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Short Communication

Levels of Indole-3-Acetic Acid in Vigorous and Genetic Dwarf Apple Trees

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Abstract. Seasonal levels of indole-3-acetic acid (IAA) present in buds, meristematic tissues, and leaves of 1-year-old shoots of two selections (vigorous and dwarf) of a F_2 generation apple population with original "Goldspur" × "Redspur" parentage were determined using gas chromatography–selected ion monitoring–mass spectrometry (GC-SIM-MS) with a ^{13}C-IAA internal standard. A comparison of IAA levels through one growing season was made in the shoots of two trees differing in growth. The levels of IAA in the leaves of the vigorous tree were higher in the early and late growing seasons, as compared to those in the dwarf tree. Levels of IAA in buds of the dwarf tree shoots were found to be higher than in those of the vigorous tree shoots.

Over the past 30 years there has been a dramatic increase in the planting of dwarfed apple trees. Tree size is controlled by grafting genetically vigorous cultivars onto size-controlling clonal rootstocks. Recently, interest has been expressed in the production of genetically reduced-sized apple trees by breeding dwarf cultivars (Faust and Zagaja 1984). The mechanism responsible for dwarfing of these trees is not yet understood. The existing knowledge concerning the role of auxins (IAA) in the dwarfing of the grafted apple tree has been summarized by Lockard and Schneider (1981). Some studies of growth substances in genetically dwarfed trees have been done, including the measurement of auxin-like substances for two growing seasons by the *Avena* curvature bioassay in shoots and vascular tissues of apple hybrids having different

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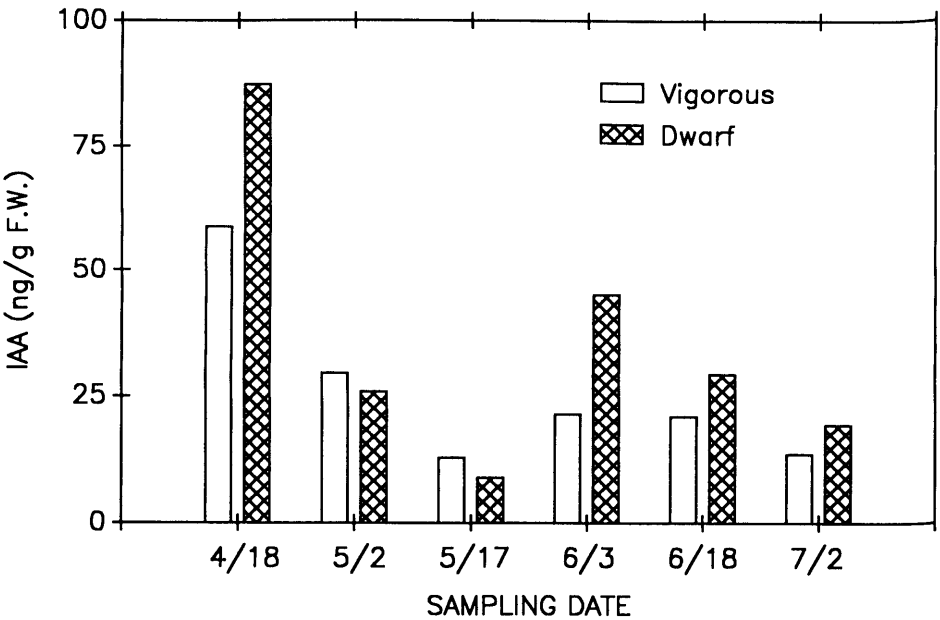

Fig. 1. IAA levels in buds and meristematic tissues from 1-year-old shoots of apple at different times during the growing season as measured by GC-SIM-MS.

growth patterns (Grochowska et al. 1984a). Determination of auxin levels by gas chromatographic (GC) analysis of indole-3-acetic acid (IAA) has also been done for a small number of these apple trees, sampled once (Terry et al. 1983).

This preliminary study of one growing season has been done to investigate changes in IAA levels in buds and leaves throughout the season in apple trees having vigorous and dwarf growth habits. The analytical technique, gas chromatography–selected ion monitoring–mass spectrometry (GC-SIM-MS), allowed corroboration of the earlier studies.

