

UNIVERSITY OF NEW ENGLAND

**DEVELOPING A MULTI-SPECIES WEED-CONTROL THRESHOLD
MODEL FOR HIGH-YIELDING IRRIGATED COTTON**

A Dissertation submitted by
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For the award of Doctor of Philosophy

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Certification of Dissertation

I certify that the ideas, experimental work, results, analyses and conclusions reported in this dissertation are entirely my own effort, except where otherwise acknowledged. I also certify that the work is original and has not been previously submitted for any other award.

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REFLECTION ON A PhD JOURNEY

This PhD draws together and develops research undertaken over more than a decade, culminating in an exciting, new multi-species weed control threshold model for high-yielding cotton, extending the Critical Period for Weed Control far beyond any previous application of this concept.

It has for me been a long and challenging learning process, and one which has developed my skills as an experienced field scientist, to now be able to develop mathematical models to interpret field results, as well as developing a model-based weed management tool for farmers.

The field experiments began in 2002, using two mimic weeds (the cultivated plants sunflower and Japanese millet), a range of weed densities and multiple times of weed establishment and weed removal. A third mimic weed; mungbean, was added in later years.

The first phase of my PhD process involved working through the complex data base of approximately 40,000 values collected from these experiments over seven years, and finding and fixing typographical and numerical errors, such as plant height data on one occasion entered in a spreadsheet using millimetres as the unit of measurement, whereas all other height data was entered using centimetres as the measurement. This proved to be a much more involved task than I had expected, taking many months, with some errors only becoming obvious during the modelling phase of my work.

The second phase involved exploring the best way to present my data, with many alternatives to the critical period for weed control concept tested and rejected.

At about this time I underwent my confirmation of candidature interview, at which I was challenged regarding my unstated assumption that my findings using mimic weeds could be related to competition from real weeds. While driving home from the interview, I realised that some additional data from small plots that had been collected early in the experimental process probably held the answer to this question.

This began my first detour for my thesis plan, with this relatively simple set of data being very challenging to analyse and present in a coherent story. I sought biometrical help for this process, but was not satisfied with the analysis my biometrician suggested. This paper took around two years to write, with many versions back and forth to my supervisors. My first “final” draft eventually sailed through the system until the eighth internal reviewer

challenged the approach I had used for the analysis, threw it out and I was back to square one, wondering if my project was really worth the effort. A second version of the paper, using a totally different and more conventional approach to the analysis, was initially rejected for publication but later accepted after some alterations. This second analytical approach formed the basis for later papers. To my great satisfaction, this paper substantiated that mimic weeds could be substituted for real weeds in competition experiments and also opened the possibility for developing a multi-species competition model. My first major hurdle had been passed.

At about this time I was also introduced to the R statistical software, starting with a workshop run by the University of New England. This software is not user-friendly but is very powerful and has been an essential tool for analysis and graphing for my papers.

So, back to my thesis plan. I began working on analysing the data for the first of my mimic weeds (sunflower) and soon found that the data from this single weed was a paper by itself. Thus began my second, major detour from my thesis plan, with what I thought would be a single paper combining the results from all three mimic weeds becoming three published papers, one for each weed.

Analysis and modelling for the sunflower paper was time consuming, also involving learning to use the R statistical package but proceeded until it became obvious that I needed to expand my model to include weed density. Easily said, but how was I to do this? I could find nowhere in the literature where this had been done using the critical period for weed control concept, but it had been done using some other approaches. However, I was unable to make any of these alternative models work for me, with most papers not giving enough detail for me to understand their process. I also again sought assistance from a biometrician but to no avail. I spent many weeks exploring the literature and possible approaches for my analysis until, one day, after many trials and many errors, I attempted to add an additional term into the Gompertz equation (one of the standard equations used with the critical period for weed control model) and it worked. It seemed to describe my data – but was it a valid approach? When I demonstrated my solution to my biometrician, his response was “yes, you have just added in one of the higher-order terms”, and dismissed my months of frustrated searching as just an obvious step. The struggle to find this solution was the second major hurdle for my research.

My next two papers flowed from this second paper, with a lot of commonality between the results for the three mimic weeds, but also many interesting differences. My final paper brought together the findings from the four preceding papers, establishing a multi-species weed control threshold model which is directly applicable in Australian cotton production, and opening the door for this approach to be used with a range of other crops.

In many ways, the simplicity of my final, multi-species model seems to make the whole process appear obvious, but it was far from obvious, and the elegance of the final model belies the numerous pitfalls along the way. There were many points along my path where my approach seemed too flawed to be of value, with many previously published papers failing to develop critical period relationships that were applicable to more than a single species, site and season. I owe huge thanks to the many agricultural scientists and cotton growers who have established an Australian crop production system where cotton yields are largely limited only by temperature and sunlight (assuming irrigation water is readily available), making this crop so predictable and this modelling approach feasible.

When I look back on my adventure, there are three things that stand out.

1. Believe in the data. If the data is telling a strong story, keep working with the data until you find a way to describe the story. If there isn't a method to do this, you just might have to create the method. My inclusion of additional terms in the logistic and Gompertz equations I used for my models had not been done before in weed science, as far as I can tell, but it opens a doorway in the models that greatly expands their potential applications.
2. Be prepared to question the status quo. Whilst I will always have enormous respect for the agricultural scientists who have gone before me, I have learned that when they say something can't be done, that may only mean that it hasn't yet been done. Old "rules" don't necessarily apply to new situations in agriculture. I don't expect that a multi-species weed threshold model is possible in many rain-fed cropping systems, where crop yields are most closely related to prevailing seasonal conditions. However, the Australian cotton production system is different, where crop production is driven by temperature and soil moisture is maintained through irrigation. This difference makes the crop very predictable and more complex modelling feasible.
3. In science it doesn't matter how long the research takes. A sound idea is worth pursuing and should be pursued until the data answers the question, one way or the

other.

The work presented in this thesis doesn't represent a final answer for Australian cotton production, but rather a doorway for future weed control models. There are still many unanswered questions around a broadly applicable weed threshold model for Australian cotton, and many opportunities to apply this modelling approach in other crops and other situations. My advice to those who may follow is: it can be done, but you need to identify the driving constraints and ensure they are included in the model. For Australian cotton, the main constraint is temperature, or heat units, which were readily incorporated in my model. For other situations, the solution may not be so simple, but that doesn't mean it can't be done.

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ABSTRACT

Glyphosate tolerant and resistant weeds are becoming increasingly problematic in cotton fields in Australia, necessitating a return to a more integrated weed management (IWM) system. Ideally, an IWM system will combine the judicious use of herbicides with mechanical and cultural approaches to weed control, to optimise production, and minimise selection for herbicide resistance and negative off-target impacts. The development of an IWM system for cotton can be facilitated by identifying the critical period for weed control (CPWC), a concept that enables cotton growers to optimize the timing of their weed control inputs. CPWC models are commonly developed for specific weed and crop combinations, but the results from these studies are generally specific to the site, season and species tested. No CPWC model, applicable to multiple weed species, has been developed thus far. In order to explore the potential to create a multi-species CPWC model, applicable to a range of weeds in high-yielding, fully irrigated Australian cotton, we developed CPWC models using mimic weeds with widely differing morphological and physiological traits. We then combined the information from our individual mimic weeds and from this data, developed, and tested, a multi-species weed control model. Thus, we achieved the aim of this research, to test the hypothesis that a statistically valid multi-species weed-control threshold model for weeds of irrigated cotton could be developed, integrating aspects of weed size and density, and that such a model could be applied across seasons.

Crop plants have been used as mimic weeds to substitute for real weeds in many competition studies. These mimic weeds have the advantages of availability of seed, uniform germination and growth, and potentially conferring better experimental controllability and repeatability. However, the underlying assumption that the competitive effects of mimic weeds are similar to real weeds had not been tested. We compared a range of morphological traits (plant height, node and leaf number, leaf area, leaf size and dry weight) between the mimic weeds and real weeds: Japanese millet vs. awnless barnyard grass; mungbean vs. bladder ketmia; and sunflower vs. fierce thornapple. The impact of these mimic and real weeds on cotton plant height, node and leaf number, leaf area, leaf size, dry weight, lint yield and ginning percentage was also assessed. There were similarities and differences between the mimic and real weeds, but their impact on cotton lint yield was most closely associated with weed height and dry weight at mid-season. We concluded from this research that mimic weeds could be satisfactorily substituted for real weeds in competition experiments where seasonal and

environmental conditions are not limiting, such as with fully irrigated cotton, provided the plants have similar dry weight and height at mid-season, or one can account for the differences in dry weight and height. A more generalised relationship estimating the yield loss of high yielding, irrigated cotton from weed competition over a range of weed dry weights and heights was defined, allowing the competitive effects of a range of weeds to be extrapolated from the results of mimic weeds.

Based on our findings that mimic weeds could be satisfactorily substituted for real weeds in competition experiments, field studies were conducted over six seasons to determine the critical period for weed control (CPWC) in high-yielding, irrigated cotton, using sunflower, Japanese millet, and mungbean as mimic weeds. Mimic weeds were planted with or after cotton emergence at densities of: 1, 2, 5, 10, 20 and 50 plants m^{-2} (sunflower); 10, 20, 50, 100 and 200 plants m^{-2} (Japanese millet); and 1, 3, 6, 15, 30 and 60 plants m^{-2} (mungbean). Weeds were added and removed at approximately 0, 150, 300, 450, 600, 750 and 900 growing degree days (GDD) after planting.

High levels of intraspecific and interspecific competition occurred at the highest weed densities, with increases in weed biomass and reductions in crop yield not proportional to the changes in weed density. The data were fitted to extended Gompertz and logistic curves including weed density as a covariate, allowing a dynamic CPWC to be estimated for the observed weed densities. Using a 1% yield-loss threshold, the CPWC extended from before crop emergence through to 836 GDD, and full-season, from before crop emergence through to crop harvest for one and 35 or more sunflower plants m^{-2} , respectively. Japanese millet also competed strongly with cotton, with season-long competition resulting in an 84% reduction in cotton yield with 200 Japanese millet plants m^{-2} . Using a 1% yield-loss threshold, the CPWC commenced at 65 GDD, corresponding to 0 to 7 days after crop emergence (DAE), and ended at 803 GDD, 76 to 98 DAE with 10 Japanese millet plants m^{-2} , and 975 GDD, 90 to 115 DAE with 200 Japanese millet plants m^{-2} . Similarly, mungbean competed strongly with cotton, with season-long interference at densities of 60 mungbean plants m^{-2} resulting in an 84% reduction in cotton yield. Using a 1% yield-loss threshold, the CPWC extended for the full growing season of the crop at all weed densities. The minimum yield loss from a single weed control input was 35% at the highest weed density of 60 mungbean plants m^{-2} . The relationship for the critical time of weed removal was further improved by substituting weed biomass for weed density in the relationship.

Using data from these field studies, a multi-species CPWC model was developed from the combined data sets for sunflower, Japanese millet and mungbean competing in cotton, using

weed height and weed biomass as additional descriptors in the models. Comparison of observed and predicted relative cotton lint yields from the multi-species CPWC model demonstrated that the single model reasonably described the competition from these three very different mimic weeds, opening the possibility for cotton growers to use a multi-species CPWC model to determine the timing of weed control inputs in cotton fields.

Application of our multi-species weed threshold model in Australian cotton will facilitate the optimisation of the timing of weed control inputs and the prioritisation of fields for weed control. This will lead to further improved crop yields and improved economic returns to cotton growers, with benefits flowing through to other crops in the cropping system. Further, our approach should be applicable to other intensively farmed, fully irrigated crops, where crop yields are not highly influenced by environmental variability, such as many of the horticultural crops.

INTRODUCTION

The invasion and establishment of unwanted plants is the bane of all field cropping systems, whether they be cropping monocultures or more diverse systems (Holm et al. 1977). These unwanted plants of cropping, collectively categorised as ‘weeds’, are often plants common to the broader environment and have a wide range of characteristics, varying from prostrate, sometimes rhizomatous or stoloniferous plants, through to large herbs or shrubs that can be metres in height. Weeds commonly compete with crop plants for nutrients, light and water, can harbour pests and diseases, reduce machinery and irrigation efficiency, lead to product contamination, and can be toxic or injurious, potentially harming the crop, animals or humans (Holm et al. 1977). Most weeds have one or more of these characteristics and range from major weeds that can have serious negative impacts on the crop even at very low densities, through to minor weeds that can be tolerated in the crop at low or even moderate densities. Noogoora burr (*Xanthium occidentale* Bertol.) is an example of a major weed of cotton that can have serious negative impacts on the crop even at very low densities, reducing yield, contaminating lint, and damaging picking machinery, while red pigweed (*Portulaca oleracea* L.) has a minimal impact on crop yield when present at low or even moderate densities. The weed control threshold for Noogoora burr in Australian cotton was established at 1 weed in 195 m of cotton row (Charles et al. 1998), an extremely low weed density. Presence in excess of the threshold will necessitate management inputs to control the weed to avoid economic damage to the crop; damage which would exceed the cost of controlling the weed (Knezevic et al. 2002).

1.1 The Australian Cotton Industry

The modern Australian cotton industry began in the Wee Waa district of New South Wales in 1961, with 120 ha of cotton planted. Weeds were an impediment to cotton production from the outset, with yellow vine (*Tribulus micrococcus* Domin) a major problem in this first crop. Only 26 ha of the crop were harvested, with the remainder lost to weeds and flooding (Jones and Shaw 2014; Marshall 2015). The first large-scale crop of 1700 ha was planted in 1963 at Auscott, between Wee Waa and Narrabri, but again only 400 ha of the crop was harvested, with the remainder lost to unmanageable weed competition. These crops were fully irrigated using furrow irrigation (water running down furrows between cropping rows), and this has remained the most commonly used production system for cotton in Australia.

1.2 Fully Irrigated and Rain-fed Cotton

Cotton production in the Australian industry has continued to be based around furrow irrigation, with four to six or more irrigation events common in many seasons. Hot, fine days, with ample water and high levels of soil fertility, together with careful management of pests, have combined to produce the world's highest cotton yields in most seasons (Dowling 2019). However, reduced access to irrigation water due to ongoing droughts has limited yields in some seasons, such as the 2010–2011, 2016–2017 and 2018–2019 seasons (Dowling 2019). A shortage of irrigation water and inadequate rainfall during the cotton-growing season during these years resulted in a large reduction in Australia's average yields, with some crops only partially-irrigated. Many of these partially-irrigated crops received insufficient irrigation water later in the season when plants were filling bolls, the most critical stage of crop growth, leading to reduced yields of cotton lint.

Some areas of non-irrigated, rain-fed cotton are planted in most seasons, especially in the more favored areas, such as north-eastern NSW and the Darling Downs of south-eastern Queensland, where high levels of stored plant available soil water are often present at planting. Yields from these crops are variable and often much lower than the yields from fully irrigated crops, with yields largely driven by in-crop rainfall. Larger areas of rain-fed cotton are typically planted in wetter seasons, especially if cotton-lint prices are above the long-term average.

1.3 Herbicide Use and Weed Management in Cotton

The introduction of a range of residual herbicides, in combination with cropping rotations, improved management of weeds in fallow, within-crop inter-row cultivation, and hand-hoeing, allowed the cotton industry to develop an integrated approach to weed management. The residual herbicides used included: diuron; fluometuron; metolachlor; pendimethalin; prometryn; and trifluralin, with combinations of up to five of these herbicides commonly applied within a season. As a result, weeds become a more manageable problem on many cotton fields, and the weed seed-bank declined over time (Charles et al. 2004). Nevertheless, by 1989, weed management was still costing the industry around \$187 ha⁻¹, with in-crop herbicides costing \$76 ha⁻¹, and hand hoeing costing \$67 ha⁻¹ (Charles 1991), equivalent to \$409, \$166 and \$146/ha, respectively in 2020 dollars, adjusted for inflation (Webster 2020). This cost of \$187 ha⁻¹ for weed control represented around 10% of the growing costs of cotton (Cotton Australia 2020). The five most important weeds in cotton crops at that time were: Noogoora burr; nutgrass (*Cyperus rotundus* L.); Bathurst burr (*X. spinosum* L.);

Chinese lantern (*Physalis* spp.); and cowvine (*Ipomoea lonchophylla* J.M Black) (Charles 1991).

Competition from weeds continued to be a major issue on many newly-developed cotton fields, which often inherited heavy burdens of hard-seeded weeds from their prior land use. Heavy infestations of Noogoora burr, Bathurst burr and fierce thornapple (*Datura ferox* L.) were common, especially around creeks and on wetter areas in fields that had formally been used for extensive grazing by sheep. However, by the end of the 1990s, the regular inputs of multiple residual herbicides applied pre- and at-planting and post-crop emergence, together with inter-row cultivation, hand-hoeing and fallow weed management, had led to very low levels of weed pressure through much of the Australian cotton industry (Charles et al. 2004). Banding of residual herbicides became increasingly common by the end of the 1990s as a way of reducing herbicide input costs, with at-planting herbicides commonly applied on a 50% band behind the planter, with the 50 cm (50%) of the furrow (inter-crop area) not treated at the time of crop planting (Charles et al. 2004). The use of these multiple weed control inputs went together with an attitude of very low tolerance towards weeds by most cotton growers. Table 1 shows a typical weed control program prior to the introduction of transgenic cotton varieties with tolerance to glyphosate (Charles et al. 1995). Additional herbicides, cultivation and hand-hoeing inputs were used on fields with higher weed pressure.

Table 1. A typical weed control program prior to the introduction of cotton varieties with tolerance to glyphosate. Source: Charles et al. (1995).

Time of input	Input	
August	glyphosate	
September	trifluralin	incorporated by cultivation
at-planting	fluometuron	incorporated by the planter
November	inter-row cultivation	
	hand-hoeing	
December	inter-row cultivation	
	diuron	incorporated by cultivation
	hand-hoeing	
April	picking	
June	heavy cultivation	
July	2,4-D	

1.4 Changes in the Cotton Cropping System

Reductions in the number of cultivation passes and the use of glyphosate in the farming system became more common towards the end of the 1990s, with glyphosate increasingly used as the herbicide of choice in cropping fallows, often replacing cultivation passes (Charles et al. 2004). The use of glyphosate as an in-crop herbicide also became more common, applied between the crop rows through shielded-sprayers, purpose-built equipment, designed to prevent the herbicide contacting cotton foliage. These applications were targeted to manage weeds, such as nutgrass, that were not well controlled using a more traditional weed management program (Charles 1995).

The Australian cotton industry saw the introduction of the first cotton varieties with tolerance to glyphosate (Roundup Ready[®] varieties) in the 2001–2002 season. These varieties allowed glyphosate to be applied over-the-top of the crop from first emergence until the fifth node of crop growth. The Roundup Ready trait was replaced in the 2007–2008 season by the more robust Roundup Ready[®] Flex (RRF) trait that improved the crop's tolerance to glyphosate in later stages of crop growth, allowing glyphosate to be used over-the-top of the crop at all stages of crop growth. The RRF technology was rapidly adopted by the industry, with approximately 99% of cotton planted over the last decade including the trait (Tony May, Monsanto Australia, personal communication, February 2017). As a consequence, glyphosate is now the most commonly used herbicide for controlling weeds in the Australian cotton industry (Werth et al. 2013).

The widespread use of glyphosate in Australia has contributed to high levels of weed control in most cotton crops and has gone hand-in-hand with increasing cotton yields over time (Dowling 2019). The problem of poor seedling establishment seen in the 1980s and 1990s was often attributed to the use of residual herbicides pre- and at-planting, in combination with the presence of plant diseases and wet, cold conditions following planting (Charles and Taylor 2004). Some crops had to be planted two or three times before satisfactory plant stands were achieved in the worst years. Poor crop establishment due to issues with herbicide damage has become a rare occurrence over the past decade, with a large reduction in the amount of residual herbicide used pre- or at-planting (Charles and Taylor 2004; Werth et al. 2013). A wider and later crop planting window in the northern and central parts of the cotton industry, enabled by the improved cotton varieties and agronomic practices now used, has also allowing growers to largely avoid planting into cold and wet conditions, further reducing potential issues with residual herbicides. Wet and cold conditions at planting

remain a potential issue for the southern cotton area, which has a shorter growing season, but the ability to grow cotton in this area without the use of pre- and at-planting residual herbicides has contributed to relatively few crop establishment issues occurring over the past decade.

1.5 Weeds in Australian Cotton

Weeds remain ever-present pests of all cotton production in Australia, with glyphosate tolerant and glyphosate resistant weeds becoming increasingly problematic over the last decade due to over-reliance on glyphosate in the farming system, combined with a reduction in the use of other weed control tactics (Koetz 2019a; Werth et al. 2013). Many of these weeds are small- to medium-sized, broadleaf plants, such as bellvine (*Ipomoea plebeia* R. Br.), bladder ketmia (*Hibiscus trionum* L.), common sowthistle (*Sonchus oleraceus* L.), cowvine, and dwarf amaranth (*Amaranthus macrocarpus* Benth.). Werth et al. (2013) surveyed 19 Australian fields planted to cotton and found 40 weed species remaining after weed-control inputs, of which three species were large broadleaf weeds, 10 species were grass weeds and the other 27 species were small- to medium-sized broadleaf weeds. These weeds either emerged after the last in-crop weed-control input or were not controlled by the input. The glyphosate-resistance status of the weeds from these fields was not tested, but four of these species were amongst those known to have developed resistance to glyphosate (Heap 2019), including the two species most commonly found in these fields, flaxleaf fleabane (*Conyza bonariensis* L.) and common sowthistle.

Charles (2015) surveyed 73 cotton fields in the southern Australian cotton area between 2013 and 2015 and recorded 49 weed species remaining after weed-control measures had been undertaken, with an average weed density of 1.2 weeds m⁻². Of these weeds, 43 species were small- to medium-sized, broadleaf weeds. Six of the species observed in these fields were amongst those known to have developed resistance to glyphosate in Australia. Most of these species were present at relatively low densities of less than one plant m⁻², but higher densities were observed, with an average of eight red pigweed m⁻² recorded in one field during 2015. Another field had an average combined weed density of greater than 12 weeds m⁻² in 2015, with bladder ketmia and awnless barnyard grass (*Echinochloa colona* (L.) Link), each present at greater than five plants m⁻². Of the large weed species present: prickly lettuce (*Lactuca serriola* L.); spear thistle (*Cirsium vulgare* (Savi) Ten.); and variegated thistle (*Silybum marianum* (L.) Gaertn.), prickly lettuce is known to have developed resistance to glyphosate. Three of the grass species found in these surveys have also

developed resistance to glyphosate: annual ryegrass (*Lolium rigidum* Gaudin); awnless barnyard grass; and windmill grass (*Chloris truncata* R.Br.).

1.6 The Need for a Weed Control Threshold for Cotton

The increase in weed issues in cotton over the last decade has necessitated a return to a more integrated approach to weed management on many farms (an approach not just reliant on one tool; glyphosate, to control weeds), with the increasing use of residual herbicides, inter-row cultivation, spot spraying and hand hoeing (Koetz 2019b). One of the tools which could facilitate the adoption of an integrated weed management (IWM) system would be a weed control threshold, defining a trigger for initiating weed control, enabling cotton growers to optimise the timing of their control inputs (Knezevic and Datta 2015; Knezevic et al. 2002; Kores and Norsworthy 2015). A weed control threshold establishes the point at which the reduction in crop yield caused by weed competition equals the cost of controlling the weeds. Weed control is justified on economic grounds beyond this point.

A weed control threshold would help cotton growers better balance the need to control weeds before the weeds set seed and before weed competition significantly impacts crop yields, against the economic and practical imperatives to minimise inputs. By so doing, growers would protect crop yields, while reducing costs and reducing negative effects on production and the environment due to the unintended impacts of herbicides and other tools used for weed control (Taylor et al. 2004). However, with such a large number of weed species impacting cotton fields, it would be impractical to develop weed control thresholds for each species. To be of practical value, a weed control threshold would need to be based on a generalised model, applicable to groups of weed species, or ideally, all weeds.

