Since tryptophan might serve as a precursor for both neutral and acidic auxins, the amounts of tryptophan in unpollinated and pollinated ovaries were determined (Stokes *et al.* 1945; Difco Laboratories 1948; Nitsch 1951) at successive time intervals. Unfortunately, no precursor function can be ascribed to tryptophan due to the disparity, in absolute amounts, between it and the auxins. However, the results in Table 1 show that although there was a slight increase in tryptophan content in the unpollinated ovary tissue following anthesis, it was in the free or unbound form. In the interval between pollination and fertilization Lund (1956*b*) reports only a slight increase in total tryptophan content, most of it in the free form, whereas the increase in tryptophan following fertilization was entirely in the protein form. However, in the present experiments there was a greater increase in the protein tryptophan than in the free tryptophan, both before and after fertilization, indicating that in addition to effects on auxin production, the pollen is effective in stimulating amino acid metabolism and protein synthesis.

References

Bennet-Clark, T. A., and Kefford, N. P. (1953).—*Nature* **171**: 645.

Bentley, J., and Bickle, A. (1952).—*J. Exp. Bot.* **3**: 424.

Cartwright, P., Sykes, J. T., and Wain, R. L. (1956).—In "Chemistry and Mode of Action of Plant Growth Substances." (Ed. R. L. Wain and F. Wightman.) (Butterworths Scientific Publications: London.)

Difco Laboratories (1948).—"Media for the Assay of Vitamins and Amino Acids." Pamph. No. 128. (Difco Laboratories: Detroit, Mich.)

Lund, H. (1956*a*).—*Amer. J. Bot.* **43**: 562.

Lund, H. (1956*b*).—*Plant Physiol.* **31**: 334.

Muir, R. M. (1947).—*Proc. Nat. Acad. Sci., Wash.* **33**: 303.

Muir, R. M., and Hansch, C. (1953).—*Plant Physiol.* **28**: 218.

Nitsch, J. P. (1951).—*C.R. Soc. Biol., Paris* **145**: 1809.

Stokes, J., Guness, M., Dwyer, I., and Caswell, M. (1945).—*J. Biol. Chem.* **160**: 35.

DIFFERENTIAL SPECIFICITY EXHIBITED BY TWO GERMINATION INHIBITORS PRESENT IN *ECHIUM PLANTAGINEUM* L.*

By L. A. T. Ballard† and A. E. Grant Lipp†

The widespread occurrence of seed-germination inhibitors which come from sources external to seeds, and which apparently control germination by inhibiting embryo growth (mainly the radicle) once it has started (Moewus and Schader 1951; Mayer and Evenari 1952) is well appreciated. Examples of this class of inhibitor have been detected both in the juices or extracts of fruits or other structures derived from parts of flowers (Evenari 1949; Moewus, Moewus, and Schader 1951), and in vegetative structures (Konis 1947; Winter and Sievers 1952; Yardeni and Evenari

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1952; Bublitz 1953; Winter and Bublitz 1953). It is also recognized that they are largely non-specific, in that a given inhibitor may react differently against different seeds, as well as a given seed showing different sensitivity to different inhibitors (Evenari 1949; Koller 1955; Toole *et al.* 1956). It does not appear to have been explicitly stated that both these aspects of non-specificity may be observed in a single source. Evidence to this effect is presented in this communication.

TABLE 1

INHIBITION OF SEED GERMINATION BY WATER AND ALCOHOL FRACTIONS OF E. PLANTAGINEUM EXTRACT

Concentration of both fractions equivalent to 0·1 g dry weight of *E. plantagineum* per ml

Species	Inhibition* by:	
	Water Fraction	Alcohol Fraction
Echium plantagineum L.	++	±
Cucumis sativus L.	+++	±
Cynoglossum australe R.Br. var. *drummondii* (Benth.) Brand	+++	±
Triticum aestivum L. 'Koala'	+++	±
Brassica sp. 'Mustard Greens'	+++	+
Avena sativa L. 'Victory'	++	±
Lycopersicon esculentum Mill. 'Marglobe'	++	±
Lolium rigidum Gaud.	+	±
Trifolium subterraneum L. 'Mount Barker'	+	±
Lepidium hyssopifolium Desv.	±	±
Medicago tribuloides Desr.	±	±
Allium cepa L. 'White Pearl'	±	±
Daucus carota L. 'Chantenay'	+	+
Marrubium vulgare L.	+++	++
Brassica campestris L. 'Purple Globe'	+++	++
Phalaris tuberosa L.	++	++
Salvia verbenaca L.	+++	+++
Lactuca sativa L. 'Imperial 847'	+++	+++
Trifolium glomeratum L.	++	+++
Lepidium sativum L.	±	+++

* Germination of treated seeds as per cent. of control germination. +++, <25 per cent.; ++, 25–50 per cent.; +, 50–75 per cent.; ±, >75 per cent.