Materials and Methods

F_2 progeny produced from sib crossings of *Malus domestica* Borkh. selections with "Goldspur" × "Redspur" parentage were used in this study. These 8-year-old trees, growing in an experimental plot at the USDA, Agricultural Research Center, Beltsville, Maryland, varied greatly in growth habit. One large tree (4.5 m) of vigorous growth with shoot lengths in mid June (10 cm) similar to cultivated apple trees, and one small tree, (1.5 m) of dwarf growth with short internodes (3 cm) were sampled. For the first sampling (4/18) terminal and lateral buds were removed from ten 1-year-old shoots from each tree. In subsequent samplings, terminal and lateral meristematic tissues from shoots were collected, quickly weighed, and frozen in liquid nitrogen. Leaf sampling consisted of collecting fully expanded leaves (10–15 g). Replicate samples were not taken to avoid exhaustion of the plant material during the period of sampling. Fruit was collected from the vigorous tree (10–15 g).

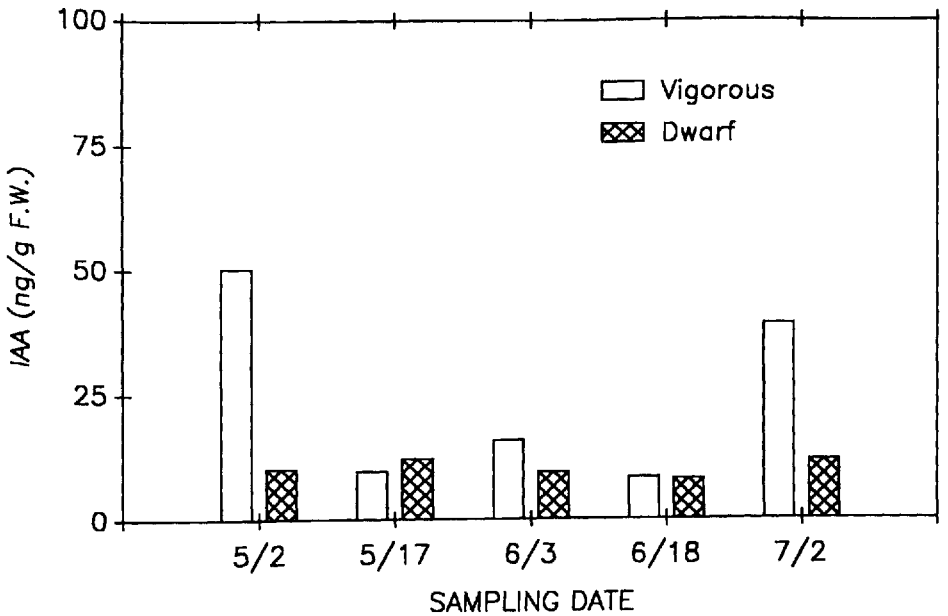

Fig. 2. IAA levels in leaves from 1-year-old shoots of apple at different times during the growing season as measured by GC-SIM-MS.

IAA was analyzed by a procedure involving solvent partitioning, HPLC purification, and methylation with diazomethane. Quantitation of IAA levels was determined using GC-SIM-MS analysis (Cohen et al. 1986), which required the addition of $^{13}C_6$-IAA as the internal standard during the initial extraction of the plant material. Triplicate injections were made for each sample and average values were reported.

Results and Discussion

The presence of IAA in buds and leaves of 1-year-old apple shoots sampled during the spring growth period was confirmed by use of GC-SIM-MS procedure. The monitoring of the ions m/z 130 and 189 for the methyl ester of IAA, and m/z 136 and 195 for the methylated internal standard, enabled a quantitative comparison to be made with earlier studies of IAA levels in apple shoots. Levels of IAA found in the buds, meristematic tissues, and leaves of the shoots of trees with high and low growth rates are shown in Figs. 1 and 2. These data are indicative of trends in changes in IAA levels, and multiple samples would be required for statistically valid analyses. Buds of both tree types have relatively high concentrations of IAA in mid April. It should be noted, however, that the mid-April levels may be influenced by the presence of bud scales which were not removed prior to extraction. Following mid April, IAA levels in the meristematic tissues from both tree types decreased and remained at low and relatively equal levels, with the exception of a noticeable increase in the dwarf tree at the June 3 collection date. This increase in the IAA level was not

Table 1. Weights of apple bud and meristematic tissues obtained from ten 1-year-old "Goldspur" × "Redspur" shoots.

