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Relative phytotoxicity of parthenium weed (*Parthenium hysterophorus* L.) residues on the seedling growth of a range of Australian native and introduced species

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Abstract. The invasive herbaceous species *Parthenium hysterophorus* L. (Asteraceae), commonly known as parthenium weed has rapidly become a significant weed in more than 30 countries. Parthenium weed litter taken from the introduced biotypes was relatively more phytotoxic than that taken from biotypes coming from the native range when tested on lettuce and this may indicate one reason for invasion success. However, no significant difference was observed in phytotoxicity to lettuce seedling growth when two Australian biotypes of parthenium weed were compared, one invasive and one non-invasive, indicating that invasiveness was not associated with litter phytotoxicity in all cases. Residue from the invasive parthenium weed biotype had a greater phytotoxic effect upon Australian native pasture grass species relative to the introduced pasture grass species with buffel grass (*Cenchrus ciliaris* L.) and bull Mitchell grass (*Astreble sequarrosa* C.E. Hubb) showing the greatest tolerance to parthenium weed phytochemicals. When compared with residue taken from plants that has a range of phytotoxic capacity, parthenium weed residue was considered to be only moderately phytotoxic suggesting that the phytotoxicity of its residue may not be the main reason for the plants invasive trait.

Additional keywords: invasion, pasture grasses, weed biotypes.

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Introduction

Parthenium weed (*Parthenium hysterophorus* L.) is an important invasive weed that is native to the tropical and sub-tropical America (Dale 1981). It has been accidentally introduced into more than 40 countries around the world (Shi *et al*. 2015) where it has had a serious adverse impact upon rangeland productivity, agricultural crop production, human and animal health and the biodiversity of natural communities (Chippendale and Panetta 1994; Navie *et al*. 2005; Adkins and Shabbir 2014). Many new regions around the world are also under considerable threat from parthenium weed invasion (McConnachie *et al*. 2011). Therefore, it is essential to understand the invasive characteristics of this weed which may include the plant's rapid growth rate and heavy seed production (Reddy *et al*. 2007), its genetic diversity (Mahadevappa 2009; Hanif 2014), its competitiveness (Khan *et al*. 2014), tolerance to stressful growth conditions (Williams and Groves 1980) and its allelopathic competence (Lewis *et al*. 1987; Ramesh *et al*. 2003; Shinwari *et al*. 2013).

Allelopathy is a well-defined physiological trait possessed by some crops and native plant species, but especially by invasive weeds (Pisula and Meiners 2010). It is a biological phenomenon involving the release of chemicals that may cause a stimulatory, but more often an inhibitory effect upon another plant's growth, its reproduction and/or its survival. Residues produced through natural senescence and decomposition of plant tissues can deliver large quantities of allelochemicals to the soil (Belz *et al*. 2007) where they can be incorporated. Research has been undertaken on the impact of parthenium weed tissue extracts on the germination (Channappagoudar *et al*. 2005; Verma and Rao 2006; Maharjan *et al*. 2007; Netsere and Mendesil 2011; Hu *et al*. 2013) of some species, but relatively little work has addressed the impact of parthenium weed residues on germination or seedling growth of other plants.

Multiple biotypes of parthenium weed are known to exist around the world and differences exist in their invasion success (Hanif 2014). These differences in invasiveness may be due to variability in the allelopathic competence, in particular the phytotoxic nature of their litter. Of particular interest would be a comparison between the phytotoxic nature of litter produced by plants coming from the native range and those from the introduced range. The novel weapons hypothesis (Callaway and Aschehoug 2000) suggests that certain invasive plants will only produce potent allelochemicals once they have been introduced to a new area. This atypical

production of unique allelochemicals then helps those invading plants to gain a foothold in the new location.

Parthenium weed has been accidently introduced to Australia on two separate occasions. The first introduction occurred at Toogoolawah in south-east Queensland in ~1945, which resulted in the formation of the small, non-invasive Toogoolawah population (Parsons and Cuthbertson 1992). The second introduction was in 1958 into central Queensland when a contaminated pasture seed lot imported from the USA was sown on a property near Clermont (Haseler 1976). This second, highly invasive Clermont population has now spread over 600 000 ha of Queensland whereas the Toogoolawah population has spread over just a few ha.

The most biologically relevant bioassay for evaluating the natural release of phytotoxic substances from plant residues is the sandwich bioassay (Fujii *et al*. 2003). Using this bioassay technique, Shinwari *et al*. (2013) have shown a moderate to high phytotoxic effect of parthenium weed litter on the growth of lettuce seedlings (86% reduction in shoot growth) when compared with that produced by other known allelopathic species such as sweet clover (*Melilotus officinalis* L.) and the four o'clock plant (*Mirabilis jalapa* L.), which reduced shoot growth by 98% and 87%, respectively. The advantage of the sandwich bioassay is that it is easy to undertake and can be applied to numerous target species all in one short time. However, as the phytotoxic interaction between residues and the test species, is affected by the environment (Zhang *et al*. 2013) the conditions under which the sandwich bioassay is undertaken needs to be calibrated for the residues of each species to be studied (Fujii *et al*. 2004).

