VARIATION OF WHEAT AND SUBTERRANEAN CLOVER CULTIVARS IN SUSCEPTIBILITY TO VULPIA RESIDUE PHYTOTOXICITY

Min An, **J. E. Pratley**, T. Haig, and P. Jellett

Centre for Conservation Farming, Charles Sturt University, Wagga Wagga, NSW 2678

Summary. The germination and seedling growth of six cultivars of subterranean clover and wheat were tested in the presence of aqueous extracts of vulpia dry matter residues. Significant differences in tolerance were obtained among cultivars of both species. The order of increasing susceptibility of cultivars was: for wheat, Ford, Darter, Rosella, Dollarbird, Janz and Vulcan; for subterranean clover, Trikkala, Seaton Park, Karridale, Clare, Woogenellup and Junee.

Introduction

It is documented that phytotoxicity of plant residues on crops, pastures, vegetables and weeds is genotype-specific (4, 8, 12). Such selective toxicity reflects the differential inherent resistance of different genotypes exposed to plant residues (6, 9).

Vulpia (*Vulpia* spp.) is a component of many poor quality pastures in the winter rainfall areas of southern Australia and can severely reduce grain yields of winter crops through its allelopathic effects (10, 11). Such effects are variable, due to environmental effects and differential species susceptibility. This report investigates the variation in cultivar response within subterranean clover and wheat as a means for managing allelopathy of vulpia through cultivar selection.

Materials and Methods

A Petri dish bioassay procedure to measure the responses by test plants to a prepared aqueous extract from vulpia residue was employed to investigate the susceptibility of cultivars of wheat and subterranean clover to vulpia residues.

Test cultivars. Six cultivars for each of wheat (Vulcan, Dollarbird, Janz, Rosella, Ford and Darter) and subterranean clover (Trikkala, Clare, Junee, Seaton Park, Woogenellup and Karridale) were chosen for susceptibility tests.

Preparation of aqueous extracts. *V. myuros* residues were collected at Wagga Wagga, oven dried at 40oC for 72 hr for storage in dry conditions. The residues were ground in a Wiley mill to pass a 0.1 mm screen. An aqueous extract was prepared by soaking 100 g ground vulpia residues in a 2L Erlenmeyer flask with 1000 mL distilled water for 5 days in the dark at 20oC. Extracts were filtered and squeezed through two layers of cheesecloth, filtered by vacuum filtration on a Buchner funnel with a #1 Whatman filter paper and centrifuged for 30 min at 3900 rpm. The supernatant was decanted. The residues were re-extracted by adding a further 500 mL distilled water and soaking for one hour. The supernatant from this was recovered as before and added to the first filtrate. The two extracts were mixed and several dilutions made, ie. full strength (100%), 0.01, 1, 10, 25, 50, and 75% dilutions (% v/v).

Bioassay. Bioassay was carried out in 9 cm plastic Petri dishes lined with one #1 Whatman filter paper. Every dish contained twenty seeds of the appropriate cultivar. The seven concentrations of the extract, ie. full strength, 75, 50, 25, 10, 1, 0.01 (% v/v) were added in separate Petri dishes. Five mL of each concentration were used for wheat, and 4 mL for subterranean clover. Control treatments received the same volume of distilled water as for extract volumes. The Petri dishes were kept in a dark incubator at 24oC. All treatments were arranged in a randomised complete block design with three replications. To eliminate the possible effect of time lag and seed quality itself on germination homogeneity, seed germination counts were recorded 48 hr later. At this time at least 50% of seeds in the control had germinated. Seeds were considered germinated if the radicle had emerged 2 mm from the seed coat.

Subsequently, the radicle and coleoptile or hypocotyl lengths (mm) of germinated seeds were measured. All data were expressed as percentage of the control.

