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Impact of insects and fungi on doublegee (*Emex australis*) in the Western Australian wheatbelt

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Abstract. Biotic influences on doublegee (*Emex australis* Steinheil) seed production were investigated as a precursor to the introduction of new insect biological control agents for this weed, and to investigate the cause of doublegee decline in the northern and central wheatbelt of Western Australia since 1990. The symptoms of the decline are doublegee plants of reduced size with distorted leaves, collapsed stems, and smaller achenes (the spiny seed-bearing fruit) that crumble when mature. Three sites were investigated in 1992 by surveys for insects and fungi, and insect and fungus exclusion experiments.

Emex stem blight (*Phomopsis emicis* R. G. Shivas) was present at the 3 study sites. The Watheroo site had comparatively high levels of dock aphids (*Brachycaudus rumexicolens* Patch) on doublegee plants, the Badgingarra site had a comparatively high density of dock sawfly (*Lophyrotoma analis* Costa) on doublegee, and very few insects were present on doublegee at the Wongan Hills site. Viruses were not detected in samples of plants showing the effects of decline.

The exclusion experiment showed a significant effect of removing insects and fungi on achene dry weight at the Watheroo site. There was no treatment effect at the Badgingarra and Wongan Hills sites. The biology of the fungus and the aphid lead to the conclusion that the primary cause of doublegee decline is the dock aphid. This indicates that biological control against *E. australis* might be achieved by using insects that indirectly affect seed quality.

Additional keywords: Brachycaudus rumexicolens, *Phomopsis emicis*, biological control, seed biology.

Introduction

During 1990, farmers in the Western Australian wheatbelt reported that one of their major weeds, doublegee (*Emex australis* Steinheil), demonstrated a range of symptoms that came to be called doublegee decline. The symptoms observed included smaller plants with distorted leaves, collapsed stems, and smaller achenes (the spiny seed-bearing fruit) that crumbled when mature. Samples of plants forwarded to Agriculture Western Australia showed the presence of the fungus *Phomopsis emicis* R. G. Shivas (emex stem blight) (Shivas 1992) and *Brachycaudus rumexicolens* Patch (dock aphid) (Berlandier and Scott 1993). Both organisms have host ranges restricted to *Emex* and related Polygonaceae species, and appear to have arrived accidentally in Australia. The presence of aphid populations is generally not obvious on plants, and the fungus was known to damage only mature plants with stems (Shivas and Scott 1993), which lead to doubts as to the primary cause of doublegee decline.

Doublegee is a weed of crops and pastures, and its spiny achenes contaminate grain and cause injury to livestock (Gilbey and Weiss 1980). Biological control agents have been sought in southern Africa and in the Mediterranean region, leading to the introduction of the weevils *Perapion antiquum* (Gyllenhal) and *Lixus ferrugatus* Boheman. Both species appear to have not survived and further biological control agents are being sought (Scott and Shivas 1990; Scott 1992). Since doublegee is a target for biological control in Australia, it is necessary to establish whether potential biological

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control agents or previously unrecognised organisms are already present on this weed. In this paper we report on a survey of 3 study sites and exclusion experiments, to establish the causes of doublegee decline, and to consider the likely impact that organisms already present might have on future biological control attempts.

Methods

Study sites

Three study sites, a farm near Watheroo (30° 10′ S, 166° 03′ E) and research stations near Badgingarra (30° 20′ S, 155° 33′ E) and Wongan Hills (30° 48′ S, 116° 37′ E), were chosen to conduct the exclusion experiments. The sites were located in paddocks with a history of doublegee infestation. Livestock and pesticides were excluded from the area surrounding the experimental plots.

The farm near Watheroo was one of the first to be observed with doublegee decline. An area of sandy loam soil maintained free of herbicide on the edge of a wheat field was selected for the exclusion experiments. Doublegee had germinated in the trial area during February/March following early rains. Because the doublegee plants were present in a strip next to the wheat field, the experiment was arranged in 5 blocks of five 0·5 by 1·0 m quadrats each allocated to a randomised treatment within each block. The treatments started 12 June 1992 when plants were rosettes, and coincided with a second and less abundant seedling emergence. Plants were sprayed at 3-weekly intervals until 24 September for a total of 6 sprays. Plants had senesced by 15 October and were harvested on 20 October 1992.