The aims of this study were to first test the 'sandwich' bioassay as a way of assessing parthenium weed plant residues for their phytotoxic competence. The second aim was to compare the phytotoxicity of residues coming from plants coming from the native and introduced range. The third was to examine the phytotoxic potential of the residues from the two, invasive and non-invasive, Australian biotypes. The fourth was to determine the impact of parthenium weed residues on the seedling growth of a range of Australian native and non-native species and lastly, to compare the phytotoxic competence of parthenium weed residues against several known allelopathic plants.

Materials and methods

Test species

Lettuce (*Lactuca. sativa* L. cv. Great Lakes) seed, used as the test species throughout this study, was obtained from Mr Fothergill's Seeds Pty Ltd (South Windsor, NSW, Australia). Seed of other test species (Table 1) was obtained from various seed companies and parthenium weed and giant rat's tail grass were obtained from the University of Queensland seed collection. All seed lots were stored in a seed room (at 15 ± 1°C and 15 ± 3% relative humidity) to maintain viability and vigour.

Before use, all seed lots were X-rayed to determine the proportion of filled seed (Faxitron, Faxitron Bioptics, LLC, Tucson, AZ, USA) and, if necessary, seed lots were cleaned to give lots with >90% seed fill. Prior to use, a germination test was undertaken on all seed lots. This comprises three replicate lots of 30 seeds from each species, that were placed onto an agar medium (10 g L^{-1} w/v; Sigma-Aldrich, Castle Hill, NSW, Australia; 15 mL per dish) contained in 9-cm-diameter plastic Petri dishes, the dishes were sealed around their edges with Parafilm and incubated at 25/20°C (day/night) using a 12/12-h photoperiod (light intensity of 100 µmol m^{-2} s^{-1}) for 14 days in a germination incubator (Thermoline, Sydney, NSW, Australia). The percentage of germinated seed was recorded, and only seed lots yielding >90% germination were used in the following studies.

Preparation of leaf residues

Parthenium weed seeds were germinated in a University of California compost contained within seedling trays, moistened to field capacity with tap water and incubated at 25/20 ± 2°C (day/night) with a 12/12-h photoperiod (light intensity of ~800 µmol m^{-2} s^{-1}). When seedlings were ~2 cm tall they

Table 1. The test species used in sandwich bioassay studies to assess the allelopathic nature of parthenium weed residues, showing their status within Australia, their common name and scientific name

Status within Australia	Species	Scientific name
Introduced	African lovegrass	*Eragrostis curvula* L.
	Buffel grass	*Cenchrus ciliaris* L.
	Creeping blue grass	*Bothriochloa insculpta* L.
	Giant rats' tail grass	*Sporobolus pyramidalis* L.
	Green panic grass	*Panicum maximum* Jacq.
	Lambs quarters	*Chenopodium album* L.
	Liverseed grass	*Urochloa panicoides* P.Beauv.
	Rhodes grass	*Chloris gayana* L.
	Tall finger grass	*Digitaria milanjiana* Stapf
Native	Bull Mitchell grass	*Astreble squarrosa* C.E.Hubb
	Cotton panic grass	*Digitaria brownii* L.
	Curly windmill grass	*Enteropogon acicularis* L.
	Forest blue grass	*Bothriochloa bladhii* (Retz.) S.T. Blake
	Kangaroo grass	*Themeda triandra* Forssk.
	Pitted blue grass	*Bothriochola decipens* (Hack.) C.E.Hubb
	Queensland blue grass	*Dichanthium sericeum* R.Br
	Weeping grass	*Microlaena stipoides* L.

were transplanted into 14-cm-diameter plastic pots filled with University of California compost moistened to field capacity and the seedlings allowed to continue their growth under the same conditions as described above. The upper leaves from 60- to 90-day-old plants were collected, placed within paper bags and immediately oven-dried at 35 ± 1°C for 4 days. All dried leaf samples were placed into in a single large paper bag and stored in the seed room (15 ± 1°C and 15 ± 3% relative humidity) until used 1 month later (unless otherwise stated). Immediately before use in the sandwich bioassays, the dried parthenium weed leaves were hammer milled (Apex Construction Ltd., Greensborough, Vic., Australia) so to pass through a 0.5-mm screen.

The sandwich bioassay

Agar (7.5 g L^{-1} w/v, Sigma-Aldrich) was autoclaved (20 min at 121°C) then cooled to ~45°C. At this point Amphotericin B antibotic solution (Sigma-Aldrich; 10 mL of 250 µg mL^{-1}) was stirred into the liquid agar. Aliquots (5 mL) of the agar solution was then added to each well of six well culture plates (Corning Inc., New York, USA) under sterile conditions in a laminar air flow hood. Once cooled, 50 mg of hammer milled parthenium weed residue was evenly spread onto the agar surface of three wells of each plate (unless otherwise stated) and a further aliquot (5 mL) of liquid agar then added to all six wells of the plate to form a sandwich. Once cooled, seven surface sterilised (2%, v/v; sodium hypochlorite solution applied for 5 min followed by three rinses in sterile water) lettuce seeds were sown onto the top of the agar of each well. Then, a second plate base was inverted over the top of the first to act as a 'lid' and providing a space into which seedling growth could occur. Finally, the edges of all plates were closed with Parafilm and the plates placed into a germination incubator (Thermoline, Australia) set at a 25/20°C (day/night) thermoperiod (unless otherwise stated) with a 12/12-h day/night photoperiod (light intensity ~100 µmol m^{-2} s^{-1} unless otherwise stated).

