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Effects of environmentally hazardous chemicals on the emergence and early growth of selected Australian plants

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Abstract

The effect of soil-incorporated copper, tri-allate, and anthracene on the emergence and early growth of three Australian native species (*Banksia ericifolia*, *Casuarina distyla* and *Eucalyptus eximia*) and three crop species (*Avena sativa*, *Cucumis sativus* and *Glycine max*), was assessed using OECD Test Guideline 208. The crop species are sensitive species used in overseas phytotoxicity testing, and their responses were compared with those of the native species. Seeds were grown in pots in a glasshouse in a sandy loam soil at the chemical concentrations of 0, 10, 100, 1000 and 2000 mg kg^{-1}. LC50 and EC50 values were determined for each species. The most sensitive species was the monocotyledon *A. sativa*, while among the five dicotyledons *C. distyla* was most sensitive. All three chemicals delayed emergence and affected seedling growth. The results indicate that the conditions of the OECD Test Guideline can be met under Australian conditions, but that the Guideline requires modification for use with Australian native species.

Introduction

Various toxicity tests have been developed for the screening of chemicals, the tests being designed to assess their impact on the environment. Although most toxicity tests are aquatic (Thurston *et al.*, 1985), some tests for terrestrial systems have recently been developed. In particular, the Organisation for Economic Co-operation and Development (OECD) has developed guidelines for the screening of chemicals, which enable phytotoxicity of soil-incorporated substances to terrestrial higher plants to be assessed (OECD, 1984).

Overseas phytotoxicity data are very fragmented, even for the recommended crop test species (Fletcher *et al.*, 1985), and the published results are of unknown applicability under Australian climatic and soil conditions. Native species have been included in the project since there is virtually no information on the effects of any environmentally hazardous chemical on Australian native plants. The comparative sensitivity of test plants and Australian natives are unknown.

The aim of this project has been to test the applicability of OECD Test Guideline 208 to Australian plants and soils using copper, tri-allate and anthracene and, in addition, to investigate to what extent can one particular species be used as a surrogate for others in the Australian environment.

Materials and methods

The experiment was carried out in accordance with the OECD Test Guideline 208 (OECD, 1984), unless otherwise specified.

Test species

The test species were: *Banksia ericifolia* L.f. (heath banksia); *Casuarina distyla* Vent. (she-oak);

Eucalyptus eximia Schau. (yellow bloodwood); *Avena sativa* L. cv Coolabah (oat); *Cucumis sativus* L. cv Green Gem (cucumber); and *Glycine max* (L.) Merr. cv Forrest (soybean).

The native species were chosen because of their abundance in the Sydney region, their readily visible seeds and their capacity to germinate at any season. The crop plants chosen are locally used varieties of species for which some phytotoxicity data are available (Fletcher *et al.*, 1985).

Soil

The soil used was a sandy loam collected from a little-disturbed eucalypt forest at the Forestry Commission of New South Wales Research Centre at West Pennant Hills (near Sydney). The test soil had a silt/clay fraction of 12.4% (w/w), an organic matter content 2%, and pH 5.5.

Chemicals

The chemicals used were copper ($CuSO_4 \cdot 5H_2O$: 1% stock solution in distilled water), a heavy metal of known phytotoxicological significance (Merry *et al.*, 1986; Walsh *et al.*, 1972); tri-allate (S-2, 3, 3 -trichloroallyl diisopropylthiocarbamate: 1% stock solution in ethanol), a selective pre-emergent herbicide whose residues can accumulate in soil (Smith, 1971); anthracene (a polycyclic aromatic hydrocarbon: dissolved to appropriate concentrations in ethanol or acetone), accorded a high priority for investigation for its hazardous properties by the Australian Environment Council (SPCC, 1983).

Soil preparation

Soil was prepared in 5- or 10-kg batches with appropriate dilutions of chemicals added at the rate of 1 l per 5 kg soil and mixed for 20 minutes in a tumbler (Cubex International Shrinkage Tester, Floataire Ltd, England) of 50 l capacity, rotating at 60 rpm. Final concentrations were, respectively 0, 10, 100 and 1000 mg kg^{-1} air-dried soil, plus 2000 mg kg^{-1} for copper and tri-allate only, because at this concentration anthracene was not soluble. Before planting, the soil samples were dried for 24 hours, to allow evaporation of the solvent.

Test conditions

Batches of 10 seeds were planted in 100-mm diameter pots, with four replicates of each treatment, and arranged in a randomised complete block design in a glasshouse. In winter, warming trays were use to maintain the soil temperature at 23–25°C. In summer, when temperatures ranged from 23–35°C, heating trays were not necessary. Seeds were grown with supplementary light of 2800 lux intensity and with a 16 hour photoperiod. Pots were hand-watered once or twice a day with a mist-spray of distilled water, and emergent seedlings were counted daily.

