THE EFFECT OF SOME SUBSTITUTED PHENOXYACETIC ACIDS ON THE RESPIRATION OF THREE SPECIES OF FERN

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CHAPTER I

INTRODUCTION.

The problem of control of weeds in agricultural land has long confronted man, and toxic chemicals have been employed for half a century in eradication or suppression of weeds. The last two decades have seen a tremendous expansion in the use and variety of applications, principally because of the development of translocated organic compounds of a growth regulatory type which very often are highly selective.

Some indication of the interest in chemical weed control, for example with the substituted phenoxyacetic acids, can be seen in the fact that in the U.S.A. alone over twenty million pounds of 2.4-D is manufactured annually. The volume of research has likewise been tremendous. Norman et al (1), reviewing the field in 1950 covered some three hundred and fifty new papers for the previous year on phytotoxicity.

The mechanism of action of growth regulatory type herbicides is an important problem, particularly in so far as it may throw light on the physiology of plant growth and development, and their control by plant hormones.

The general approach is still largely empirical, involving a high degree of speculation in the selection and synthesis of new compounds followed by trial and error tests in the laboratory, glasshouse and field. Detailed physiological studies have been limited and it is only by such study that the principles of toxic action can be laid down.

This study is an attempt to examine one small facet, the effect of plant regulators on respiration, of a complex and rapidly expanding field of endeavour.

(i) Purpose of the Study

The purpose of the study is to compare the actions of an homologous series of substituted phenoxyacetic acids on the endogenous respiration of three species of Pteridophyta. Experiments are designed to show the presence of differences or similarities between the respiratory levels of the three species of Pteridophyta, together with any significant changes induced by the application of the plant regulators.

The three substituted phenoxyacetic acids used were -

(a) Para-4-chlorophenoxyacetic acid. (4-CPA)
(b) 2,4 - dichlorophenoxyacetic acid. (2,4 - D)
(c) 2,4,5 - trichlorophenoxyacetic acid. (2,4,5 - T)

Of the three species of fern examined *Pteridium aquilinum* has an underground rhizome, *Paesia scaberula* and *Microsorium diversifolium* have an above ground rhizome. A brief description of their appearance and habit as described by Dobbie (2) are as follows.

(a) *Pteridium aquilinum var esculentum.* (Forst. f.)

Commonly called "Bracken Fern" or by the Maoris "Rahurahu". In the Genus Pteridium the rhizome possesses hairs but no scales. The rhizome has an underground habit of growth. After germination the primary axis bears about seven to nine leaves, then it forks and each shank burrows down into the soil. The shanks will fork again, unequally so that in a short time a single plant can cover a wide area while its rhizomes, being underground, are well protected. When the older parts rot beyond the area of branching two individuals are left. It thus multiplies principally by vegetative propagation. The rhizome is thick, creeping below the ground producing numerous scattered fronds. Stipes are variable in length, stout, rigid, erect, smooth and shining. Fronds are usually from two to six feet long, sometimes ten to twelve feet, stiff and harsh, green to reddish green, lighter below. Sori usually extending round entire margins of fertile segments. Outer indusium formed by reflexed leaf margin, inner often very poorly developed.

It is abundant everywhere in New Zealand except in dense forests. Found from sea level to four thousand feet.

(b) Paesia scaberula. (A. Rich).

Commonly called "Scented fern", "Lace fern" or "Hard fern". It belongs to a genus of terrestrial ferns with long creeping above ground rhizomes and finely cut fronds, usually with a zig-zag rachis. Sori long with true inner indusia, the outer being formed by the reflexed frond margin. The rhizome is wide creeping, rigid wiry and clothed with chestnut brown scales. Stipes four to twelve inches long, sinuous, rigid, erect yellow-brown, rough to the touch, more or less bristly. Fronds nine to eighteen inches high, rarely more, by four to nine inches broad, pale yellow-green, somewhat harsh to the touch. Midrib sinuous, rough. Both stipes and fronds are extremely hairy and glandular on both sides. The product of the glands gives the frond its characteristic stickiness. The sori are usually very copious when present. It grows on sunny banks to the exclusion of other plants. Found from sea level to two thousand five hundred feet.

(c) Microsorium diversifolium. (Willd) Synonomous with Polypodium diversifolium (Willd).