The location of the six well culture plates within the germination incubator was randomly changed each day. After 2 days the plates were opened and the seedlings thinned to five uniformly growing seedlings per well. The plates were then reclosed and incubated for a further 5 days under the same conditions. Following a total of 7 days growth the seedlings were gently removed from the agar, washed with tap water and the length of the shoot and root measured as root/shoot length was correlated with root/shoot dry weight (Supplementary material fig. 1, available at journal's website). From this, and in comparison to the control, the phytotoxicity of the treated seedlings was determined using the following equation:

$$\text{Growth Inhibition (\%)} = 100(\%) - \text{Relative Growth Rate (RGR)(\%)}$$

The RGR was calculated from the linear equation RGR (%) = b (treatment)/b (control) × 100, where b is the y intercept from the linear regression equation.

Experimental design and data analysis

Unless otherwise stated, a completely randomised design was used in all experiments with three replicate culture plates per treatment. The experiments were repeated over time using residue from two different sets of 60- to 90-day-old parthenium weed plants. In all cases, no significant differences were found between duplicate experiments, so the two datasets were pooled and it is pooled data that is presented. Prior to presentation, all datasets were transformed using a general linear model, with test species and treatment as the main blocks, using the Minitab statistical package. Furthermore, two sample *t*-tests were undertaken on all root and shoot length datasets to examine differences between treatments.

Standardisation of the assay

In order to determine an appropriate amount of parthenium weed residue to use in the sandwich bioassay, an experiment was undertaken using the standard protocol (as described above) but with different quantities of parthenium weed leaf residue. Lettuce seed was sown onto prepared six well culture plates containing 0, 10, 20, 30, 40, 50 or 60 mg of 1-month-old parthenium weed residue collected from either 60-day old Clermont or Toogoolawah (Australian biotypes) plants and then incubated under a 25/20°C (day/night) thermoperiod and a 12/12-h (day/night) photoperiod with a light intensity of ~100 µmol m^{-2} s^{-1}. The inhibition of seedling growth was determined after 7 days of incubation.

The optimal incubation temperature for the sandwich bioassay was determined by sowing lettuce seed onto prepared six well culture plates containing 50 mg (determined from the first standardisation assay) of 1-month-old parthenium weed residue (Clermont biotype) and then incubated at 20/15, 25/20, or 30/25°C (day/night) with a 12/12-h (day/night) photoperiod and a light intensity of ~100 µmol m^{-2} s^{-1}. The inhibition of seedling growth was determined after 7 days of incubation.

Optimum light intensity for the sandwich bioassay was determined by sowing lettuce seed onto prepared six well culture plates containing 50 mg of 1-month-old parthenium weed residue (Clermont biotype) and then incubated at 25/20°C (day/night) and a 12/12-h (day/night) photoperiod (determined from the second standardisation assay) using light intensities of 0, 100 or 800 µmol m^{-2} s^{-1} created using two germination incubators and one growth chamber, respectively. The inhibition of seedling growth was determined after 7 days of incubation.

Comparison of a range of parthenium weed biotypes

In order to determine the impact of a range of parthenium weed biotypes residue on lettuce seedling growth, an experiment was undertaken using the standard protocol (as described above) but with dried leaf residues coming from several different parthenium weed biotypes. Lettuce seed was sown onto the prepared six well culture plates containing 50 mg of 1-month-old parthenium weed residue from biotypes sourced from USA, Mexico, Argentina (both native range), Australia (Clermont and Toogoolawah), China and Vietnam (from introduced range). The prepared culture plates were incubated at 25/20°C (day/night) under a 12/12-h (day/night) photoperiod using a light intensity of 100 µmol m^{-2} s^{-1} and undertaken in a germination incubator. The inhibition of seedling growth was determined after 7 days of incubation.

The impact on a range of pasture species

The phytotoxic effect of parthenium weed residues was investigated using the standard sandwich bioassay protocol (as described above) against a range of pasture grass species in place of lettuce. Surface sterilised seeds of nine introduced pasture grass species (Table 1) were sown onto prepared culture plates containing 50 mg of 1-month-old parthenium weed leaf (Clermont biotype). Sandwich bioassays were incubated at 25/20°C (day/night) with a 12/12-h (day/night) photoperiod using a light intensity of 100 µmol m^{-2} s^{-1} in a germination incubator. The inhibition of seedling growth was determined after 7 days of incubation.