Pest control thresholds have been widely used in cotton production in Australia, starting with the introduction of SIRATAC, a pest-threshold based tool introduced in the 1980s for managing heavy infestations of insecticide resistant helioverpa (*Helicoverpa armigera* and *H. punctigera*) (Hearn and Bange 2002). Since then, pest control thresholds have been adopted for all major insect and mite pests of cotton in Australia, with individual thresholds developed for each species or group of closely related species (Grundy 2019). The need for individual thresholds has been necessitated by the widely varying impacts of different insects. Thrips (*Thrips tabaci*, *Frankliniella schultzei*, *F. occidentalis*), for example, can cause unacceptable early-season damage to cotton, but at low numbers, can be beneficial in the crop later in the season as they are key predators of spider-mite eggs (*Tetranychus urticae*), another major pest species (Grundy 2019). Spider mites are generally a later-season pest, with

the threshold modified according to the expected length of the growing season for the differing cotton growing regions. Hence, very different thresholds are applied to thrips and mites as these pests impact the cotton crop in very different ways.

A multi-species weed control threshold should, at least conceptually, be simpler to develop than multi-species insect thresholds, as most weeds have similar competitive effects on a crop, with the level of damage due to plant competition most closely related to the time of weed emergence (relative to crop emergence) and duration of competition, weed density, and weed size (Askew and Wilcut 2001, 2002a, 2002b; Cortés et al. 2010; Fast et al. 2009; Korres and Norsworthy 2015; Ma et al. 2016; Scott et al. 2000; Webster et al. 2009). The impact of weed competition on a crop can also be affected by factors such as seasonal variation in weed species and density (Bukun 2004), soil moisture (Tingle et al. 2003; Vencil et al. 1993), soil fertility (Robinson 1976; Tursun et al. 2015), row spacing (Tursun et al. 2016), and crop health (Buchanan et al. 1977; Webster and Davis 2007). However, in fully irrigated cotton production in Australia, most of these factors are maintained as closely as possible to optimum, such that these factors should normally have little influence on the crop's response to weed competition. Hence, a multi-species weed control threshold model for irrigated cotton in Australia might be possible if the model is able to account for the time of weed emergence, duration of weed growth, weed density, and weed size.

1.7 The Concept of Critical Period for Weed Control

One feasible approach to determining the optimal timing for managing asynchronous weed germinations in a glyphosate-tolerant cotton crop to which glyphosate can be applied over the top from emergence to 22 nodes of crop growth (generally late in the season, depending on the rate of crop growth), is to define the critical period for weed control (CPWC) (Bukun 2004). The CPWC is the period of the season during which weeds need to be controlled to ensure yield losses due to weed competition do not exceed an identified yield-loss threshold (Fast et al. 2009; Korres and Norsworthy 2015; Webster et al. 2009). The yield-loss threshold is normally based on the cost of the weed control option to be used. The CPWC is determined for each weed and crop combination and relates to a specific level of weed competition (Bridges and Chandler 1987; Papamichail et al. 2002; Tursun et al. 2016). It is influenced by factors such as the time of weed and crop emergence (Webster et al. 2009), seasonal variation (Bukun 2004; Tingle et al. 2003), plant nutrition (Buchanan and McLaughlin 1975; Tursun et al. 2015), and row spacing (Buchanan et al. 1977; Rogers et al. 1976; Tursun et al. 2016).

The CPWC is determined by identifying the critical time for weed removal (CTWR), the critical weed-free period (CWFP), and the yield-loss threshold. The CTWR is the period after crop emergence during which weed competition can be tolerated without causing a yield loss exceeding the yield-loss threshold. The CWFP is the minimum period after crop emergence during which the crop must be maintained weed free to prevent a yield loss exceeding the threshold. The combination of the CTWR and the CWFP with the yield-loss threshold can be used to define the CPWC (Korres and Norsworthy 2015).

The CPWC has traditionally been determined for each weed and crop combination, but these experiments are both labor intensive and time consuming when multiple weeds are considered. Complications such as hard-seeded weeds and asynchronous weed germination can further confound the experiments. Alternatively, the CPWC can be determined for naturally occurring, mixed weed populations, although the results from these experiments are likely to be season and site specific (Buchanan et al. 1977), with different combinations of weed species, germination timings, and weed pressure occurring in each experiment (Bukun 2004; Korres and Norsworthy 2015; Ma et al. 2016; Papamichail et al. 2002; Tursun et al. 2015; Webster et al. 2009). Both approaches are challenging to apply to cotton production in Australia, where more than 70 weed species can be commonly found in the field and at least 40 species are considered to be problematic, ranging from prostrate weeds such as yellow vine, to potentially very large species, such as fierce thornapple, sesbania (*Sesbania cannabina* (Retz.) Poir.), and Noogoora burr (Werth et al. 2013). Other large weeds, such as Budda pea (*Aeschynomene indica* L.) and velvetleaf (*Abutilon theophrasti* Medik.) are also present in some regions.

1.8 Use of Mimic Weeds

An alternative approach for competition experiments is to use a “mimic” weed in place of naturally occurring weed populations. A mimic weed is normally a readily available cultivated species with a high germination rate and low seed dormancy. Many species have been used as mimic weeds, including barley (*Hordeum vulgare* L.) (Strydhorst et al. 2008); sunflower (*Helianthus annuus* L.) (Charles and Taylor 2007); Japanese millet (*Echinochloa esculenta* (A.Braun) H.Scholz) (Wu et al. 2010); oats (*Avena sativa* L.) (Brain et al. 1999), perennial ryegrass (*Lolium perenne* L.) (Afifi and Swanton 2012), rapeseed (*Brassica napus* L.) (Vollmann et al. 2010), white mustard (*Sinapis alba* L.) (Didon and Boström 2003; Lotz et al. 1996), and wheat (*Triticum aestivum* L.) (Cerrudo et al. 2012).

Mimic weeds are often chosen from crop species of the same genus as the real weeds for which they substitute: oats substituting for wild oat (*Avena fatua* L.) (Brain et al. 1999), and Japanese millet for awnless barnyard grass (Wu et al. 2010). When closely related species are not available, species of different genera that display similar morphological characteristics have been used, such as sunflower substituting for fierce thornapple (Charles and Taylor 2007).

Where nutrients and water are not limiting, weeds primarily compete for light (Didon and Boström 2003; Ma et al. 2016). Consequently, plant height, leaf structure and orientation, photosynthetic rate and growth rate are the primary morphological characteristics associated with plant competitiveness in well fertilized, fully irrigated crops (Didon 2002; MacRae et al. 2013), as is the situation for most cotton grown in Australia. Thus, mimic weeds are generally chosen to have a similar height and structure to the real weed, although other factors can be important, such as a similarity in sensitivity to herbicides (Brain et al. 1999).

Using a mimic weed in competition studies has many advantages, including greater experimental controllability and repeatability (Strydhorst et al. 2008), as the use of a mimic weed generally brings with it the ability to achieve more uniform weed germination, growth, density and distribution (Lotz et al. 1996; Vollmann et al. 2010). Using mimic weeds allows experiments to be conducted to examine aspects of competition such as the effects of crop variety (Didon and Boström 2003), soil nutrition and herbicide tolerance (Brain et al. 1999) without the confounding effects of uneven weed emergence, growth, density and distribution. Alternatively, mimic weeds can be valuable to examine the effects of weed emergence, growth, density or distribution, while keeping the other factors constant (Charles and Taylor 2007). Post-experiment issues, such as the on-going germination of hard-seeded weeds from seeds produced during the experiments (Walker et al. 2010), may also be reduced or largely eliminated by using mimic weeds.

1.9 A CPWC for Australian Cotton

No multi-species CPWC has yet been established for weeds in cotton. Large weeds, for example, can be competitive in cotton at relatively low densities, whereas low densities of small weeds may cause no detectable damage. Ma et al. (2016) found that velvetleaf densities as low as 0.25 plants m^{-1} of row reduced cotton growth and delayed crop development. Charles et al. (1998) reported even lower densities of Noogoora burr and fierce thornapple causing economic damage in cotton in Australia, with thresholds at 1 weed per 195 m and 1 weed per 73 m cotton row for Noogoora burr and fierce thornapple, respectively. As weed

density increases, generally the level of competition also increases, further reducing crop yields (Buchanan and Burns 1971; Ma et al. 2016; MacRae et al. 2013). However, intraspecific competition between weed plants also increases with increasing weed density and there is often a concurrent decrease in weed biomass per plant, although at a slower rate than the increase in weed density (Ma et al. 2016). This reduction in weed biomass per plant may not be reflected in other measures of weed size, such as weed height. Ma et al. (2016) found that an increase in velvetleaf density led to shorter, thinner cotton plants, but taller velvetleaf plants, even though weed biomass per plant declined. MacRae et al. (2013) observed a linear relationship between weed density and weed biomass m^{-1} of row, with no evidence of a reduction in Palmer amaranth (*Amaranthus palmeri* S. Watson) plant size with increasing weed density from two to 10 weeds m^{-1} of row.

Using data from three seasons, Charles and Taylor (2007) attempted to develop a multi-species CPWC model for Australian cotton with sunflower and Japanese millet as mimic weeds at a range of weed densities. They were able to fit CPWC models to each weed in each season and at each weed density, but could not generalise the relationships over species, densities or seasons. Charles and Taylor (2008) used a statistical approach to extrapolate the data for each season and weed density to produce a weed threshold model for Australian cotton based on the stage of crop growth, weed density and weed type for the categories: grass weed; medium-sized broadleaf weed; and large broadleaf weed. These weed control models of Charles and Taylor (2008) have been used by the Australian cotton industry over the past decade, but are of limited value as they are not predictive, they do not allow for differences in weed size beyond the three broad categories, they are not easily applied to mixed weed populations, and they rely on the accuracy of field sampling, which can be problematic in large fields, with individual cotton fields often in excess of 100 ha. It is important that both weed density and weed size is reported in future weed competition experiments to reduce site and seasonal variability in the results.

1.10 The Importance of Weed Size and Density in Competition Studies

Weed size is an important aspect of weed competition that has not been fully accounted for in many competition studies (Smith et al. 1990a). Buchanan et al. (1977) established both the CPWC and the minimum density of prickly sida (*Sida spinosa* L.) required to reduce the yield of cotton. Bridges and Chandler (1987) did the same for Johnsongrass (*Sorghum halepense* (L.) Pers.) and Tingle et al. (2003) for Paddy melon (*Cucumis melo* L.). However, in none of

these studies did the researchers consider the interaction of weed density or size and the CPWC.

In many other studies, the importance of weed density and size has largely been disregarded. Cardoso et al. (2011), for example, reported the relative abundance of weed species in a naturally occurring population, but did not report the actual weed density or biomass of the weeds, even though these measures were used to determine the relative abundance of the weeds. In many earlier studies, only the range of species present or the average weed density for the experiment was reported, often averaged over seasons (Buchanan and Burns 1970; Buchanan and McLaughlin 1975; Rogers et al. 1976; Snipes et al. 1987). Other researchers have recorded weed biomass (Papamichail et al. 2002; Webster et al. 2009), which is correlated with weed density (Ma et al. 2016; MacRae et al. 2013), but have not reported weed density. Fast et al. (2009), for example, reported Palmer amaranth biomass over time, but only gave the weed density averaged over four seasons.

This failure to fully account for weed density and size in many previous studies may be one of the factors leading to the site and seasonal variability in the results of many of these competition experiments (Korres and Norsworthy 2015; Ma et al. 2016). Other factors contributing to seasonal variability may include species differences and differences in weed emergence patterns (Fast et al. 2009; Robinson 1976a), rainfall (Rogers et al. 1976; Tingle et al. 2003), the presence of plant diseases (Buchanan et al. 1977), soil nutrition, and other soil constraints (Buchanan and McLaughlin 1975; Tursun et al. 2015). It is important that both weed density and weed size are reported in future weed competition experiments to reduce site and seasonal variability in the results.

1.11 Applying the Findings from Competition Studies in Fully-Irrigated Cotton

Although weed and crop interactions can be defined relatively easily in field experiments, all too often the results cannot be easily generalized to develop industry recommendations, because of the combination of diverse sites and season-specific factors. However, many of these constraints are less problematic in a fully-irrigated crop, such as most cotton grown in Australia, where crop and weed germination is usually initiated by irrigation, and soil nutrition and moisture are largely nonlimiting throughout the season. The impact of seasonal temperature differences between field sites and seasons can also be diminished by using growing degree days (GDD) as the measurement of time (Knezevic and Datta 2015; Tursun et al. 2016; Webster et al. 2009).

Alternatively, it would seem that more sophisticated crop growth models could be used to overcome these limitations which generally cause models to be site and season specific, by modelling crop and weed growth and including a wide range of environmental factors. In practice, however, Deen et al. (2003) found that increasing model complexity did not improve model accuracy and did not justify the additional levels of complexity involved.

While the inclusion of weed density is an improvement in weed:crop competition models, weed density alone can be a poor descriptor of weed competitiveness, especially in studies with naturally occurring weed populations, comprising of a range of weed species with differing competitiveness. In a comparison of data from nine sites with collectively 19 site:years of data, McDonald et al. (2004) found that 60% of the observed variation in crop yield could be accounted for by soil moisture and early-season temperature. Clearly, the rate of weed and crop growth needs to be accounted for in the competition model, especially with mixed weed populations. Competition models using measures such as weed ground cover, leaf area, biomass, or relative leaf area, rather than weed density, should result in more robust competition models (Kropff and Spitters 1991; Lutman et al. 1996; Lotz et al. 1996; Wilson et al. 1995), but these measures have not been commonly included in competition models for cotton crops. Where a measure of weed size has been used, this measure has most commonly been weed biomass. The inclusion of weed biomass in the competition model has overcome the issue of site and season variability in some studies (Askew and Wilcutt 2001; Charles et al. 1998; Fast et al. 2009; Smith et al. 1990a, 1990b), but not in other studies (Askew and Wilcutt 2002a; Clewis et al. 2008; Rowland et al. 1999; Scott et al. 2000; Snipes and Mueller 1992; Thomas et al. 2007).

There is also evidence that the CPWC approach may be too coarse to detect some of the early season effects of weeds, missing the impact of weeds on the growth of crop seedlings (Page et al. 2009). Nevertheless, the CPWC approach has merit in irrigated cotton production where weed management decisions are made not just on the basis of weed competition and economic thresholds, but also after consideration of prevailing weather conditions, the efficacy of the control tool, irrigation scheduling and resource availability. Given the accepted industry practice of planting cotton into a weed-free field and the complexity of management decisions made on a field-by-field basis, any lack of early-season precision in defining weed:crop competition would not negate the value of a weed-control threshold throughout the remainder of the cropping season (Buchanan et al. 1980).

The development of a competition model that is applicable over sites and seasons would seem more feasible in an industry, such as the Australian cotton industry, where a perennial,

indeterminate crop is primarily grown under full irrigation with abundant soil nutrition and the influence of many of the factors that typically limit competition models is minimised (Charles and Taylor 2007).

1.12 The Aim of this Research

The aim of this research was to test the hypothesis that a statistically valid multi-species weed-control threshold model for weeds of irrigated cotton could be developed, integrating aspects of weed size and density, and that such a model could be applied across seasons. The research sought to achieve this aim by first exploring the potential to use mimic weeds to define the CPWC in fully irrigated Australian cotton and developing CPWC models for each of three weeds, then developing and testing a multi-species CPWC model from these data. Data collected over six seasons were used to ensure the results from this research are not season specific. These aims are achieved in the following five studies published in Weed Technology in 2019 and 2020.

While the research was based on the use of mimic weeds and does not answer all the questions raised in this introduction, it does represent a large step forward in defining weed control thresholds of value to the cotton grower in Australia.

1.13 Outputs from the Research

This research outlines the results from a series of experiments over six summer seasons from 2002 to 2015.

The work starts with a small plot experiment which compares the competitive effects on cotton of three mimic weeds: Japanese millet; mungbean; and sunflower, with three weeds: awnless barnyard grass; bladder ketmia; and fierce thornapple, respectively. The comparison validates the use of mimic weeds in the subsequent research and was published as:

The value of using mimic weeds in competition experiments in irrigated cotton. Charles GW, Sindel BM, Cowie AL, Knox OGG (2019) Weed Technol 33:601–609.

Three papers flow from the initial work, exploring weed competition and developing dynamic (changing with time/crop growth) CPWC models individually for sunflower, Japanese millet and mungbean in fully irrigated cotton, each building on the findings of the preceding paper. The fourth paper on mungbean competition develops a dynamic CPWC model, which includes both weed density and weed biomass. These studies were published as follows:

Determining the critical period for weed control in high-yielding cotton using common sunflower as a mimic weed. Charles GW, Sindel BM, Cowie AL, Knox OGG (2019) *Weed Technol* 33:800–807

Determining the critical period for grass control in high-yielding cotton using Japanese millet as a mimic weed. Charles GW, Sindel BM, Cowie AL, Knox OGG (2020) *Weed Technol* 34:292–300, and

Determining the critical period for broadleaf weed control in high-yielding cotton using mungbean as a mimic weed. Charles GW, Sindel BM, Cowie AL, Knox OGG (2020) *Weed Technol* 34:689-698

The final paper integrates the results from the preceding three papers, firstly developing CPWC models including weed density and/or weed biomass for individual species, and then developing a multi-species CPWC model using weed height and weed biomass as descriptors. The paper concludes by testing the observed and predicted relative yields for each species and the combined data set. This paper was published as follows:

Developing a multi-species weed competition model for high-yielding cotton. Charles GW, Sindel BM, Cowie AL, Knox OGG (2020) [published ahead of print August 28, 2020]. [10.1017/wet.2020.97](https://doi.org/10.1017/wet.2020.97)

The value of using mimic weeds in competition experiments in irrigated cotton

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Nomenclature:

Bladder ketmia, *Hibiscus tridactylites* Lindl.; common sunflower, *Helianthus annuus* L.; cotton, *Gossypium hirsutum* L.; fierce thornapple, *Datura ferox* L.; Japanese millet, *Echinochloa esculenta* (A. Braun) H. Scholz; junglerice, *Echinochloa colona* (L.) Link; mungbean, *Vigna radiata* (L.) R. Wilczek

Keywords:

dry weight; leaf area; lint yield; plant height; morphology; model weeds

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Abstract

Crop plants have been used as mimic weeds to substitute for real weeds in competition studies. These mimic weeds have the advantages of availability of seed, uniform germination and growth, and the potential to confer better experimental controllability and repeatability. However, the underlying assumption that the competitive effects of mimic weeds are similar to real weeds has not been tested. We compared a range of morphological traits (plant height, node and leaf number, leaf area, leaf size, and dry weight) between the mimic weeds and real weeds: Japanese millet vs. junglerice, mungbean vs. bladder ketmia, and common sunflower vs. fierce thornapple. The impact of these mimic and real weeds on cotton was also assessed. There were similarities and differences between the mimic and real weeds, but impact on cotton lint yield was most closely associated with weed height and dry weight at mid-season. Mimic weeds may be satisfactorily substituted for real weeds in competition experiments where seasonal and environmental conditions are not limiting, such as with fully irrigated cotton, provided the plants have similar dry weight and height at mid-season. Alternatively, one can account for the differences in dry weight and height. We define here a generalized relationship estimating the yield loss of high-yielding, irrigated cotton from weed competition over a range of weed dry weights and heights, allowing extrapolation from the results with mimic weeds to the competitive effects of a range of weeds.

Introduction

Field experiments assessing the impact of weeds on crop growth and yield have been conducted for many crops and a wide range of weeds. These experiments generally fall into two categories: first, those using naturally occurring weeds, often hampered by uneven distribution and density of weed species and by staggered germination (Cardoso et al. 2011; Elezovic et al. 2012); and second, those using sown weeds, often of a single weed species and usually at specific densities (Ma et al. 2016; MacRae et al. 2013).

The second approach using sown weeds, often of a single species, offers the advantage of giving detailed information on the impacts of individual weed species. However, this approach can be challenging to undertake, as it can be difficult to obtain the large quantities of weed seed that may be required for field experiments. In addition, it can be difficult to get weeds to germinate at the densities and times required for an experiment, often because of low germination rates and variable seed dormancy (Strydhorst et al. 2008). These issues can be overcome by transplanting weeds into the field (Ma et al. 2016; MacRae et al. 2013), but this process is labor-intensive and the survival rate of transplanted weeds can be poor in hot, dry conditions, as is often the case over much of the period when cotton is grown in Australia.

An alternative approach for competition experiments is to use a “mimic” weed, which is normally a readily available cultivated species with a high germination rate and low seed dormancy. Among the many species used as mimic weeds are barley (*Hordeum vulgare* L.) (Strydhorst et al. 2008), common sunflower (Charles and Taylor 2007), Japanese millet (Wu et al. 2010), oats (*Avena sativa* L.) (Brain et al. 1999), perennial ryegrass (*Lolium perenne* L.) (Afifi and Swanton 2012), rapeseed (*Brassica napus* L.) (Vollmann et al. 2010), white mustard (*Sinapis alba* L.) (Didon and Boström 2003; Lotz et al. 1996), and winter wheat (*Triticum aestivum* L.) (Cerrudo et al. 2012).

Mimic weeds are often chosen from crop species of the same genus as the real weeds for which they substitute—oats substituting for wild oat (*Avena fatua* L.) (Brain et al. 1999) and Japanese millet for junglerice (Wu et al. 2010). When closely related species are not available, species of different genera that display similar morphological characteristics have been used, such as common sunflower substituting for fierce thornapple (Charles and Taylor 2007).

Where nutrients and water are not limiting, weeds primarily compete for light (Didon and Boström 2003; Ma et al. 2016). Consequently, plant height, leaf structure and orientation, photosynthetic rate, and growth rate are the primary morphological characteristics associated with plant competitiveness in well-fertilized, fully irrigated crops (Didon 2002; MacRae et al. 2013), as is the situation for most cotton grown in Australia. Thus, mimic weeds are generally chosen to have a similar height and structure to the real weed, although other factors can be important, such as a similarity in sensitivity to herbicides (Brain et al. 1999).

The use of a mimic weed in competition studies offers many advantages, such as greater experimental controllability and repeatability (Strydhorst et al. 2008). Mimic weed cultivation generally involves the ability to achieve more uniform weed germination, growth, density, and distribution (Lotz et al. 1996; Vollmann et al. 2010). Experiments utilizing mimic weeds can be conducted to examine aspects of competition such as the effects of crop variety (Didon and Boström 2003), soil nutrition, and herbicide tolerance (Brain et al. 1999), without the confounding effects of uneven weed emergence, growth, density, and distribution. Alternatively, mimic weeds can be valuable to examine the effects of weed emergence, growth, density, or distribution, while keeping the other factors constant (Charles and Taylor 2007). Post-experiment issues, such as the ongoing germination of hard-seeded weeds from seeds produced during the experiments (Walker et al. 2010), may also be reduced or largely eliminated by using mimic weeds.

The studies thus far, however, have relied on the often-unstated assumption that the competitive effect of the mimic weed is similar to that of a real weed. This assumption is central to these studies but has not previously been examined in the scientific literature. The aim of the current study was to test this assumption (that a mimic weed can have a competitive effect comparable to that of a real weed), comparing the morphological traits of the mimic and real weeds, and their competitive effects on cotton morphology and lint yield.

The weeds used in the study—bladder ketmia, fierce thornapple, and junglerice—are species commonly problematic in cotton fields in Australia (Werth et al. 2013) that represent a range of weed morphological types: a medium-sized broadleaf weed, a large broadleaf weed, and a grass weed, respectively. Bladder ketmia, fierce thornapple, and junglerice were compared with the mimic weeds: mungbean, common sunflower, and Japanese millet, respectively.

Materials and methods

Experiments were conducted in the three growing seasons, 2004–2005, 2006–2007, and 2007–2008, in bins constructed from fibrous cement sheets, each bin with surface dimension 1.0 m by 0.9 m, and 0.5 m deep, fully exposed to the external environment, at the Australian Cotton Research Institute, Narrabri (30.12°S, 149.36°E, elevation 201 m). Bins were filled with a sand and peat moss mix (2:1 by volume), with a drainage hole in each bin. Bins were arranged in eight rows, oriented north–south, and irrigated to saturation by overhead sprinklers in rows on a weekly basis. A slow-release complete plant fertilizer was applied at planting at the recommended rate, and bins were top-dressed every 2 wk with nitrogen at 18 kg ha⁻¹ in the form of granulated urea (46% N). Weed seeds of bladder ketmia, fierce thornapple, and junglerice were collected from multiple plants from nearby cotton fields over the 2002–2003 and 2003–2004 seasons.