The study area at Badgingarra was a sheep pasture on a laterite soil. Doublegee decline had not previously been reported from the area, but its symptoms were evident at the start of the study. The paddock had been used as sheep pasture in previous years. The 5 treatments were allocated in a completely randomised design of 25 (each 0·5 by 1·0 m) quadrats in an area of 10 by 10 m inside a 20 by 20 m area that was fenced to exclude sheep. At the start of the spraying schedule on 24 July the quadrats contained plants that had germinated in February–March. There was no subsequent emergence. The quadrats were sprayed at 3-weekly intervals up to 15 October (total of 5 times) and harvested on 26 October at the onset of plant senescence.

The study area at Wongan Hills was a sheep pasture in previous years. In 1992 it was cultivated in early July and sown with wheat. Doublegee decline occurred in the paddock in previous years. A completely randomised experiment in a 10 by 10 m area similar to the Badgingarra site was set up on 31 July 1992 when doublegee seedlings were present. The quadrats were sprayed at 3-weekly intervals up to 22 October (total of 5 sprays). Plants were harvested on 12 November at the onset of plant senescence.

Insect and plant growth and abundance

On each visit to a study site the doublegee plants in the exclusion experiment were observed for the presence of insects and pathogens. In addition, five 0·5 by 0·5 m quadrats were located randomly inside the 5-m perimeter of the experimental area. Doublegee plants in these quadrats were severed from the taproot and collected in plastic bags. In the laboratory, the plants were washed 3 times in hot tap water (60°C) and sieved (0·25-mm mesh) each time to remove aphids and other insects, which were identified and counted. The washed plants were oven-dried at 43°C for 7 days and weighed.

Treatments and sampling

The number of plants in each quadrat was counted at the start of the experiment. All plants other than doublegee were hand weeded from the quadrats on each visit. At Badgingarra, however, the quadrats were dominated by subterranean clover (*Trifolium subterraneum* L.) and it was not possible to weed all small plants out of the quadrats. The 5 treatments were untreated control, sprayed with tap water, insecticide Nitofol (methamidophos, 1 mL/1 L tap water), fungicide Benlate (benomyl, 1 g/1 L tap water), and combined fungicide and insecticide (ratio 1 : 1). Plants were sprayed to runoff using hand-held 5-L spray cans with manual pumps. Nitofol was chosen because it is a broad spectrum insecticide and miticide and because it is effective against insecticide-resistant forms of *Myzus persicae* (Sulzer) (green peach aphid), which are found in the study areas. Benlate was chosen because it is a broad spectrum systemic fungicide. At harvest all doublegee plants were collected in paper bags and processed as described below. The soil surface within each quadrat was swept to collect achenes fallen from the plants. The sweepings were sieved through mesh (0·5 mm) to remove sand and other debris.

Sample analyses

In the laboratory, achenes were threshed from stems and the number of plants was counted. The plant material and sweepings were combined then sieved (0·5 mm mesh) to remove soil and finer debris. A subsample was taken from which all the current season's achenes were extracted. The comparatively uncommon achenes from previous seasons (identified by their dark colour) were also swept into the sample from the soil surface. These were excluded from the subsample. The subsamples averaged 577±182 achenes (mean±s.d., $n = 75$). The subsample residue (pieces of leaves and stems, stones, and other debris), the subsample achenes, and the remaining bulk sample were weighed to give an estimate of the total number of achenes in the quadrat. Twenty achenes were removed to be analysed for fungi, then the achene sample was dried for 48 h at 43°C then weighed to obtain the dry weight per achene.

Pathogens

Five leaves from plants at Watheroo with symptoms of doublegee decline were collected and scanned for viruses by using leaf dip transmission electron microscopy (TEM). Isolation of other pathogens was not attempted because the only pathogen symptoms observed on the doublegee plants corresponded to those of *Phomopsis emicis* (Shivas 1992; Shivas *et al*. 1994). After the plant material had been harvested, 20 seeds from each quadrat were dissected from their achene. The seeds were surface sterilised in sodium hypochlorite (2% available chlorine) for 3 min, rinsed in sterile distilled water, and plated onto potato dextrose agar. Plates were incubated at 25°C in the dark. After 5 days the plates were examined for the presence of *P. emicis*. Subcultures were taken to confirm the identity of the fungus.