Data analysis. The inhibition area was calculated using MicroOrigin software over the range of treatments between plant response of the control (ie. 100 %) and the phytotoxicity curve for each cultivar (Fig. 1). Thus

$$\text{Inhibition area} = 100\int_{CT}[100 - f(C)]\, dC$$

where C is the extract concentration in % v/v, CT is the threshold concentration for inhibition. Comparison were then made based on the concept of "inhibition index" which was defined as the percentage of the maximum area inhibited. Thus:

$$\text{Inhibition index} = \frac{\text{Inhibition area}}{100\int_{0}^{100} dC} \times 100$$

Large index values indicate cultivars to be sensitive, whilst small values indicate tolerance. The inhibition index data were subjected to analysis of variance using MicroStat. Significant differences among cultivars were tested at the 5% level using Duncan's multiple range test.

Principal components analysis (5) was also used to assess the overall response of a cultivar to vulpia residue extracts. The inhibition indices for germination and seedling growth for each cultivar were combined into a weighted average of germination and root inhibition indices, which was computed using the percentage of germination and root inhibition in the first principal component.

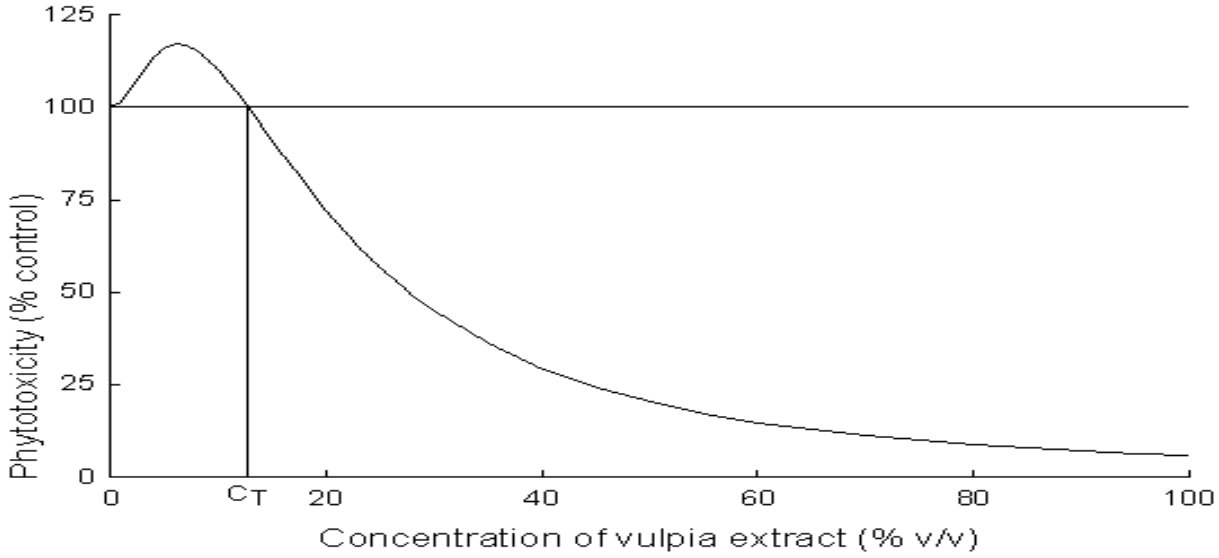

Figure 1. Diagrammatic representation of data analysis for cultivar response to extract concentrations. The shaded section represents the inhibition area for a cultivar in response to vulpia extracts. CT is the threshold concentration for inhibition.