Experimental design

The experiments used a factorial design with six species and five weed densities, with four replicates. Cotton and weeds were planted in a single row 0.9 m in length running north to south along the center of each bin on October 13 of each year. All bins were planted with cotton at 15 seeds m⁻¹, and weeds were planted to achieve 0, 1, 2, 5, and 10 plants m⁻¹ for common sunflower and fierce thornapple; 0, 3, 6, 15, and 30 plants m⁻¹ for mungbean and bladder ketmia; and 0, 10, 20, 50, and 100 plants m⁻¹ for Japanese millet and junglerice—one real or mimic weed species per bin. These target populations represented moderate to heavy levels of weed pressure, as typically seen in Australian cotton fields. Populations were thinned to the target numbers of weeds 6 wk after planting. The removed weeds were selected to optimize spacing between the remaining weeds. Bins were hand-weeded regularly to remove other weeds.

Data collection

The number of cotton plants and corresponding weed plants were recorded in each bin at mid-season, 90 d after planting (DAP), and at cotton harvest, 150 DAP. Plant height, the number of leaves, and the node number (broadleaf plants only) were recorded at mid-season on five cotton and five weed plants in each bin chosen randomly (all weeds were measured in treatments with fewer than five weeds per bin). Two weed plants were removed from each bin at this stage, 90 DAP, after cutting at ground level. Leaf area, leaf size, and aboveground plant weight of the weeds was recorded (a single weed was removed and measured in treatments with only one weed per bin). This removal was done after the end of the critical period for weed control (Korres and Norsworthy 2015). Leaf area was measured using a Li Cor LI-3100 area meter (LI-COR Inc., Lincoln, NE). Plants were weighed after drying at 70 C for at least 72 h in a forced-air oven. Weed and cotton height, cotton node number, and the aboveground plant dry weight of the cotton were recorded at cotton harvest. Once all bolls had opened, seed cotton was hand-picked from all cotton plants in each bin and was ginned using a single-saw gin to determine cotton lint yield.

Statistical analysis of paired datasets

Data for each of the morphological traits of both weeds and cotton, the cotton lint yield, and the ginning percentage were compared for the effects of year, density, and species by ANOVA for each real weed and mimic weed pair separately (junglerice vs. Japanese millet, bladder ketmia vs. mungbean, and fierce thornapple vs. common sunflower) using R statistical software (R version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria), with a significance level of $P \leq 0.05$. Treatment means for each weed pair were averaged over density and separated using Fisher's protected LSD test at $P \leq 0.05$, where density was not a significant effect in the analysis. Data were presented separately where density was a significant effect without an effect of species. Figures were used to present relationships where a significant effect of both density and species was indicated ($P \leq 0.05$). Linear (Equation 1), allometric (Equation 2), exponential (Equation 3), and hyperbolic (Equation 4) models were fit to these data and the Akaike information criterion used to select the model with best fit. These equations were as follow:

$$y = a + bx \quad (1)$$

Table 1. Comparisons of morphological traits for each weed and mimic weed pair at mid-season (90 d after planting), and plant height at cotton harvest (150 d after planting).

Weed species ^a	Plant height	Nodes ^b	Leaves	Leaf area	Leaf size	Dry weight	Plant height
	cm	No. plant ⁻¹		cm ² plant ⁻¹	cm ² leaf ⁻¹	g plant ⁻¹	cm
Junglerice	132 a	—	44	356	10.4		122
Japanese millet	91 b	—	34	441	11.3		101
Bladder ketmia	72	11.2 a	100 a	483 b	4 b	26.7 b	85
Mungbean	80	9.5 b	29 b	2,080 a	131 a	55.8 a	84
Fierce thornapple	100 b	8.3 b	67				145 b
Common sunflower	170 a	35.0 a	60				179 a

^a There was no significant ($P \geq 0.05$) effect of weed density. Within each pair, means followed by differing letters are significantly different according to Fisher's protected LSD test at $P \leq 0.05$.

^b Node number was not recorded for the grasses.

where y is the morphological trait, ginning percentage, or lint yield; x is the weed density; a is the intercept of the line; and b is the slope of the line,

$$y = ax^b \quad (2)$$

where y is the morphological trait, ginning percentage, or lint yield; x is the weed density; a and b are constants,

$$y = a + be^{cx} \quad (3)$$

where y is the morphological trait, ginning percentage, or lint yield; x is the weed density; a , b , and c are constants, and

$$y = \frac{ax}{b+cx} \quad (4)$$

where y is the morphological trait, ginning percentage, or lint yield; x is the weed density; a is the upper asymptote; b is a constant.

Standard errors of the means were determined using the delta method in R. The quality of the fit of the models was assessed using the coefficient of determination (r^2).

Statistical analysis of combined data set

The data sets for the six species were combined. The effects of weed species, density, and year on the cotton lint yield were examined for the combined data set using ANOVA. Density and year were not significant effects in the analyses, with differences in lint yield related only to species. To explore any associations between the morphological traits and cotton lint yield, a linear model (Equation 1) was developed, relating cotton lint yield per meter to weed species and the combination of each of the morphological traits per meter. Data for each trait were first tested for normality and log transformed where indicated by the analysis. Both transformed and nontransformed traits were included in the regression, allowing the results of the regression to select the relationship with the best fit. Traits that were not significantly correlated with cotton lint yield were progressively deleted from the analysis using the stepwise linear regression method. A minimal model derived from the analysis containing only those traits significantly ($P < 0.05$) correlated with lint yield was fit to the data. This process, using stepwise linear regression, was repeated to explore the association between the morphological traits and relative cotton lint yield.

Linear (Equation 1) and exponential (Equation 3) regression models were fit to the relationships for lint yield and relative lint yield, and the Akaike information criterion used to select the model with best fit. The quality of the fit of the models was assessed using the coefficient of determination (r^2).

Results and discussion

The species used as mimic weeds in this study were chosen because they had at least some physical resemblance to the real weeds selected for the study, with broad similarities in plant height, dry weight, and architecture. However, in-season measurements showed many differences between the real and mimic weeds, as described below.

Japanese millet vs. junglerice

Junglerice plants were taller than Japanese millet plants at mid-season, but no difference in plant height was observed at cotton harvest (Table 1). There were no effects of species or density on leaf number, leaf area, or leaf size at mid-season (Table 1). Dry weight at mid-season decreased with increasing weed density, but there was no effect of species (Table 2).

Increasing weed density reduced the height of the accompanying cotton plants at mid-season, with no difference between the species (Table 2). There was no effect of weed species or density on cotton leaf number at mid-season, or cotton height, node number, dry weight, or ginning percentage at harvest (Table 3). However, junglerice reduced cotton's node number at mid-season by one node when compared with Japanese millet (Table 3). The presence of either weed caused a reduction in lint yield of 30% or more at all densities, with junglerice reducing lint yield by 16% more than Japanese millet at the highest weed density—a 53% yield reduction compared with a 37% reduction for Japanese millet (Figure 1). The greater reduction in lint yield at the highest density may be related to the greater height at mid-season of the junglerice plants when compared with Japanese millet.

The junglerice in this study was quite different from the plants reported by Awan et al. (2014), who used junglerice collected from fields in Indonesia. At a similar weight, the Indonesian junglerice plants had many more leaves and much greater leaf area than the plants in our study. This disparity in leaf number and leaf area can be attributed to the plasticity of this species, to the very different selection environments of rice production in Indonesia, and to

Table 2. Comparisons of Japanese millet and junglerice dry weight, and the effect of these weeds on cotton height at mid-season (90 d after planting), over weed densities of 10 to 100 plants m^{-1} of crop row.

Weed species ^a	Weed dry weight				Cotton height			
	10 m^{-1}	20 m^{-1}	50 m^{-1}	100 m^{-1}	10 m^{-1}	20 m^{-1}	50 m^{-1}	100 m^{-1}
	g $plant^{-1}$				cm			
Junglerice	55.2 a	29.4 ab	19.5 ab	17.2 b	51.9 ab	47.4 b	45.1 b	43.0 b
Japanese millet	37.4 a	33.3 ab	22.0 ab	15.1 b	63.0 a	50.7 ab	45.0 b	46.5 b

^a The analysis showed no effect of species. Means followed by differing letters are significantly different according to Fisher's protected LSD test at $P \leq 0.05$.

Table 3. Responses of cotton morphological traits and ginning percentage to competition from weed and mimic weed pairs. Measurements were taken at mid-season, and at cotton harvest, 90 and 150 d after planting, respectively.

Weed species ^a	Mid-season			Harvest			
	Plant height cm	Nodes No. $plant^{-1}$	Leaves	Plant height cm	Nodes No. $plant^{-1}$	Dry weight g $plant^{-1}$	Ginning %
Junglerice		9.8 b	15.7	73	16.9	23.0	40.1
Japanese millet		10.9 a	18.3	74	17.8	30.1	40.7
Bladder ketmia	69	12.8	26.0	83	18.4	38.2 a	40.2
Mungbean	67	12.5	23.4	82	18.0	32.3 b	40.7
Fierce thornapple				80 a	18.8 a	36.9 a	40.3 b
Common sunflower				61 b	15.0 b	15.5 b	41.8 a

^a There was no significant ($P \geq 0.05$) effect of weed density. Within each pair, means followed by differing letters are significantly different according to Fisher's protected LSD test at $P \leq 0.05$.

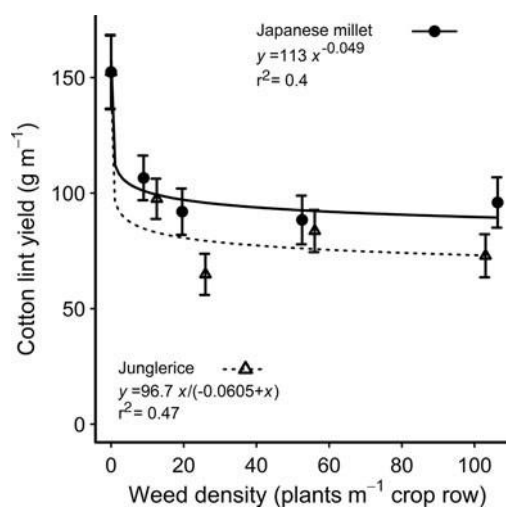


Figure 1. Reduction in cotton lint yield from competition with Japanese millet and junglerice over densities of 10 to 100 weeds m^{-1} of crop row. The parameters of the equations are shown within each figure. Vertical bars indicate 1 SEM.

the more open canopy of the broad-acre cropping system of the Australian cotton industry. Phenotypic plasticity among *Echinochloa* spp. has been reported elsewhere (Claerhout et al. 2016; Danquah et al. 2002; Tabacchi et al. 2006). Altop and Mennan (2011), for example, reported variations of up to 100% in plant height, leaf area, and dry weight of *E. crus-galli* (L.) Beauv. ECHCG samples from Turkey.

Given the known plasticity of the *Echinochloa* genus, the similarity of morphological traits between the Japanese millet and the junglerice used in our study indicates that Japanese millet has value as a mimic weed to substitute for junglerice. However, the variety of Japanese millet we used was less competitive than our junglerice and therefore not an ideal mimic weed to be substituted for junglerice. A more aggressive millet variety may be more suitable for future studies.

Mungbean vs. bladder ketmia

Mungbean and bladder ketmia plants remained similar in height throughout the season but varied in node number, leaf number, leaf area, leaf size, and dry weight at mid-season (Table 1). Bladder ketmia had two more nodes and three times more leaves at mid-season than did mungbean. However, the mungbean leaves were 34 times larger than the bladder ketmia leaves, and mungbean had 4 times the leaf area compared with bladder ketmia at mid-season. Mungbean plants were double the weight of the bladder ketmia plants.

There was no effect of weed species or density on plant height, node number, leaf number, or ginning percentage of the cotton plants (Table 3). However, mungbean reduced the dry weight of the cotton plants at harvest by 15% when compared with the bladder ketmia, with no effect of weed density. The presence of both weed species reduced cotton lint yield compared with the weed-free plots, but there was no effect of species (Table 4).

The bladder ketmia used in this study had a large number of small, deeply lobed leaves, with low specific leaf area compared with mungbean. Bladder ketmia is a variable species (Johnson and Craven 2013), with plants from different regions varying

Table 4. Response in cotton lint yield to the presence of bladder ketmia and mungbean at densities of 0 to 30 m⁻¹ of crop row.

Weed species ^a	Lint yield				
	0 m ⁻¹	3 m ⁻¹	6 m ⁻¹	15 m ⁻¹	30 m ⁻¹
	g m ⁻¹				
Bladder ketmia	159 a	137 abc	143 abc	108 c	117 bc
Mungbean	154 ab	119 bc	113 c	107 c	123 abc

^aThe analysis showed no effect of species. Means followed by differing letters are significantly different according to Fisher's protected LSD test at $P \leq 0.05$.

greatly in their morphological traits (Johnson 2003). The large number of small leaves is typical of bladder ketmia, with the plants in our study mid-range in size (height, leaf number, and leaf area) when compared with those in the study of Johnson (2003). Mungbean was more similar in leaf size to *Hibiscus verdcourtii* Craven, another closely related weed also common in Australian cotton fields (Werth et al. 2013).

Given the differences in node number, leaf number, leaf size, leaf area, and dry weight between the two species, mungbean would not seem to be a suitable mimic weed to substitute for bladder ketmia. However, the real and mimic weeds had similar competitive effects on the cotton, with no differences in their effect on cotton lint yield or their impact on other cotton morphological traits, other than a small difference in cotton dry weight at harvest. On this basis of a similar competitive effect, mungbean is an acceptable substitute for bladder ketmia.

Common sunflower vs. fierce thornapple

Fierce thornapple plants were shorter than common sunflower both at mid-season and at cotton harvest, although the difference in height diminished with time (Table 1). Fierce thornapple was a more open, branched plant and had only a quarter the number of nodes compared with common sunflower at mid-season, but the same number of leaves (Table 1). The common sunflower used in these experiments was a commercial variety ('Hysun 38') and produced a single head on the main stem, with no branching. There was a large disparity in leaf size at mid-season, with common sunflower leaves 18 times larger than fierce thornapple leaves at the lowest weed density, diminishing to 9 times larger at the highest density (Figure 2A). Plant density had almost no effect on fierce thornapple leaf size at mid-season, whereas common sunflower leaf size declined by 60% as weed density increased. The disparity in leaf size resulted in common sunflower having 11 times the leaf area of fierce thornapple at the lowest density at mid-season, declining to 4 times the leaf area at the highest density (Figure 2B). Weed dry weight was affected by both species and density. Common sunflower plants were four times heavier than fierce thornapple plants at mid-season for the lowest weed density, with no difference at the highest weed density (Figure 2C).

The presence of common sunflower had greater negative impact on the morphological traits of the accompanying cotton than did fierce thornapple at all weed densities and over the range of traits measured. Crop height at mid-season was reduced 47% more by one common sunflower per meter, than by one fierce thornapple, declining to 58% more at 10 weeds m⁻¹ of crop row (Figure 3A). At cotton harvest, the reduction in crop height was still 19 cm, or 24%, with no effect of density remaining (Table 3). Fierce thornapple had little impact on the node number of the cotton. At mid-season, the cotton plants had four fewer

nodes when competing with common sunflower compared with fierce thornapple at 1 weed m⁻¹, declining to six fewer nodes at 10 weeds m⁻¹ (Figure 3B). Cotton competing with common sunflower still had approximately four fewer nodes than cotton competing with fierce thornapple at cotton harvest (Table 3). Common sunflower reduced the leaf number per meter of the cotton by around 57% more than did fierce thornapple at mid-season over the range of weed densities (Figure 3C). Cotton plants were 60% lighter at harvest when competing with common sunflower, compared with plants competing with fierce thornapple (Table 3). These differences combined to cause a 41% greater reduction in lint yield when cotton competed with common sunflower compared to cotton competing with fierce thornapple at 1 weed m⁻¹, declining to a 50% yield reduction at 10 weeds m⁻¹ (Figure 3D). The fierce thornapple plants in our study weighed 91 to 236 g plant⁻¹ at mid-season for 10 and 1 plant m⁻¹ of crop row, respectively—much less than the plants reported in Charles et al. (1998), which averaged 1,100 ± 300 g dry weight plant⁻¹—but were similar in height to the plants in Charles et al. (1998) at 140 ± 10 cm (Table 1). This difference in weight was associated with heavy insect injury suffered by the plants in the current study, especially later in the season. This level of insect injury was not seen in fierce thornapples in the previous study, as insecticides were heavily used to manage insects in the cotton crop where these weeds were located. As a consequence of this heavy insect injury, the fierce thornapple in our study was less competitive than the plants in the earlier study and less competitive than the common sunflower, which suffered minimal insect injury. The common sunflower plants were similar in weight to the plants reported by Massignam et al. (2009), which at maturity ranged from 200 to 400 g plant⁻¹, but smaller in size than the fierce thornapple plants reported in Charles et al. (1998).

Given these differences in node number and branching, leaf size and area, and impact on cotton yield, the common sunflower variety chosen was not an ideal mimic weed to substitute for fierce thornapple. It is likely that a shorter, branched variety of common sunflower would have been more suitable for this study.

Cotton lint yield and the morphological traits

Selecting a mimic weed that closely resembles a real weed in both its morphological traits and its impact on the crop is an ideal that may be difficult to achieve in practice. Japanese millet, for example, is closely related to junglerice and appears to be a good substitute for this weed, but in our study, it caused around 15% less reduction in cotton lint yield compared with junglerice. This difference between Japanese millet and junglerice could not be overcome by adjusting the density of the mimic weed within the range of densities used in our study (Figure 1). Yet, for the cotton grower, it is the impact of the weed on the crop's yield that is the most important measure of the weed's competitive effect on the crop. Hence, in practical terms, a mimic weed that has the same impact on crop yield as the real weed is arguably a suitable substitute in competition experiments even if it differs in other morphological traits. Such was the case with bladder ketmia and mungbean, where despite the many differences in their morphological traits, the two species had similar impacts on cotton lint yield (Table 4). From this observation, we conjectured that differences or similarities in some of the morphological traits we measured were more strongly associated with reductions in cotton lint yield than were differences in other traits.

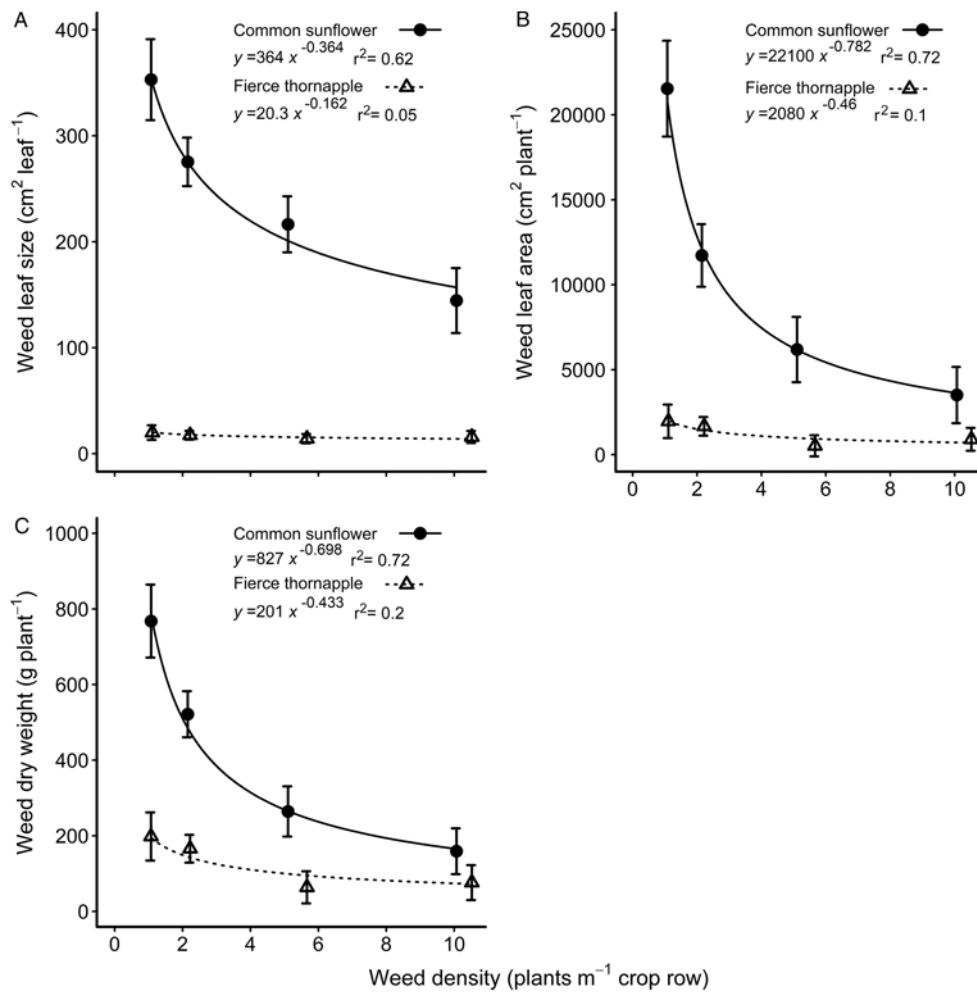


Figure 2. Comparisons of common sunflower and fierce thornapple leaf size (A), leaf area (B), and aboveground dry weight (C) at mid-season (90 d after planting) over densities of 1 to 10 weeds m⁻¹ of crop row. The parameters of the equations are shown within each figure. Vertical bars indicate 1 SEM.

To explore associations between morphological traits of a weed and reductions in crop yield, data sets over all weeds (real and mimic weeds) were combined and stepwise linear regression was used to determine which of the morphological traits was most significantly correlated with the reductions in cotton lint yield ($P \leq 0.05$). Of the traits measured, a combination of weed height and weed dry weight at mid-season were most significantly correlated ($P < 0.001$) with reductions in cotton lint yield, with neither species (real or mimic weed), plant density, nor year significant components of the regression. The relationship between cotton lint yield and a competition index combining weed height and weed dry weight at mid-season is shown in Figure 4. These relationships were further generalized by comparing relative cotton lint yield (lint yield as a percentage of the weed-free yield) with weed height (Figure 5A) and weed dry weight (Figure 5B) at mid-season, and developing a new combined competition index incorporating weed height and dry weight (Figure 5C).

The importance of weed dry weight in our findings was consistent with the observations of Charles et al. (1998), Goldberg and Landa (1991), and Ma et al. (2016), and effectively integrates many of the other components of competitiveness. Dry weight is often used as a measure of competitive ability (Awan et al. 2014; Brain et al. 1999; Cerrudo et al. 2012). Although weed density was not directly an important effect in our combined analysis, it

had a significant effect on weed dry weight (g m⁻¹) of all weed pairs, with dry weight per meter increasing as density increased. Hence, weed density was incorporated in the relationship as a component of weed dry weight, with dry weight a more significant indicator of reductions on crop yield than weed density per se. Plant height also significantly correlated with weed competitiveness (Didon 2002; MacRae et al. 2013), as plants primarily compete for light in situations where water and nutrients are not limiting. This correlation emphasizes the need to ensure that real weeds and their mimic analogues are as similar in height throughout the season as possible. Didon (2002) also observed that early growth rate and leaf angle can be important traits in competition for light, and these may have been additional factors in the differing responses of junglerice and Japanese millet. Our measurements were less detailed than that of Didon (2002), not allowing this level of scrutiny.

