Allelopathy Journal 24 (1): 67-76 (2009)
Table: -, Figs : 5

0971-4693/94 US $ 5.00
International Allelopathy Foundation 2009

Allelopathic assessment of annual ryegrass (*Lolium rigidum*): Bioassays

R. AMINI, M. AN[*1,2], J. PRATLEY[2] and S. AZIMI[3]

Department of Agronomy and Plant Breeding,
College of Agriculture, University of Tabriz, Tabriz.-51666-16471. Iran.
Email: ramini58@yahoo.com , man@csu.edu.au

(Received in revised form: May 20, 2009)

ABSTRACT

A laboratory-based root exudates bioassay was conducted to assess the allelopathic potential of annual ryegrass against wheat using the equal- compartment-agar method. The allelopathic effects of different growing times of ryegrass (2, 4, 6, 8, 11 and 15 days) was investigated on wheat. Results indicated that co-growth of annual ryegrass and wheat reduced the root and shoot length of wheat. Ryegrass growth for 6-8 days was most inhibitory to wheat root growth. Increasing ryegrass density beyond 20 plants per beaker did not increase the allelopathic activity of annual ryegrass. To validate the allelopathic effects between the ryegrass and wheat, activated charcoal was added to the growth medium (2 % v/v), it increased the inhibition of wheat root and shoot length. Thus, the inhibitory effect of annual ryegrass on wheat root and shoot length was chemically directed through its root exudates, inhibition was greater on wheat roots than on wheat shoots.

The allelopathic effect of ryegrass root exudates was evaluated on root and shoot growth of four wheat cultivars, (WW14192, Ventura, Sunco and Janz). The allelopathic effect of ryegrass differed on root and shoot growth of wheat cultivars, the greatest effect was on root and shoot growth of cultivar WW14192. However, the trends were similar for all cultivars.

Key words: Activated charcoal, allelopathy, annual ryegrass, bioassay, *Lolium rigidum*, root exudates, *Triticum aestivum*, wheat,

INTRODUCTION

In allelopathy, plants provide themselves with a competitive advantage by releasing the phytotoxins into the nearby environment (29). Allelopathy occurs through the release of chemicals from one plant specie affecting other species growing in its vicinity, usually to their detriment (3). Allelopathy has potential for integrated weed management (5,14,24,25,30,31). Allelochemicals involved in this phenomenon are released through volatilization, leaching from leaves, degradation of plant residues and root exudation (28). Root exudates are one of the largest direct inputs of plant chemicals into the rhizosphere environment, and therefore root exudates also likely the largest source of allelochemical inputs into the soil environment (8). Recent achievements to understand the genetic control and transfer of crop allelopathy for weed management have been reviewed (4,36).

* Correspondence author, [1] Environmental and Analytical Laboratories, Faculty of Science, Charles Sturt University Locked Bag 588, Wagga Wagga, NSW 2678, Australia. [2]EH Graham Centre for Agricultural Innovation, Charles Sturt University, Locked bag 588, Wagga Wagga, NSW 2678, Australia.[3] Department of Plant Protection, College of Agriculture, University of Tehran, Karaj, P.O. Box 4111, Tehran,

The annual ryegrass (*Lolium rigidum*) is major winter weed in southern Australia. Its infestation at 200 plant/m^2 caused 20-50 % yield loss in wheat, in a strongly competitive cultivar and the yield loss was more with poorly competitive one (36). San Emeterio *et al.* (32) evaluated the allelopathic potential of L. *rigidum* on L. *multiflorum* Lam., *Dactylis glomerata* L., and *Medicago sativa* L. Germination was less sensitive to allelopathy than seedling growth. Seeds of L. *rigidum* adversely affected the seedling growth and development of three species, but especially that of Italian ryegrass (L. *multiflorum*). Shoot extracts of L. *rigidum* mostly stimulated shoot growth. Although some studies have reported stimulatory effects on shoot growth (23,34), but most studies show inhibitory effects (30). Stimulatory effects at low concentrations of allelochemicals may become inhibitory at higher concentrations (20, 23). *Chenopodium murale* root exudates also reduces the wheat growth in agar medium. The root and shoot length was reduced by 44 % and 32 %, respectively, whereas, seedling weight was reduced by 52 % (6). Several fine fescue species (*Festuca* spp.) produced detectable amounts of m-tyrosine (nonprotein amino acid) as the major active component in root exudates that had phytotoxic effects on other species (7).