Results

Both seed germination and root growth of all cultivars were significantly affected by the vulpia extract (Fig. 2). All cultivars showed characteristic responses to vulpia extracts, ie. inhibition at high concentrations,

stimulation or no effect at low concentrations (1). Analysis of variance revealed significantly different inhibition responses between cultivars (Table 1). For germination of wheat, Ford was the most sensitive, while Rosella was the most tolerant, other cultivars were intermediate in response. For germination of subterranean clover, little or no significant variation among cultivars were found. Ford and Junee seeds were particularly sensitive to residue extract at the full strength concentration. In contrast to seed germination there was some exchange of tolerance positions among cultivars in seedling responses. For example, Ford wheat was ranked as tolerant in terms of seedling inhibition, but its germination response ranked it as sensitive. In general, seed germination was less inhibited by vulpia extract than root growth. The order of susceptibility of germination and seedling growth responses in subterranean clover were more consistent than those in wheat. In the analysis of the overall effect marked differences occurred between cultivars. Ford wheat, and Trikkala subterranean clover were the most tolerant, while Vulcan wheat, and Junee subterranean clover were the most sensitive. Other cultivars were intermediate.

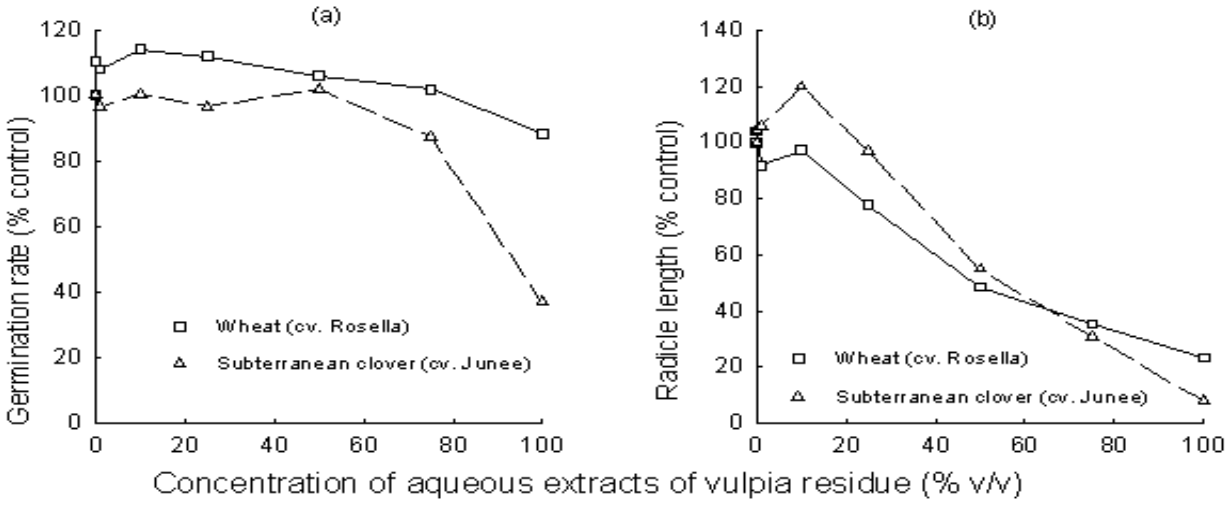

Figure 2. Representive germination and radicle responses of cultivars to aqueous extracts of vulpia residues. (a) Cultivar germination; (b) radicle elongation.

Table 1. Susceptibility of wheat and subterranean clover cultivars to vulpia toxicity as measured by the inhibition index

			Inhibition index*		
	Sensitivity	Cultivar	Germination	Root	Overall**
Wheat	Tolerant	Ford	26.9b	27.9 a	27.8 a
		Darter	6.4a	36.6 b	32.2 ab
		Dollarbird	4.8a	41.2 bc	35.9 b
		Rosella	1.5a	44.2 bc	38.0 b

		Janz	4.1a	48.1 cd	41.7 bc
	Sensitive	Vulcan	11.0ab	53.5 d	47.3 c
Subterranean	Tolerant	Trikkala	9.0 a	9.3 a	9.3 a
clover		Seaton Park	15.8 a	31.2 b	28.9 b
		Karridale	9.0 a	34.7 bc	31.0 bc
		Clare	16.1 a	39.1 c	35.7 bc
		Woogenellup	16.1 a	39.3 c	35.9 bc
	Sensitive	Junee	12.8 a	41.9 c	37.6 c