Selecting mimic weeds

From our findings, we conclude that mimic weeds should be chosen that match as closely as possible the height and dry weight at mid-season of the real weeds, with attention to changes in these traits throughout the season. Varietal variation in these traits may provide for closer matching among commercial crop varieties. In a fully irrigated cotton crop with good crop nutrition, the

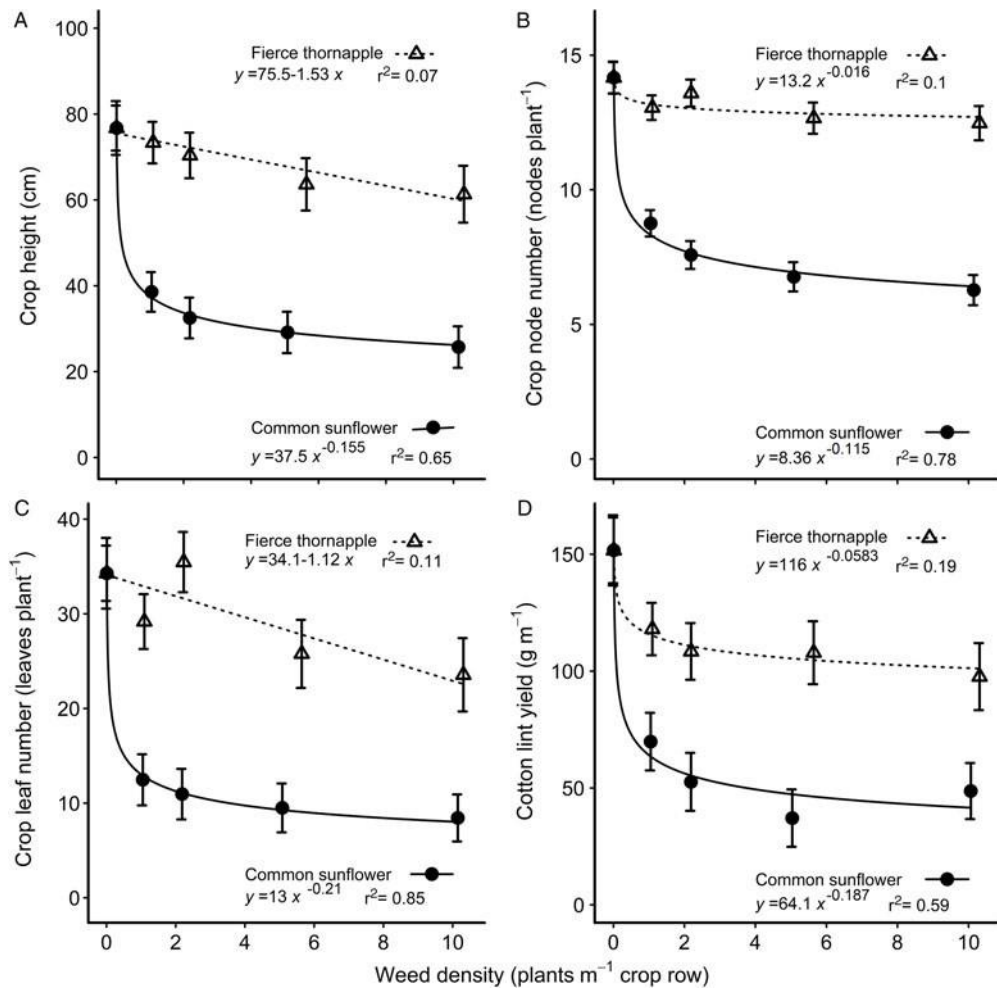


Figure 3. Effect of competition from common sunflower and fierce thornapple on cotton height (A), node number (B), and leaf number (C) at mid-season (90 d after planting), and the lint yield at cotton harvest (D) over densities of 1 to 10 weeds m⁻¹ of crop row. The parameters of the equations are shown within each figure. Vertical bars indicate 1 SEM.

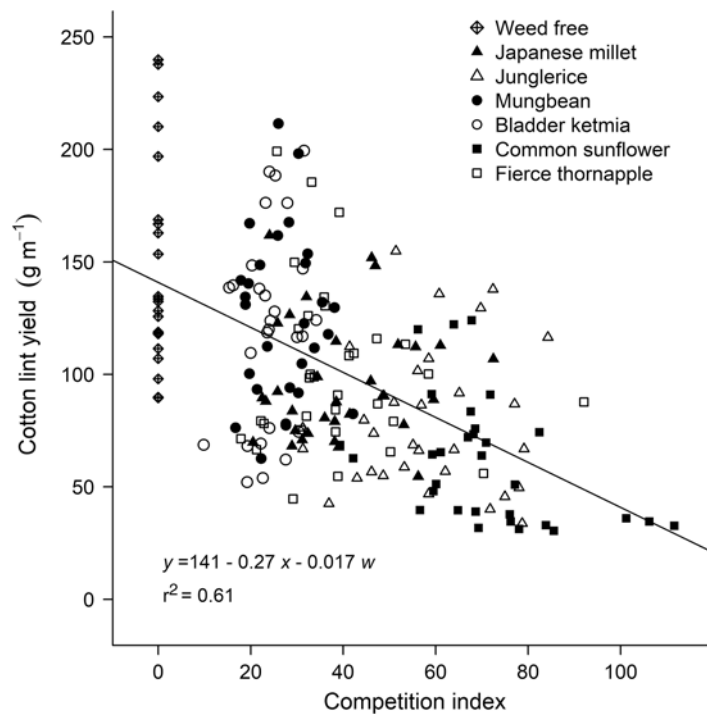


Figure 4. Cotton lint yield (y) was correlated with a competition index derived using stepwise regression. The competition index combined weed height (x) and dry weight in g m⁻¹ (w) at mid-season, 90 d after planting. The parameters of the equation are shown within the figure.

Table 5. The relationship between weed height and dry weight at mid-season (90 d after planting) of the mimic weeds, mungbean, Japanese, millet and common sunflower, and estimated relative yield of irrigated cotton using the regression equation $y = 91.3 - 0.167x - 0.0122w$, where y is the lint yield relative to the weed-free yield, x is the weed height, and w the weed dry weight

Species	Density	Height	Dry weight	Estimated relative lint yield
	Plants m^{-1}	cm	$g m^{-1}$	%
Mungbean	3	73	192	77
Mungbean	6	80	454	72
Mungbean	15	80	515	72
Japanese millet	20	88	466	71
Japanese millet	10	85	607	70
Mungbean	30	89	685	68
Japanese millet	50	96	658	67
Japanese millet	100	95	1,230	60
Common sunflower	1	183	857	50
Common sunflower	2	165	1,170	50
Common sunflower	5	169	1,440	46
Common sunflower	10	165	1,680	43

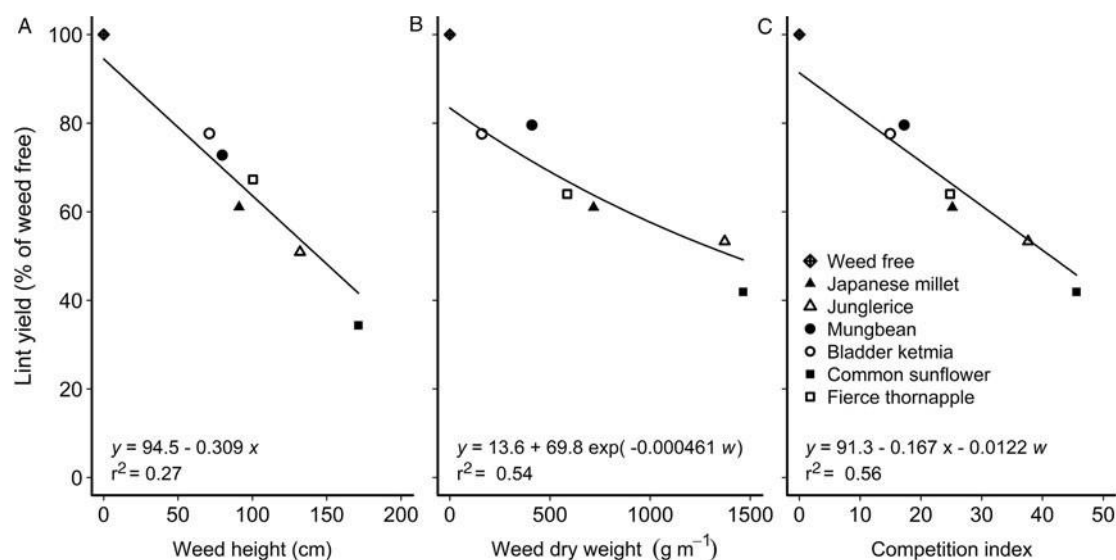


Figure 5. Cotton lint yield (y) as a percentage of the weed-free yield (y) was correlated with (A) weed height (x), (B) weed dry weight (w), and (C) the combination of height (x) and dry weight (w) at mid-season, 90 d after planting. The equations of the lines are shown within each figure.

competitive differences between plants as dissimilar as common sunflower and junglerice could be largely related to differences in plant height and dry weight. This difference allows the possibility of mimic weeds being substituted for a range of weeds in irrigated cotton, provided one can account for the differences in these traits. The mimic weeds in our study covered a range from a height of 73 cm and dry weight of $192 g m^{-1}$ for 3 mungbeans m^{-1} at mid-season, reducing the estimated yield of the accompanying cotton to 77% relative to weed-free cotton, through a height of 165 cm and dry weight of $1,680 g m^{-1}$ for 10 common sunflower plants m^{-1} , reducing the cotton yield to 43% (Table 5). The results from these mimic weeds could be used to estimate the yield loss for any weed in this height and dry-weight range. Data from other mimic weeds would be needed to define the relationship outside this range.

When comparisons are being made where seasonal variation can have a larger impact on crop production, such as in rain-fed cropping, this accounting for differences in height and dry weight may

require comparisons between real and mimic weeds in each experimental season to allow for seasonal variations (Halford et al. 2001). Where seasonal and environmental conditions are not limiting, such as with fully irrigated cotton, it seems likely that mimic weeds can be satisfactorily substituted for real weeds, provided the plants have similar height and dry weight at mid-season, or one can account for the differences in height and dry weight. From our results in fully irrigated cotton, it would seem that it is unnecessary to study the competitive effects of a full range of weed species, as results could be extrapolated from fewer species. However, differences in root biomass and architecture were not considered in our study where rooting depth was constrained. Differences between weeds in root biomass and architecture may be of more importance in the field, especially where soil moisture and nutrition are limiting. Future studies should be undertaken in the field where rooting depth and architecture are not constrained by artificial barriers, as may have occurred in our study.

This work has implications for competition experiments in well-fertilized, fully irrigated cotton, where competition studies have previously often been undertaken on a species-by-species basis. It may be possible to extend our experimental approach using mimic weeds to develop a multi-species weed competition model, where species differences are accounted for using differences in plant height and dry weight. This possibility warrants further testing in the field.

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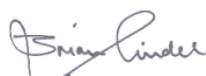
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Candidate

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We, the Research PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

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	Annette L. Cowie	10%
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Determining the critical period for weed control in high-yielding cotton using common sunflower as a mimic weed

Graham W. Charles¹, Brian M. Sindel², Annette L. Cowie³ and Oliver G. G. Knox⁴

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Abstract

Field studies were conducted over six seasons to determine the critical period for weed control (CPWC) in high-yielding cotton, using common sunflower as a mimic weed. Common sunflower was planted with or after cotton emergence at densities of 1, 2, 5, 10, 20, and 50 plants m^{-2} . Common sunflower was added and removed at approximately 0, 150, 300, 450, 600, 750, and 900 growing degree days (GDD) after planting. Season-long interference resulted in no harvestable cotton at densities of five or more common sunflower plants m^{-2} . High levels of intraspecific and interspecific competition occurred at the highest weed densities, with increases in weed biomass and reductions in crop yield not proportional to the changes in weed density. Using a 5% yield-loss threshold, the CPWC extended from 43 to 615 GDD, and 20 to 1,512 GDD for one and 50 common sunflower plants m^{-2} , respectively. These results highlight the high level of weed control required in high-yielding cotton to ensure crop losses do not exceed the cost of control.

Introduction

Cotton varieties with the glyphosate tolerance trait were first introduced to the Australian cotton industry in the 2000–2001 season and were rapidly adopted by the industry, with approximately 99% of cotton planted over the last decade including this trait (May, Monsanto Australia, personal communication). The widespread use of glyphosate in Australian cotton has led to high levels of weed control being achieved and has gone hand in hand with increasing yields, such that Australia continues to have the highest average cotton yields in the world (Dowling 2016).

However, an increasing number of weed species have developed resistance to glyphosate since 2000 (Heap 2019), and weeds continue to be troublesome for cotton growers. The development of resistance to glyphosate has primarily occurred because of overreliance on glyphosate as the main method of weed control in fallows between cropping phases, and these resistant weeds have now become problematic during the cropping phase (Thornby et al. 2013). Weeds have been generally well managed in cotton, with most weeds present at densities well below one plant m^{-2} after weed control operations; volunteer cotton often is the only exception to this rule (Werth et al. 2013).

Farmers have managed their weeds in cotton by adopting a low tolerance to weeds in crop and preventing seed set of weeds that survive a management operation, driving the seed bank down. This attitude of low tolerance to weeds, however, is becoming increasingly difficult to maintain on cotton farms as the occurrence of glyphosate-resistant weeds increases. Although a range of alternative herbicides remains available to cotton growers, none is as effective as glyphosate, and overreliance on the alternative chemistries will almost certainly result in weeds also developing resistance to these options. Managing weeds in irrigated cotton is made more difficult by asynchronous germination events, often making it challenging to determine the optimal timing for weed management inputs.

One approach to determining the optimal timing for managing asynchronous weed germinations in a glyphosate-resistant cotton crop to which glyphosate can be applied over the top from emergence to 22 nodes of crop growth is to define the critical period for weed control (CPWC) (Bukun 2004). The CPWC is the period of the season during which weeds need to be controlled to ensure yield losses due to weed competition do not exceed an identified yield-loss threshold (Fast et al. 2009; Korres and Norsworthy 2015; Webster et al. 2009). The yield-loss threshold is normally based on the cost of the weed control option to be used. The CPWC is determined for each weed and crop combination and relates to a specific level of weed competition (Bridges and Chandler 1987; Papamichail et al. 2002; Tursun et al. 2016). It is

influenced by factors such as the time of weed and crop emergence (Webster et al. 2009), seasonal variation (Bukun 2004; Tingle et al. 2003), plant nutrition (Buchanan and McLaughlin 1975; Tursun et al. 2015), and row spacing (Buchanan et al. 1977; Rogers et al. 1976; Tursun et al. 2016).

The CPWC is determined by identifying the critical time for weed removal (CTWR), the critical weed-free period (CWFP), and the yield-loss threshold. The CTWR is the period after crop emergence during which weeds can be allowed to compete with the crop without causing a yield loss exceeding the yield-loss threshold. The CWFP is the minimum period after crop emergence during which the crop must be maintained weed free to prevent a yield loss exceeding the threshold. The combination of the CTWR and the CWFP with the yield-loss threshold can be used to define the CPWC (Korres and Norsworthy 2015).

The CPWC has traditionally been determined for each weed and crop combination, but these experiments are both labor intensive and time consuming when multiple weeds are considered. Complications, such as hard-seeded weeds and asynchronous weed germination, can further confound the experiments. Alternatively, the CPWC can be determined for naturally occurring, mixed weed populations, although the results from these experiments are likely to be season and site specific (Buchanan et al. 1977), with different combinations of weed species, germination timings, and weed pressure occurring in each experiment (Bukun 2004; Korres and Norsworthy 2015; Ma et al. 2016; Papamichail et al. 2002; Tursun et al. 2015; Webster et al. 2009). Both approaches are challenging to apply to cotton production in Australia, where more than 70 weed species can be commonly found in the field and at least 40 species are considered to be problematic, ranging from prostrate weeds such as puncturevine (*Tribulus terrestris* L.), to potentially very large species, such as large thornapple (*Datura ferox* L.), sesbania [*Sesbania cannabina* (Retz.) Poir.], and common cocklebur (*Xanthium strumarium* L.) (Werth et al. 2013). Other large weeds, such as Indian jointvetch (*Aeschynomene indica* L.) and velvetleaf (*Abutilon theophrasti* Medik.) are also present in some regions.

No CPWC has been established for large broadleaf weeds in high-yielding cotton in Australia. Recent work by Charles et al. (2019) demonstrated the potential for using mimic weeds to define weed competition, and this approach could be applied to determining the CPWC. Use of a mimic weed could give the advantages of better control over weed density, more uniform weed emergence, and better experimental repeatability. Ideally, mimic weeds should closely resemble their weedy analogues, allowing the results to be directly translated from mimic weed to real weed.

Large weeds can be competitive at relatively low densities. Ma et al. (2016) found that velvetleaf densities as low as 0.25 plants m^{-1} of row reduced cotton growth and delayed crop development. Charles et al. (1998) reported even lower densities of common cocklebur and large thornapple causing economic damage in cotton in Australia. As weed density increases, generally the level of competition also increases, further reducing crop yields (Buchanan and Burns 1971; Ma et al. 2016; MacRae et al. 2013). However, intraspecific competition between weed plants also increases with increasing weed density and there is often a concurrent decrease in weed biomass per plant, although at a slower rate than the increase in weed density (Ma et al. 2016). This reduction in weed biomass per plant may not be reflected in other measures of weed size, such as weed height. Ma et al. (2016) found that an increase in velvetleaf density led to shorter, thinner cotton plants, but taller velvetleaf plants, even though weed biomass per plant declined. MacRae et al. (2013) observed a linear

relationship between weed density and weed biomass m^{-1} of row, with no evidence of a reduction in Palmer amaranth (*Amaranthus palmeri* S. Watson) plant size with increasing weed density from two to 10 weeds m^{-1} of row.

Weed density is an important aspect of weed competition that has not been fully accounted for in many competition studies (Smith et al. 1990). Buchanan et al. (1977) established both the CPWC and the minimum density of prickly sida (*Sida spinosa* L.) required to reduce the yield of cotton. Bridges and Chandler (1987) did the same for johnsongrass [*Sorghum halepense* (L.) Pers.] and Tingle et al. (2003) for smellmelon (*Cucumis melo* var. *dudaim* Naud.). However, in none of these studies did the researchers consider the interaction of weed density and the CPWC.

In many other studies, the importance of weed density has largely been disregarded. Cardoso et al. (2011), for example, reported the relative abundance of weed species in a naturally occurring population, but did not report the actual weed density or biomass of the weeds, even though these measures were used to determine the relative abundance of the weeds. In many earlier studies, only the range of species present or the average weed density for the experiment was reported, often averaged over seasons (Buchanan and Burns 1970; Buchanan and McLaughlin 1975; Rogers et al. 1976; Snipes et al. 1987). Other researchers have recorded weed biomass (Papamichail et al. 2002; Webster et al. 2009), which is correlated with weed density (Ma et al. 2016; MacRae et al. 2013), but have not reported weed density. Fast et al. (2009), for example, reported Palmer amaranth biomass over time, but only gave the weed density averaged over four seasons.

This failure to fully account for weed density and biomass in many previous studies may be one of the factors leading to the site and seasonal variability in the results of many of these competition experiments (Korres and Norsworthy 2015; Ma et al. 2016). Other factors contributing to seasonal variability may include species differences and differences in weed emergence patterns (Robinson 1976a), environmental differences (Fast et al. 2009), rainfall (Rogers et al. 1976; Tingle et al. 2003), the presence of plant diseases (Buchanan et al. 1977), soil nutrition, and other soil constraints (Buchanan and McLaughlin 1975; Tursun et al. 2015).

Although weed and crop interactions can be defined relatively easily in field experiments, all too often the results cannot be easily generalized to develop industry recommendations, because of the combination of diverse sites and season-specific factors. However, many of these constraints are less problematic in a fully irrigated crop, such as most cotton grown in Australia, where crop and weed germination is usually initiated by irrigation, and soil nutrition and moisture are largely nonlimiting throughout the season. The impact of seasonal temperature differences can also be diminished by using growing degree days (GDD) as the time measurement (Tursun et al. 2016; Webster et al. 2009). The objective of this study was to determine the CPWC for a large, mimic broadleaf weed in high-yielding irrigated cotton over a series of seasons and evaluate the impact of weed density on the CPWC.

Materials and Methods

Field studies were conducted over six seasons at the Australian Cotton Research Institute, Narrabri (30.12°S, 149.36°E; elevation 201 m), using commercial cotton cultivars Sicot 289 RRI in 2003–2004; Sicot 289 BR in 2004–2005; Sicot 80 BRF in 2005–2006, 2006–2007, and 2007–2008; and Sicot 71 BRF in 2015–2016. The soil was a heavy alluvial clay (fine, thermic,

smectitic, Typic Haplustert). Cotton was planted at 15 seeds m^{-2} on September 30, 2003; October 4, 2004; October 19, 2005; October 6, 2006; October 8, 2007; and October 21, 2015. The cotton was grown on raised hills, 1 m apart in fully irrigated fields and fertilized with 180 kg N ha^{-1} , in line with commercial practices. Irrigation was scheduled according to computer modelling of the crop's requirements. Common sunflower, 'Hyleic 43,' was planted in the 2003–2004, 2004–2005, and 2005–2006 seasons and 'Hysun 38' was planted in the later seasons at the specified densities and times in rows adjacent to and offset from the cotton rows by 100 mm. Plots were otherwise maintained weed free with trifluralin (Triflur X, 480 g ai L^{-1} ; Nufarm Australia, Melbourne, Victoria, Australia) at 1.1 kg ha^{-1} , incorporated at preplanting. Glyphosate (Roundup Ready Herbicide, 690 g kg^{-1} ; Monsanto Australia, Melbourne, Victoria, Australia) at 1 kg ha^{-1} was applied POST as necessary over weed-free plots (2004–2005 season and later), and hand hoeing was performed as needed.

Experimental Design

The experiments used a split-plot design within a randomized, complete block design with four replications. Main plots were times of weed planting and subplots were times of weed removal and weed densities. Subplots were 4 rows wide (4 m) by 10 m long. Common sunflower seed was planted to achieve 0, 1, 2, 5, 10, 20, and 50 plants m^{-2} , planted with the crop or at predetermined POST periods. Times of weed planting and weed removal were measured in GDD, using 15.5 C as the base temperature (Tursun et al. 2016). Times of weed planting and removal were targeted to occur at 150, 300, 450, 600, 750, and 900 GDD, but actual times were influenced by factors such as rainfall and irrigation scheduling. Not all weed densities and times of weed planting and removal occurred in all seasons, with weed emergence sometimes delayed by inadequate surface soil moisture at the time of planting, and not all target weed densities were achieved in all seasons.

The density of established weeds was counted on 1 m of row in each plot at the time of weed removal. Cotton was harvested at the end of each season using a modified commercial harvester with a single picking head, recording seed-cotton yield from the two central rows of each plot. Subsamples from one row were ginned using a single-saw gin to determine lint yield.

Statistical Analysis

Data were analyzed by ANOVA with replicate, year, time of weed interference and removal, and weed density as factors, using R, version 3.4.2, statistical software (Foundation for Statistical Computing, Vienna, Austria) with a significance level of $P < 0.05$. Analysis indicated no significant year effect or year interactions on relative lint yield (lint yield relative to the weed-free control in each season), allowing the data sets from the six seasons to be combined. Relative lint yield was significantly related to time of weed removal and interference, and weed density ($P < 0.001$). Data were then grouped into density categories such that the average density of each group equated as closely as possible to the nominal densities of 1, 2, 5, 10, 20, or 50 common sunflower plants m^{-2} . Relative lint yield was regressed as a function of the time of weed removal or interference within each nominal weed density.

The effect of weed interference at each nominal weed density was modelled using the Gompertz function (Equation 1) (Korres and Norsworthy 2015):

$$y = a \exp^{-\exp^{b(T-c)}} \quad [1]$$

where y is the yield as a percentage of the weed-free control, a is the upper asymptote (constrained to 100%), b and c are constants, and T is the cumulative degree days since planting. T (Equation 2) was defined as:

$$T = \sum \frac{(t_{min} + t_{max})}{2} - t_b \quad [2]$$

where t_{min} and t_{max} are the daily minimum and maximum air temperatures, respectively, and t_b is the base temperature of 15.5 C (Bukun 2004).

The effect of weed removals at each nominal weed density was modelled using the logistic function (Equation 3) (Korres and Norsworthy 2015):

$$y = \frac{a}{1 + \exp^{b(T-c)}} \quad [3]$$

where y is the yield as a percentage of the weed-free control, a is the upper asymptote, b and c are constants, and T is the cumulative degree days since planting.

These functions were extended to include actual weed density as a covariate. The extended Gompertz function (Equation 4) was:

$$y = a \exp^{-\exp^{b(T-c+dTW)}} \quad [4]$$

where d is an additional constant and W is the observed weed density.

The extended logistic function (Equation 5) was:

$$y = \frac{a}{1 + \exp^{b(T-c+dTW)}} \quad [5]$$

where d is an additional constant and W is the observed weed density.

Data for weed and crop height, and weed biomass were analyzed by ANOVA with replicate, year, time of weed removal, and weed density as factors, using a significance level of $P < 0.05$. Analysis indicated all year effects and year interactions were fully accounted for by the time of weed removal, allowing the data sets from the six seasons to be combined. Data were grouped into density categories and modelled using the Gompertz function (Equation 1) and an exponential model (Equation 6), and the Akaike information criterion used to determine the model of best fit for the data. The exponential model was:

$$y = a + b \exp^{cT} \quad [6]$$

where y is crop height in cm, or weed or crop biomass in $g \text{ plant}^{-1}$ or $g \text{ m}^{-2}$; a , b , and c are constants, and T is the cumulative degree days since planting.