Sometimes called "Hound's Tongue". A broad leaved bright green species climbing over rocks and trees with a thick creeping above ground rhizome; very irregular in shape and size; the large round sori of a bright orange and most conspicuous. The rhizome is long, stout, creeping, often sea green in colour covered with black specks. Stipes jointed to the rhizomes two to eight inches long, stout, erect, smooth and glossy. Fronds numerous, scattered along the rhizome bright green, stiff and firm in texture, polished and shining, varying greatly in shape, sometimes three to nine inches long by one and a half to two inches broad, quite entire; sometimes six to eighteen inches long by three to nine inches broad deeply cut. Veins netted and conspicuous in young fronds. Sori numerous, large, round, orange-red, forming a single row on each side of the midrib between margins and midrib or sometimes nearer the former. Found from sea level to three thousand feet everywhere, in the shade or sun.

(ii) The Importance of the Problem

Pteridium aquilinum one of the most successful and widespread species of the family Pteridophyta, is a major weed in unploughable hill country regions of Northern England and Scotland, South Eastern Australia and throughout New Zealand, more particularly in the hill regions of the North Island. Up to the present time no successful method of eradicating it has been discovered for unploughable country. Cultivation of the soil appears to be the only effective method of providing control, by breaking up and exposing the network of underground rhizomes which enable the plant to recover from the very severest mutilation of foliage.

Chemical treatment has so far been unsuccessful. Contact weed-killers such as sulphuric acid and sodium chlorate are effective only in such high concentrations that their use over a large area is uneconomic. Braid (3) (4) and Bates (5) using sodium chlorate found that two to three tons per acre could effectively kill Bracken in localised areas but there was no evidence to suggest any translocation of the herbicide through the plant and deep seated rhizomes were apt to escape. Conway and Stephens (6) using 3-p-chlorophenyl-1, 1 dimethylurea (CMU), Egler (7) and Holly et al (8) using 2 methyl 4 chlorophenoxyacetic acid (MCPA), 2,4-D and 2,4,5-T acids together with mixtures of MCPA and 2,4-D, and 2,4D and 2,4,5-T at a wide range of concentrations and at a number of seasons of the year found, that although the fronds could in some cases be destroyed, the rhizomes in every case remained unaffected. Rhizomes and fronds developing later in the season or in the year following treatment were normal in appearance and did not differ in number or height from those on untreated areas.

A preliminary spraying trial was carried out in the upper Tiritea valley, near Palmerston North, in May 1954 (Autumn) on Pteridium aquilinum and Paesia scaberula using commercial formulations (Butoxyethanol-esters) of 4-CPA, 2,4-D and 2,4,5-T, in concentrations of two pounds, one pound and half a pound acid equivalents per acre. Circular plots four feet in diameter were sprayed and in the case of Paesia scaberula a ditch six inches deep was opened around the plots to separate treated and untreated rhizomes.

After four weeks Paesia scaberula showed moderate epinastic effects in young frond growth after treatments of 4-CPA, 2,4-D and 2,4,5-T at two pounds. AE/acre and 2,4-D and 2,4,5-T at one pound AE/acre. However the plants recovered and by July there was nothing to indicate any effect of the treatments.

Pteridium aquilinum showed an even smaller response. 2,4,5-T at concentrations of two pounds, one pound and one half a pound gave mild epinastic effects to the rachis and in some cases thickening of the stipe of the frond. Beyond this, treatment was of no significance and the plants rapidly recovered.

From these results a conclusion might be made that there is a high degree of resistance to attack by plant regulators in the growing regions of the rhizome of Pteridium aquilinum and that attack through the fronds is not sufficiently effective to ensure complete control of the plant.

Examination of the physiological factors involved in the successful application of plant regulators as herbicides reveals that the regulator must first enter the plant, be translocated to the site of action and then take place in a reaction or reactions which will ultimately bring about the death of the plant. In the particular cases of the three species of Pteridophyta ultimately examined, the fronds are quite heavily cutinised, more particularly Pteridium aquilinum. Norman et al (1) have shown that stomata appear to be important though not exclusive means of entry for oils particularly those of low surface tension; further that aqueous solutions do not ordinarily penetrate through open stomata. Diffusion through the cuticle is probably the usual means of entry.