The use of crop cultivars with elevated allelopathic activity could reduce the need for commercial herbicides in early season application, while in late season, weed control may be provided by the crop competitiveness. Allelopathy is a two-way interaction, therefore, ryegrass allelopathic effects on wheat are through release of allelochemicals and thereby affecting the wheat establishment.

A screening bioassay method, the 'equal-compartment-agar method' (ECAM), has been developed to assess the wheat seedling allelopathy on ryegrass (35). We used this method to evaluate the allelopathic effect of ryegrass on wheat cultivars by determining the relative tolerance of cultivars to allelochemicals released by ryegrass. This study aimed to evaluate the chemical interaction of root exudates between annual ryegrass and wheat seedlings and to compare four wheat cultivars challenged by annual ryegrass.

MATERIALS AND METHODS

Cultivars for testing

In this study, three experiments were conducted during November 2007 to April 2008. Test wheat cultivars were: WW14192, Ventura, Sunco and Janz, obtained from the Australian winter cereals collection. Ryegrass seeds were provided from commercial sources. Wheat and ryegrass seeds were surface sterilised by soaking in 2.5% sodium hypochlorite solution for 15 min followed by 5 rinses in sterilised distilled water. The surface-sterilised seeds of wheat and ryegrass were incubated in light at 25°C for 48 and 72 h, respectively, to germinate. Germinated seeds were used for bioassay experiments.

Experiment 1. The effects of ryegrass growing time

The ECAM (Equal Compartment Agar Method) developed by Wu *et al.* (35) was used to evaluate the allelopathic potential of annual ryegrass against wheat. Twelve pre-germinated ryegrass seeds were uniformly selected and sown on the aseptic agar surface with the embryo upwards, in 3 rows (1cm apart) on one-half of a glass beaker (7.5 cm dia and 12 cm depth, 500 ml) prefilled with 30 ml of 0.3% water agar. The beakers were sealed with parafilm and kept in a controlled growth cabinet [light/dark 13 h/11 h and 25°C /13°C.]

The fluorescent light intensity in the cabinet was $3.56 \pm 0.16 \times 10^3$ lux. After the growth of ryegrass seedlings for 2, 4, 6, 8, 11 and 15 days, 12 pre-germinated seeds of wheat were transplanted on the other half of the agar surface in 3 rows. A piece of pre-autoclaved white paperboard was inserted across the centre and down the middle of the beaker with the lower edge of the paperboard kept 1 cm above the agar surface. The entire beaker was thus divided into 2 equal compartments, to separately grow the wheat and ryegrass seedlings. Competition above the agar surface between wheat and ryegrass was thus avoided by confining plants within their own compartment. However, the roots of ryegrass can freely enter the wheat compartment and thus any allelochemicals produced and released by ryegrass seedlings can diffuse throughout the entire agar medium to affect the wheat growth. After wheat sowing, the beakers were again wrapped with parafilm and placed back in the growth cabinet for continuous growth of further 10 days. The growth of wheat alone was used as the control.

In Experiment-II, ryegrass was grown for 2, 4, 6, 8, 11 and 15 days and then removed from the beakers. Thereafter 12 pre-germinated wheat seeds were transplanted into the beakers and grown for 10 days alone without ryegrass. Wheat seedlings without ryegrass pre-treatment were used as control.

Experiment 2. The effects of activated charcoal

To determine the allelopathic effect between the ryegrass and wheat, 2% (v/v) activated charcoal (0.140 gr/beaker) was added to the agar in the beakers. Activated charcoal absorbs organic compounds such as allelochemicals and therefore, the allelopathic effect if present will be reduced where activated charcoal is added to water agar medium. Different densities of ryegrass seeds used were 4, 8, 12, 16, 20, 24, 35, 50, 70 and 100 plants per beaker. The germinated ryegrass seeds were transplanted into the beakers at mentioned densities. Also the germinated seeds of ryegrass were transplanted into the beakers at the same mentioned densities without adding activated charcoal to the agar in the beakers as no charcoal treatment. All germinated ryegrass seeds (transplanted at different densities in the beakers with and without adding activated charcoal) incubated for seven days under the conditions previously mentioned. Seven days after incubation, 12 pre-germinated wheat seeds were added to all the beakers and incubated for another 10 days.