Measurements of achene and seed size

Fifty achenes covering the range of sizes observed were selected from the subsample of achenes from the third control quadrat at Watheroo. A similar sample was selected from the third combined insecticide and fungicide treated quadrat. The width and length of each achene and length of spine

were measured with vernier callipers (Fig. 1). The achene was weighed and the seed extracted then weighed. There is one seed per achene. The seed colour (white, beige, and brown indicating an increasing degree of maturity) was noted.

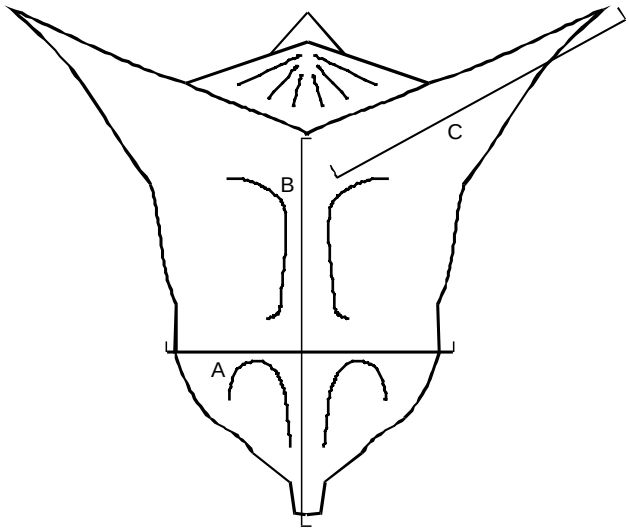

Fig. 1. Measurements taken on doublegee achenes. A, achene width; B, achene length; C, spine length.

Statistical analyses

The computer package STATISTIX (Version 4) was used for statistical analysis. All analyses using ANOVAs were previously found to have equal variance using Bartlett's test. *A posteriori* comparison of means was made by l.s.d. (T) pairwise comparisons. The Kruskal-Wallis ANOVA was used to analyse non-parametric data. Linear regressions were compared by the ANACOV procedures in the computer package STATISTICA for Windows.

Results

Growth and abundance of plants and insects at the study sites

The number of plants per m^2 declined slightly throughout the study, but did not fall below 20 plants/m^2 (Fig. 2). The second germination at Watheroo was rapidly lost from the population. The other sites had slow decline in numbers. The experimental plots had a similar plant density, indicating that the process of weeding did not increase plant density. There were comparable numbers of plants per quadrat at each study site at the start of the experiment (Table 1). Overall, Watheroo had 68 plants/m^2, Badgingarra had 90 plants/m^2, and Wongan Hills had 64 plants/m^2 before treatments were applied. The fungicide and insecticide treatments had no effect on plant number present at harvest (Table 1). Overall, Watheroo had 64 plants/m^2 and both Badgingarra and Wongan Hills had 48 plants/m^2 that survived to the end of the experiment.