* Means identified in the same column by the same letter are not significantly different at the 5% level, Duncan's new multiple-range test;

** Weighted average of germination and root inhibition indices. The weight is 14.6% for the germination, 85.4% for root.

Discussion

Germination can be inhibited and delayed by allelopathic effects (2, 16). It has been consistently documented that seed germination is less sensitive than seedling growth to plant residue toxicity (3, 8). It is also common that the order of the germination response is different from seedling responses (8, 9, 13). Therefore, to comprehensively assess plant tolerance, consideration of all processes measured is desirable. Principal component analysis used in this study showed that the inhibition area was highly correlated (r2 is in the range 0.87 to 0.96) to the first principal component of the response profiles (Fig. 1), indicating that it is a statistically efficient summary of the response profiles (2).

The results presented here indicate differential susceptibility of cultivars within species to extracts from vulpia dry matter residues. Pederson (1985) reported that genotypes of white clover (*Trifolium repens* L.) showed marked differences in tolerance to leaf extracts of tall fescue in terms of their germination and seedling growth. Similar findings were also reported by other researchers with different test plant species and plant residues (4, 15) suggesting that such responses are genetically based (7, 9). Selection of tolerant cultivars to compete with vulpia in the field may be a realistic management strategy and needs to be further investigated. The findings of this experiment are sufficiently aligned with those of others to indicate that the bioassay techniques and subsequent data analyses are useful as a screening methodology for assessing allelopathy.

Acknowledgments

The financial support of the Grains Research & Development Corporation is gratefully acknowledged. Vulpia residues were provided by Dr. A. Leys, wheat seeds by Dr. Fisher, Agricultural Research Institute, Wagga Wagga.

References

1. An, M., Johnson, I. R. and Lovett, J. V. 1993. J. Chem. Ecol. 19 (10), 2379-2388.

2. An, M. 1995. Ph.D. thesis, Charles Sturt University, Wagga Wagga, NSW.

3. Barnes, J. P. and Putnam, A. R. 1987. J. Chem. Ecol. 13 (4), 889-906.

4. Dusky, J.A., Bewick, T. A. and Shilling, D. G. 1994. Allelopathy Journal 1 (2), 15-19.

5. Mardia, K.V., Kent, J.T. and Bibby, J.M. 1979. Multivariate Analysis (Academic Press), London. pp. 213-254.

6. Mersie, W. and Singh, M. 1987. Plant and Soil 98, 25-30.

7. Narwal, S.S. and Gupta, K.1992. In: Allelopathy in Agroecosystems (Eds. P. Tauro and S.S. Narwal), India. pp. 25-28.

8. Nielson, K.F., Cuddy, T.F. and Woods, W.B. 1960. Cana. J. Plant Sci. 40, 188-197.

9. Pederson, G.A. 1985. Proc. XV Int. Grasslands Congr., Kyoto, Japan, 323-324.

10. Pratley, J.E. 1989. Proc. 5th Aust. Agronomy Conf., Perth, WA. pp. 472.

11. Pratley, J.E. and Ingrey, J. D. 1990. Proc. 9th Aust. Weeds Conf., Adelaide, 436-439.

12. Read, J.J. and Jensen, E.H. 1989. J. Chem. Ecol. 15, 619-628.

13. Sahid, I.B. and Sugau, J.B. 1993. Weed Sci. 41, 303-308.

14. Steel, R.G.D. and Torrie, J.H. 1981. Principles and Procedures of Statistics, 2nd edition (McGraw-Hill Book Co.), Auckland. 633 pp.

15. Weston, L.A., Burke, B.A. and Putnam, A.R. 1987. J. Chem. Ecol. 13(3), 403-421.

16. Williams, R.D. and Hoagland, R.E. 1982. Weed Sci. 30, 206-212.