The amount of regulator that enters is a function of the time and area of contact; both factors are related to the degree of wetting of the surface and the contact angles of discrete droplets (Fogg (9)). Paesia scaberula has a very rough frond surface and thus might be expected to provide a poor surface for entry of both aqueous solutions and oil formulations.

Non-polar molecules penetrate the cuticle (Lipoidal in nature) more readily than polar molecules. Weak acids in general are absorbed in the form of undissociated molecules rather than as ions, (9).

Several important physiological factors therefore control the entry of a plant regulator.

Once having successfully penetrated the plant, a herbicide may either act by rapidly killing the superficial cells thus preventing further spread and action unless simple diffusion takes place (very slow), or by acting more slowly, thus becoming more widely distributed. Substances entering via the root are transported by the transpiration stream. Transport from the leaf is with the products of photosynthesis (sugars). (Mitchell and Brown (10), Weaver and De Rose (11)).

If we assume that plant regulators can penetrate the cuticle of Bracken fronds and thus enter the translocatory stream it could be further assumed that they would then be carried to points of sugar storage or utilisation such as the rhizome or primary meristem of the rhizome. Does any further reaction take place at these sugar ⇌ starch metabolic centres?

In the case where Bracken foliage has been destroyed without apparently affecting the rhizome, the non-effectiveness may be due to the plant regulator never reaching the rhizome because the pathway of translocation is destroyed by direct herbicidal action of high concentrations of plant regulator within cells.

The other alternative is that if the plant regulator is applied in a low enough concentration to penetrate the cuticle, enter the translocatory stream without damaging cells and then penetrate to a site of action in the rhizome, it may be in such low concentrations in the rhizome as to be ineffectual.

By eliminating the two steps, (a) of entry and (b) translocation of plant regulators, any direct effects on the rhizome can be studied. It was with this aim in mind that the present study was carried out.

There are many interrelated processes which can be involved in a phytotoxic response to an applied plant regulator. Metabolism may be stimulated to the point where rapid growth and cell division deplete the plant of food reserves and photosynthesis is unable to supply enough energy for the plant to survive. Resistance of the plant to micro-organisms may be lowered and its death hastened by pathogens. On the other hand death may occur through direct inhibition of vital processes such as photosynthesis, respiration, water and salt accumulation.

Cell division, and changes in water uptake and growth generate a concomitant change in respiration. It was for this reason that a measure of respiratory rate was used to indicate any effects of the plant regulators on the rhizomes of the three species of fern studied.

(iii) Definition of Terms Used

The following three terms are used in agreement with a recent (12) conference on nomenclature of plant growth substances and denote:-

A. Plant regulators.

"Organic compounds other than nutrients which in small amounts promote, inhibit or otherwise modify any physiological process in plants." 4,CPA, 2,4-D and 2,4,5-T are therefore included in this definition.

B. Auxin.

"A generic term for compounds characterised by their capacity to induce elongation in shoot cells. They resemble Indole-3-acetic acid in physiological action. Auxins may and do affect other processes besides elongation, but elongation is considered critical. Auxins are generally acids with an unsaturated cyclic nucleus or their derivatives."

C. Anti-auxins.

"Are compounds which inhibit competitively the action of auxins."

Respiration. In this text, respiration refers to aerobic respiration as measured by changes in the level of oxygen uptake.

Phytotoxic concentrations of Plant regulators.

All concentrations which inhibit respiration (i.e. decrease oxygen uptake) are considered to be phytotoxic in the sense that normal death of tissue is accelerated.

Physiological concentrations of Plant regulators.

Denote all other concentrations used whether they have a stimulatory effect or none at all on tissue respiration.

Rhizome.

A stem, generally of root like appearance, having a diageotropic position, either above or below the ground (13).

Meristems. The definitions are those adopted by Eames and MacDaniels (14).

Promeristem.

The region of new growth in a plant body where the foundation of new organs or parts of organs is initiated. The remainder of the meristem represents the early stages of the tissues formed by the promeristem. No term exists for this partly developed region in which segregation of tissues is beginning but cell division continues freely.

Apical Meristem.

Initiation of growth is by one or more cells (a solitary cell in Pteridophytes) situated at the tip of the organ which maintain their individuality and position and are called apical initials or cells. Promeristem consists in part therefore of apical meristem.

Primary Meristems.

Those that build up the fundamental primary part of the plant and consist in part of promeristem. (There is no secondary development in ferns.)