and Food. This work was supported in part by the Lord Rank Research Centre, High Wycombe, Bucks., U.K.

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The Metabolism of 4-Chloro-2-Methylphenoxyacetic Acid in Plants

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The results described below were obtained from an investigation into the metabolism of the selective herbicide MCPA* in rape (*Brassica napus* var. *arvensis* var. Early Giant), a susceptible species, and in red campion (*Melandrium rubrum*) and peas (*Pisum sativum* var. Progress no. 9), two species that possess some resistance to the herbicide.

The presence of two ether-soluble metabolites, rigorously identified in peas as 4-chloro-2-hydroxymethylphenoxyacetic acid (hydroxy-MCPA) (Collins & Gaunt, 1970) and *N*-(4-chloro-2-methylphenoxyacetyl)-L-aspartic acid (MCPA-aspartic acid), was confirmed in rape and red campion by using radioactive-isotope-dilution analysis. A further ether-soluble metabolite, which was detected in peas, has not been identified.

A β-glycoside of hydroxy-MCPA was detected in all species and tentatively identified as 4-chloro-2-(β-D-glucopyranosidomethyl)phenoxyacetic acid. A β-linked sugar ester of MCPA was detected in rape and tentatively identified as 4-chloro-2-methylphenoxyacetyl-β-D-glucose. A further minor unidentified β-glycoside was detected in peas.

MCPA-aspartic acid, hydroxy-MCPA and the β-glucoside of the latter were synthesized and examined for auxin activity by using the *Avena* first-internode and pea third-internode bioassays. MCPA-aspartic acid showed growth-promoting effects similar to those of MCPA. Hydroxy-MCPA possessed some auxin activity at higher concentrations (10mg/l), but its β-glucoside was virtually inactive. After foliar applications to intact plants of each species hydroxy-MCPA was completely inactive. MCPA-aspartic acid, however, produced phytotoxic effects comparable with those produced by MCPA itself.

Foliar applications of ^{36}Cl- or *carboxy*-^{14}C-labelled MCPA were made to 21-day-old plants of each species and the distribution of MCPA and its metabolites was followed during the next 14 days. In rape about 50% of the label that entered the treated leaves was rapidly translocated to the rest of the plant. In peas and red campion translocation was markedly less extensive.

Differences were also evident in the patterns of metabolism in the three species. Rape showed conversion of MCPA, with MCPA-aspartic acid as the major product. In peas and red campion hydroxy-MCPA and the β-glycosides were predominant.

It seems probable that the differences in phytotoxicity of MCPA with these plants can be largely attributed to differential translocation and to a smaller extent to differential metabolism.

* Abbreviation: MCPA, 4-chloro-2-methylphenoxyacetic acid.

D.J.C. is indebted to Ranks–Hovis–McDougall for financial support. This work was in part supported by grants from the Agricultural Research Council and Imperial Chemical Industries Ltd.

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The Subcellular Distribution of Tocopherols in the Green Leaves of *Pisum sativum*

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Previous studies on the subcellular distribution of tocopherols in plant tissues (Bucke, 1969; Dilley & Crane, 1963; Lichtenthaler, 1966; Peake, 1970) have been restricted by the limited ability of differential centrifugation to resolve cellular organelles and by the low capacity of density-gradient centrifugation in conventional rotors. Also, the recoveries of tocopherols after homogenization and centrifugation have often been very low. This could be attributable to the destructive action of tocopherol oxidase, an enzyme found in many plant tissues (Barlow & Gaunt, 1968).

In the present work the zonal rotor has been used to achieve large-capacity density gradients. Effective inhibitors of tocopherol oxidase have been incorporated in homogenization and centrifugation media. Green-leaf homogenates were fractionated into three fractions in the zonal rotor: a nuclear fraction, chloroplast fraction and a mixture of mitochondrial, microsomal and supernatant fractions. The last was then subjected to differential centrifugation at 20000*g* and 100000*g* to separate the three parts. Each fraction was analysed for tocopherols and related isoprenoid lipids.