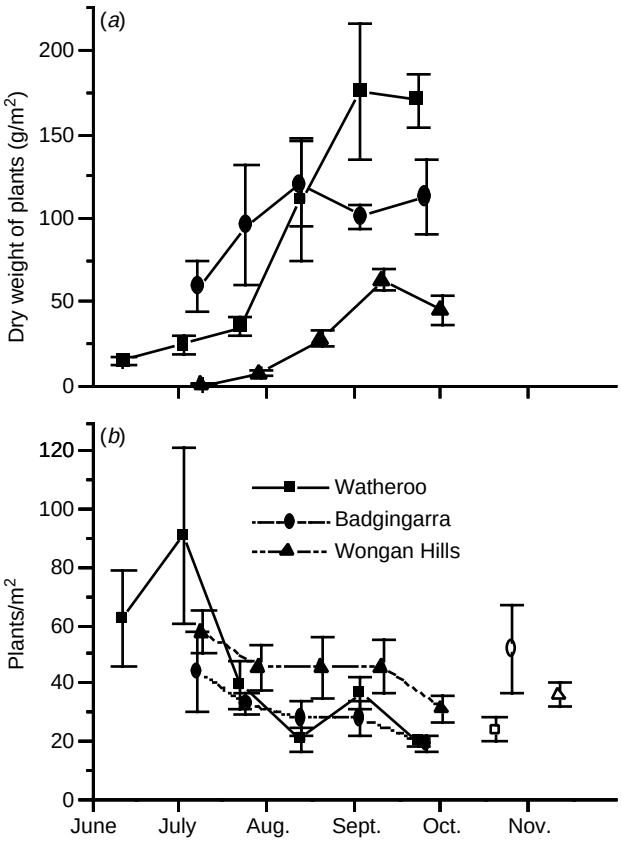

Fig. 2. Growth of doublegee at 3 sites during 1992: (a) mean dry weight (±s.e.m.) per m^2, (b) mean number (±s.e.m.) per m^2. Open symbols show the number of plants at harvest.

Table 1. Mean (±s.d.) number of plants per 0·5 m^2 quadrat at the start of the experiment and at harvest

ANOVA not significant for each site

Treatment	Watheroo	Badgingarra	Wongan Hills
	Start of experiment		
Control	24±9	52±33	36±8
Water only	40±19	48±34	31±7
Insecticide	30±15	33±25	27±12
Fungicide	50±34	50±41	32±9
Insecticide+fungicide	30±20	43±37	36±4
F	1·26	0·23	0·97
	Harvest		
Control	26±14	22±14	27±9
Water only	32±18	22±14	23±6
Insecticide	30±13	22±10	22±6
Fungicide	42±26	29±21	21±4
Insecticide+fungicide	29±15	23±16	26±8
F	0·61	0·21	0·67

Fig. 2 shows the seasonal progression of aboveground dry weight of doublegee plants at the 3 sites. At the start of observations the plants at Watheroo were rosettes. At Badgingarra the plants were more advanced, having produced stems. At Wongan Hills

the earlier germination was eliminated by cultivation and this study is based on the seedlings that subsequently germinated. Watheroo plants were the largest, producing a maximum of about 175 g above-ground dry matter/m^2. Plants at Badgingarra produced up to about 120 g above-ground dry matter/m^2 and those at Wongan Hills produced up to about 63 g above-ground dry matter/m^2.

The most abundant phytophagous species at Watheroo was *B. rumexicolens* (dock aphid). Populations of dock aphids peaked at 230/m^2 in July and then declined to around 40/m^2 in August and September. Very few dock aphids were found at Badgingarra and at Wongan Hills and they were only present in low numbers at harvest. At Badgingarra the most obvious phytophagous insect on doublegee was the dock sawfly *Lophyrotoma analis* Costa (Hymenoptera : Pergidae). Larvae at densities of around 15/m^2 were present throughout July and August, then declined to about 10/m^2 in September. Adult sawflies were observed on each visit. Eggs were present in the first and last samples, but absent over winter. Sawfly eggs were collected in the first sample from Watheroo but all stages were absent thereafter. The sawfly was not found at Wongan Hills, although the area is within the distribution of the insect (J. K. Scott, unpubl. obs.). Few other insects were found on the plants. Thrips species were commonly collected but no associated damage was observed and they were probably visiting the flowers. Cicadellids, pentatomids, and lepidoptera larvae were collected, none of which appeared to be damaging to the plants.

The only pathogenic fungus observed on the plants was *P. emicis*. Before senescence the fungus was evident as spots on leaves and stems. During senescence, lesions developed and stems collapsed. No virus particles were observed in TEM grids prepared from plants that showed signs of doublegee decline at Watheroo.

Insect and fungus exclusion experiment

Mid-way through the experiment at Watheroo, plants in the control and water treatment only plots were visibly smaller and with redder stems and leaves than the plants in other treatments, which were green. This difference was not obvious towards the end of the experiment after the spring flush of growth. The difference between treated and control plots was not observed at Badgingarra or at Wongan Hills.

There were significantly more aphids in the control and water-treated quadrats than in the pesticide treatments at Watheroo (Table 2). The levels of aphid infestation were very low and did not differ between treatments at Badgingarra and Wongan Hills. The mean number of aphids per g dry weight of plant was 0·07–0·53 per treatment at Badgingarra and 0·16–1·14 per treatment at Wongan Hills.