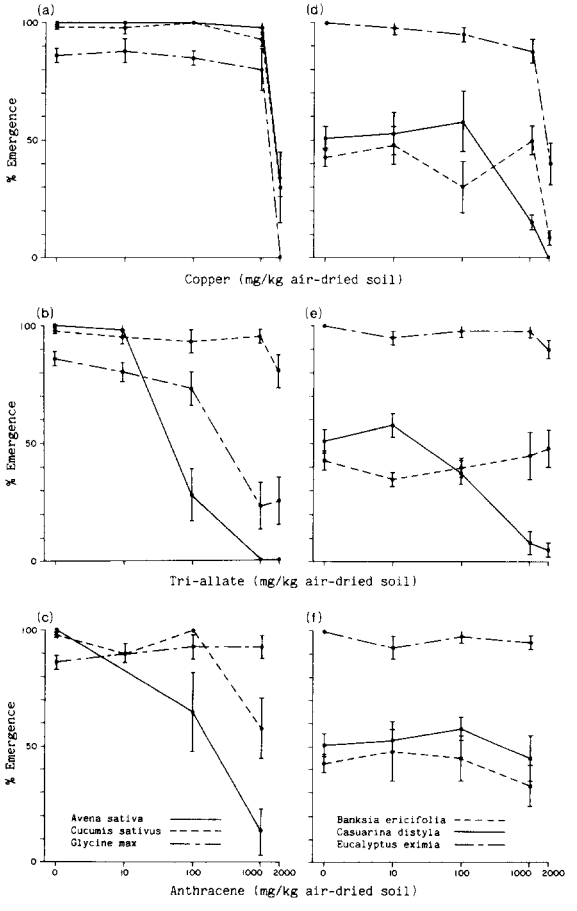

Fig. 1. The mean percentage emergence of crop species (**a**)–(**c**) and Australian native species (**d**)–(**f**) under increasing concentrations of test chemicals. *Vertical bars* represent standard error of the mean, n = 12 for controls, n = 4 for treatments.

Table 1. Estimated LC50 values of test species for each chemical with their 95% confidence limits. Values are expressed as mg chemical/kg air-dried soil

Species	Copper		Tri-allate		Anthracene	
	LC50 (mg/kg)	95% conf. limits	LC50 (mg/kg)	95% conf. limits	LC50 (mg/kg)	95% conf. limits
Avena sativa	1765	1575–1955	80	70–90	525	385–665
Cucumis sativus	1725	1210–2240	> 2000		> 1000	
Glycine max	1140	935–1340	1150	905–1395	> 1000	
Banksia ericifolia	1520	775–2270	> 2000		> 1000	
Casuarina distyla	580	395–770	655	435–875	> 1000	
Eucalyptus eximia	1845	1435–2255	> 2000		> 1000	

In accordance with OECD Guideline 208, plants were harvested two weeks after 50% of the controls had germinated, except for *B. ericifolia* and *C. distyla* where germination did not reach this level. The latter two species were grown for 32 and 56 days respectively. At harvest, shoots were cut as close as possible to the soil surface and weighed.

Definitions

The following definitions were used (OECD, 1984).

Emergence: The appearance of the seedling above the surface of the soil.

LC50: The concentration at which the change in emergence is 50% that of the controls.

EC50: The concentration at which the change in growth is 50% that of the controls.

The LC50 values were estimated by probit analysis and the EC50 values were estimated by linear regression of the plant fresh weight on chemical concentration (Baker and Nelder, 1978).

Results

Final emergence percentages

The final percentage emergence rates are shown in Fig. 1, and the estimated LC50 values in Table 1.

Copper reduced emergence in all species. The effect of tri-allate was more varied, with *A. sativa* more sensitive than any of the dicotyledonous species. This result was expected because tri-allate is used as a pre- and post-seeding herbicide for the control of weed grass species. The effect of anthracene was particularly variable, with *G. max* and *E. eximia* unaffected, while *A. sativa* was again the most sensitive species.

Delays in emergence

Delays in emergence are typical of a subtle toxic effect (Guderian *et al.*, 1985), and such delays may be the only perceptible effect of a chemical at intermediate concentrations. Figure 2 illustrates the effects of the three chemicals on the timing of seedling emergence.

A comparison of Fig. 1 with Fig. 2 reveals the trend that delays in emergence occurred at concentrations lower than those producing a major decline in germination rates, and may be used as an indicator of germination reduction.

Growth effects

The effect on post-emergence seedling growth is summarised as EC50 values in Table 2.

The three chemicals appeared to cause large reductions in growth in *A. sativa*. Among the dicotyledonous species, *C. distyla* and *C. sativus* were most affected, showing similar reductions in growth. The remaining three species were sensitive to copper and tri-allate. The much slower growth rates of the woody native species may mask early detection of phytotoxic effects on growth of seedlings.

Certain phytotoxic symptoms were observed in seedlings. These included: malformed cotyledons or coleoptiles; elongated petioles; severe hooking of hypocotyls; shoot apex not freed from testa; stunted growth and some chlorosis.