Results and Discussion

Cotton Lint Yield and Weed Density

Cotton yields varied over the six seasons, with average lint yields for the weed-free plots of 1,800, 2,110, 2,450, 1,540, 2,250, and 2,110 kg lint ha^{-1} , in the 2003–2004, 2004–2005, 2005–2006, 2006–2007, 2007–008, and 2015–016 seasons, respectively.

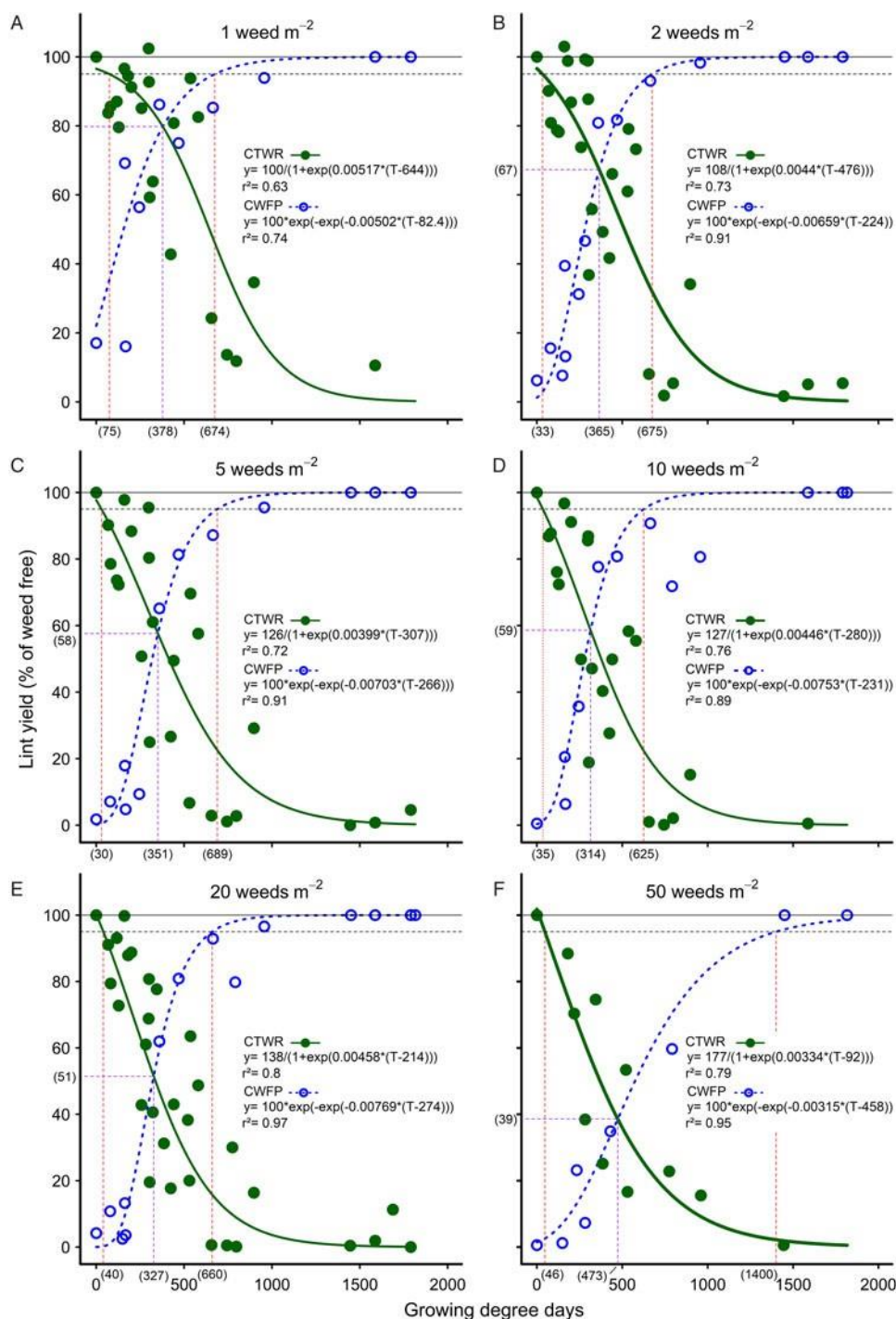


Figure 1. The influence of common sunflower interference durations—critical timing for weed removal (CTWR; green lines) and critical weed-free periods (CWFP; dashed blue lines)—on the cotton lint yield for densities of (A) 1, (B) 2, (C) 5, (D) 10, (E) 20, and (F) 50 weeds m^{-2} . Parameters of the Gompertz (CTWR) and logistic (CWFP) functions are shown within the figures, where y is the lint yield and T the cumulative degree days since planting. Data points for the relationships are treatment means. The horizontal solid lines indicate the weed-free yield and the horizontal dashed lines give a nominal 5% yield-reduction threshold. The critical period for weed control (CPWC) is defined by the upper intersection of the CTWR and CWFP lines with the threshold. The limits of the derived CPWC curves are shown by the vertical dashed red lines and values bracketed below the x-axis. The point of minimal yield loss is shown by the dashed purple lines and bracketed values.

These yields were close to the average yield of Australian cotton for these years (Dowling 2016).

Common sunflower competed strongly with cotton. The CTWR curves fit to each weed density showed that when the weed germinated with the crop, high levels of competition commenced

soon after crop emergence, with the curves rapidly declining toward 100% yield loss around mid-season (Figure 1). Season-long interference resulted in no harvestable cotton at densities of five or more common sunflower plants m^{-2} . Even at the lowest density of 1 m^{-2} , common sunflower still competed strongly with cotton,

with season-long interference reducing cotton lint yield by 89%. Weeds that emerged early in the season after crop emergence were still highly competitive, as shown by the CWFP curves.

An arbitrary 5% lint-yield reduction threshold was applied (Ghosheh et al. 1996), such that the CPWC was defined by the upper intersection of the CTWR and CWFP curves with the 5% threshold at each weed density. The critical periods so derived ranged from 75 to 674 GDD at one common sunflower plant m^{-2} to 40 to 660 GDD at 20 common sunflower plants m^{-2} and 46 to 1,400 GDD at 50 common sunflower plants m^{-2} (Figure 1A, 1E, and 1F, respectively). These results show little effect of weed density on the lower limit of the CPWC or on the upper limit of the CPWC at densities of one to 20 common sunflower plants m^{-2} .

This lack of response to weed density, however, appears to be an artefact resulting from the form of the curves used. Webster et al. (2009) proposed that another way of measuring the relative competitiveness of weed and crop was to consider the point of minimum yield loss from a single weed-control measure, that is, the point where the weed removal and weed interference curves intersect. This comparison shows that the point of minimum yield loss declined consistently with increasing weed density, from 20% yield loss with one common sunflower plant m^{-2} (Figure 1A) to 61% yield loss with 50 common sunflower plants m^{-2} (Figure 1F), corresponding to 378 GDD and 139 g weed biomass m^{-2} , and 473 GDD and 547 g weed biomass m^{-2} , respectively (Figure 2D). This result with one common sunflower plant m^{-2} was very similar to the results reported by Webster et al. (2009) for Benghal dayflower (*Commelina benghalensis* L.) competing in cotton, where, for June-planted cotton in 2004, the point of minimum yield loss of 19% occurred at 174 g weed biomass m^{-2} and 373 GDD.

Nevertheless, although there was a consistent decline in the point of minimum yield loss with increasing weed density, there was no consistent effect of weed density on the CPWC for one to 20 weeds m^{-2} . This lack of consistent response in our CPWC relationships reflects two components. First, the derived CPWC was very sensitive to the shape of the fitted curves as they approached the threshold. The upper end of the CTWR curve changed noticeably at the lowest density, resulting in an increase in the start of the CPWC at one common sunflower plant m^{-2} compared with other densities. Had a lower threshold been chosen, at say, 20% lint yield loss, the change in the critical period would have been much more clearly correlated to changing weed density, increasing from no critical period for one common sunflower plant m^{-2} to 148 to 934 GDD with 50 common sunflower plants m^{-2} . This sensitivity to the shape of the curves as they approach the asymptote is fundamental to the type of curves used (i.e., logistic and Gompertz curves) and has not been an issue in most previous work, where the CPWC commenced several weeks after crop emergence and the maximum observed yield loss was much less than 100% (Cardoso et al. 2011; Korres and Norsworthy 2015; Webster et al. 2009). We overcame this issue for the Gompertz curves by constraining the curves to asymptote at 100%. Such a constraint applied to the logistic curves, however, distorted the curves unacceptably. Consequently, the issue of sensitivity to curve shape is an inherent problem with logistic curves when highly competitive weeds are present, crop damage commences at or soon after crop emergence, and the maximum crop damage approaches 100% yield loss.

The second reason for the lack of response to weed density in the CPWC relationships is that the competitive effect of the weeds is not directly proportional to the density of weeds, because of

increasing intraspecific competition between the weeds with increasing weed density. Thus, the level of competition experienced by the crop increased at a slower rate than the rate of increase of the weed density. This effect became increasingly important as weed density increased.

Plant Height and Biomass

There was little difference in weed height (Figure 2A) or weed biomass (Figure 2C) with increasing weed density up until approximately 300 GDD. Later in the season, weed height and biomass per plant decreased as weed density increased, but the changes were far less than the changes in weed density, as was observed with velvetleaf in cotton (Ma et al. 2016). At mid-season (800 GDD), weed biomass approximately doubled, from 850 g m^{-2} to 1,553 g m^{-2} , as weed density increased 50-fold (Figure 2C). At the same time, weed height declined by only 21% (Figure 2C), but weed biomass per plant decreased by 96% from 841 g $plant^{-1}$ at 1 $plant\ m^{-2}$, to 34 g $plant^{-1}$ at 50 weeds m^{-2} , a 25-fold change in biomass. Similarly, crop height at mid season was reduced by 48% by a single common sunflower plant m^{-2} , from 73 cm to 38 cm (Figure 2B). Increasing the weed density 50-fold to 50 common sunflower plants m^{-2} only reduced crop height by an additional 30% to 16 cm. Crop biomass at mid season was reduced by 73% by a single common sunflower plant m^{-2} , from 312 to 86 g m^{-2} (Figure 2D). Increasing the weed density 50-fold to 50 common sunflower plants m^{-2} only reduced crop biomass by an additional 22% to 20 g m^{-2} .

The decrease in common sunflower biomass per plant with increasing weed density followed the trend reported for johnson-grass (Bridges and Chandler 1987) and velvetleaf up to nine weeds m^{-1} crop row (Ma et al. 2016) but was a much steeper curve than reported in these studies. At 300 GDD, the curve declined from 121 g $plant^{-1}$ with one common sunflower plant m^{-2} , reaching an asymptote of 14 g $plant^{-1}$ and reaching 95% of this asymptote (20 g $plant^{-1}$) at 20 weeds m^{-2} (Figure 3A). By 600 GDD, the initial part of the curve had become steeper again, reaching 95% of the asymptote of 55 g $plant^{-1}$ at 11 weeds m^{-2} (Figure 3B). The curve became increasingly steep with time, by 900 GDD reaching 95% of the asymptote of 78 g $plant^{-1}$ at five weeds m^{-2} (Figure 3C). These steeper curves relate to the much higher levels of competition in our experiments, with common sunflower densities of up to 50 weeds m^{-2} and mid-season weed biomass of up to 1,550 g m^{-2} (Figure 2D). With velvetleaf densities of up to 25 m^{-2} and end-of-season weed biomass of greater than 1,100 g m^{-2} , Cortés et al. (2010) also observed much steeper relationships than reported by Bridges and Chandler (1987) and Ma et al. (2016), although not as steep as in the current study, probably due to the lower levels of weed biomass in the earlier studies.

The steepness of the curves in our studies (Figure 3) indicates that very high levels of both intraspecific and interspecific competition were occurring at the higher weed densities (Firbank and Watkinson 1985; White and Harper 1970). Self-thinning of the common sunflower population could be expected at these high weed densities (Deng et al. 2012), as was observed by Robinson (1976a) at much lower weed biomass levels than occurred in the current study. However, no reductions in either the common sunflower or cotton densities were observed over the course of the experiments beyond the mortality level of the plots with the lowest weed density, with plant survival probably enhanced by regular irrigation and high levels of soil nitrogen (Robinson 1976b).

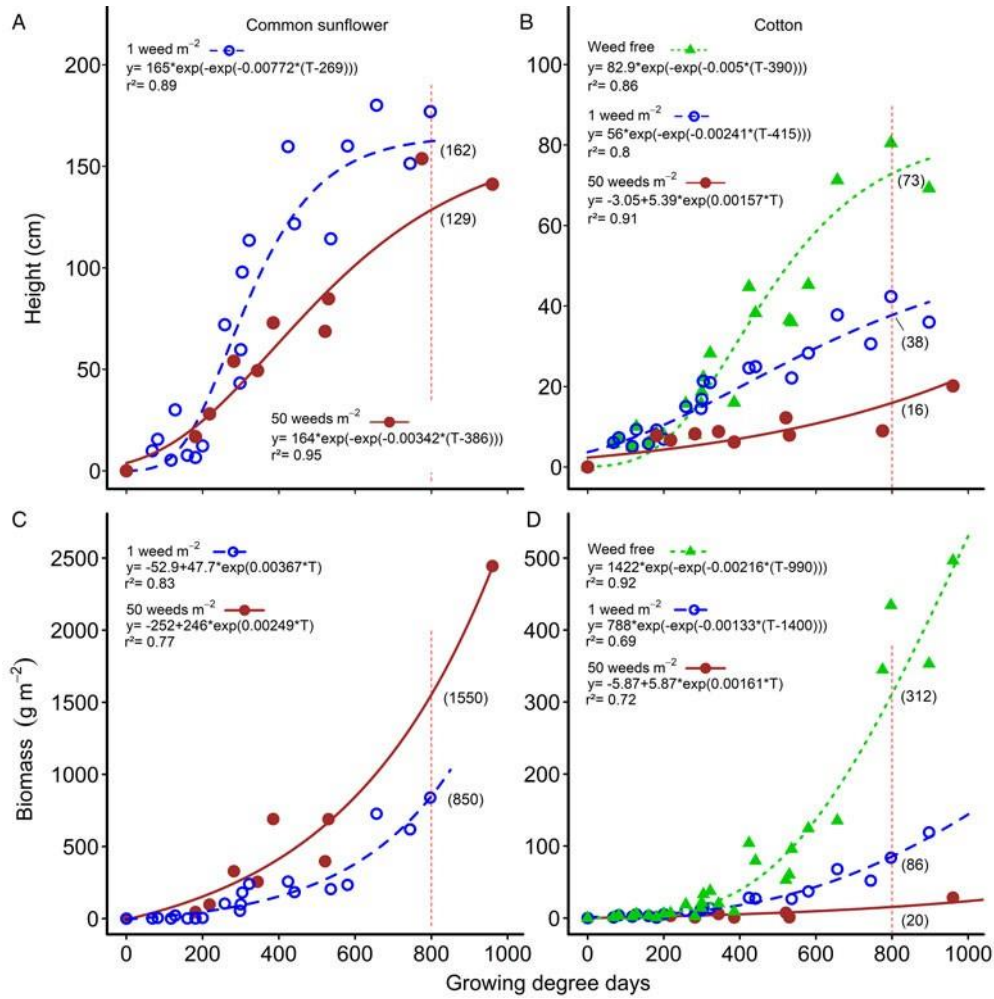


Figure 2. Changes in common sunflower and cotton height (A and B, respectively) and above-ground biomass (C and D, respectively) over the growing season for weed densities of 0 (weed free), 1, and 50 m⁻². Parameters of the models are shown within the figure. Data points for the relationships are treatment means. Height and biomass values at mid season (800 growing degree days) are indicated by the vertical red dashed lines and bracketed values.

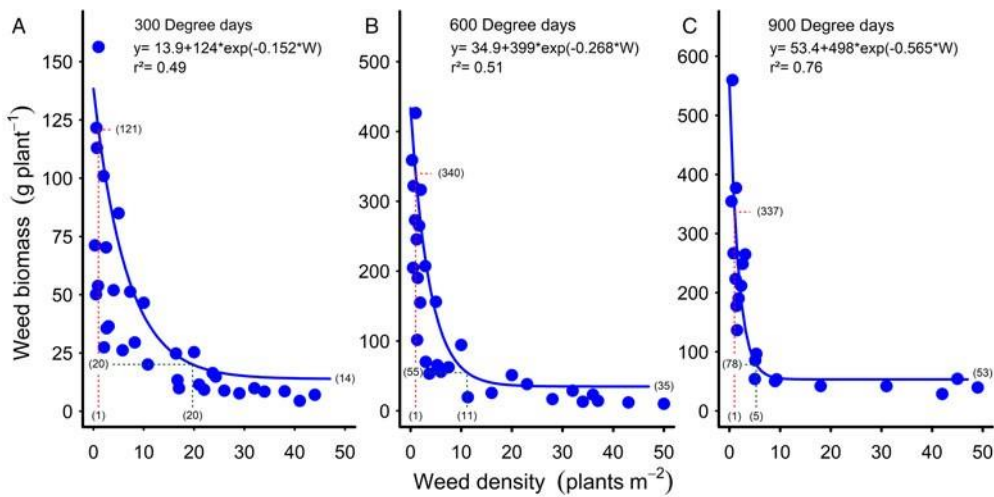


Figure 3. Reduction in common sunflower dry biomass with increasing weed density at (A) 300, (B) 600, and (C) 900 growing degree days. Parameters of the models are shown within the figures. Data points for the relationships are treatment means. Biomass values at one plant m⁻² are indicated by the red dashed lines and bracketed values. Green dashed lines and bracketed values show the biomass and plant density at 95% of the asymptote. The weed biomass asymptote values are bracketed at the ends of the curves.

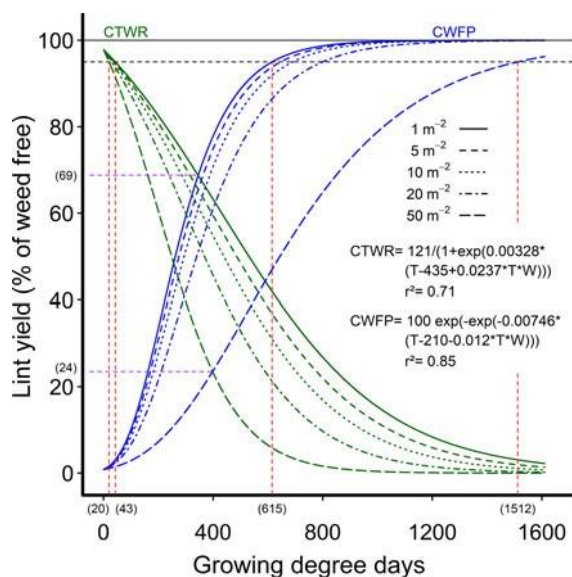


Figure 4. Dynamic relationships showing the influence of common sunflower interference durations—critical timing for weed removal (CTWR) and critical weed-free periods (CWFP)—on the relative cotton lint yield, using extended Gompertz (CTWR) and logistic (CWFP) functions including weed density as a covariate. Parameters of these models are shown within the figure. The derived relationships for common sunflower densities of 1, 5, 10, 20, and 50 m^{-2} are presented as examples. The horizontal solid line indicates the weed-free yield and the horizontal dashed line gives a nominal 5% yield-reduction threshold. The critical period for weed control (CPWC) is defined by the intersection of the CTWR and CWFP lines with the threshold. The CPWC for one and 50 common sunflowers m^{-2} are shown by dashed red lines. The limits of the CPWC are bracketed below the x-axis. The point of minimum yield loss from a single weed control input at one and 50 weeds m^{-2} is shown by the dashed purple lines and bracketed numbers.

Dynamic Relationships for Cotton Lint Yield

As an alternative approach to address the lack of consistent response of the CPWC to weed density at a 5% yield-loss threshold, we fit the data to extended Gompertz and logistic curves that included weed density as a covariate in the equations. These extended equations allowed a CPWC to be calculated for all densities in the experimental range of one to 50 common sunflower plants m^{-2} . The equations also had the advantage of using actual weed densities, rather than the grouped densities of the earlier equations, making more efficient use of the data and creating a dynamic CPWC that can be more easily applied in the field. The CPWC estimated by these equations increased from 43 to 615 GDD for one common sunflower plant m^{-2} to 20 to 1,512 GDD for 50 common sunflower plants m^{-2} , using a 5% yield-reduction threshold (Figure 4).

The lower limit of the dynamic CPWC commenced between PRE and 11 d after crop emergence (DAE) for one common sunflower plant m^{-2} , and between PRE and 6 DAE for 50 common sunflower plants m^{-2} over the six experimental seasons. The CPWC ended at 615 GDD (63 to 81 DAE) for one common sunflower plant m^{-2} and 1,512 GDD (136 DAE to full season) for 50 common sunflower plants m^{-2} , with the CPWC ending after the application of the first crop defoliant in two of the six seasons. The start of our dynamic CPWC using a 5% yield-loss threshold was earlier than has been reported in many previous studies, commencing at or prior to crop emergence in four of six seasons. With a mixed population including the large weed common cocklebur, Bukun (2004) reported the start of the CPWC at 100 to 150 GDD with a 5% yield-loss threshold, although

that CPWC commenced before planting when Bukun (2004) assigned a 2.5% threshold. Snipes et al. (1987) reported the start of the CPWC 14 to 28 d after planting with approximately eight common cocklebur plants m^{-2} , similar to our 6 to 21 d after planting for eight common sunflower plants m^{-2} , but many others recorded the start of the CPWC between 19 and 31 DAE (Cardoso et al. 2011; Fast et al. 2009; Korres and Norsworthy 2015; Papamichail et al. 2002).

The end of the CPWC was also later in our study than has been reported in many previous studies, extending late into the growing season for 50 common sunflower plants m^{-2} (1,512 GDD, or 136 DAE or more), with most other studies reporting the end of the CPWC at 42 to 84 DAE (Bukun 2004; Cardoso et al. 2011; Korres and Norsworthy 2015; Papamichail et al. 2002; Tursun et al. 2015). Tursun (2016), for example, calculated the end of the CPWC at 67 and 60 DAE, but the weeds were also less competitive in Tursun's study, with a biomass of 1,340 and 1,310 g m^{-2} at 1,000 GDD (2012 and 2013, respectively), compared with 1,550 g m^{-2} mid season for 50 common sunflower plants m^{-2} in our study. Bukun (2004) calculated the end of the CPWC at 77 to 84 DAE, but again, the weeds were less competitive, at 1,150 to 1,330 g m^{-2} at 1,000 GDD in this study. The CPWC extended to season long when a 2.5% threshold was used (Bukun 2004).

This combination of high weed densities, a highly competitive weed, and a high-yielding crop is a major contributing factor to the extended length of the dynamic CPWC observed in our studies. Cotton is an indeterminate plant, able to compensate for a high level of early-season damage (Wilson et al. 2003). However, the ability to compensate for damage is associated with delayed crop growth and crop maturity. It appears from our work that a high-yielding cotton crop is less able to compensate for damage later in the season than would be expected on the basis of earlier work, generally on much lower-yielding crops. Our crops, averaging 5,180 kg seed cotton ha^{-1} , were more sensitive to mid- and late-season damage from weed competition than has previously been reported, resulting in an extended CPWC.

The very high densities of a larger weed used in these experiments, of up to 50 common sunflower plants m^{-2} , also contributed to the prolonged dynamic CPWC observed, but increasing weed density did not have a strong influence on the CPWC, with high-yielding cotton sensitive to even a single large common sunflower plant m^{-2} . A doubling in weed density from five to 10 weeds m^{-2} increased weed biomass at 800 GDD by only 10% (Figure 2C) and the length of the dynamic CPWC by 3 to 6 d (Figure 4). This limited response to increasing weed density is consistent with the finding of Ma et al. (2016), who observed yield reductions from as few as 0.25 velvetleaf plants m^{-2} , but recorded little change in cotton yield, with densities doubling from four to eight weeds m^{-2} .

Implications for High-Yielding Crops

We conclude that high-yielding cotton crops are very sensitive to competition from a large broadleaf weed such as common sunflower, with the CPWC commencing at or near crop emergence. The duration of the CPWC extended to mid season or later, depending on weed density and the level of the yield-loss threshold chosen. We applied an arbitrary 5% yield-loss threshold to our data, but where the target weed is susceptible to glyphosate in a glyphosate-resistant cotton crop, a cost-based yield-loss threshold of less than 1% could be applied on the basis of 2019 commodity prices in Australia. A 1% yield-loss threshold extended the CPWC

from before crop emergence through to 836 GDD (78–101 DAE) for one weed m⁻², and from before crop emergence through to crop harvest for densities of 35 weeds m⁻² and higher.