Table 2. Effect of treatments on the mean number (±s.d.) of dock aphids present on doublegee plants at the final harvest at Watheroo

ANOVA on log-transformed number +1 of aphids/g plant dry weight: $F = 20{\cdot}24$, 24 d.f., $P < 0{\cdot}001$. Treatment means followed by the same letter are not significantly different at $P = 0{\cdot}05$

Treatment	Aphid number
Control	19·3±12·4a
Water only	14·9±8·8a
Insecticide	1·0±1·1b
Fungicide	2·3±1·2b
Insecticide+fungicide	1·0±1·0b

The mean percentage of seed infested with *P. emicis* was 0–29% per treatment at Badgingarra, 9–28% at Watheroo, and 0–7% at Wongan Hills. The treatment with fungicide produced variable results. The percentage of seeds infected with *P. emicis* was not significantly different between treatments at Watheroo and Wongan Hills, although it was least in quadrats not treated with fungicide (Table 3). The zero score for fungicide and insecticide combined treatment at Badgingarra resulted in a significant difference between treatments. The Kruskal-Wallis ANOVA was not significant once the zero score was removed. The data for Badgingarra and Watheroo indicate that watering (as in water and insecticide treatments) may have increased the incidence of fungi in the seed (Table 3).

Table 3. Effect of the treatments on the mean percentage (±s.d.) of seed infected by *P. emicis* at the three field sites

Treatment	Watheroo	Badgingarra	Wongan Hills
Control	15±11	14±14	3±5
Water only	18±12	28±16	2±5
Insecticide	28±15	29±16	7±6
Fungicide	9±11	11±11	0
Insecticide+fungicide	12±14	0	7±8
Kruskal-Wallis statistic	5·9	13·0	7·3
P	n.s.	*	n.s.

* $P < 0{\cdot}05$; n.s., not significant.

The number of achenes produced per quadrat at the 3 study sites did not differ (Table 4). Very high numbers of achenes were produced at Watheroo, where the average was 8765/m^2. The maximum number recorded, in a fungicide-treated quadrat, was equal to 17 460 achenes/m^2. At Badgingarra the average number of achenes was 4112/m^2 and at Wongan Hills 6496/m^2. At Watheroo the weight per achene was significantly greater in treated quadrats than in the controls (untreated and water only) (Table 4). Single pesticide treatments produced achenes 50% heavier,

Table 4. Effect of the treatments on mean (±s.d.) achene number per 0·5 m^2 quadrat and dry weight (mg) per achene at the three field sites

Treatment means for Watheroo followed by the same letter are not significantly different

Treatment	Watheroo	Badgingarra	Wongan Hills
	Number of achenes		
Control	3925±2682	1329±1088	2746±640
Water only	3827±1583	1737±905	3211±989
Insecticide	4251±1603	2348±1382	3336±697
Fungicide	5163±2402	2554±1387	3475±853
Insecticide+fungicide	4748±2393	2316±1163	3475±324
F	0·34	0·90	0·84
P	n.s.	n.s.	n.s.
	Dry weight (mg) per achene		
Control	18·8±3·9a	38·1±4·7	35·1±3·9
Water only	23·2±4·8a	36·7±4·7	34·6±1·4
Insecticide	32·1±3·3b	44·0±8·2	31·7±2·0
Fungicide	30·9±1·2b	45·9±6·8	36·4±2·5
Insecticide+fungicide	40·7±3·4c	41·6±5·7	34·7±3·2
F	29·47	1·97	2·05
P	***	n.s.	n.s.

*** $P < 0·001$; n.s., not significant.

and the combined treatment doubled achene weight. Even so, the combined treatments at Watheroo were equivalent to the controls at Badgingarra and Wongan Hills where achene weight did not differ between treatments and controls.

Relationship between achene and seed size

The seed dry weight was significantly related to characteristics of the achene in both the control and pesticide-treated sample from Watheroo. Achene dry weight was the best predictor of seed dry weight (Table 5). Figs 3 and 4 show the relationship between seed dry weight and achene dry weight or width in control and treated quadrats. In both quadrats the heavier and wider achenes contained mature (brown) seeds. Achenes with mature seeds weighed >30 mg and were >3·5 mm wide.