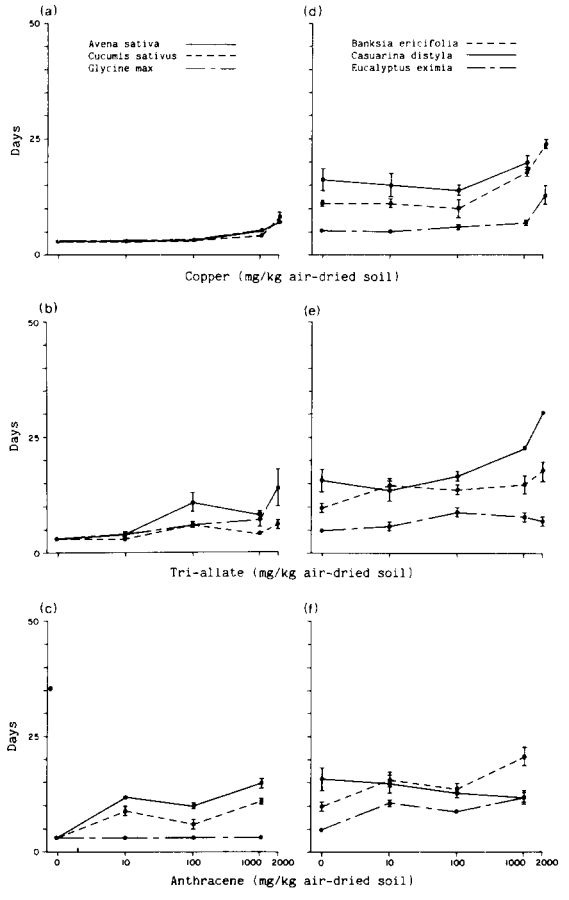

Fig. 2. Effect of test chemicals on time to first emergence for crop species (**a**)–(**c**) and Australian native species (**d**)–(**f**). *Vertical bars* represent standard error of the mean, n = 12 for controls, n = 4 for treatments.

Discussion

Test conditions

The results indicate that the conditions of Test Guideline 208 can be met in NSW. However, some modification needs to be made for Australian plant and soil conditions.

Test Guideline 208 requires an 80% final germination rate in the controls, and calls for harvest at least two weeks after the 50% emergence of controls, so as to provide results on early growth. This is a readily achievable requirement for crop varieties. The 80% germination requirement is impractical for many native species, however, because they exhibit variable dormancy (Mott and Groves, 1981; Richards and Beardsell, 1987) and other wide genotypic variability (Boland *et al.*, 1980; Turnbull and Doran, 1987) even though they may be sensitive to chemicals, as indicated by the results reported here for *C. distyla.* As neither *B. ericifolia* nor *C. distyla* attained a 50% germination rate in the control plants, the Guideline needs to be modified for the testing of Australian native species. The Guideline would similarly need to be modified for the testing of wild plant populations elsewhere in the world.

Phytotoxic symptoms

Tri-allate was the most phytotoxic of the three chemicals tested, as indicated by LC50 and EC50 values. Copper gave the most consistent and uniformly phytotoxic response, and is a non-specific phytotoxin. Anthracene caused the least reductions in growth but did, nevertheless, produce distinctive growth abnormalities and significant delays in emergence in all species except *G. max* and *C. distyla.*

A. sativa was found to be the most sensitive species overall. However, *C. distyla* was apparently at least as sensitive as *C. sativus*, which is regarded as the most sensitive dicotyledonous crop test spe-

Table 2. Estimated EC50 values of test species for each chemical with their 95% confidence limits. Values are expressed as mg chemical/kg air-dried soil

Species	Copper		Tri-allate		Anthracene	
	EC50 (mg/kg)	95% conf. limits	EC50 (mg/kg)	95% conf. limits	EC50 (mg/kg)	95% conf. limits
Avena sativa	535	425–655	3	2–5	30	20–45
Cucumis sativus	540	435–660	50	30–80	720	225–1655
Glycine max	550	370–765	415	275–580	> 1000	
Banksia ericifolia	610	220–1195	> 2000		> 1000	
Casuarina distyla	205	60–480	30	10–80	> 1000	
Eucalyptus eximia	560	440–700	2000	1280–2920	> 1000	

cies (Fletcher *et al.*, 1985). The other two native species, *B. ericifolia* and *E. eximia*, were slightly more resistant to the chemicals tested than the two crop species, *C. sativus* and *G. max*.

Scope of test

One of the aims of this project is to seek to find a suitable species for chemical screening assays, that is, the ideal surrogate plant for the Australian environment. To date, there is little information on this form of phytotoxicity testing, and no information on comparative sensitivity of Australian native and crop species.

Difficulties, also, arise because crop species have more uniform responses and generally higher germination rates than native species do. The genotypic variation among seed of a native species in the wild is enormous compared with that of a carefully bred crop variety. Furthermore, the provenance, seasonal conditions of production and collection, age of seed and plant health, can cause great variation in germination rates and can also be expected to affect the response of plants to toxicants as well.

The inclusion of native species in any Australian phytotoxicity testing is essential. Since this is the first attempt at assessing native species, a much larger database needs to be established before ecotoxicological assessments can be made, and any species confidently used as a surrogate for Australian plants.

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