Experimental

Air-dried, ground aerial parts of *Echium plantagineum* were extracted overnight at room temperature with 80 per cent. ethanol and filtered. The extraction was repeated twice and the filtrates combined. The alcohol was removed under reduced pressure at a temperature not greater than 50°C, and the residue taken up in water. This *crude extract* was fractioned by passage down a column of activated carbon and "Hyflo Supercel", and the eluate and water washings combined to give a *water fraction*. The extruded column was boiled for approximately 1 min in 80 per cent.

ethanol, filtered, the alcohol removed under reduced pressure, and the residue taken up in water to give an *alcohol fraction*. Extracts or fractions to be chromatographed were applied as streaks to Whatman No. 1 filter paper and chromatograms developed in ethanol–water (80 : 20 v/v). The activities of extracts, fractions, and chromatograms were assessed by the germination responses of the seeds of species listed in Table 1. These were set out on filter paper moistened with distilled water, extract, or fraction, or on strips cut from chromatograms moistened with distilled

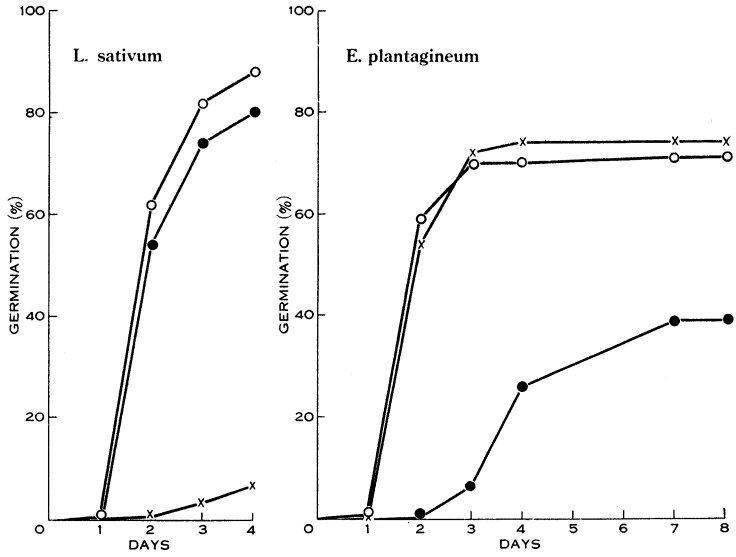

Fig. 1.—Germination of *E. plantagineum* and *L. sativum* seeds in distilled water (○), water fraction (●), and alcohol fraction (×), both fractions at a concentration equivalent to 0·1 g dry weight of *E. plantagineum* per ml.

water. The seeds of *Trifolium subterraneum* L. and *Medicago tribuloides* Desr. were incubated in the dark at 20°C, and the remainder at 25°C. Those of *Salvia verbenaca* L., after 2 days in the dark at 25°C, were transferred to artificial light at 20°C (8 hr per day at approximately 1500 f.c.).

Evidence for Two Inhibitors

From typical results presented in Figure 1 it is seen that the water fraction reduces germination of *E. plantagineum* to about half that of the water control, but has no significant effect on *Lepidium sativum* L. germination. The alcohol fraction behaves conversely.

On chromatograms of crude extract, an inhibitor of *E. plantagineum* is found at R_F 0·3, and of *L. sativum* at R_F 0·7–0·8 (Fig. 2). When chromatograms from both fractions are assayed separately, *E. plantagineum* is inhibited at R_F 0·3–0·4 in the water-fraction chromatograms and nowhere in those of the alcohol fraction; *L. sativum* is inhibited at R_F 0·7–0·8 in the alcohol-fraction chromatograms and nowhere in those of the water fraction.