Experiment 3. Allelopathic effects of ryegrass on different wheat cultivars

The four test cultivars of wheat were pre-germinated and transplanted into beakers that had previously grown ryegrass for 7 days at densities of 4, 8, 12, 24 and 35 plants per beaker.

Experimental design and measurements

The experiments were arranged in a randomised complete block design with 3 replicates. After 10 days of co-growth of ryegrass with wheat in the growth cabinet, the longest root and shoot length of wheat seedlings were measured.

Statistical analysis

Experimental data were subjected to analysis of variance using MSTATC and Minitab and the treatment means were tested separately using standard error. The data that were used in ANOVA met the assumptions such as normality and homogeneity of variance and did not require transformation.

RESULTS AND DISCUSSION

Experiment 1. Ryegrass growing time

Wheat root length

After the removal of ryegrass from the agar medium, wheat root length was significantly reduced, due to the chemicals present in the agar (Figure 1). Ryegrass growth for 2 days significantly decreased the wheat root length and the inhibition increased with ryegrass growing time up to maximum inhibition at 11 days. Increasing the ryegrass growing time beyond 15 days, the ryegrass root exudates were only slightly inhibitory. Thus the allelopathic activity of annual ryegrass was time-related and maximum inhibition was between 8 to 15 days of growth.

In co-growth experiment, root exudates of ryegrass significantly reduced the wheat root length and the reduction was greater than when ryegrass was removed before transplanting of wheat. There was no significant difference between the 2 and 4 days of ryegrass growing time, and ryegrass growth for 6 and 8 days caused maximum inhibition in wheat root growth. Increasing ryegrass growing time up to 15 days had no further significant inhibition effects. Thus when annual ryegrass grew for 11 days, it had greatest inhibition effects on wheat root growth and increasing the growing time beyond this time had no further significant effect on inhibitory activity, possibly due to degradation of ryegrass root exudates, thereby decreasing the inhibition effects of ryegrass. Huang *et al.* (12) also found that concentration of allelopathic compounds was greatest between 6-8 growing days and after this time their concentration declined. One explanation could be associated with the limited half-life of these compounds in agar medium. For example, Macias *et al.*, (21) found that DIMBOA has a half-life of 5.3 hr at 28 ^{0}C at pH 6.75 and decomposes to the more stable MBOA that also has inhibitory activity, but to a lesser degree. The decline in allelopathic compounds toward the end of experimental period could be due to resorption by the growing ryegrass plants as observed in wheat (17) and *Agropyron repens* (11). The root exudates of goosefoot (*Chenopodium murale*) in agar medium reduces the wheat root length by up to 44 % relative to the control (6). Wheat root growth was inhibited by root exudates of ragweed (*Ambrosia trifida*) collected in the rhizophore zone (18). Aqueous extracts of *Wedelia trilobata* L. reduces the rice root growth and weight per plant (26).

Wheat shoot length

In both conditions (co-growth and ryegrass removed), ryegrass root exudates significantly reduced the wheat shoot length (Fig. 2). In ryegrass removed experiment, increasing ryegrass growing time had no significant effect on wheat shoot length and there was no significant difference among the ryegrass growing times. But in co-growth experiment, ryegrass significantly reduced the wheat shoot length. Increasing the ryegrass growing time up to 6 days, reduced the wheat shoot length. One explanation is that the allelopathic compounds produced by ryegrass have low half-life and decompose in a short time and thus their concentration decreases in the growth medium. Therefore the presence of ryegrass in the growth medium is necessary for its complete inhibitory effect on wheat shoot growth. The root exudates have lesser inhibitory effects on shoot growth than on root growth (6,9,23). By increasing the ryegrass growing time beyond 6 days, the inhibition effect of

