Growth Analysis and Plant Hormone Studies in Apple (*Malus sylvestris* Mill.)

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at

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To My parents, Soon Ja, Jin Soo, Si Young, and Jin Hyoung.

Since we are assured that the all-wise Creator has observed the most exact proportions, of number, weight and measure, in the make of all things; the most likely way therefore, to get any insight into the nature of those parts of the creature, which come within our observation, must in all reason be to number, weigh and measure.

"God has not only comprehended the dust of the earth in a measure, and weighed the mountains in scales, and the hills in a balance, Isai. Xl. 12." (Hales, 1727).

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Abstract

Part I

Growth analysis studies

Previous data on gravitational effects on shoot growth and flowering have been inconsistent. Attempts have been made to investigate shoot growth and flowering on shoots with a 3/8 phyllotaxis in 90 cm and 150 cm laterals. These were bent at different times to the horizontal or to a pendulous position in apple varieties Red Delicious and Granny Smith on MM 106 rootstocks grown under a semi-intensive system on a commercial orchard in the major apple growing area of Hastings. The four treatments comprised: horizontal or pendulous bending during the dormant period, at petal fall, second cover stage and with normal vertical laterals as controls.

Horizontal bending increased total shoot growth and flowering relative to the vertical controls in the 90 cm treatment in both varieties. There seemed to be a tendency to decrease total shoot growth when the time of bending was later in the season and no differences in flowering occurred among the horizontal bending treatment. On the other hand shoot growth was relatively constant in all treatments in the 150 cm treatment. A very significant increase in flowering, however, was found in the petal fall pendulous bending. In the dormant period pendulous bending there was a slight effect on the flower promotion relative to the verticals.

The production of laterals and flower buds was always more pronounced on the upper side of the bent shoots, with an intermediate on the flanks, and greatly inhibited effects on the lower side, indicating a steep linear relationship from the lower to the upper during the dormant period treatment in all experiments. Generally, the percentages of shoot growth and flower production were increased from the dormant period bending to the petal fall, second cover and to the vertical control.

The greatest increase in shoot length and increased percentage of flowering in all experiments were found in the apical whorl zone, and these further decreased from the 1st to the 2nd, and to the 3rd whorl; this was the case for shoot growth and flowering in the first whorl was not increased due to the inherent properties in Red Delicious. The shoot growth and flowering at the different whorls in 150 cm length laterals bent pendulously in Red Delicious showed a quadratic relationship due to the longer shoots in the apical and the arch position on the shoot when bent at the 5th whorl in all treatments. But at the 5th whorl flowering was reduced considerably, because of substantial lateral growth.

In order to describe the growth relationship between shoot volume and total leaf area an index based on the ratio of vegetative and reproductive responses was established e.g. vegetative 10.83 and reproductive 19.80-24.40.

The relationship of shoot growth and flowering are discussed in terms of a hormone balance theory.

Abstract

Part II

Plant hormone studies

In order to establish a ratio of different plant hormones for an understanding of physiological phenomena, appropriate extraction procedures are required for especially apple leaves which are rich in phenolic compounds and other inhibitors. Therefore extraction procedures and purification were examined using ^{14}C-IAA and ^{3}H-zeatin.

Loss of ^{14}C-IAA during extraction procedures was due to a high pH in the aqueous phase during solvent partitioning. The final recovery of ^{14}C-IAA was 3.8% at pH 8.0 and 81.1% at pH 2.5 through solvent partition and column chromatography. ^{14}C-IAA was chromatographed on a silica gel-celite column and a Sephadex LH-20 column, giving 80% recovery in 30 ml elution volume around the main peak and 90% recovery in 20 ml elution volume around the main peak respectively. Nearly 100% recovery from a Sephadex G-10 column was obtained. 50-57% recovery of ^{14}C-IAA was obtained in cellulose thin layer chromatography at the Rf of IAA, and no loss of ^{14}C-IAA occurred during 3 days storage in a dark cabinet.

The partition coefficient of ^{3}H-zeatin at pH 8.3 was 13.12 with ethyl acetate and 0.488 with n-butanol; at pH 2.5, 108-89 with ethyl acetate and 16.73 with n-butanol. Backwashing can recover ^{3}H-zeatin from ethyl acetate phase which was partitioned at pH 2.5. 80% recovery of ^{3}H-zeatin in the first 1,000 ml was obtained from Sephadex G-10 and Dowex 50 W x 8. 88.6% recovery of ^{3}H-zeatin could be obtained in a 20 ml peak using Sephadex LH-20 eluted with 95% EtOH containing 0.001 M HCl. The behaviour of ^{3}H-zeatin was studied in paper chromatography and cellulose, DEAE cellulose and silica gel, thin layer chromatography, about 82-60% of ^{3}H-zeatin the Rf of ^{3}H-zeatin being recovered.