Comparison to a range of other known phytotoxic species

Mature leaves, from chinaberry tree (*Melia azedarch* L.), lychee (*Litchi chinensis* Sonnerat), tamarind (*Tamarindus indica* L.), orchid tree (*Amhersita nobilis* Wall.) and fiddlewood (*Citharexylum spinosum* L.), were collected from the Brisbane Botanic Garden (Mt Coo-tha, Brisbane, Qld, Australia) then immediately oven-dried at 35 ± 1°C for 4 days. Dried leaf samples were individually placed in to paper bags, well mixed and placed in a constant temperature seed room (15 ± 1°C and 15 ± 3% relative humidity) before being hammer milled so to pass through a 0.5-mm screen 1 month later. To evaluate the impact of these residues and that of parthenium weed (Clermont biotype) on lettuce seedling growth, an experiment was undertaken using the standard protocol and the six types of residue. Lettuce seed was sown onto prepared six-well culture plates containing 50 mg of 1-month-old residue coming from each of the six species and incubated at 25/20°C (day/night) under a12/12-h (day/night) photoperiod using a light intensity of 100 µmol m^{-2} s^{-1} created using a germination incubator. The inhibition of seedling growth was determined after 7 days of incubation.

Results

Standardisation of the assay

Impact of the amount of residue applied

At all quantities used in the sandwich bioassays, parthenium weed residues suppressed both root and shoot elongation of lettuce. In all cases, the root growth was inhibited more than the

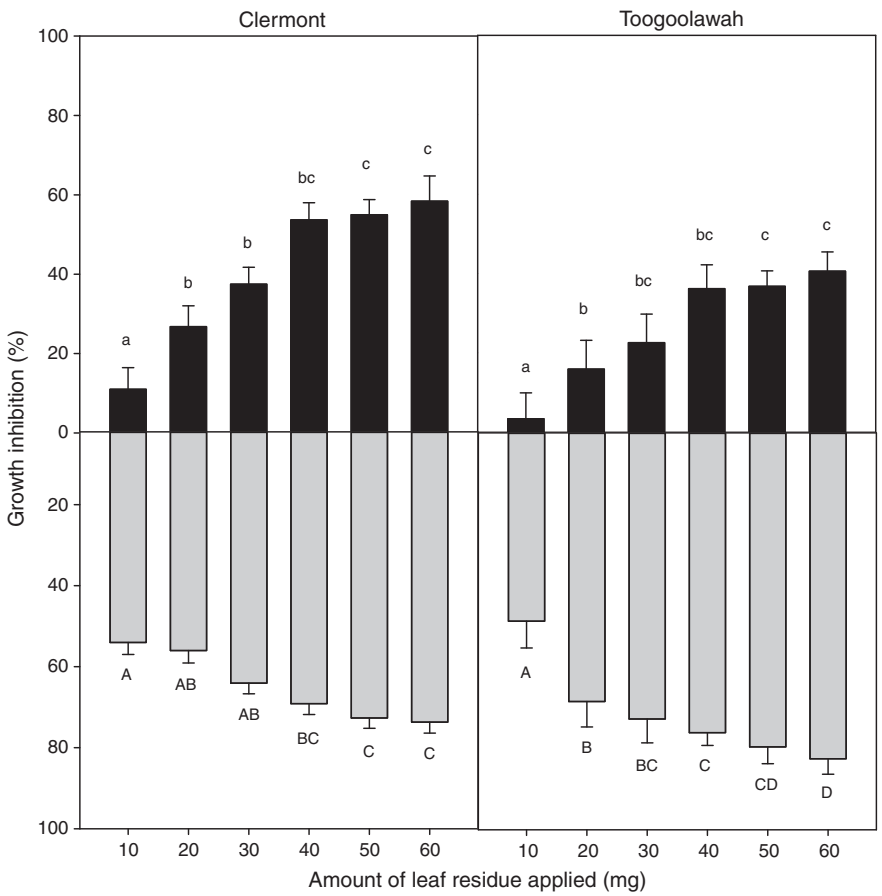

Fig. 1. The percent root (light grey) and shoot (black) growth inhibition of lettuce seedlings when growing with leaf residues from parthenium weed (Clermont or Toogoolawah biotypes). Error bars represent two standard deviations from the mean as calculated for nine replicates of five seedlings and from duplicate experiments for each biotype. Means within tissue type and biotype that do not share the same letter are significantly different from one another at $P > 0.05$.

shoot growth (Fig. 1). The greatest inhibition of lettuce seedling growth was provided by 40, 50 and 60 mg of residue, with no significant differences between these three amounts (Fig. 1). A residue amount of 50 mg was selected for use in all further sandwich bioassay experiments.

Impact of the temperature of incubation

Parthenium weed residues, at all incubation temperatures examined, suppressed both root and shoot growth of lettuce. In all cases, the root growth was inhibited relatively more than shoot growth (Fig. 2). The greatest inhibition of lettuce seedling growth was observed at 25/20 and 30/25°C, with no significant differences in growth inhibition achieved between these two day/night temperature regimes (Fig. 2). A day/night temperature regime of 25/20°C was therefore selected for use in all further bioassay experiments.

Impact of the light of incubation

Parthenium weed residues, at all illumination conditions used, suppressed both the root and shoot growth of lettuce. In all cases, the root growth was inhibited more than shoot growth (Fig. 3). The greatest inhibition of lettuce seedling growth was provided by darkness while statistically similar levels of inhibition were observed at 100 and 800 $\mu mol\ m^{-2}s^{-1}$ light intensity (Fig. 3). A light intensity of 100 $\mu mol\ m^{-2}\ s^{-1}$ was selected for use in all further bioassay experiments.

