Seed Dormancy in *Acer*:
Endogenous Germination Inhibitors and Dormancy in *Acer pseudoplatanus* L.

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Summary. Dormant seeds of *Acer pseudoplatanus* L. contain two zones of inhibition on paper chromatograms in “10:1:1” as detected by the lettuce and cress seed germination, and the wheat coleoptile bioassays. One zone at Rf 0.6-0.8 was partitioned into ethyl acetate at acid pH and was shown to contain ABA by its behaviour on GLC and isomerization under ultra-violet light. The other zone at Rf 0.9 was detected only in the germination bioassays and was partitioned into ethyl acetate over a range of pH indicating the presence of one or more neutral compounds.

The inhibitors present in the embryo of dormant sycamore seeds inhibited the germination of non-dormant sycamore seeds at relatively low concentrations. A comparison with the effects of application of exogenous ABA indicated that endogenous ABA could not solely account for the inhibitory activity of seed extracts, which appeared to be due partly to the presence of ABA and partly to that of neutral compounds present in the embryo. Leaching treatments that removed dormancy led to a decrease in the level of inhibitors present mainly in the basic fraction. The exogenous application of kinetin to dormant sycamore seeds increased germination whereas gibberellic acid had no effect. Similar responses were obtained with lettuce seeds inhibited by the basic fraction of dormant sycamore seeds.

It is suggested that an inhibitor-cytokinin interaction may be involved in the dormancy of sycamore seeds.

Introduction

Previous experiments on dormancy in seeds of *Acer pseudoplatanus* L. indicated that whole fruits and seeds with the testa intact require a period of chilling at 5°C before dormancy is broken whereas bare embryos germinate immediately at 20°C on moist filter paper without pretreatment (Webb and Wareing, 1972). Studies on the manner in which the testa imposed dormancy on the embryo indicated that restriction on oxygen uptake, water uptake, mechanical restriction of embryo enlargement and the presence of germination inhibitors in the testa are not limiting factors at the early stages of dormancy. However, results from leaching experiments suggest that dormancy is the result of the restriction by the testa to the outward diffusion of a germination inhibitor(s) present in the embryo (Webb and Wareing, 1972).
This paper describes experiments on the role of endogenous germination inhibitors in the dormancy of seeds of the sycamore maple.

Material and Methods

Seed Storage and Germination. Sycamore fruits were collected from single open-grown trees in the Botany Gardens, Aberystwyth, in November, 1969. The fruits were air-dried and stored in sealed containers at 2–5 °C.

Seeds were germinated on two layers of Whatman No. 3 filter paper moistened with 7 ml of distilled water in petri dishes at 20 °C. Protrusion of the radicle through the covering structures was used as the criterion for germination.

Extraction of Growth Regulators. Growth regulators were extracted by a modification of the methods of Hayashi and Rappaport (1962). The seed material was homogenized in a Virtis blender with cold redistilled 80% methanol (10 ml per gram fresh weight). The slurry was continuously stirred for 12 h at 2 °C and then filtered under vacuum. The residue was then re-extracted twice more with the same amount of methanol as before. The residue was dried at 80 °C for 24 h and the dry weight recorded. The combined methanol extracts were reduced to aqueous and frozen at -12 °C for approximately 18 h to precipitate chlorophyll. The aqueous fraction was thawed and centrifuged at 20,000 g for 30 min to remove suspended matter. The aqueous fraction was adjusted to pH 8.0 with 5% NaHCO₃ and partitioned 4 times with equal volumes of redistilled ethyl acetate. The bulked ethyl acetate fractions were dried over anhydrous Na₂SO₄ and reduced to a small volume. This constituted the “Basic” fraction. The aqueous fraction was then adjusted to pH 2.5 with 1.0 N HCl and partitioned as before with equal volumes of redistilled ethyl acetate. The bulked fractions were dried over anhydrous Na₂SO₄ and reduced to a small volume. This constituted the “Acidic” fraction with the “Aqueous” fraction remaining.

Chromatography and Bioassay. The partitioned extracts were strip loaded onto Whatman No. 1 chromatography paper and developed in a descending manner in the solvent system isopropanol:ammonia (sp g 0.88):water (10:1:1). The chromatograms were run 30 cm in the solvent, air-dried and cut into Rf zones for bioassay.

In some cases the extracts were tested for inhibitory activity using germination of non-dormant sycamore seeds as the bioassay. However, because of the difficulty in storing sycamore seeds for long periods in this state the germination of lettuce seeds var. Grand Rapids was used routinely for the bioassay of germination inhibitors. Fifty lettuce seeds were added to petri dishes containing the segment of the chromatogram to be tested. This was moistened with 1.0 ml of distilled water. The seeds were germinated under continuous light at 25 °C. Germination was recorded after 48 h. In all of the experiments reported the results are the means of at least two bioassays run at different times.

The germination of cress seeds var. Plain were also used to detect inhibitory activity of extracts. Fifty seeds were added to each dish with 1.0 ml of distilled water and placed in continuous light at 25 °C. Germination was recorded after 48 h.

In some experiments the extracts were assayed for inhibitory activity by means of the wheat var. Atle coleoptile “straight-growth” assay following the procedure of Bentley and Housley (1954).

Results

Germination Inhibitors in Dormant Sycamore Seeds

Previous work had shown that dormancy in sycamore fruits was the result of the presence of germination inhibitors in the embryo which