Table 5. Relationship between achene parameters and seed dry weight (mg) at Watheroo

Achene measurement (X)	Regression	R^2	P
	Control		
Width (mm)	$3·4X-6·8$	0·66	<0·001
Length (mm)	$2·7X-2·7$	0·67	<0·001
Spine length (mm)	$3·1X-10·5$	0·54	<0·001
Dry weight (mg)	$0·17X-0·4$	0·79	<0·001
	Insecticide and fungicide		
Width (mm)	$7·7X-17·4$	0·81	<0·001
Length (mm)	$5·3X-15·4$	0·77	<0·001
Spine length (mm)	$5·5X-16·64$	0·58	<0·001
Dry weight (mg)	$0·27X-0·32$	0·90	<0·001

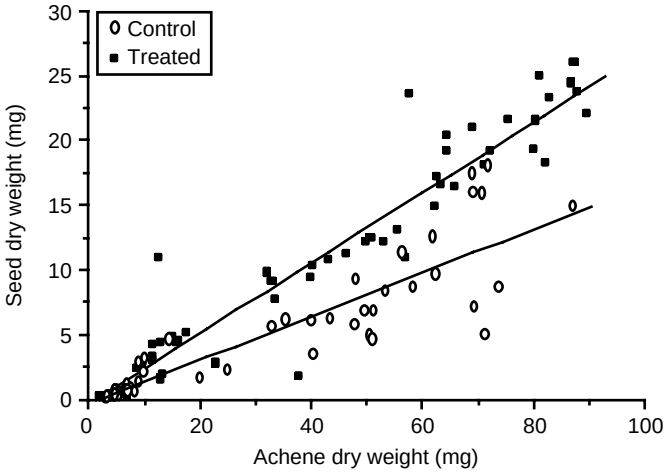

Fig. 3. Relationship between achene dry weight and seed dry weight in Watheroo control quadrat 3 and insecticide and fungicide treated quadrat 3. ANCOVA: overall regression, $F = 45·2$, d.f. = 97, $P < 0·001$; between regressions, $F = 455·6$, d.f. = 97, $P < 0·001$; test for parallel slopes, $F = 31·1$, d.f. = 96, $P < 0·001$.

Discussion

Chemical-based exclusion experiments, although offering better evidence than observations, still have serious shortcomings (Crawley 1989). The potential problems include phytotoxic and fertiliser effects of the pesticide, the removal of predators of the herbivores, and effects on the soil (Crawley 1989). The results from Badgingarra and Wongan Hills, where there was no difference between treated and control quadrats, support the conclusion that the insecticide and fungicide treatments did not have phytotoxic or fertiliser effects

on plant growth. Benlate is known to affect aphid survival and reproduction (Akhtar and Van Emden 1992). This is supported by the results from Watheroo where dock aphids were reduced in all pesticide treatments. The treatments may also have affected the degree of predation on insects, the presence of aphid-attacking fungi, and potential interactions between insects and pathogens.

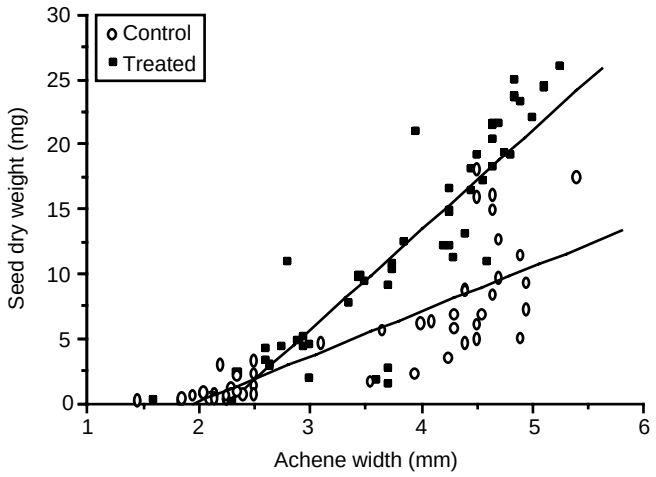

Fig. 4. Relationship between achene width and seed dry weight in Watheroo control quadrat 3 and insecticide and fungicide treated quadrat 3. ANCOVA: overall regression, $F = 11{\cdot}6$, d.f. = 97, $P < 0{\cdot}001$; between regressions, $F = 187{\cdot}1$, d.f. = 97, $P < 0{\cdot}001$; test for parallel slopes, $F = 21{\cdot}5$, d.f. = 96, $P < 0{\cdot}001$.