Comparison of a range of parthenium weed biotypes

Parthenium weed residues from all biotypes examined suppressed both root and shoot growth of lettuce (Fig. 4). In all cases, root growth was inhibited more than shoot growth. The greatest inhibition of lettuce seedling growth was provided by residues from the Chinese and Vietnamese biotypes, with no significant differences noted between these two biotypes (Fig. 4). As a group, the inhibition of seedling growth (root and shoot) produced by biotypes from the species' native range were less than that of biotypes from the regions where they were introduced.

The impact on a range of pasture species

Parthenium weed residues suppressed both root and shoot growth of nine introduced and eight native pasture species, as well as its own seedlings (Fig. 5). In all cases, root growth was more inhibited than shoot growth. As a group, the inhibition of lettuce seedling growth (root and shoot) was greater for the native species than for the introduced species (Table 2). Of the introduced species, the shoot growth of African lovegrass, buffel grass and giant rat's tail grass, and the root growth of buffel grass and parthenium weed, were the least inhibited in the presence of parthenium weed residues. Of the native species, the shoot and root growth of bull Mitchell grass and weeping grass were the

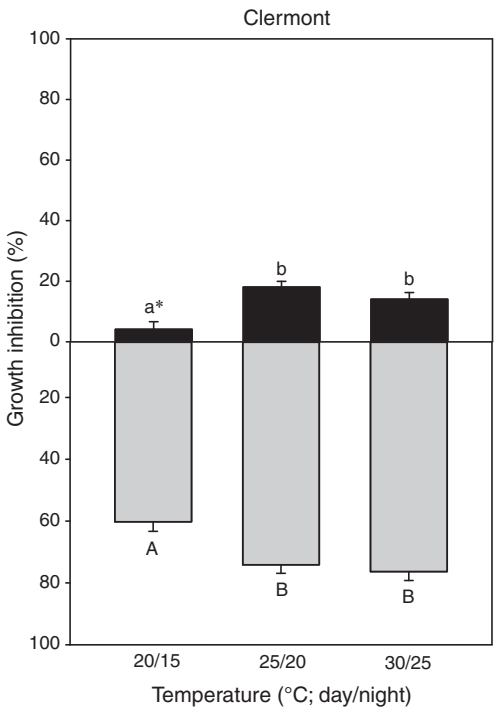

Fig. 2. The percent root (light grey) and shoot (black) growth inhibition of lettuce seedlings when growing with leaf residues from parthenium weed (Clermont biotype) under three day/night temperature regimes. Error bars represent two standard deviations from the mean as calculated for nine replicates of five seedlings and from duplicate experiments. Means within the same tissue types that do not share the same letter are significantly different from one another at $P > 0.05$. * indicates growth stimulation relative to other treatments.

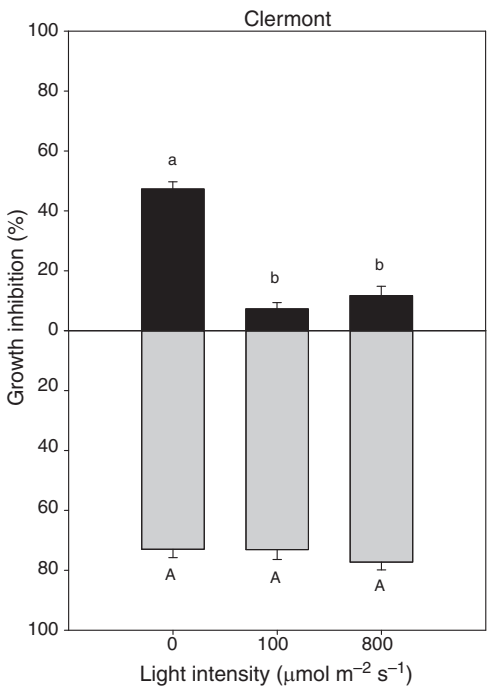

Fig. 3. The percent root (light grey) and shoot (black) growth inhibition of lettuce seedlings when growing with leaf residues from parthenium weed (Clermont biotype) under one of three light regimes. Bars represent two standard errors of the mean and calculated for nine replicates of five seedlings and from duplicate experiments. Means within the same tissue type that do not share the same letter are significantly different from one another at $P > 0.05$.