Species Response

Differential sensitivity to the two inhibitors is indicated by the results of Table 1. Germination responses depend on external inhibitor concentration; at the concentration employed most species are like *E. plantagineum* in being more sensitive to the water-fraction inhibitor, and only *Trifolium glomeratum* approaches *L. sativum* in being more sensitive to the alcohol-fraction inhibitor. Others are either relatively insensitive, or equally sensitive, to both inhibitors.

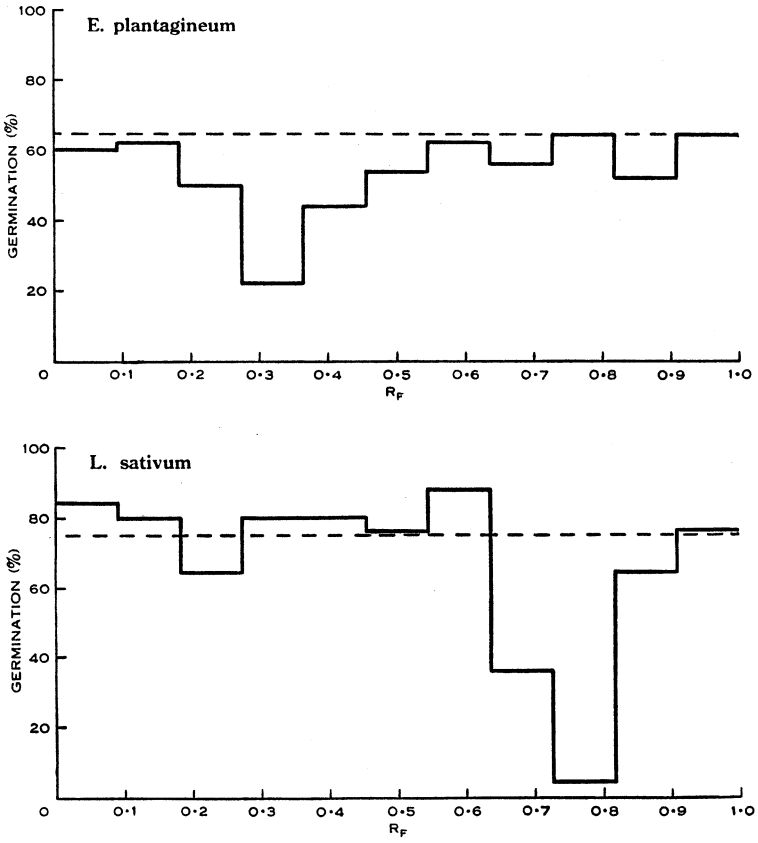

Fig. 2.—Germination on day 3 of test of *E. plantagineum* and *L. sativum* seeds on strips cut from chromatograms of crude extract. Broken lines represent mean germination on paper through which pure solvent had passed.

The inhibitors present in aerial parts of *E. plantagineum* are distinguishable chemically by their behaviour on carbon columns and on paper chromatograms, and biologically by the differential responses to them of seeds of a number of species. Both inhibitors are water soluble, stable to alcohol, and to temperatures of at least 50°C, and to prolonged storage at −10°C. They have not been further characterized.

References

BUBLITZ, W. (1953).—*Naturwissenschaften* **40**: 275–6.

EVENARI, M. (1949).—*Bot. Rev.* **15**: 153–94.

KOLLER, D. (1955).—*Bull. Res. Coun. Israel* **4**: 379–87.

KONIS, E. (1947).—*Palest. J. Bot.* J **4**: 77–85.

MAYER, A. M., and EVENARI, M. (1952).—*J. Exp. Bot.* **3**: 246–52.

MOEWUS, F., MOEWUS, L., and SCHADER, E. (1951).—*Z. Naturforsch.* 6B: 261–70.

MOEWUS, F., and SCHADER, E. (1951).—*Ber. dtsch. bot. Ges.* **64**: 124–9.

TOOLE, E. H., HENDRICKS, S. B., BORTHWICK, H. A., and TOOLE, V. K. (1956).—*Annu. Rev. Pl. Physiol.* **7**: 299–324.

WINTER, A. G., and BUBLITZ, W. (1953).—*Naturwissenschaften* **40**: 416.

WINTER, A. G., and SIEVERS, E. (1952).—*Naturwissenschaften* **39**: 191–2.

YARDENI, D., and EVENARI, M. (1952).—*Phyton* **2**: 11–16.