears to assure entry and distribution of chemicals that may not be taken up by spray application.

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## Calcium Crystals in Apple Stem, Petiole and Fruit Tissue<sup>1</sup>

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**Abstract.** Single crystals and clusters of crystals or druses found by polarized light microscopy in tissues of *Pyrus malus* L. cv. Jonathan were found to contain Ca using the electron microprobe. Crystals insoluble in 20% acetic acid occurred in cells adjacent to the vascular tissues near the pedicel in mature fruit and in dormant flower buds, stems, petioles, shoot apex, roots and callus tissue. Because of deposition of calcium as crystals, calcium supplies to cortical cells of apple fruit may be limited and may result in an increased incidence of internal breakdown due to low Ca levels in those cells.

A positive relationship between oxalic acid concn in the stem and susceptibility to a Ca-deficiency disorder in *Nicotiana tabacum* L. is described by Brumagen and Hiatt (1). They suggest that because Ca is precipitated by high levels of oxalic acid in the upper stalks and young leaves of susceptible cultivars, insufficient Ca

reaches the apex of the plant which then expresses symptoms of Ca deficiency.

Chang et al. (2) found that symptoms of a Ca-deficiency disorder in young tobacco leaves increased with temp from 21 to 30°C. The Ca content of the stems increased with temp suggesting that Ca was immobilized in the stems at higher temp. A similar phenomenon with maize seedlings was observed by Walker (6). At 21°C soil temp, leaves appeared normal. At each 1° increment, symptoms were proportionately more severe. The concn of Ca in maize shoots did not indicate a Ca deficiency level, yet Ca concn in the extreme half of the blades of the youngest 2 leaves was in the deficiency range. Whether the immobilization of Ca in maize and tobacco was related to crystal formation was not made clear by either Chang et al. (2) or Walker (6). Simons (5) found similar appearing crystals in apple phloem tissues but did not chemically analyze them.

Microprobe examination of mature fruit of 'Jonathan' apple revealed the presence of numerous Ca crystals near

the vascular bundles (Fukuda, H., unpublished results). Since a Ca deficiency in the fruit cortex has been related to internal breakdown of the fruit, a study was undertaken to locate and characterize the crystals and to examine their possible role in causing the deficiency.

All apple tissues for cryostat sectioning were cut with a razor blade and immediately mounted and frozen in Optimum Cutting Temperature Compound (OCT -15° to -30°C, Fisher Scientific Company), according to the method of Rasmussen et al. (4). Sections of 16  $\mu$  thickness were cut on a cryostat at -20°, dried in air, and examined under plane polarized light.

Crystals were extracted from shoots on 1-year-old seedlings by macerating the tissues in a mortar and pestle with a few ml of water. Druse crystals survived this treatment with no apparent change. Drops of material thus prepared were placed on slides and treated with 20% acetic acid or 0.1 N HCl.

The procedures employed in examination for crystals by microprobe are described by Rasmussen et al. (4). Sections for microprobe analysis were mounted on polished carbon disks. Identification of elements present in typical crystals was accomplished by scanning the X-ray spectrum emitted during bombardment of the crystal with an electron beam. Oscillograms taken at

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