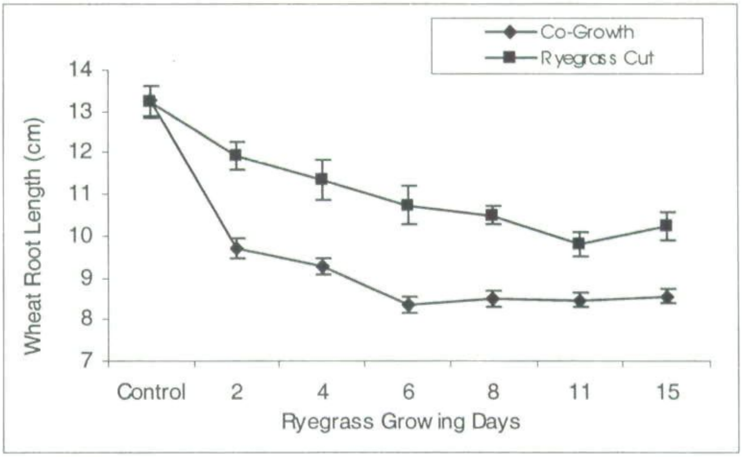

Figure 1. Effects of ryegrass growing days on wheat root length in co-growth and ryegrass removal experiment. Bars indicate the standard error of observations for each treatment.

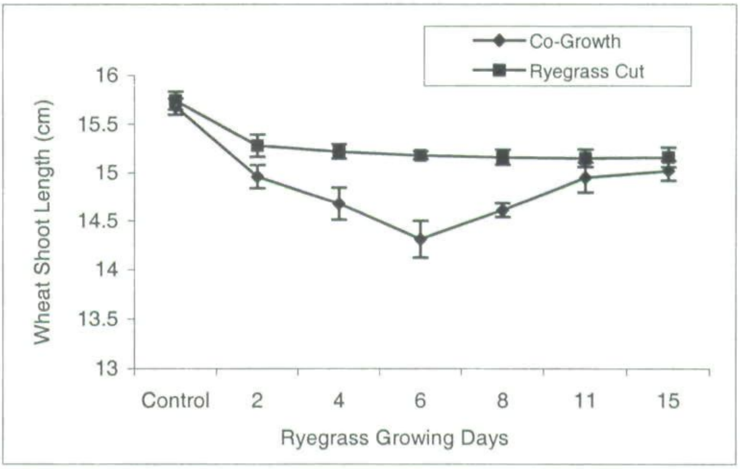

Figure 2. Effects of ryegrass growing days on wheat shoot length in co-growth and ryegrass removal experiment. Bars indicate the standard error of observations for each treatment.

annual ryegrass on wheat shoot length decreased. There was no significant difference in wheat shoot length when ryegrass grew for 2, 11 and 15 days (Fig. 2). Singh *et al.* (33) showed that wheat shoot length reduced significantly in soils that contain root exudates of bill goat weed (*Ageratum conyzoides*).

The ryegrass effect on wheat growth in the co-growth experiment was greater than in ryegrass removal experiment. The presence of annual ryegrass in growth medium intensifies its inhibitory effects on wheat root and shoot. The effect of ryegrass root exudates on wheat root growth was greater than its effect on wheat shoot growth and is consistent with previous studies.The root exudates of goosefoot *Chenopodium murale* reduced the wheat shoot length and that this effect was less than its effect on wheat root length (6). Khanh *et al*. (16) reported that dodder extract was most inhibitory to root and shoot length of radish (*Raphanus sativus* L.) and root length of lettuce (*Lactuca sativa* L.). The most tolerant was shoot length of alfalfa (*Medicago sativa* L.) and lettuce (*Lactuca sativa* L.). Root exudates represent the largest direct inputs of plant chemicals into the rhizosphere environment (8) and we found that ryegrass root exudates were more inhibitory to wheat root than its shoot. As root elongation is a critical trait in plant establishment, the ryegrass allelopathy has the potential to prevent the growth and development of wheat plants.

Experiment 2. Activated charcoal

In the absence of activated charcoal, ryegrass inhibited the wheat root growth and this effect increased significantly with increasing ryegrass density up to 20 plants per beaker. Increasing ryegrass density beyond 20 had no effect on wheat root length (Fig. 3). Addition of activated charcoal to the growth medium substantially decreased the inhibitory effect of annual ryegrass on wheat root growth. This result indicates that ryegrass roots have released some allelopathic compounds inhibitory to wheat root growth and activated charcoal has absorbed these compounds, thereby, reducing the allelopathic effects of annual ryegrass on wheat. Increasing the wheat root length in presence of activated charcoal in the growth medium indicated that root exudates of annual ryegrass contains the allelopathic compounds.

Numerous researchers have used activated charcoal to adsorb the allelopathic compounds to demonstrate the presence of allelopathic activity (10,13,19,22,27).