	Sampling date					
Shoots	4/18	5/2	5/17	6/3	6/18	7/2
Vigorous (g fresh wt)	42.7[a]	6.5	2.8	2.5	1.4	1.2
Dwarf (g fresh wt)	9.9[a]	4.1	2.0	2.1	1.4	1.2

[a] Buds present before leaf expansion occurred. Remainder of samples are meristematic tissues collected after leaf expansion.

associated with the increase in weight of the terminal and lateral meristematic tissues in early June (Table 1).

The IAA levels in the apple leaves were highest in the young leaves, diminished rapidly, and remained at a relatively low level through early July. The young leaves of the vigorous tree in May (5/2) had a higher level of IAA than did those of the dwarf tree. This difference in the hormone level was found again in July (7/2).

The decrease in the IAA concentration in leaves of the apple shoots of both growth patterns in this study is not in agreement with earlier *Avena* curvature auxin bioassay data obtained from the same group of trees (Grochowska et al. 1984a). However, there may be a phenotypic difference since the dwarf trees studied earlier had extremely short internodes, whereas the trees in this study did not have this extreme growth pattern. The results of the earlier study indicated a seasonal increase in auxin level in shoots from 25–64 ng/g for vigorous trees and an essentially constant 74–79 ng/g auxin concentration for dwarfed trees. The possibility also exists that other endogenous substances, such as phenolics, may have affected the bioassay determinations, resulting in a higher estimation of IAA content. The concentrations of IAA found in our GC-SIM-MS study are reasonably close to the values given in the earlier GC study (Terry et al. 1983), where 53 ng/g IAA was found in buds of vigorous apple trees.

Levels of auxin close to those reported here were also found in a similar study of the "McIntosh" apple (Grochowska et al. 1984b), where higher levels of auxin (20–30 ng/g IAA equivalent) were found in the shoots in May followed by diminishing levels through the season to approximately 10 ng/g. These data contrast with the bioassay study of dwarf apple cultivars (Grochowska et al. 1984a), where auxin levels were relatively low in the spring and increased throughout the growing season.

Only high-growth rate trees produced fruit. IAA levels were measured in the young developing apples (Table 2). The highest level of IAA (11 ng/g) was found in the young fruit (5/2), and levels diminished as the fruit developed further. These levels are considerably less than those reported in a study where the occurrence of a seasonal (late May) IAA peak during apple fruit development (300–900 ng/g) was found for two commercial varieties (Ebert and Bangerth 1985). Interferences in the quantitation of IAA by fluorescence measurement have been previously reported and may result in the finding of elevated IAA levels (Sandberg et al. 1987).

Table 2. IAA levels in developing apple fruit as measured by GC-SIM-MS.

Sampling date	5/2	5/17	6/3	6/18	7/2
IAA (ng/g fresh wt)	11.1	4.8	0.9	2.2	0.8
Fruit weight (g)	10.0	10.0	15.0	15.0	15.0

Determinations by bioassay of auxin levels in fruit trees during the growing season have been done on peaches (Cristoferi and Filiti 1981). Higher levels of auxin-like substances in tree shoots were found in the spring with lower levels found in the fall. More auxin was found in the shoots of "Cresthaven," a tall (vigorous) peach, compared to lower levels in "Bonanza," a dwarf cultivar.

The data obtained in this GC-SIM-MS study of vigorous and dwarf apple trees indicate that the highest seasonal levels of IAA occur in leaves on the earliest sampling date. These results are comparable to those of the bioassay studies of "McIntosh" apple and peach cultivars. Higher IAA levels were found in the leaves of the vigorous tree as compared to those in the dwarf tree leaves during this early growth period, which is similar to the auxin levels found in the two comparable peach cultivars. Later, the levels of IAA in the leaves diminished from an early seasonal maximum, as was found in the auxin levels in the bioassay studies.

The data on IAA levels in the buds (meristematic tissues) indicated relatively high levels in both "Goldspur" × "Redspur" F_2 apple trees in April, followed by a decline in May. An increase in IAA levels in meristematic tissues of the shoots occurred during the period of terminal bud set in June. No bioassay studies on the fluctuation in auxin levels in apple buds have been done to our knowledge.

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