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

A thesis submitted in partial fulfilment of the
requirements for the degree of Doctor of Philosophy

at

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Massey University
Palmerston North
New Zealand

Heung Sub Park

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

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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AbstractPart 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.

AbstractPart 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 ^3H -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 ^3H -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 ^3H -zeatin from ethyl acetate phase which was partitioned at pH 2.5. 80% recovery of ^3H -zeatin in the first 1,000 ml was obtained from Sephadex G-10 and Dowex 50 W x 8. 88.6% recovery of ^3H -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 ^3H -zeatin was studied in paper chromatography and cellulose, DEAE cellulose and silica gel, thin layer chromatography, about 82-60% of ^3H -zeatin the Rf of ^3H -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, GA₁, GA₃, GA₄, GA₅, GA₇, GA₉, GA₁₃ 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, GA₃ and GA₁ but reduced the ABA peak by half and the GA₉ 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)trifluoroacetamide |
| BuOH | n-butanol |
| CKs | Cytokinins |
| CHCl ₃ | Chloroform |
| CH ₃ Cl | Methyl chloride |
| DC-11 | Silicone grease |
| EDTA | Ethylenediamine 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 ₂ HPO 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 ₂ CO ₃ | Anhydrous sodium carbonate |

| | |
|---|-------------------------------------|
| Na HCO ₃ | Sodium bicarbonate |
| NaOH | Sodium hydroxide |
| Na ₂ SO ₄ | Anhydrous sodium sulphate |
| Na ₂ S ₂ O ₅ | Sodium pyrosulfate |
| NH ₄ OH | Ammonium hydroxide |
| OV-1 | Methyl silicene |
| PYR | Pyridine |
| PVP | Polyvinylpyrrolidone |
| QF-1 | 50% trifluoropropyl 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 |