From the dynamic model, the minimum yield loss where a single weed control input was made during the season increased from 31% for one weed m⁻² to 76% for 50 weeds m⁻² (Figure 4), far exceeding a cost-based yield-loss threshold estimated at less than 1%. Consequently, where large broadleaf weeds are present, a high level of weed control must be maintained throughout much of the cropping season in high-yielding cotton to ensure crop losses do not exceed the cost of weed control. Although densities of fewer than one weed m⁻² were not considered in our study, previous work has shown that season-long competition from densities as low as 0.005 large, broadleaf weeds m⁻² can cause economic damage (Charles et al. 1998) in Australian cotton, and weeds present at lower densities still need to be controlled to preserve fiber quality, to avoid difficulties at harvest, and to help manage herbicide resistance by driving down the weed seedbank over time (Korres and Norsworthy 2015).

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Determining the critical period for grass control in high-yielding cotton using Japanese millet as a mimic weed

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Abstract

Field studies were conducted over five seasons from 2004 to 2015 to determine the critical period for weed control (CPWC) in high-yielding, irrigated cotton using a competitive mimic grass weed, Japanese millet. Japanese millet was planted with or after cotton emergence at densities of 10, 20, 50, 100, and 200 plants m⁻². Japanese millet was added and removed at approximately 0, 150, 300, 450, 600, 750, and 900 degree days of crop growth (GDD). Data were combined over years. Japanese millet competed strongly with cotton, with season-long interference resulting in an 84% reduction in cotton yield with 200 Japanese millet plants m⁻². The data were fit to extended Gompertz and logistic curves including weed density as a covariate, allowing a dynamic CPWC to be estimated for densities of 10 to 200 Japanese millet plants m⁻². Using a 1% yield-loss threshold, the CPWC commenced at 65 GDD, corresponding to 0 to 7 d after crop emergence (DAE), and ended at 803 GDD, 76 to 98 DAE with 10 Japanese millet plants m⁻², and 975 GDD, 90 to 115 DAE with 200 Japanese millet plants m⁻². These results highlight the high level of weed control required throughout the cropping season in high-yielding cotton to ensure crop losses do not exceed the cost of weed control.

Introduction

Glyphosate resistance is a problem in many grass and broadleaf weeds in Australia, with the first case of a glyphosate-resistant weed, rigid ryegrass (*Lolium rigidum* Gaudin), confirmed in 1996 (Preston 2019). Since then, glyphosate resistance has been confirmed in an additional nine grass and seven broadleaf weeds, with resistant weeds being troublesome in a range of situations, including cropping, fallows, fence lines, and roadways in Australia (Preston 2019). Many of these resistant weeds are difficult to control in the northern farming region of eastern Australia where the cotton industry is established.

Glyphosate-resistant junglerice [*Echinochloa colona* (L.) Link.] was first confirmed in a cotton field in Australia in 2009 (Werth et al. 2010). Glyphosate-resistant weeds have since become widespread throughout the cotton industry, with glyphosate-resistant junglerice, feather fingergrass (*Chloris virgata* Sw.), windmillgrass (*Chloris truncata* R. Br.), hairy fleabane (*Erigeron bonariensis* L.), rigid ryegrass, and annual sowthistle (*Sonchus oleraceus* L.) present in or surrounding many fields. Glyphosate-resistant liverseedgrass (*Urochloa panicoides* P. Beauv.) and sweet signalgrass [*Brachiaria eruciformis* (Sm.) Griseb.] are also present in the region where cotton is grown.

Managing glyphosate-resistant weeds is challenging for many cotton growers, especially where weeds have established on surrounding properties or irrigation infrastructures, providing a continuing source of reinfestation that may be outside the control of the cotton grower. In-field management of these weeds has become increasingly reliant on the use of alternative weed management tactics, such as herbicides with different modes of action, interrow cultivation, and hand hoeing, with the aim of preventing seed set of any weeds that survive a glyphosate application and driving the weed seedbank down over time (Thornby et al. 2013).

Before the development of glyphosate resistance, grass control had not been a major issue in the Australian cotton industry, as grass weeds were readily controlled using a range of herbicides, including glyphosate. Only junglerice was listed among the 15 most numerous weeds of cotton in 2001 (Charles et al. 2004) and again in 2010 (Werth et al. 2013). However, with the development of glyphosate resistance, grass weeds have become increasingly problematic in the Australian cotton-farming system (Thornby et al. 2013). Glyphosate-resistant junglerice

and feather fingergrass were present in 23% and 26%, respectively, of cotton fields (Otto 2018). Over the last decade, these plants have been managed using a range of control tactics, with heavy reliance on the acetyl-coA carboxylase (ACCase)-inhibiting herbicides applied over the top of cotton and other broadleaf crops. Consequently, the incidence of resistance in junglerice and feather fingergrass to both glyphosate and the ACCase-inhibiting herbicides is increasing in the cotton system (Otto 2018; Tout 2012; A Jalaludin, Queensland Department of Agriculture and Fisheries, personal communication).

Rigid ryegrass, primarily a winter-growing weed, was not previously an issue in cotton, as it did not grow through the hotter part of the season in the cotton-growing regions. However, with cotton production expanding into cooler, more southerly areas of eastern Australia, cotton is now being grown in areas where rigid ryegrass with resistance to multiple modes of herbicidal action is present. In addition, some ecotypes of rigid ryegrass appear to be changing, and populations of this “winter” weed can now be found actively growing in cotton fields in midsummer and are spreading into the more northerly parts of the cotton-producing region, infesting 11% of cotton fields (Otto 2018). Anecdotally, plants have been found in cotton with resistance to all but one of the herbicidal modes of action currently registered for grass control in cotton in Australia.

Glyphosate-resistant feather fingergrass and windmillgrass are also becoming increasingly common in cotton fields, and it is likely to be only a matter of time before liverseedgrass and sweet signalgrass become problematic in cotton. With increasing reliance on alternative chemistries such as the ACCase-inhibiting herbicides to control these glyphosate-resistant grass weeds, it can be expected that populations of these and other commonly occurring grasses will also develop resistance to these alternative modes of herbicidal action in the near future.

The initial response of many farmers to a spray failure is to increase the rate of the herbicide or to apply an alternative herbicide, often with the same herbicidal mode of action. However, extensive experience from within Australia and around the world has shown that continuous reliance on herbicides from a single mode-of-action group leads to more failures and promotes further resistance (Powles 2008). Thornby et al. (2013) have shown that by adopting an integrated approach to weed management, the development of herbicide resistance in weeds in cotton can be delayed for many years. To achieve this, it is essential that weeds are controlled before they set seed and that any survivors of a weed management input are controlled using an alternative input, driving down the weed seedbank over time (Thornby et al. 2013).

The timing of weed control inputs can be challenging with species such as junglerice, for which a main flush of emergence may occur at planting, but successive germination flushes typically occur throughout the season. Consequently, these weeds often need to be controlled on multiple occasions throughout the season, after rainfall or irrigation events that trigger weed germinations. Ideally, weed control activities are triggered by the growth stage of the weeds, ensuring weeds are controlled before they set seed, or before this point, triggered by an economic threshold. The adoption of a control threshold ensures weeds are managed before they suppress crop growth and cause economic loss that exceeds the cost of control. Additionally, the control threshold needs to be dynamic, responding to the stage of crop growth, as previous research has shown that crops are more sensitive to early-season weed competition than late-season competition (Charles et al. 2019a; Korres and Norsworthy 2015; Webster et al. 2009).

Establishing a dynamic economic threshold for weed control can be done using the critical period for weed control (CPWC), which identifies the period during crop development when the crop is sensitive to weed competition, such that the damage caused by weed competition exceeds the cost of controlling the weeds (Fast et al. 2009; Korres and Norsworthy 2015; Webster et al. 2009). The CPWC is determined by identifying the critical time for weed removal (CTWR), the critical weed-free period (CWFP), and the yield-loss threshold. The CTWR is the period after crop emergence during which weeds can be allowed to compete with the crop without causing a yield loss exceeding the yield-loss threshold. The CWFP is the minimum period after crop emergence during which the crop must be maintained weed-free to prevent a yield loss exceeding the threshold. The combination of the CTWR and the CWFP with the yield-loss threshold defines the CPWC (Charles et al. 2019a; Korres and Norsworthy 2015).

The CPWC has been established for a range of weeds in cotton, but generally in lower-yielding cotton crops. Charles et al. (2019a) used common sunflower (*Helianthus annuus* L.) as a large, mimic broadleaf weed at densities of 1 to 50 plants m^{-2} and found that at the highest weed density, the CPWC extended to almost the full season in high-yielding cotton (averaging 2,040 kg lint ha^{-1} on weed-free plots), much longer than had been observed in previous studies on lower-yielding crops (Bukun 2004; Cardoso et al. 2011; Korres and Norsworthy 2015).

As an alternative to undertaking experiments using naturally occurring weeds, a range of mimic weeds have been used in competition experiments, as they have the advantages of greater experimental controllability and repeatability, with better control over weed density and more uniform weed emergence (Charles et al. 2019b). Mimic grass weeds used in competition experiments have included barley (*Hordeum vulgare* L.) (Strydhorst et al. 2008), Japanese millet (Wu et al. 2010), oats (*Avena sativa* L.) (Brain et al. 1999), perennial ryegrass (*Lolium perenne* L.) (Afifi and Swanton 2012), and winter wheat (*Triticum aestivum* L.) (Cerrudo et al. 2012). A mimic weed is generally chosen that has morphological characteristics similar to those of the real weed for which it is being substituted and is often of the same genus as the real weed. Charles et al. (2019b) compared the competitive effects of junglerice and the mimic weed Japanese millet in irrigated cotton and found that although both real and mimic weeds had similar morphological characteristics (plant height, leaf number per plant, leaf area per plant, and dry weight per plant), junglerice was more competitive with cotton, causing a greater reduction in cotton lint yield than did Japanese millet at the highest observed density of 100 plants m^{-1} of crop row. No differences were found at 50 and 10 weeds m^{-1} . These findings demonstrated that while Japanese millet was not a perfect substitute for junglerice, experimental results from competition experiments using Japanese millet as a mimic weed can give valuable insight into the effect of a competitive grass weed such as junglerice in cotton.

The impact of a competitive grass weed on high-yielding cotton has not previously been reported. However, earlier work has shown that grasses can be very competitive in lower-yielding cotton crops. Bridges and Chandler (1987) recorded reductions of seed cotton yield approaching 100% from season-long competition with johnsongrass [*Sorghum halepense* (L.) Pers.] in cotton yielding approximately 1,400 to 3,300 kg seed cotton ha^{-1} , with the level of yield reduction increasing with increasing johnsongrass density. Similarly, Keeley and Thullen (1991a) reported seed-cotton yield reductions of up to 100% from season-long competition with barnyardgrass [*Echinochloa crus-galli* (L.) Beauv]. Brown et al. (1985)

observed seed-cotton yield reductions of up to 80% from high densities of bermudagrass [*Cynodon dactylon* (L.) Pers.], although lesser reductions in yield were noted by Keeley and Thullen (1991b) and Vencill et al. (1993).

The objective of this study was to determine the CPWC for a competitive grass weed in high-yielding, irrigated cotton over a series of seasons, using Japanese millet as a mimic weed, and to evaluate the impact of Japanese millet density on the CPWC.

Materials and Methods

Field studies were conducted over five seasons at the Australian Cotton Research Institute, Narrabri (30.12°S, 149.36°E, elevation 201 m) using commercial cotton cultivars 'Sicot 289 BR' in 2004 to 2005; 'Sicot 80 BRF' in 2005 to 2006, 2006 to 2007, and 2007 to 2008; and 'Sicot 71 BRF' in 2015 to 2016. The soil was a heavy alluvial clay soil (fine, thermic, smectitic, Typic Haplustert). Cotton was planted at 15 seeds m⁻² on October 4, 2004; October 19, 2005; October 6, 2006; October 8, 2007; and October 21, 2015. The cotton was grown on raised hills, 1 m apart, in fully irrigated fields using furrow irrigation and fertilized with 180 kg N ha⁻¹, in line with commercial practices. Irrigation was scheduled according to computer modeling of the crop's requirements. Japanese millet 'Shirohie' was planted at the specified densities and times in rows adjacent to and offset from the cotton rows by 100 mm. Plots were otherwise maintained weed-free. Glyphosate (Roundup Ready® herbicide, 690 g kg⁻¹; Monsanto Australia, Melbourne, VIC, Australia) at 1 kg ai ha⁻¹ was applied POST as necessary over plots that were weed-free, and hand hoeing was performed as needed.

Experimental Design

The experiments used a split-plot design within a randomized, complete block with four replications. Main plots were times of weed planting, and subplots were times of weed removal and weed densities. Subplots were 4 rows wide (4 m) by 10 m long. Japanese millet was sown to achieve 0, 10, 20, 50, 100, and 200 plants m⁻², planted with the crop or at predetermined POST periods. Times of weed planting and removal were measured in growing degree days (GDD), using 15.5C as the base temperature (Bukun 2004). Time (T) was defined as:

$$T = \sum \frac{(t_{min} + t_{max})}{2} - t_b \quad [1]$$

where t_{min} and t_{max} are the daily minimum and maximum air temperatures, respectively, and t_b is the base temperature.

Times of weed planting and removal were targeted to occur at around 150, 300, 450, 600, 750, and 900 GDD, but actual times were influenced by factors such as rainfall and irrigation scheduling. Not all weed densities and times of weed planting and removal occurred in all seasons, with weed emergence sometimes delayed by inadequate surface soil moisture at the time of planting, and not all target weed densities were achieved in all seasons.

The density of established weeds was recorded on 1 m of row in each plot at the time of weed removal. Plant height and above-ground biomass were recorded on 10 cotton and weed plants at the time of weed removal. Plants were weighed after drying at 70 C for at least 72 h in a forced-air oven. The values used for statistical analysis were averages of the data from these 10 plants. Cotton was harvested at the end of each season using a modified

commercial harvester with a single picking head, recording seed-cotton yield from the central two rows of each plot. Subsamples from one row were ginned using a single-saw gin to determine ginning percentage and lint yield.

Statistical Analysis

Data were analyzed by ANOVA with replicate, year, time of weed interference and removal, weed density, and cotton variety as factors using R v. 3.4.2 statistical software (R Foundation for Statistical Computing, Vienna, Austria) with a significance level of $P < 0.05$. Analysis indicated no significant year or cotton variety effect or interactions on relative lint yield (lint yield relative to the weed-free control in each season), allowing the data sets from the five seasons to be combined. Relative lint yield was significantly ($P < 0.001$) related to time of weed removal and interference and weed density. Accordingly, data were grouped into density categories, such that the average density of each group equated as closely as possible to the nominal density of 10, 20, 50, 100, or 200 Japanese millet plants m⁻². Relative lint yield was regressed as a function of the time of weed removal or interference within each nominal weed density.

The effect of weed interference at each nominal weed density was modeled using the Gompertz function (Korres and Norsworthy 2015):

$$y = a \exp^{-\exp^{b(T-c)}} \quad [2]$$

where y is the yield as a percentage of the weed-free control, a is the upper asymptote (constrained to 100%), b and c are constants, and T is the cumulative degree days since planting.

The exponential curve:

$$y = a + b \exp^{cT} \quad [3]$$

was applied where the shape of the curve did not allow the Gompertz function to be fit.

The effect of weed removals at each nominal weed density was modeled using the logistic function:

$$y = \frac{a}{1 + \exp^{b(T-c)}} \quad [4]$$

where y is the yield as a percentage of the weed-free control, a is the upper asymptote, b and c are constants, and T is the cumulative degree days since planting.

These functions were extended to include actual weed density as a covariate. The extended Gompertz function was:

$$y = a \exp^{-\exp^{b(T-c+dW)}} \quad [5]$$

where d is an additional constant, and W is the observed weed density. The interaction term TW was not included, as it did not improve the fit of the relationship, as indicated by the Akaike information criterion (AIC).

The extended logistic function was:

$$y = \frac{a}{1 + \exp^{b(T-c+dTW)}} \quad [6]$$

where d is an additional constant, and W is the observed weed density. The interaction term TW was used, as it gave a better fit, as indicated by the AIC.

The extended exponential curve was:

$$y = a \exp^{-\exp^{b(T-c+dTW)}} \quad [7]$$

with the interaction term TW again giving a better fit, as indicated by the AIC.

Data for weed and crop height and weed biomass were analyzed using ANOVA with replicate, year, time of weed removal and weed density as factors, using a significance level of $P < 0.05$. Analysis indicated all year effects and year interactions were fully accounted for by the time of weed removal, allowing the data sets from the five seasons to be combined. Data were grouped into density categories and modeled using the Gompertz function (Equation 2) and the exponential model (Equation 3), and the AIC was used to determine the model of best fit for the data. The extended Gompertz (Equation 5) and exponential model (Equation 7) were fit to the data, including actual weed density as a covariate in the calculations, and the model of best fit was determined using the AIC.

Results and Discussion

Cotton Lint Yield and Weed Density

Cotton yields averaged 5,190 kg seed cotton ha^{-1} and 2,070 kg lint ha^{-1} for the weed-free plots over the five seasons, similar to the average Australian yield for these years and well above the yields reported for most previous competition studies in cotton (Barnett and Steckel 2013; Bukun 2004; Cardoso et al. 2011; Fast et al. 2009; Korres and Norsworthy 2015).

Japanese millet competed strongly with the cotton, with season-long interference resulting in an 84% lint yield reduction with 200 Japanese millet plants m^{-2} (Figure 1E). This yield reduction was less than the 97% loss reported by Keeley and Thullen (1991a) for season-long competition with barnyardgrass with densities of 180 and 287 plants m^{-2} , and the 100% yield loss reported for large crabgrass [*Digitaria sanguinalis* (L.) Scop.] in the row at midseason densities of 250 plants m^{-2} , but greater than was reported for bermudagrass in cotton (Brown et al. 1985; Keeley and Thullen 1991b; Vencill et al. 1993). Even at the lowest density of 10 plants m^{-2} , Japanese millet in the current study competed strongly with cotton, with season-long interference reducing cotton lint yield by 49% (Figure 1A).

Comparison of the weed removal and weed interference (CTWR and CWFP) curves over increasing weed density suggests the level of weed competition increased with increasing weed density, with the maximum observed yield losses increasing. In addition, there was a decline in the point of minimum yield loss from a single weed control measure (the intersection point of the weed removal and weed interference curves) with increasing weed density (Webster et al. 2009). The point of minimum yield loss declined from 10% yield loss with 10 Japanese millet plants m^{-2} (Figure 1A) to 26% yield loss with 200 Japanese millet plants m^{-2} (Figure 1E).

An arbitrary 5% lint yield-loss threshold was applied (Ghosheh et al. 1996), such that the CPWC was defined by the upper intersection of the CTWR and CWFP curves with the 5% threshold for each weed density (Figure 1A–E). The critical periods so derived extended from 87 to 275 GDD with 10 Japanese millet plants m^{-2} through 96 to 668 GDD with 200 Japanese millet plants m^{-2} (Figure 1A and E). Thus, there was an increasing trend in the upper limit of the critical period with increasing weed density, but no

consistent trend in the effect of weed density on the lower limit of the critical periods.

This lack of consistent response in the lower limit of the CPWC relationships to weed density was also observed by Charles et al. (2019a) with sunflower used as a mimic weed competing in high-yielding cotton. Charles et al. (2019a) concluded the lack of response reflected two components. First, the sensitivity of the derived CPWC to the shape of the fitted curves, with the shape of the curves as they approach the yield-loss threshold changing with increasing weed density, leading to anomalous results when the curves intersect the threshold at or soon after crop emergence. This issue with the shape of the curves changing with changes in weed density was exaggerated in our data, as we were unable to fit logistic curves to the data for 10 and 20 weeds m^{-2} , with exponential curves replacing the logistic curves at these lower weed densities. This change in curve type changed the shapes of the curves as they approached the yield-loss threshold.

Second, Charles et al. (2019a) observed that the competitive effect of the weeds was not directly proportional to the density of weeds, due to increasing intraspecific competition with increasing weed density. Thus, at high weed densities, increasing weed density made proportionally less difference to the level of weed competition experienced by the crop, as shown by the effect of increasing weed density on cotton biomass in our data, where at the end of the season (1,600 GDD), the presence of 10 Japanese millet plants m^{-2} reduced the crop biomass by 22% compared with the weed-free control from 1,110 to 857 g m^{-2} respectively (Figure 2D). Increasing the weed density an additional 20-fold to 200 Japanese millet plants m^{-2} only reduced the crop biomass by an additional 18% to 608 g m^{-2} . At higher weed densities, increases in weed density resulted in relatively small changes in the competitive effect on the crop, and these effects became increasingly difficult to separate from the background variation.

Plant Height and Biomass

Increasing weed density caused a small reduction in weed height, with a 20-fold increase in weed density from 10 to 200 Japanese millet plants m^{-2} reducing weed height at midseason (800 GDD) by 19%, from 131 to 106 cm, respectively (Figure 2A). This reduction in weed height is similar to the findings of Charles et al. (2019a), who observed up to a 24% reduction in midseason weed height with increasing densities of common sunflower in a high-yielding cotton crop. A reduction in weed height with increasing weed density was not observed with tropic croton (*Croton glandulosus* L. var. *septentrionalis* Muell. Arg.) (Askew and Wilcut 2001). Those authors reported that a tropic croton density of 3.5 plants m^{-1} of crop row reduced crop yield by 46% and crop height by 5 cm, but increasing density of tropic croton did not reduce the weed's height. Similarly, Scott et al. (2000) observed a reduction in crop yield and crop height, but reported no response in weed height with increasing densities of jimsonweed (*Datura stramonium* L.) in cotton. Askew and Wilcut (2002) also observed no impact on weed or cotton height from increasing densities of ladythumb (*Persicaria maculosa* Gray). They proposed this lack of response was due to the slow early-season growth rate of ladythumb, which was shorter than the crop until approximately 70 d after planting (DAP). Similarly, Bryson (1987) found no effect of increasing hemp sesbania [*Sesbania herbacea* (Mill.) McVaugh] density on either crop or weed height, but again the hemp sesbania was shorter than the crop until approximately 55 DAP. This was not the case in our data, where the Japanese millet emerged with

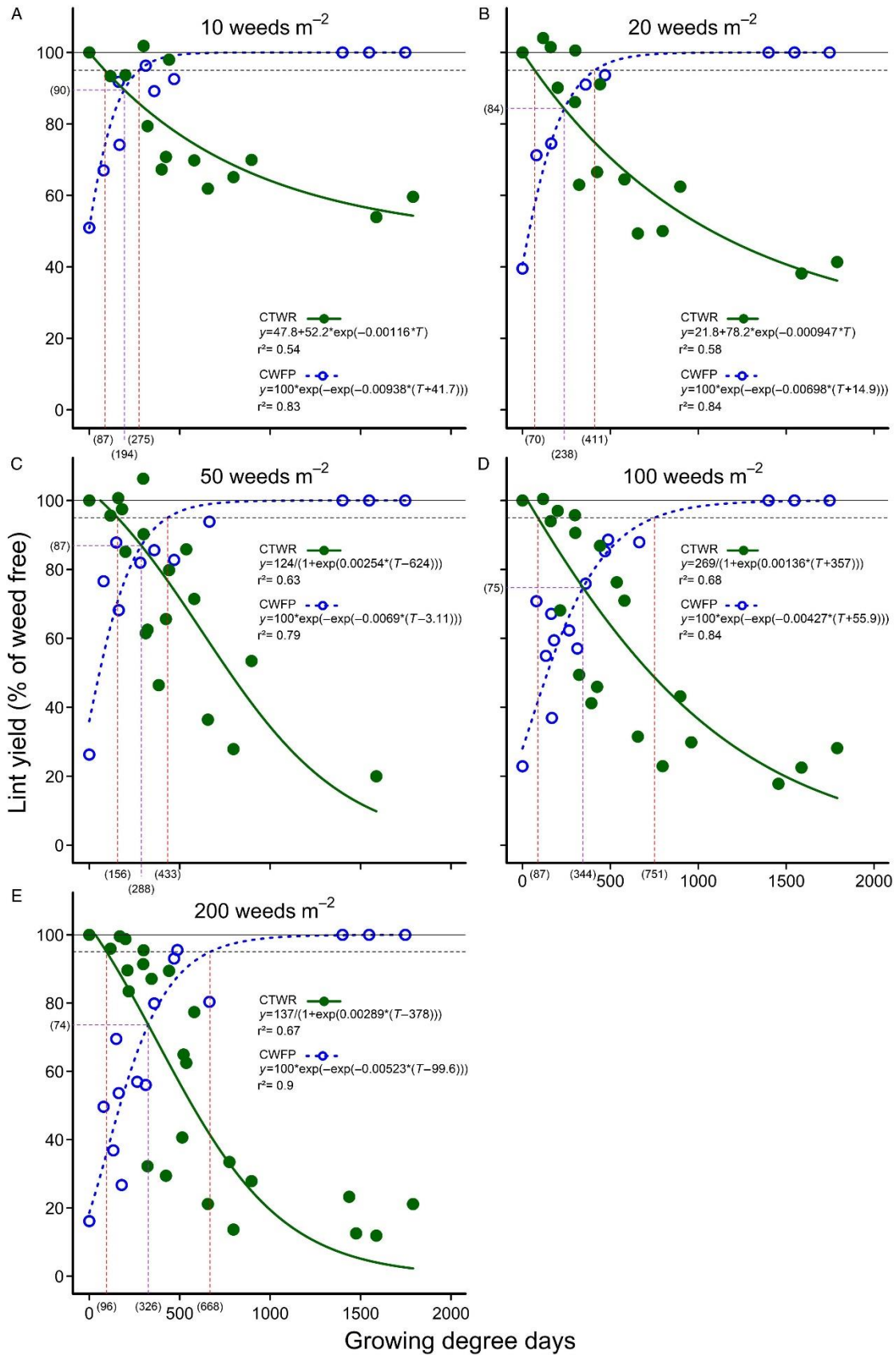


Figure 1. The influence of Japanese millet interference durations—critical time for weed removal (CTWR; green lines) and critical weed-free period (CWFP; blue lines)—on the relative cotton lint yield reduction. Parameters of the exponential and Gompertz (CTWR) and logistic (CWFP) functions are shown within the figures, where y is the lint yield and T the cumulative growing degree days since planting. Data points for the relationships are treatment means. The horizontal solid lines indicate the weed-free yield and the horizontal dashed lines give a nominal 5% yield-reduction threshold. The critical period for weed control (CPWC) is defined by the upper intersection of the CTWR and CWFP lines with the threshold. The limits of the derived CPWC curves are shown by the vertical dashed red lines and values bracketed below the x axis. The point of minimum yield loss is indicated by the dashed purple lines and bracketed values.