Experiment 3. Ryegrass effects on wheat cultivars

Root length

The increasing ryegrass density up to 35 plants/beaker reduced the wheat root length of all test cultivars (Fig. 4). The responses of cv. Ventura, Sunco and Janz were similar and were not significantly different among ryegrass densities. At density of 35 plants/beaker of ryegrass, cv. Sunco had the longest roots, thus this cultivar was most tolerant to the allelopathic effects of root exudates of ryegrass. However the response of root length in cv. WW14192 to ryegrass root exudates was significantly different from other three cultivars. In this cultivar, increasing the ryegrass density caused maximum reduction in wheat root length. Increasing the ryegrass density up to 20 plants per beaker significantly reduced the root length in this cultivar. Thus cv. WW14192 was the most susceptible to the allelopathic effect of ryegrass root exudates. An *et al*. (2) demonstrated that vulpia possessed strong allelopathic characteristics and its residues were toxic to seedling growth of wheat cultivars. Khanh *et al*. (16) reported that carrot (*Ducus carota* L.) and radish (*Raphanus sativus* L.) were most sensitive to the allelopathic effects of dodder (*Cuscuta hygrophilae* H. Pearson) extract and alfalfa (*Medicago sativa* L.) and lettuce (*Lactuca sativa* L.) were the most tolerant. Leaf extracts of *Solanum lycocarpum* significantly reduced the root growth and inhibited the root hair and lateral root differentiation in sesame seedlings. The roots were more affected than shoots (15). Ryegrass inhibited the wheat root elongation and among the wheat cultivars, there were different responses to ryegrass root exudates.

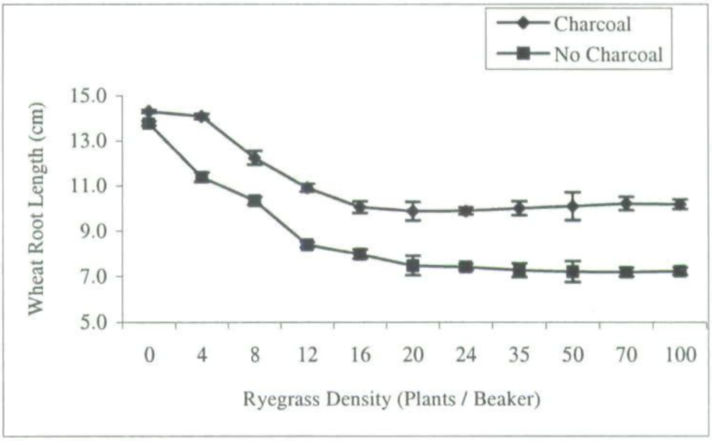

Figure 3. Effects of activated charcoal and ryegrass density on wheat root length. Bars indicate the standard error of observations for each treatment.

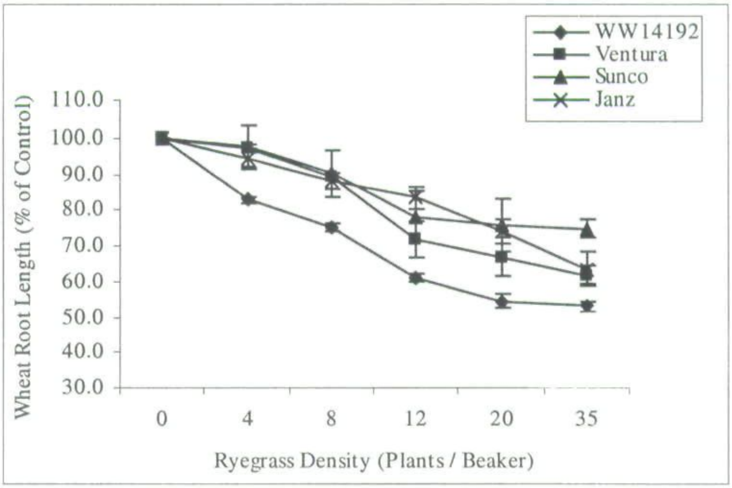

Figure 4. Effects of annual ryegrass density on wheat root length of different wheat cultivars. Bars indicate the standard error of observations for each treatment.