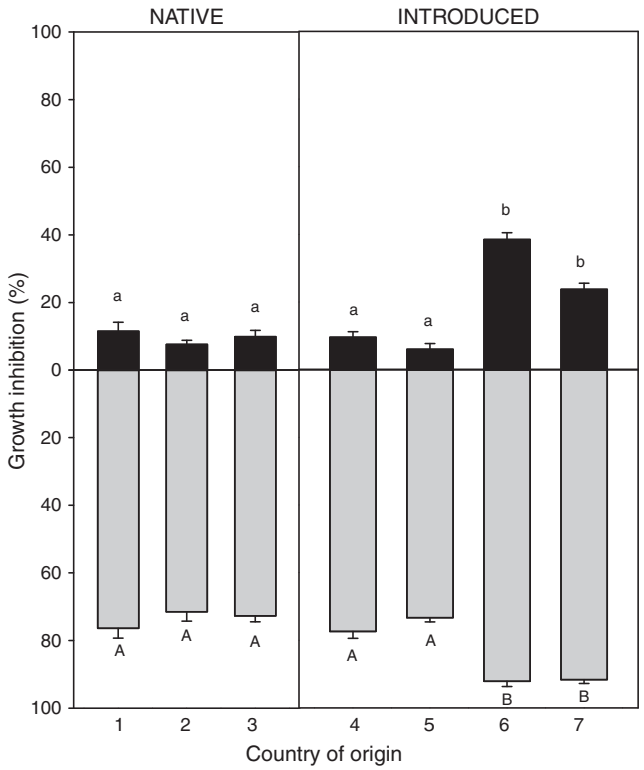

Fig. 4. The percent root (light grey) and shoot (black) growth inhibition of lettuce seedlings when grown with leaf residues from one of six parthenium weed biotypes (viz. (1) USA, (2) Mexico, (3) Argentina, (4) Australia (Clermont), (5) Australia (Toogoolawah), (6) China and (7) Vietnam). Error bars represent two standard deviations from the mean as calculated for nine replicates of five seedlings and from duplicate experiments. Means within tissue parts that do not share the same letter are significantly different from one another at $P > 0.05$.

least inhibited. The combined shoot and root growth of African lovegrass, buffel grass, giant rat's tail grass, and bull Mitchell grass were least affected whereas the growth of green panic, kangaroo grass and weeping grass were most affected (Fig. 5).

The impact of a range of residue materials

The residues from all plant species tested suppressed both the root and shoot growth of lettuce (Fig. 6). In all cases, root growth was inhibited more than shoot growth. The greatest inhibition of growth was achieved by residue from tamarind, whereas the plant species least inhibitory to lettuce seedling growth was the orchid tree. The inhibition of growth by parthenium weed litter was intermediate between these two extremes. All these findings were consistent with those from previous work (Fujii *et al*. 2004).

Discussion

The 'sandwich' method as developed by Fujii *et al*. (2003) and used in several earlier studies (Anjum *et al*. 2010; Shinwari *et al*. 2013; Akhtar *et al*. 2014) has proven to be a very pragmatic technique for studying the phytotoxic effect of parthenium weed residues on lettuce seedling growth and that of a wide range of other species. The residues prepared from mature leaves of parthenium weed were inhibitory to the growth of young seedlings of all 18 species studied (viz. 16 pasture and two weed species). This indicates that the residues produced by parthenium weed leaves, when in sufficient quantities, will inhibit the growth of neighbouring seedlings including those of its own species (Fig. 5). This observation is similar to that of several previous studies which reported parthenium weed residues to be highly suppressive to the growth of lettuce seedlings (Shinwari *et al.* 2013) and that of other weeds (Tefera 2002). As seen in these two previous studies, root growth in this present study was inhibited more than shoot growth, which is presumably because the roots are the first tissue to intercept and absorb the allelochemicals coming from the residues.

As seen before (Hanif 2014), the inhibition of lettuce seedling growth increased as the quantity of parthenium weed residues used in the bioassay increased (Fig. 1). As 50 mg of residue per well gave a quantifiable determination of growth inhibition, and because it was also the amount used in Fujii *et al*. (2004) initial studies, 50 mg of residue per well was selected as the amount to use in all subsequent experiments. In terms of what may accumulate in the field, 50 mg per well corresponds to approximately the amount of residue that would be produced onto the soil surface by a senescing parthenium weed population growing at a density of ~30 plants m^{-2}, which in fact represents a typical density of parthenium weed plants (Nguyen 2011; Dhileepan 2012; Hanif 2014). Given that a field-representative load of parthenium weed residue has been shown to reduce the growth of newly emerging seedlings of a wide range of species in the laboratory it can be assumed that similar effects would be observed in an invaded plant community. Combined with resource competition, the growth of neighbouring seedlings would be significantly affected by parthenium weed residues, at best the seedling would have low vigour, and eventually die, resulting in a reduction of community biodiversity as has been seen before by Belgeri (2013).

Relative phytotoxic activity of international biotypes

Significant differences in the ability to inhibit lettuce seedling growth were seen in the residue samples taken from parthenium weed plants coming from different parts of the world. It is interesting to note that residues from plants coming from its native range (Mexico, USA and Argentina) were no more or no less phytotoxic than those from the two introduced Australian biotypes. However, the introduced parthenium weed biotypes from China and Vietnam were significantly more growth inhibitory than the biotypes from the native range. Therefore, the novel weapons hypothesis (Ni *et al*. 2012), which suggests allelochemicals are only produced in plants outside of their native range cannot be fully supported by this study on leaf residues. The greater phytotoxic activity of parthenium weed residues from two biotypes (China and Vietnam) from the introduced range suggests that the increased phytotoxic capacity of these biotypes may have contributed to their invasive success in those locations. Additional studies are now necessary to evaluate the phytotoxic activity of residues from more biotypes collected from within the species' introduced and native range.