The pesticide treatments show that doublegee was not subject to biotic controls at Badgingarra and Wongan Hills. At Watheroo only achene weight differed between controls and pesticide treatments. The presence and abundance of the dock aphid at this study site and during the experiment indicate that the aphid is responsible for the low achene weight and for the general symptoms of doublegee decline. The fungicide and combined insecticide and fungicide treatments show that there is also an impact on achene weight from fungi, most likely from *P. emicis*. The fungus is most damaging on plants following stem development (Shivas 1992), but it does not cause distorted leaves, and rosettes are not damaged. Consequently, the fungus is unlikely to be the primary cause of doublegee decline.

Doublegee has indeterminate growth, meaning that seed production will continue as long as the season is favourable. The first seeds are produced during the rosette phase and are the largest (Gilbey and Weiss 1980). Achenes are produced at each stem node, such that proximal achenes are more mature and larger than distal achenes, which gradate in size to senescing flowers. Maximum achene weight is reached 8 weeks after flowering (Gilbey and Weiss 1980). Since the plant is branching and expanding as the season progresses, there will be increasing numbers of younger achenes. Smaller achenes resulting from attack by dock aphids could be the result of the aphids preventing seed developing fully, through effects on plant growth. This could be one effect that the dock aphid has on the plant. A second possible effect is indicated by the presence of distorted leaves on decline-affected plants. This may be due to mechanical damage due to the insect feeding at the point of new growth. Another possibility could be the presence of chemicals in the saliva that cause damage (Miles 1989). *Emex australis,* being of southern African origin, is not the natural host of the dock aphid, which comes from North America or Europe (Berlandier and Scott 1993 give the distribution). Consequently, the plant would not be adapted to attack by the insect. The possibility that the aphid and fungus have synergistic effects on seed production and size was not established in this study.

The importance of seed size in doublegee needs to be measured. For example, it remains to be determined whether smaller and larger seeds have equal germination success. Furthermore, it may be that larger seed size in doublegee will enable seedlings to establish and survive in the common situation of 'false breaks' that occur in Mediterranean climate areas of Western Australia (Rossiter 1966). Another plant in this environment, *T. subterraneum*, also shows a wide range in seed size. Black (1958) found that seedlings from larger seeds are more competitive and more likely to survive.

The dock aphid, dock sawfly, and emex stem blight fungus were the only organisms found during this study with the potential to be widespread, damaging, and relatively restricted to doublegee. No other damaging insects or fungi were found despite the release of biological control agents in the past. The weevils *Perapion antiquum* and *Lixus ferrugatus* have been released in Western Australia for the biological control of doublegee but are thought not to have established (Scott 1992). They were not found during this survey. The weevil *Rhinoncus australis* Oke was considered by Scott and Shivas (1990) to be a possibility for introduction to Western Australia because it attacks doublegee in eastern Australia (Julien and Matthews 1980). The larvae of this insect tunnel inside stems, and previously it was the only organism known to damage doublegee consistently in Australia (Julien and Matthews 1980). However, the weevil was found to be already present throughout the south-west on *Polygonum* sp., *Rumex crispus* L., and *R. pulcher* L. (J. K. Scott, unpubl. data) but not on doublegee. The

weevil's larval stages are completed during December and January and are out of phase with doublegee growth in Western Australia.

The impact of the aphid and the fungus shows that it is possible for biological control agents to have an impact on doublegee seed production. It is evident that the actions of the dock aphid will be insufficient to control doublegee, because quadrats with aphids present still produced very large numbers of seed. Additional biological control agents will be needed to complement the damage caused by the aphid. However, the impact of the aphid on doublegee points to the usefulness of biological control agents that have high dispersal ability and which are present early in the growth of doublegee.

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