

## Characterisation of Water-Soluble Phytotoxins from *Vulpia* spp. Residues

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### Abstract

*Vulpia* residues contained water-soluble materials that were toxic to germination and to seedling growth of wheat. A colorimetric method was employed to characterise the phytotoxicity of those water-soluble compounds in *vulpia*. A significant linear correlation was found between *vulpia* extract strength and the phytotoxins measured, and a characteristic relationship between extract concentration and extract phytotoxicity. This procedure can be used as a complementary index to characterise *vulpia* toxicity.

### Key words

*Vulpia*, phytotoxicity, phenolics, weeds.

### Introduction

*Vulpia* (*Vulpia* spp.) has become an increasingly important weed among pastures and cereal crops across temperate Australia. Previous work has found that *vulpia* possesses a strong allelopathic potential, and that its residues contain water-soluble materials which are toxic to the germination and seedling growth of wheat (1). This phytotoxicity is concentration dependent. Further investigations on the chemical basis for such *vulpia* toxicity are warranted for a better understanding of *vulpia* allelopathy, and for developing management strategies, both to minimise *vulpia* damage and to bring *vulpia* under control.

Previous work has found that phenolics are the most likely phytotoxic constituents of the aqueous residue extract (2), a specific colorimetric procedure was employed as a crude step to characterise this phytotoxic group responsible for *vulpia* toxicity.

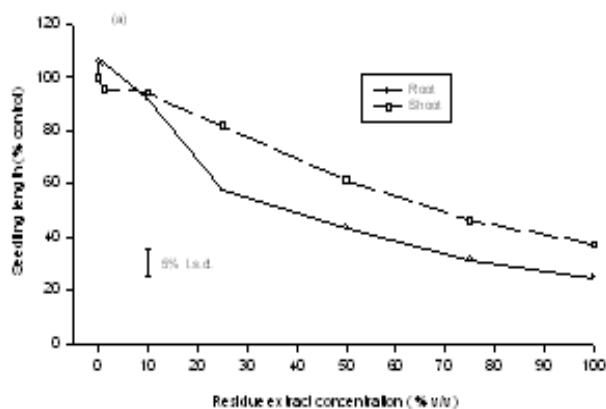
### Methods and Materials

An aqueous extract was prepared by soaking 100g of ground *vulpia* above-ground residues in 1000mL of distilled water for 5 days in the dark at 20°C. The liquid was decanted, filtered and centrifuged. The residues were re-extracted by adding another 450mL distilled water and soaking for a further one hour. The supernatant was again recovered following the same procedure as before, and then added to the first filtrate. A series of aqueous concentrations was then made by diluting the extract, viz. full strength (100%), 0.01, 1, 10, 25, 50, and 75% dilutions (% v/v), and used for bioassay. A seedling elongation bioassay was applied by using wheat (cv. Vulcan) as the test plant (1).

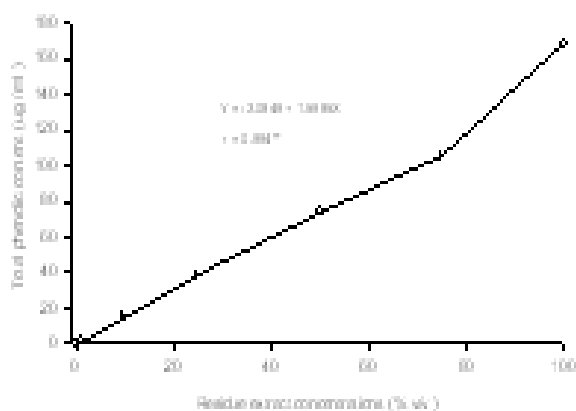
Total phenolic contents of the above aqueous extracts were estimated by colorimetric procedures based on the Folin-Ciocalteu reagent (3), which was obtained from Sigma & Aldrich Australia. Five mL of 0.01, 1, 10% treatment solutions, and 0.5mL of full strength (100%), 25, 50, and 75% treatment solutions were placed in test tubes, and 5mL and 9.5mL distilled water were added, respectively. Three mL 20% Na<sub>2</sub>CO<sub>3</sub> solution (w/v) was added to each tube, followed by 1mL Folin-Ciocalteu reagent. The solutions were mixed well, allowed to stand for 1hr at room temperature (20-25°C), and the absorbance read at 750nm in a spectrophotometer against a distilled water blank. The total phenolic substances in aqueous extracts were calculated from the calibration curve prepared from solutions of 1, 2.5, 5, 7.5, and 10 µg / mL vanillic acid.

### Results and discussion

Aqueous extracts of the vulpia residues exerted typical allelopathic effects on the wheat plants, ie. severe toxicity at high extract concentration, and low toxicity (even stimulation) at low content (Fig.1). There was a significant linear correlation between extract concentration (X) and total phenolic content (Y) in the extract (Fig. 2). Such a highly significant linear relationship, coupled with the results of Figure 1, indicated that the phenolics were the responsible agents for the allelopathic effects of vulpia residue on wheat. Phenolic compounds are well-known potential phytotoxins and exist as free forms, as esters, or as glycosides when combined with sugars, and are water soluble (4). The total phenolic content at full strength extract (100% v/v) constituted 0.1706% dry weight of vulpia residues.



**Figure 1. Dose responses of wheat to vulpia toxicity.**



**Figure 2. The concentration of aqueous residue extracts and total phenolic levels.**

## Conclusion

It is concluded that the measurement of total phenolics in vulpia residue extract by this colorimetric procedure can be used as a complementary index to characterise the phytotoxicity of certain water-soluble compounds in vulpia, and to provide useful information for further individual phytotoxin analysis by more searching analytical techniques.

## Acknowledgments

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## References

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