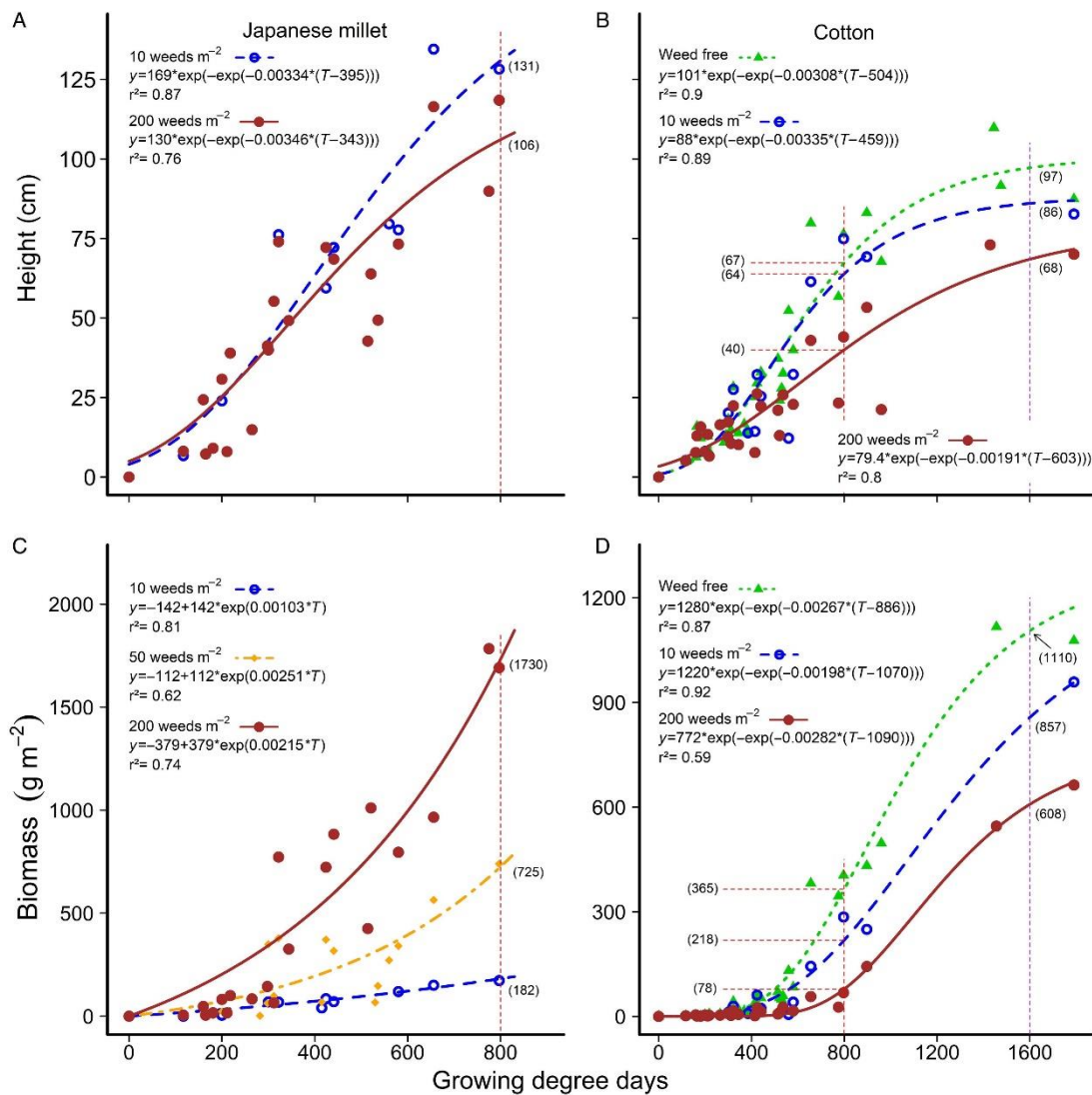


Figure 2. Changes in Japanese millet and cotton height and dry, aboveground biomass over time for densities of 0 (weed-free), 10, and 200 m^{-2} . Data points for the relationships are treatment means. Values at midseason (800 growing degree days since planting [GDD]) are indicated by the dashed red lines and bracketed values.

the crop and grew more rapidly than the crop, exceeding the height of the cotton throughout the season (Figure 2A and B). Thus, we conclude that the effect of increasing weed density on weed height relates to the dominance in height of the weed over the crop throughout the first half of the season. Where the weed is dominant throughout the season, increasing weed density results in a reduction in both crop and weed height, as we observed in our data.

Japanese millet was taller than the crop throughout the season, and around double the height of the cotton midseason, with Japanese millet 131 cm in height with 10 plants m^{-2} at 800 GDD, compared with weed-free cotton at 67 cm in height, declining to 40 cm where cotton competed with 200 Japanese millet plants m^{-2} (Figure 2B).

Crop height was reduced by increasing Japanese millet density, with weed-free cotton 27-cm taller at midseason than cotton competing with 200 Japanese millet plants m^{-2} (Figure 2B). Similarly, other studies have shown cotton height decreased in response to increasing levels of weed competition (Barnett and Steckel 2013; Charles et al. 2019a; Robinson 1976; Scott et al. 1999), although in some studies the response was only observed in some seasons or on some sites or varied with weed species (Buchanan and

Burns 1971a, 1971b; Chandler 1977). Ma et al. (2016) reported a reduction in cotton height with increasing velvetleaf (*Abutilon theophrasti* Medik.) densities, but at the same time observed an increase in velvetleaf height. These varying results suggest that species differ in their height response to increasing levels of competition, and the responses may be affected by seasonal variation. The height of corn (*Zea mays* L.), for example, rose with increasing levels of intraspecific competition to 10 plants m^{-2} , but decreased at higher densities (Abuzar et al. 2011)

Weed biomass increased with increasing weed density, but the rate of increase in biomass was less than the rate of increase in density. Midseason weed biomass increased 10-fold from 182 g to 1,730 g m^{-2} as weed density increased 20-fold from 10 to 200 Japanese millet plants m^{-2} (Figure 2C). This response was similar to the response in weed biomass with increasing densities of johnsongrass (Bridges and Chandler 1987), common sunflower (Charles et al. 2019a), and velvetleaf (Ma et al. 2016).

Crop biomass was reduced by increasing Japanese millet density, but again the response was not directly proportional to the increase in Japanese millet density. Midseason cotton biomass per meter of row was reduced by 40% by the presence of

Table 1. The start and end of the critical period for weed control (CPWC) using a 1% yield-loss threshold.

Weed density	CPWC start	CPWC end
No. m ⁻²	Growing degree days since planting	
10	39	449
20	57	644
50	81	670
100	42	1,130
200	46	979

10 Japanese millet plants m⁻² and 79% by 200 Japanese millet plants m⁻² (Figure 2D). By crop harvest, the reduction in crop biomass had lessened to 22% with 10 weeds m⁻² and 45% with 200 Japanese millet plants m⁻².

Dynamic Relationships for Cotton Lint Yield

Clearly, high-yielding cotton crops are sensitive to competition from a competitive grass weed such as Japanese millet, with the duration of the CPWC extending to midseason for densities of 100 weeds m⁻² or more, using a 5% yield-loss threshold (Figure 1D and E). However, where the target weed is susceptible to glyphosate in a glyphosate-tolerant cotton crop, a cost-based yield-loss threshold of less than 1% could be applied to the analysis, on the basis of Australian 2019 commodity prices. A 1% threshold extended the CPWC to 449 GDD with 10 weeds m⁻², and 979 GDD with 200 weeds m⁻², but the issue in our data of an inconsistent trend in the lower limit of the CPWC remained (Table 1). To address this issue of lack of consistent trend in the lower limit of the CPWC, Charles et al. (2019a) fit the relative lint yield data to extended Gompertz and logistic curves that included weed density as a covariate in the equations, allowing a dynamic CPWC to be calculated. Using this approach with our data, the CPWC estimated by these curves for a 1% yield-reduction threshold increased to be from 65 to 803 GDD with 10 Japanese millet plants m⁻², and 31 to 975 GDD with 200 Japanese millet plants m⁻² (Figure 3).

The lower limit of the dynamic CPWC corresponded to before crop emergence for 200 Japanese millet plants m⁻², to 1 to 7 d post-crop emergence (DAE) for 10 Japanese millet plants m⁻², in line with the results reported by Charles et al. (2019a) for common sunflower competing in high-yielding cotton, and Bukun (2004) for a mixed weed population using a 2.5% threshold, but earlier than most other previous studies on lower-yielding crops at 3 to 5 wk POST (Cardoso et al. 2011; Fast et al. 2009; Keeley and Thullen 1991a; Korres and Norsworthy 2015; Papamichail et al. 2002). This difference from earlier work does not appear to relate to weed size, as the earliest start of the lower limit coincides with weed emergence, but rather to greater sensitivity of higher-yielding cotton to early weed competition in combination with the low threshold adopted. Increased sensitivity to early weed competition in high-yielding cotton crops could be related to the high levels of early fruit retention of these crops (Bange et al. 2008) and consequently reduced compensatory ability. However, Wilson et al. (2003) showed that although high-yielding crops had a high level of early fruit retention, first position retention rarely exceeded 60%, and plants were still able to compensate for early-season damage. Hence, it is difficult to explain why the lower limit of the CPWC is at crop emergence in our data.

The upper limit of the dynamic CPWC corresponded to 76 to 98 DAE for 10 Japanese millet plants m⁻², and 90 to 115 DAE for

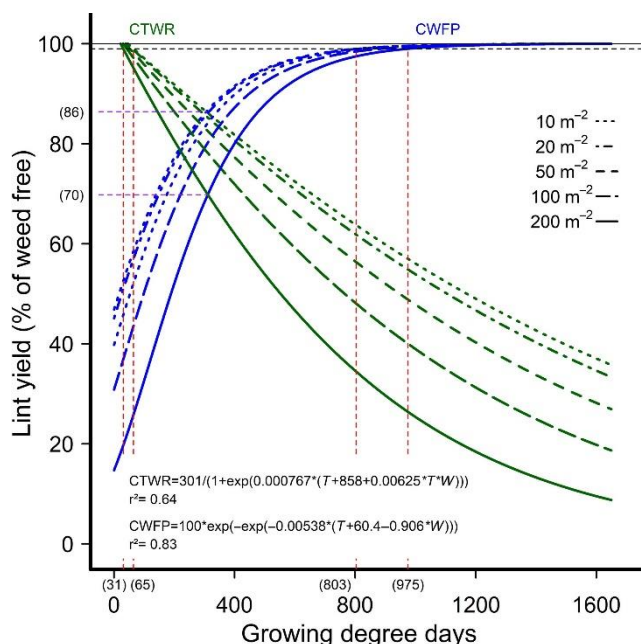


Figure 3. Dynamic relationships showing the influence of Japanese millet interference durations—critical timing for weed removal (CTWR; green lines) and critical weed-free periods (CWFP; blue lines)—on the relative cotton lint yield, using extended Gompertz (CTWR) and logistic (CWFP) functions including weed density as a covariate. Parameters of the models are shown within the figures, where y is relative lint yield, T the cumulative degree days since planting, and W the weed density. The derived relationships for the Japanese millet densities of 10, 20, 50, 100, and 200 m⁻² are presented as examples. The horizontal solid line indicates the weed-free yield, and the horizontal dashed line shows a 1% yield-reduction threshold. The critical period for weed control (CPWC) is defined by the upper intersection of the CTWR and CWFP lines with the threshold. The limits of the derived CPWC curves for 10 and 200 Japanese millet plants m⁻² are shown by dashed red lines and values bracketed below the x axis. The points of minimum yield loss from a single weed control input at 10 and 200 weeds m⁻² are shown by the dashed purple lines and bracketed values.

200 Japanese millet plants m⁻², longer than the 44 to 52 DAP reported by Korres and Norsworthy (2015) on lower-yielding cotton, or the 55 to 60 DAE observed by Tursun et al. (2015), but in line with the results of others, such as Keeley and Thullen (1991a) for barnyardgrass densities of up to 300 plants m⁻¹ of row, and Bukun (2004), Cardoso et al. (2011), and Papamichail et al. (2002), all with naturally occurring mixed weed populations. The point of minimum yield loss where a single weed control input was made during the season increased from 14% for 10 weeds m⁻² to 30% for 200 weeds m⁻², far exceeding the 1% yield-loss threshold (Figure 3).

The approach of Charles et al. (2019a) to develop a dynamic CPWC can also be applied to the weed and crop height and biomass data of Figure 2. In each case, a dynamic model including weed density improved the fit of the data, as indicated by the AIC. These dynamic models (Figure 4) allow weed and crop height and biomass to be determined for any weed density in the observed range of 10 to 200 Japanese millet plants m⁻². With these dynamic models, it may be possible to develop a more generalized, multi-species competition model, using weed biomass and height as the measures of weed competitiveness, as suggested by Charles et al. (2019b).

We conclude that where grass weeds are present at densities of 10 m⁻² or more, a high level of weed control must be maintained throughout the first half of the cropping season in high-yielding cotton to ensure crop losses do not exceed the cost of weed control.

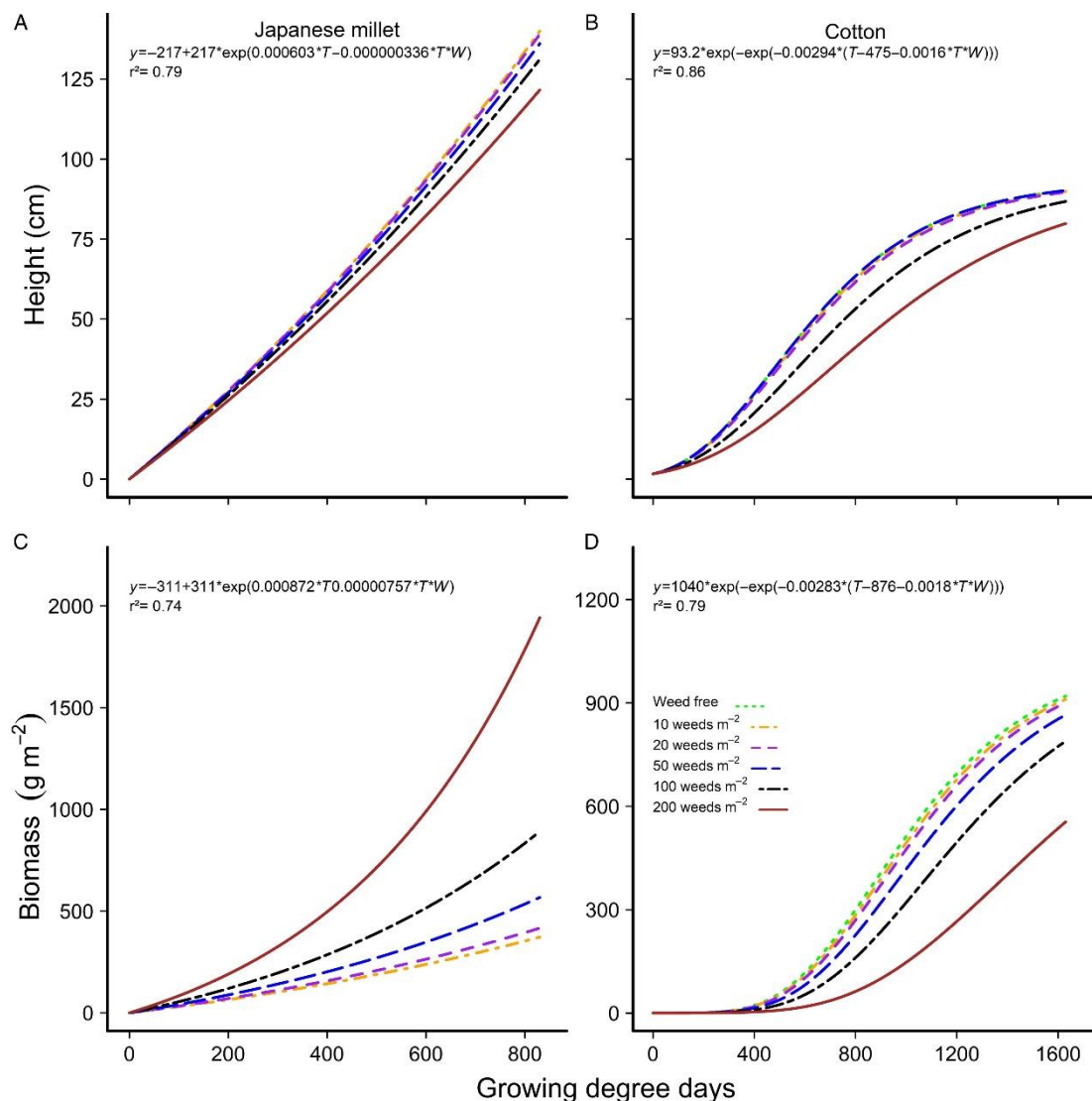


Figure 4. Dynamic relationships showing the Japanese millet and cotton height and dry, aboveground biomass over time using extended exponential and Gompertz functions including weed density as a covariate. Parameters of the models are shown within the figures, where y is plant height or biomass, T the cumulative degree days since planting, and W the weed density. The derived relationships for the Japanese millet densities of 0 (weed-free), 10, 20, 50, 100, and 200 m⁻² are shown as examples.

In addition, weeds present at low densities will still need to be controlled before they set seed to protect lint quality, to avoid difficulties at harvest, and to drive down the weed seedbank over time and help manage herbicide resistance (Korres and Norsworthy 2015; Thornby et al. 2013).

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Determining the critical period for broadleaf weed control in high-yielding cotton using mungbean as a mimic weed

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Abstract

Research using the critical period for weed control (CPWC) has shown that high-yielding cotton crops are very sensitive to competition from grasses and large broadleaf weeds, but the CPWC has not been defined for smaller broadleaf weeds in Australian cotton. Field studies were conducted over five seasons from 2003 to 2015 to determine the CPWC for smaller broadleaf weeds, using mungbean as a mimic weed. Mungbean was planted at densities of 1, 3, 6, 15, 30, and 60 plants m⁻² with or after cotton emergence and added and removed at approximately 0, 150, 300, 450, 600, 750, and 900 degree days of crop growth (GDD). Mungbean competed strongly with cotton, with season-long interference; 60 mungbean plants m⁻² resulted in an 84% reduction in cotton yield. A dynamic CPWC function was developed for densities of 1 to 60 mungbean plants m⁻² using extended Gompertz and exponential curves including weed density as a covariate. Using a 1% yield-loss threshold, the CPWC defined by these curves extended for the full growing season of the crop at all weed densities. The minimum yield loss from a single weed control input was 35% at the highest weed density of 60 mungbean plants m⁻². The relationship for the critical time of weed removal was further improved by substituting weed biomass for weed density in the relationship.

Introduction

Greater than 99% of cotton planted in Australia during the past decade has used the glyphosate-tolerance trait (Tony May, Monsanto Australia, personal communication, February 2017), with glyphosate being the most commonly used herbicide applied to these crops (Thornby et al. 2013). This widespread use of glyphosate in cotton has led to high levels of weed control and has contributed to ever-increasing crop yields, such that Australia continues to have the highest average yields of cotton in the world (Dowling 2016).

However, the heavy reliance on glyphosate for weed control in cotton has resulted in increasing issues with glyphosate-resistant weeds and a species shift toward glyphosate-tolerant weeds (Werth et al. 2013), with many of these being small- to medium-sized broadleaf weeds. Werth et al. (2013) surveyed 19 Australian fields planted with cotton and found 40 weed species remaining after weed-control inputs, three species of which were large broadleaf weeds, 10 species were grass weeds, and the other 27 species were small- to medium-sized broadleaf weeds. These weeds either emerged after the last in-crop weed-control input or were not controlled by the input. The glyphosate-resistance status of the weeds from these fields was not tested, but five of these species were among those known to have developed resistance to glyphosate (Heap 2020), including the two species most commonly found in these fields, hairy fleabane [*Erigeron bonariensis* (L.) Cronquist] and annual sowthistle (*Sonchus oleraceus* L.). Charles (2015) surveyed 73 cotton fields in the southern Australian cotton-growing area between 2013 and 2015 and recorded the presence of 49 weed species remaining after weed-control measures had been undertaken, with an average weed density of 1.2 weeds m⁻². Of these weeds, 43 species were small- to medium-sized broadleaf weeds. Five of the species observed in these fields were among those known to have developed resistance to glyphosate in Australia. Most of these species were present at relatively low densities of fewer than 1 weed m⁻², but higher densities were observed, with an average of eight common purslane (*Portulaca oleracea* L.) m⁻² recorded in one field during 2015. Another field had an average weed density of greater than 12 weeds m⁻² in 2015, with Venice mallow (*Hibiscus trionum* L.) and jungle rice [*Echinochloa colona* (L.) Link], each present at greater than 5 plants m⁻².

The aim of every cotton grower should be to control weeds before they cause economic damage to the crop (i.e., damage exceeding the cost of controlling the weeds) and before the weeds set seed, enabling the cotton grower to greatly reduce the number of seeds in the weed seedbank over time (Thornby et al. 2013). The onset of seed set can be determined by examining the weeds, but there is limited information available to growers to enable them to estimate the cost of damage caused by broadleaf weeds in high-yielding cotton crops, with the competitive ability of the weeds varying with species and depending on weed size and density (Charles et al. 2019a).

The competitive effects of some medium-sized broadleaf weeds have been determined for cotton, including Benghal dayflower (*Commelina benghalensis* L. jio) (Webster et al. 2009), black nightshade (*Solanum nigrum* L.) (Keeley and Thullen 1989), coffee senna [*Senna occidentalis* (L.) Link] (Higgins et al. 1985), ladythumb (*Persicaria maculosa* L.) (Askew and Wilcut 2002a), prickly sida (*Sida spinosa* L.) (Buchanan et al. 1977; Chandler 1977), redroot pigweed (*Amaranthus retroflexus* L.) (Buchanan and Burns 1971b; Buchanan et al. 1980), sicklepod [*Senna obtusifolia* (L.) H.S. Irwin & Barneby] (Buchanan and Burns 1971a; Buchanan et al. 1980), smellmelon [*Cucumis melo* L. var. *dudaim* (L.) Naud.] (Tingle et al. 2003), tropic croton (*Croton glandulosus* L.) (Askew and Wilcut 2001), spurred anoda [*Anoda cristata* (L.) Schltld.]], velvetleaf (*Abutilon theophrasti* Medik.), and Venice mallow (Chandler 1977). However, of these, only Venice mallow was recorded in the surveys of Australian cotton fields conducted by Werth et al. (2013). Venice mallow, black nightshade, and prickly sida were reported in the surveys of Charles (2015), but little to no information is available on the competitive ability of the remaining 40 broadleaf weed species recorded in these surveys.