Interestingly, there were no significant differences observed in the phytotoxic capacity of the residues coming from the

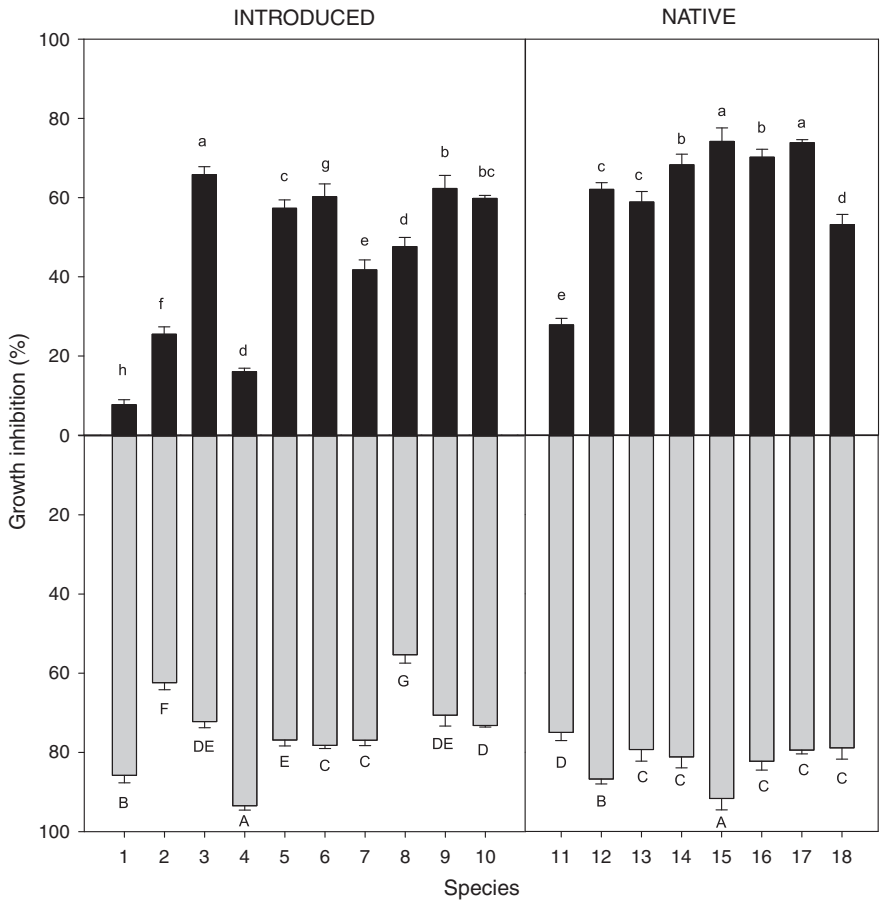

Fig. 5. The percent root (light grey) and shoot (black) growth inhibition of introduced pasture species seedlings (viz. (1) African lovegrass, (2) buffel grass, (3) creeping blue grass, (4) giant rats' tail grass, (5) green panic grass, (6) lambs quarters, (7) liverseed grass, (8) Rhodes grass, (9) tall finger grass and (10) parthenium weed – Clermont biotype) and native species (viz. (11) bull Mitchell grass, (12) cotton panic grass, (13) curly windmill, (14) forest blue grass, (15) kangaroo grass, (16) pitted blue grass, (17) Queensland blue grass and (18) weeping grass) when grown with leaf residues from parthenium weed (Clermont biotype). Error bars represent two standard deviations from the mean as calculated for nine replicates of five seedlings, and from duplicate experiments. Means within introduced and native species that do not share the same letter are significantly different from one another at $P > 0.05$.

Table 2. The percent root and shoot growth inhibition of nine introduced and eight native pasture species seedlings when grown with leaf residues from parthenium weed (Clermont biotype)

Means are shown with two standard deviations from the mean as calculated for nine replicates of five seedlings and from duplicate experiments. Means within introduced and native species that do not share the same letter are significantly different from one another at $P > 0.05$

Treatments	Average growth inhibition (%)	
	Shoot	Root
Introduced	44.41 ± 2.02a	74.50 ± 1.51a
Native	61.08 ± 2.17b	81.83 ± 2.24b

Clermont as compared with Toogoolawah biotype. As these two biotypes are known to have striking differences in their invasiveness, phytotoxicity of their leaf residues cannot be immediately considered to be one of the main factors for this invasiveness difference. However, as the Clermont biotype typically produces a much bigger (~30%) plant than the Toogoolawah biotype (Hanif 2014), the possibly exists that the bigger Clermont biotype plants will be more phytotoxic in the field than the smaller Toogoolawah biotype plants, potentially contributing to the superior invasiveness of this biotype.

Phytotoxic effect on a range of grasses and weeds found in pastures

The seedling growth of all grasses and weed species examined in this study were inhibited by parthenium weed residues (Fig. 4). This suggests that in the field seedling growth of several species would be reduced when growing in a location where parthenium weed residues had accumulated. It was noted that the inhibitory effects on seedling growth were more readily apparent in the native grasses than in the introduced grasses. To be specific, the average shoot and root growth of the native grasses was ~15% more inhibited than those of the