Shoot length

The root exudates of ryegrass reduced the shoot length of wheat, but the magnitude of reduction was less than root length (Fig. 5). Ryegrass root exudates decreased the wheat root length up to 50 %, while shoot length was reduced up to 90 % at a ryegrass density of 35 plants per beaker. Increasing the ryegrass density had no significant effect on wheat shoot length of cv. Ventura and Janz, but significantly reduced the wheat shoot length of cv. WW14192 and Sunco (Fig. 5).

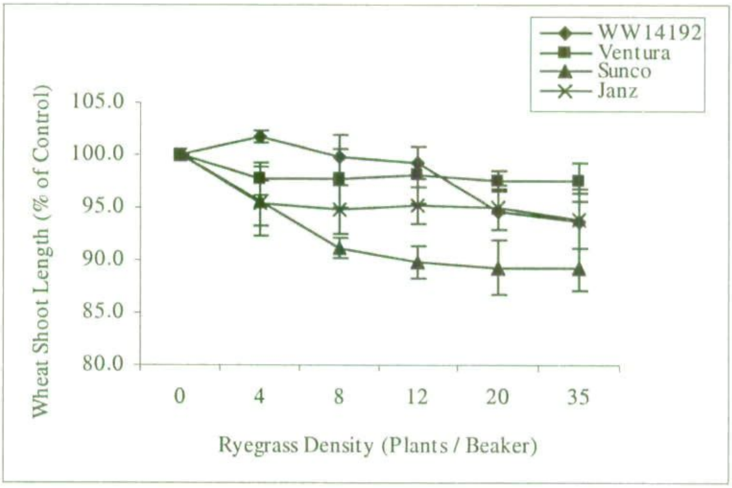

Figure 5. Effects of annual ryegrass density on wheat shoot length of different wheat cultivars. Bars indicate the standard error of observations for each treatment.

Ryegrass had greatest effect on shoot length of cv. Sunco and was least inhibitory to its root length. But in cv. WW14192, the allelopathic effect of ryegrass inhibited the root growth more than shoot growth. Kong *et al*. (18) indicated that wheat shoot growth could be significantly inhibited in *Ambrosia trifida* infested or residue amended soils. Two carotene-type sesquiterpenes, were subsequently isolated and identified from the toxic soils and both compounds were very inhibitory to wheat growth.

Generally results indicated that root exudates of annual ryegrass inhibited the root and shoot growth of wheat seedlings and this was exerted through release of annual ryegrass chemical compounds. Bertin *et al*. (7) considered several mechanisms by which M-tyrosine could inhibit root growth, including (i). direct interference with amino acid metabolism, (ii). inhibition of cell wall formation and (iii). alteration in plant hormone signalling. Addition of activated charcoal to the growth medium increased the wheat root length, indicating that the inhibitory effects of annual ryegrass on wheat growth were due to chemical compounds released by annual ryegrass. We found that wheat cultivars had different responses to root exudates of annual ryegrass and cv. WW14192 showed the maximum reduction in root length. These results indicate that wheat cultivars differed in response to the allelopathic challenge of annual ryegrass root exudates and this diversity should assist in the selection of wheat cultivars that can grow in the presence of annual ryegrass root exudates. Wheat cultivars that are more tolerant to ryegrass root exudates, should therefore grow better and wheat yield loss in the presence of annual ryegrass should be reduced. The seedling establishment stage is so important in the growing season and allelopathic activity of root exudates can suppress the growth of crop seedlings. Wheat cultivars that can grow better in initial growth stages are well positioned to dominate annual ryegrass in subsequent growth stages. Potentially, such wheat cultivars could reduce the requirement for herbicide applications. Previous studies have indicated that allelopathy can be used in integrated weed management (5,14,24,25,29,31). Given the increasing public concern about the use of synthetic herbicides, there is great need for new approaches to weed management (7). Therefore, the identification of crop cultivars with high tolerance to weed allelopathy may contribute to the development of effective and more environmentally friendly weed management systems. We can conclude that there is genetic diversity in wheat cultivars in response to the allelopathic challenge of annual ryegrass and thus more wheat cultivars need to be evaluated for this character. Use of crop cultivars with high allelopathic potential is one cultural method in integrated weed management that has the potential to help farmers reduce herbicide inputs in fields, thereby reducing costs with minimal effects on productivity.

ACKNOWLEDGEMENTS

Special thanks go to Prof. Stephen Powles and Dr Alexa Seal and all friends in the School of Agriculture of Charles Sturt University for their continued support and encouragement.

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