The recoveries of tocopherols from the homogenization and centrifugation procedure were as follows: α-tocopherol, 65%; γ-tocopherol, 82%; δ-tocopherol, 87%; α-tocopherolquinone, 66%.

The purity of each subcellular fraction was assessed by analysis of marker compounds and by

microscopy. Nuclear, chloroplast and mitochondrial fractions were almost completely resolved, as indicated by DNA, chlorophyll and ubiquinone distribution.

α-Tocopherol and α-tocopherolquinone were largely present in the chloroplasts (92% and 88% respectively). γ-Tocopherol occurred in significant quantities in all fractions, but was concentrated in chloroplasts (55%) and the microsomal fraction (24%). δ-Tocopherol was fairly evenly distributed among fractions: nuclear, 16%; chloroplast, 28%; mitochondrial, 17%, microsomal, 13%; supernatant, 26%.

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A Study of Mitochondrial Complementation in Wheat

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It has been reported that mixtures of mitochondria from various inbred lines of wheat occasionally exhibit oxidative and phosphorylative activities that are higher than those of mitochondria from either pure line, as also are the activities of mitochondria from hybrids of these lines (Sarkissian & Srivastava, 1969*a*). This phenomenon has been called 'mitochondrial complementation' (McDaniel & Sarkissian, 1966).

Attempts were made to confirm the published results of Sarkissian & Srivastava (1969*b*,*c*) by using their method for the very rapid isolation of mitochondria from varieties of wheat that, when crossed, showed heterosis. No success was achieved until a number of modifications were made to the procedure and very strict attention was paid to details of the method. A standard oxygen-electrode system was used to measure the oxygen consumption during the phosphorylation of known amounts of ADP by mitochondria isolated from each of the pure lines under consideration, and also from 1:1 mixtures of mitochondria from these lines selected in pairs and from the relevant hybrids, if available.

Measurements of mitochondrial activity in two varieties of wheat provided by the Plant Breeding Institute showed evidence of complementation, and the hybrid demonstrated positive heterosis with respect to yield of grain. One of these varieties also gave mitochondria that when mixed with particles from a third variety demonstrated negative complementation, and the hybrid showed negative heterosis. These results are judged to be sufficiently encouraging to warrant further investigations into the phenomenon of mitochondrial complementation and its relation with hybrid vigour.

This work forms part of a joint project between the Glasshouse Crops Research Institute and the Plant Breeding Institute, Cambridge. Specialized laboratory facilities at the University of Sussex were generously provided by Professor J. F. Sutcliffe.

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Allosteric Properties of Wheat-Germ Aspartate Transcarbamoylase

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Aspartate transcarbamoylase (EC 2.1.3.2), purified approx. 20-fold from commercial wheat germ, is inhibited by UMP; the enzyme is thus potentially a site for feedback regulation of the pyrimidine-biosynthetic pathway in the germinating wheat embryo. This and other features of the enzyme have been reported in a previous communication (Yon, 1971).

The same partially purified preparation has now been further characterized. Catalytic activity and sensitivity to inhibition by UMP are both pH-dependent. The most prominent feature of the pH–activity profile is a single sharp peak between pH 9.5 and 11.0, with a maximum at pH 10.5 of 0.11 μmol/min per mg of protein at 25°C. Elsewhere in the range of activity (which extends from pH 6.5 to 11.2) the activity is less than 0.04 μmol/min per mg of protein. In the range pH 6.5–7.5 the enzyme is insensitive to UMP. Above pH 7.5 sensitivity to UMP gradually increases to a maximum at pH 10.0.

Kinetic behaviour has been studied in detail at pH 10.0 and 25°C. In the absence of UMP initial-rate plots are hyperbolic for both substrates (L-aspartate and carbamoyl phosphate). In the presence of UMP kinetic plots for aspartate remain hyperbolic; the inhibition is non-competitive with respect to aspartate. However, in the presence of UMP plots for carbamoyl phosphate become sigmoidal. The degree of sigmoidicity is a function of the UMP concentration; the interaction (Hill)