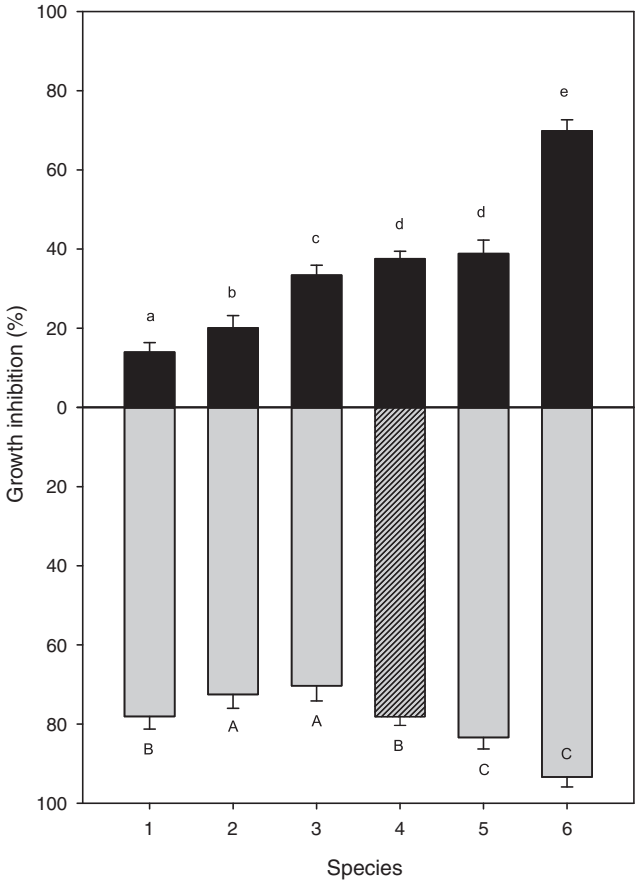

Fig. 6. The percent root (light grey) and shoot (black) growth inhibition of lettuce seedlings when grown with leaf residues from one of six species (viz. (1) orchid tree, (2) lychee, (3) chinaberry tree, (4) parthenium weed – Clermont biotype, (5) fiddlewood and (6) tamarind). The order used (left to right) is based on species response in Fujii's allelopathic species list (Fujii *et al.* 2004), with most allelopathic on right. Error bars represent two standard deviations from the mean as calculated for nine replicates of five seedlings and from duplicate experiments. Means within tissue type that do not share the same letter are significantly different from one another at $P > 0.05$.

introduced grass species. Thus, pastures comprised of mainly native grass species may be more susceptible to parthenium weed invasion than pastures comprised largely of introduced pasture species. It is also interesting to learn that parthenium weed can inhibit up to 70% of its own seedling growth. The observed differences in sensitivity to parthenium weed residues suggest that native species are more affected by parthenium weed-derived allelochemicals than the introduced pasture species (Fig. 4).

The magnitude of root growth inhibition observed among the species varied, with significantly less root growth inhibition seen for buffel grass and bull Mitchell grass relative to the other species. This may indicate a greater tolerance to parthenium weed residues in these species (Fig. 4). The seedling growth of two significant weed species, African lovegrass and giant rats' tail grass, was also quite tolerant to parthenium weed residues. Thus, assuming increased inhibition of pasture species growth with increasing parthenium weed residue mass, and analogous to results for lettuce observed in this study, at the end of the summer growing season the soil surface among and adjacent to the old parthenium weed plants will likely become covered with sufficient parthenium weed biomass to inhibit the growth of even the least sensitive seedlings emerging at that time.

Comparison of parthenium weed to other phytotoxic species

It has been argued (Shi *et al.* 2015) that one way to help determine the potency of a phytotoxic trait within a species is to compare it to other well characterised phytotoxic and non-phytotoxic species. When parthenium weed residue was compared with that of six other species, ranging from an estimated low residue phytotoxicity (*A. nobilis* Wall. and *L. chinensis* Sonnerat) to a medium (*M. azedarch* L. and *C. spinosum* L.) and a very high residue phytotoxicity (*T. indica* L.; as determined by Fujii *et al.* 2004), parthenium weed was seen to fit into a mid-range position. This indicates that when considering the phytotoxic effect of its residue, parthenium weed may not be as potent a phytotoxic plant as some have reported in the past (e.g. Batish *et al.* 2005; Belz *et al.* 2007; Khan *et al.* 2012).

Summary

These experiments provide evidence for the optimisation, or refinement, of the sandwich bioassay technique to investigate the phytotoxic activity of parthenium weed residues on lettuce seedling growth and highlight the importance of assay optimisation for each new species under study. Lettuce seedling growth exhibited different sensitivities to residues taken from parthenium weed plants coming from different biotypes, and Chinese and Vietnamese population showed a stronger inhibition of seedling growth than biotypes from both the native (USA, Mexico, Argentina) and introduced range (Australian). The greater phytotoxic effect seen in these two introduced range biotypes implies that the phototoxic activity may be related to invasive success. However, this was not the case for the Australian biotypes where one biotype was more invasive than the other. The more invasive nature of the Clermont biotype relative to the Toogoolawah biotype is therefore likely to be due to higher rates of aboveground biomass production by the Clermont biotype. As a group of 17 species, Australian native pasture species were more inhibited than introduced pasture species by parthenium weed residue. When compared with other well-known strongly and weakly phytotoxic species, parthenium weed residues sat within the middle range of the spectrum.

Conflicts of interest

The authors declare no conflicts of interest.

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