Four series of plant hormones were determined from apple leaves by ethyl acetate partitioning, Sephadex G-10 column, silica gel-celite column and cellulose thin layer chromatography of acidic fractions containing auxin-, gibberellin-, and ABA-like substances, and by butanol partitioning, Sephadex G-10, Sephadex LH-20, and DEAE cellulose thin layer chromatography for cytokinin-like substances from basic fractions. Possibly two kinds of auxin-like substances were found and possibly GA_9, GA_4, GA_5, GA_1, or GA_3 and GA_8-like substances were eluted from a silica gel-celite adsorption column. Several groups of cytokinin-

like substances were obtained from Sephadex LH-20 column chromatography, possibly zeatin, zeatin-riboside and other cytokinins were found in apple leaves.

Based on the estimation of each plant hormone from thin layer chromatography, a relative plant hormone index was established, i.e., Relative Auxin Activity Index, Relative Inhibitor Activity Index, Relative Gibberellin Activity Index, and Relative Cytokinin Activity Index, representing 6.59, 1.04, 2.64, and 8.16 respectively, the hormone giving the highest ratio being considered the dominant hormonal factor at that stage of development.

GLC techniques were also studied for plant hormone analysis, using 3% OV-1 and NAA, IAA, IPA, GA_1, GA_3, GA_4, GA_5, GA_7, GA_9, GA_{13} and ABA markers to establish retention times and detector response at the 2.5 ng level. N.O.-bis(trimethylsilyl)trifluoroacetamide (BSTFA) together with Trimethylchlorosilane (TMCS) silyl reagents produced the best peak heights for IAA, IPA, GA_3 and GA_1 but reduced the ABA peak by half and the GA_9 peak by 20%.

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Abbreviations

ABA	Abscisic acid
Alar	Succinic acid 2,2-dimethyl hydrazide
AW-DMCS	Acid washed Dimethyldichlorosilane
Axs	Auxins
BSA	(bis(trimethylsilyl)acetamide
BSTFA	N.O.-bis(trimethylsilyl)trifluroacetamide
BuOH	n-butanol
CKs	Cytokinins
$CHCl_3$	Chloroform
CH_3Cl	Methyl chloride
DC-11	Silicone grease
EDTA	Ethylene diamine tetraacetic acid
EtOH	Ethyl alcohol
Epon 1001	Exoxy resin
GA	Gibberellin
GAs	Gibberellins
GLC	Gas liquid chromatography
HCl	Hydrochloric acid
HIEFF-8BP	Cyclohexane dimethanol Apipate
HMDS	Hexamethyldisilazane
IAA	Indolyl-3-acetic acid
IAAp	Indolyl-aspartate
ICA	Indolyl-3-carboxylic acid
ILA	DL-3-(3-indolyl)lactic acid
IPA	3-(3-indolyl)-propionic acid
IPA	6-(3-methyl-2-butenylamino)adenosine (pp. 207)
msIPA	6-(3-methyl-2-butenylamino)-2-methylthioadenosine
IpyA	Indolepyruvic acid
I	Iodine
Ibs	Inhibitors
K_2HPO_4	Potassium phosphate
KI	Potassium Iodide
KOH	Potassium hydroxide
LSD	Least Significant Difference
MAAW	Methyl acetate:Acetonitrile:Ammonium hydroxide:water
MeOH	Methyl alcohol
NAA	Naphthalene acetic acid
Na_2CO_3	Anhydrous sodium carbonate

$NaHCO_3$	Sodium bicarbonate
NaOH	Sodium hydroxide
Na_2SO_4	Anhydrous sodium sulphate
$Na_2S_2O_5$	Sodium pyrosulfate
NH_4OH	Ammonium hydroxide
OV-1	Methyl silicone
PYR	Pyridine
PVP	Polyvinylpyrrolidone
QF-1	50% trifluropropyl methyl silicone
SE-30	Methyl silicone
SE-52	Methyl silicone
TLC	Thin layer chromatography
TMCS	Trimethylchlorosilane
TNH_2	Tryptamine
TOH	Tryptophol
t-RNA	transfer ribonucleic acid
TTP	Tryptophan
UC-W98	Unknown % vinyl methyl silicone
V	Volume