Of the species for which the competitive effect has been determined, the yield reductions from season-long competition range from 33% to 55% for ladythumb (Askew and Wilcut 2002a); 60% for tropic croton, with 3.5 plants m^{-1} (Askew and Wilcut 2001); and 38% to 80% for 7 sicklepod plants m^{-1} (Buchanan and Burns 1971a). Redroot pigweed reduced cotton yield by between 22% and 90% with 7 plants m^{-1} (Buchanan and Burns 1971b), prickly sida reduced yield by between 45% and 90% (Buchanan et al. 1977), and coffee senna reduced yield by between 17% and 55% with 2 to 4 plants m^{-1} (Higgins et al. 1985). Season-long competition of Venice mallow caused no significant reduction in lint yield, whereas prickly sida reduced the yield by approximately 66% and spurred anoda and velvetleaf reduced yield by nearly 100% at densities of 5 weeds m^{-1} (Chandler 1977). The high level of variation observed in a number of these experiments was attributed to factors including seasonal variation and differences between sites in soil type and disease incidence (Buchanan and Burns 1971a, 1971b; Buchanan et al. 1977; Higgins et al. 1985).

Determining the competitive effect of these weeds enables the critical period for weed control (CPWC) to be determined for each species. It also helps to define the period of the season during which the crop is most sensitive to weed competition, such that the damage caused by weed competition exceeds the cost of controlling the weeds (Charles et al. 2019b, 2019c; Fast et al. 2009; Korres and Norsworthy 2015; Webster et al. 2009). However, to our knowledge, the CPWC is yet to be determined for many of the broadleaf weeds found in Australian cotton fields. As an alternative to undertaking experiments using naturally occurring weeds, a range of mimic weeds have been used in competition experiments. Mimic weeds have the advantages of giving better control over

weed density, more uniform weed emergence, and better experimental repeatability (Charles et al. 2019a). Mimic broadleaf weeds used in competition experiments have included common sunflower (*Helianthus annuus* L.) (Charles et al. 2019a; Charles and Taylor 2007), mungbean (Charles et al. 2019a), rapeseed (*Brassica napus* L.) (Vollmann et al. 2010), and white mustard (*Sinapis alba* L.) (Didon and Boström 2003; Lotz et al. 1996). A mimic weed is generally chosen that has similar morphologic characteristics to the actual weed for which it is being substituted and is often of the same genus.

Charles et al. (2019a) compared the competitive effects of Venice mallow, a real weed, and mungbean, a mimic weed, in irrigated cotton and found that although the real and mimic weeds were dissimilar in many morphologic characteristics (i.e., node and leaf number, leaf area and size, and dry weight at midseason), they had similar competitive effects on the lint yield of cotton when competing at densities of 3 to 30 weeds m^{-1} . Charles et al. (2019a) concluded that in fully irrigated cotton, the competitive effects of a range of weeds might be satisfactorily extrapolated from the results of mimic weeds, provided the real and mimic weeds are of similar dry weight and height at midseason or the relationship accounts for differences in plant dry weight and height. They proposed a generalized relationship estimating the yield loss of high-yielding, irrigated cotton from weed competition over a range of weed species, heights, and dry weights.

Charles et al. (2019b) determined the CPWC for a large broadleaf mimic weed, common sunflower, in high-yielding cotton and found that the CPWC extended from crop emergence through to mid-season or longer, depending on weed density. The point of minimum yield loss from a single weed-control input declined from 31% for one common sunflower plant m^{-2} to 76% for 50 weeds m^{-2} . The CPWC for 50 common sunflower plants m^{-2} extended season long, from crop emergence to harvest, much longer than had been observed in previous studies on lower-yielding crops (Bukun 2004; Cardoso et al. 2011; Korres and Norsworthy 2015). Similarly, Charles et al. (2019c) reported the CPWC for a mimic grass weed, Japanese millet [*Echinochloa esculenta* (A. Braun) H. Scholz], for which the CPWC extended from crop emergence to mid-season for 10 weeds m^{-2} or more in high-yielding cotton. However, the point of minimum yield loss from Japanese millet was only 14% for 10 weeds m^{-2} , declining to 30% for 200 weeds m^{-2} . Hence, although the CPWC was similar for the two weeds at their lowest densities, the potential for yield loss was greater for a single common sunflower plant m^{-2} than for any of the observed densities of Japanese millet of up to 200 plants m^{-2} .

The objective, therefore, for this study was to determine the CPWC for a medium-sized broadleaf weed in high-yielding, irrigated cotton over a series of seasons, using mungbean as a mimic weed, and to evaluate the impact of the weed density on the CPWC.

Materials and Methods

Field studies using commercial cotton cultivars were conducted at the Australian Cotton Research Institute, Narrabri (30.12°S, 149.36°E; elevation 201 m) on a heavy alluvial clay (fine, thermic, smectitic, Typic Haplustert) soil over five seasons. Cotton was planted at 15 seeds m^{-1} on September 30, 2003, using the commercial cultivar 'Sicot 289 RRI'; on October 4, 2004, using 'Sicot 289 BR'; on October 6, 2006, and October 8, 2007, using 'Sicot 80 BRF'; and on October 21, 2015, using 'Sicot 71 BRF.' The crops were grown in line with commercial practices on raised hills, 1-m apart. Fields were fertilized with 180 kg N ha^{-1} , applied before

planting and were irrigated as required using flood irrigation triggered by computer modelling of the crop's water requirements. The mungbean cultivar 'Berken' was planted at the specified densities and times in rows adjacent to, and offset from, the cotton rows by 100 mm. Plots were otherwise maintained weed free with trifluralin (TriflurX®, 480 g L⁻¹; Nufarm Australia, Melbourne, Victoria, Australia) incorporated before planting at 1.1 kg ai ha⁻¹. Weed-free plots were maintained using glyphosate (Roundup Ready® herbicide, 690 g kg⁻¹; Monsanto Australia, Melbourne, Victoria, Australia) at 1 kg ai ha⁻¹ (2004 to 2005 season and later), and hand hoeing was performed as needed.

Experimental Design

The experiments were conducted using split plots within a randomized, complete block design with four replications within each season. Times of weed planting were main plots and times of weed removal and weed densities were subplots, each 4 rows wide (4 m) by 10 m long. Mungbean was planted with the crop or at predetermined periods after cotton emergence, sown to achieve 0, 1, 3, 6, 15, 30, and 60 plants m⁻². Times of weed planting and removal were measured in growing degree days (GDD) since planting, using 15.5 C as the base temperature (Bukun 2004), defined as:

$$T = \sum \frac{(t_{min} + t_{max})}{2} - t_b \quad [1]$$

where t_{min} and t_{max} were the daily minimum and maximum air temperatures, respectively, and t_b was the base temperature.

Weed planting and removal times were targeted to occur at 150, 300, 450, 600, 750, and 900 GDD, but actual times were influenced by factors such as rainfall and irrigation scheduling, with not all weed densities and times of weed planting and removal occurring in all seasons. Weed emergence was delayed by inadequate soil moisture on some occasions, with not all target weed densities achieved in all seasons.

At the time of weed removal, weed density was recorded on 1 m of row in each plot, and height and aboveground biomass were recorded on 10 cotton and weed plants. The values used for statistical analysis were averages of these 10 plants. Plants were weighed after drying at 70 C for at least 72 h in a forced-air oven. Cotton was mechanically harvested, and seed-cotton yield was recorded from the central two rows of each plot. A single-saw gin was used to determine ginning percentage and lint yield from subsamples from one row.

Statistical Analysis

Relative lint yield (i.e., lint yield relative to the weed-free control in each season) was analyzed by ANOVA using R statistical software, version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria) with a significance level of $P < 0.05$, with replicate, year, time of weed interference, and removal and weed density as factors. Analysis indicated no significant year effect; thus, the data sets from the five seasons could be combined. Relative lint yield was significantly related to time of weed removal and interference and weed density.

The effect of weed interference on relative lint yield at each weed density was modelled using Gompertz, logistic, and exponential functions as described by Charles et al. (2019b, 2019c), with the exponential function substituted for the logistic function where the shape of the curve did not allow the logistic function to be fit; or

where the application of the exponential function improved the fit of the data, as indicated by the Akaike information criterion (AIC). These functions were extended to include weed density as a covariate, as described by Charles et al. (2019b, 2019c).

Weed and crop height and biomass from treatments where weeds established at the start of the season were analyzed using ANOVA, with replicate, year, time of weed removal, and weed density as factors. Analysis indicated the data could be best related to weed density and the time of weed removal, with no significant year effect, allowing the data sets from the five seasons to be combined. Data were modelled using Gompertz and exponential functions, with the AIC used to determine the model of best fit. Extended Gompertz and exponential functions including weed density as a covariate were fit to the weed and crop height and biomass data, and the model of best fit was determined using the AIC. Combinations of the duration of weed competition, density, biomass, and height were tested to improve the quality of fit of the weed interference and weed removal relationships using the extended Gompertz and exponential models by including these as additional covariates in the relationships. The model of best fit was determined using the AIC.

Results and Discussion

Plant Height and Biomass

Weed and crop height were reduced by increasing weed density (Figure 1). Mungbean plant height at midseason (800 GDD) was reduced by 15% by increasing mungbean density from 1 to 60 plants m⁻² (52 and 45 cm tall, respectively), but no difference remained at the end of the season (1,600 GDD) (Figure 1A), as was observed with tropic croton (Askew and Wilcut 2001), jimsonweed (*Datura stramonium* L.) (Scott et al. 2000), and ladythumb (Askew and Wilcut 2002a).

Crop height was reduced by increasing mungbean density, with weed-free cotton 23-cm taller at midseason than cotton competing with 60 mungbean plants m⁻², 59- and 36-cm tall, respectively (Figure 1B). The difference in height increased to 36 cm at cotton harvest, with weed-free cotton 104 cm tall compared with 68 cm for cotton competing with 60 weeds m⁻². Similarly, other studies have shown cotton height decreased in response to increasing levels of weed competition (Barnett and Steckel 2013; Charles et al. 2019b, 2019c; Robinson 1976; Scott et al. 2000). The mungbean plants were shorter than the crop throughout the season at the lowest density of 1 mungbean plant m⁻², and almost half the height of the cotton at harvest, with mungbean plants 52-cm tall at midseason and 58 cm at harvest, compared with cotton plants that were 63-cm tall at midseason, increasing to 95-cm tall at harvest. The mungbean plants were taller than the crop at midseason (45 and 36 cm, respectively) at the highest weed density of 60 mungbeans m⁻², but by harvest, the cotton was taller than the mungbean plants, having reached 68 cm compared with mungbean at 56 cm (Figure 1A and 1B).

Mungbean is an intermediate-sized broadleaf mimic weed, with 76 to 650 g m⁻² above-ground, dry biomass at crop harvest, with densities of 1 and 60 plants m⁻², respectively, larger than Venice mallow (Chandler 1977; Charles et al. 2019a), but similar in weight to Benghal dayflower (Webster et al. 2009), tropic croton (Askew and Wilcut 2001) and velvetleaf and prickly sida (Chandler 1977). At the end of the season, the biomass of mungbean plants in our experiment, when competing with cotton, was only about half the

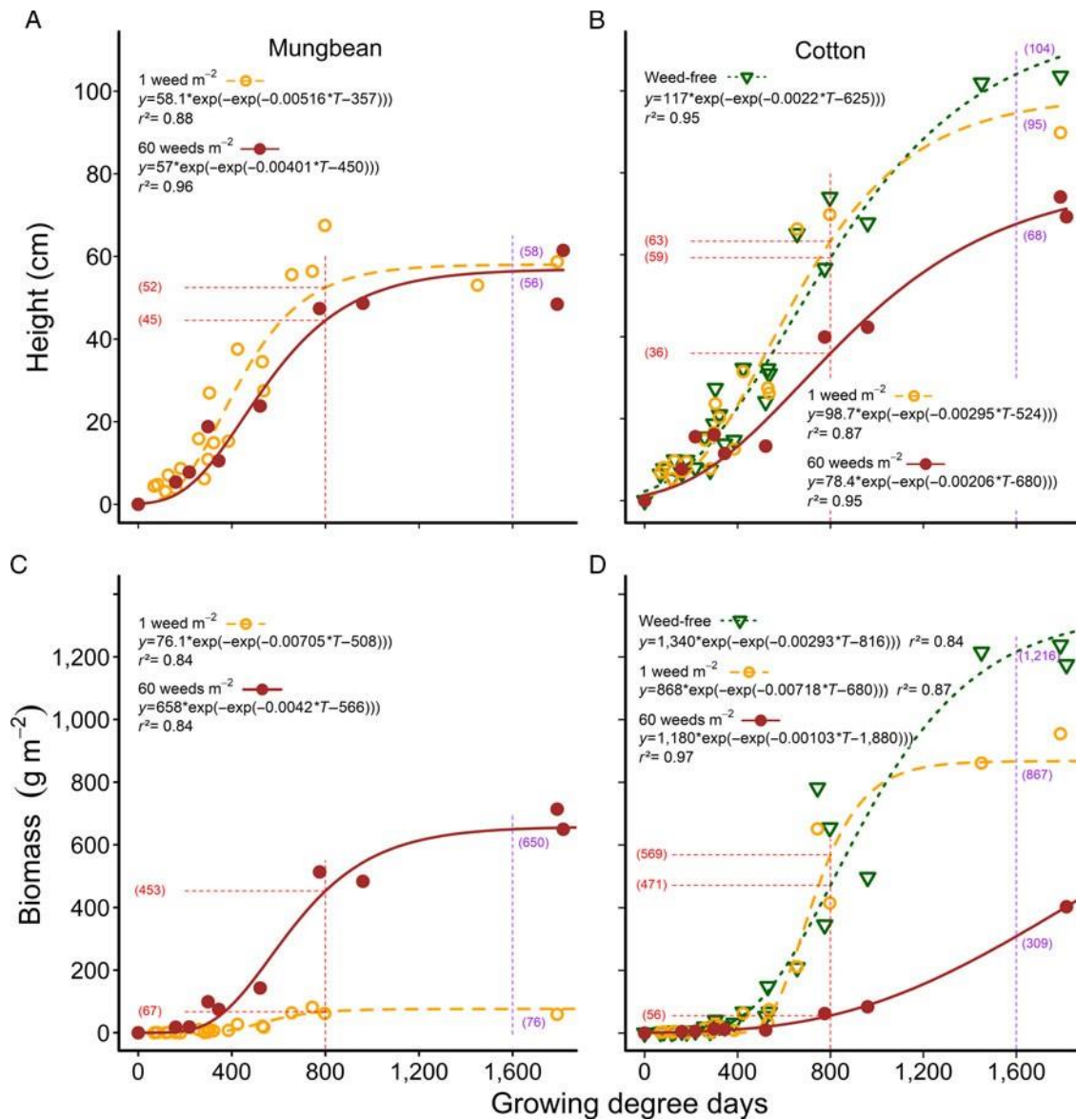


Figure 1. Graphs of (A) mungbean and (B) cotton height, and (C) mungbean and (D) cotton dry, aboveground biomass over the growing season for weed densities of 0 (weed-free), 1, and 60 plants m^{-2} . Data points are treatment means. Values at midseason (800 growing degree days [GDD]) are indicated by the dashed red lines and bracketed values, and at cotton harvest (1,600 GDD) by dashed purple lines and bracketed values. Parameters of the models are as follows: y is weed or crop height or biomass; T is the cumulative degree days since planting.

biomass of spurred anoda (Chandler 1977), pale smartweed [*Persicaria lapathifolium* (L.) Delarbre] (Askew and Wilcut 2002b), ladysthumb (Askew and Wilcut 2002a), and mixed-weed populations, including the large weeds common cocklebur (*Xanthium strumarium* L.) (Bukun 2004; Tursun et al. 2016) and jimsonweed (Tursun et al. 2015), and our mungbean was much smaller than large thornapple (*Datura ferox* L.) (Charles et al. 2019a). Mungbean biomass m^{-2} increased with increasing mungbean plant density, but the rate of increase in biomass was less than the rate of increase in density. Total weed biomass increased seven-fold at midseason, from 67 to 453 $g m^{-2}$, as weed density increased 60-fold from 1 to 60 mungbean plants m^{-2} (Figure 1C).

By the end of the season, a three-fold increase in weed density from 1 to 3 mungbean plants m^{-2} had resulted in a 2.6-fold

increase in weed biomass, from 76 to 194 $g m^{-2}$ (data not shown). However, the relationship was not linear, with a 60-fold increase in weed density from 1 to 60 mungbean plants m^{-2} resulting in only a nine-fold increase in weed biomass, from 76 to 650 $g m^{-2}$ (Figure 1C). This decline in the rate of response to increasing weed density indicated that high levels of intraspecific competition were occurring at the higher mungbean plant densities, as occurred with high densities of velvetleaf (Ma et al. 2016) and common sunflower (Charles et al. 2019c). Cortés et al. (2010) observed a similar trend with velvetleaf, where doubling the density from 1 to 2 weeds m^{-2} increased weed biomass 1.7-fold, but increasing the density 25-fold to 25 weeds m^{-2} only increased weed biomass 5.5-fold. There was no density-related decline in weed numbers over time in our treatments, indicating the weed density was not sufficiently high to cause self-thinning (Deng et al. 2012).

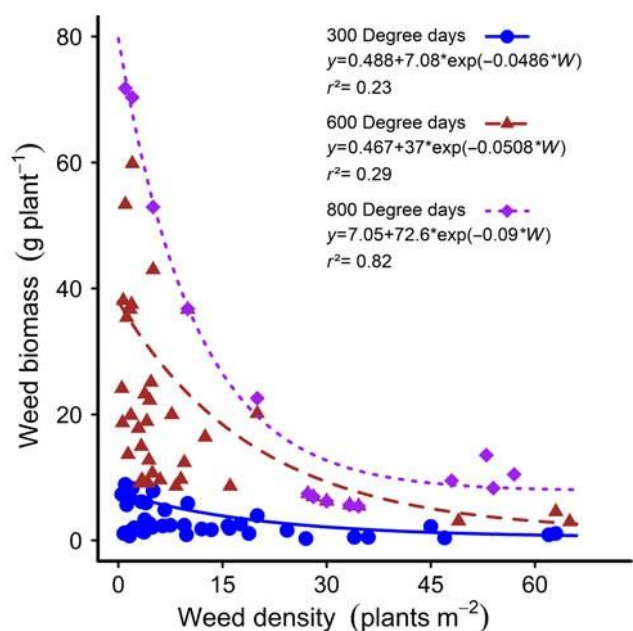


Figure 2. Reduction in mungbean aboveground biomass with increasing weed density at 300, 600, and 800 growing degree days. Parameters of the models are as follows: y is weed biomass; W is the weed density. Data points are treatment means.

The decrease in mungbean biomass plant^{-1} with increasing weed density became more pronounced over time, with the steepness of the response curves of weed density and biomass increasing with time (Figure 2). The same response was observed with velvetleaf (Cortés et al. 2010; Ma et al. 2016) and common sunflower (Charles et al. 2019c), but the curves were much steeper for these larger, more competitive weeds.

Crop biomass was reduced by increasing mungbean plant density, but again, the response was not directly proportional to the increase in mungbean density. Cotton biomass was not reduced by the presence of 1 mungbean plant m^{-2} at midseason, but was reduced by 88% by 60 mungbean plants m^{-2} (Figure 1D). Cotton biomass was reduced by 29% at cotton harvest when competing with 1 mungbean plant m^{-2} , but the reduction in crop biomass had declined from the midseason reduction of 88% to 74% when competing with 60 weeds m^{-2} .

Cotton Lint Yield and Weed Density

Cotton yields averaged 5,260 kg seed cotton ha^{-1} and 2,100 kg lint ha^{-1} for the weed-free plots over the five seasons of this study. Mungbean plants competed strongly with cotton at the highest weed densities, with season-long interference resulting in an 84% reduction in cotton lint yield with 60 mungbean plants m^{-2} (Figure 3F) and a 73% yield reduction in lint yield with 30 mungbean plants m^{-2} (Figure 3E). This level of yield loss was much greater than the 20% yield loss reported by Charles et al. (2019a) for cotton competing with 30 mungbean plants m^{-2} , where the plants were grown in bins with limited rooting depth, yielding 1,410 kg lint ha^{-1} (weed-free controls). Mungbean plants competed less strongly at lower densities, with season-long interference resulting in a 41% reduction in lint yield with 3 mungbean plants m^{-2} (Figure 3B), similar to the results for a range of other broadleaf weeds (Askew and Wilcut 2002a; Buchanan and Burns 1971a, 1971b; Buchanan et al. 1977; Higgins et al. 1985). Even at the lowest density of 1 plant m^{-2} , mungbean in the current study

reduced cotton-lint yield by 25% from season-long crop competition (Figure 3A).

Webster et al. (2009) used the point of minimum yield loss from a single control input (the intersection point of the weed removal and weed interference curves) as a way of measuring the relative competitiveness of crop and weed. However, in our data, there was no consistent trend in the point of minimum yield loss with increasing weed density, even though the maximum yield losses consistently increased with increasing weed density (Figure 3A–3F). This finding contrasts with the observations of Charles et al. (2019b, 2019c), who reported consistent reductions in the point of minimum yield loss with increasing weed density for the mimic weeds common sunflower and Japanese millet in high-yielding cotton, with average yields of 2,040 and 2,070 kg lint ha^{-1} , respectively (weed-free controls).

An alternative approach for understanding the relationship between weed competition and crop yield is to determine the critical period for weed control, using an arbitrary lint yield-loss threshold of, for example, 5% (Ghosheh et al. 1996), such that the CPWC is defined by the upper intersection of the critical time for weed removal (CTWR) and critical weed-free period (CWFP) curves with the threshold at each weed density (Figures 3A–3F). The critical periods so derived extended from 83 to 1,230 GDD with 1 mungbean plant m^{-2} to 139 to 1,040 GDD with 60 mungbean plants m^{-2} (Figure 3A and F). Thus, as was observed with the point of minimum yield loss, there was, again, no consistent trend in the critical period with increasing mungbean density, with the end of the CPWC greater for one mungbean plant than for 60 plants m^{-2} . This observation again contrasts with previous work showing strong relationships between increasing weed density and increasing yield loss (Askew and Wilcut 2001, 2002a, 2002b; Cortés et al. 2010; Higgins et al. 1985; Ma et al. 2016; MacRae et al. 2013; Scott et al. 2000) over cotton yields ranging from 4,550 to 1,040 kg seed cotton ha^{-1} (Cortés et al. 2010; Higgins et al. 1985).

A lack of consistent response in the CPWC relationships with increasing weed density was also observed by Charles et al. (2019b, 2019c), with common sunflower and Japanese millet used as mimic weeds in high-yielding cotton. They concluded that the lack of response in the lower limit of the CPWC could be attributed, at least in part, to the sensitivity of the derived CPWC to the shape of the fitted CWTR curves, with the shape of the curves as they approach the yield loss-threshold changing with increasing weed density. This change in curve shape, in turn, led to anomalous results when the curves intersected the threshold at or soon after crop emergence (Charles et al. 2019b, 2019c). The issue with the shape of the curves changing with changes in weed density was exaggerated in our data where exponential curves were used to describe the relationships for the lower weed densities, because it was not possible to fit logistic curves to the data for 1, 3, 6, and 15 weeds m^{-2} . However, logistic curves were fit to the 30 and 60 weeds m^{-2} relationships, because these curves best described these data. This change in curve type appeared to contribute to the lack of consistent results for both the point of minimum yield loss and the start of the CPWC in our data.

Charles et al. (2019b, 2019c) also noted that increasing weed density did not have a large additional effect on the CPWC at higher weed densities, because the competitive effect of the weeds was not directly proportional to the density of weeds, due to increasing interspecific competition between the weeds with increasing weed density. Consequently, at higher weed densities, increasing weed density made proportionally less difference to

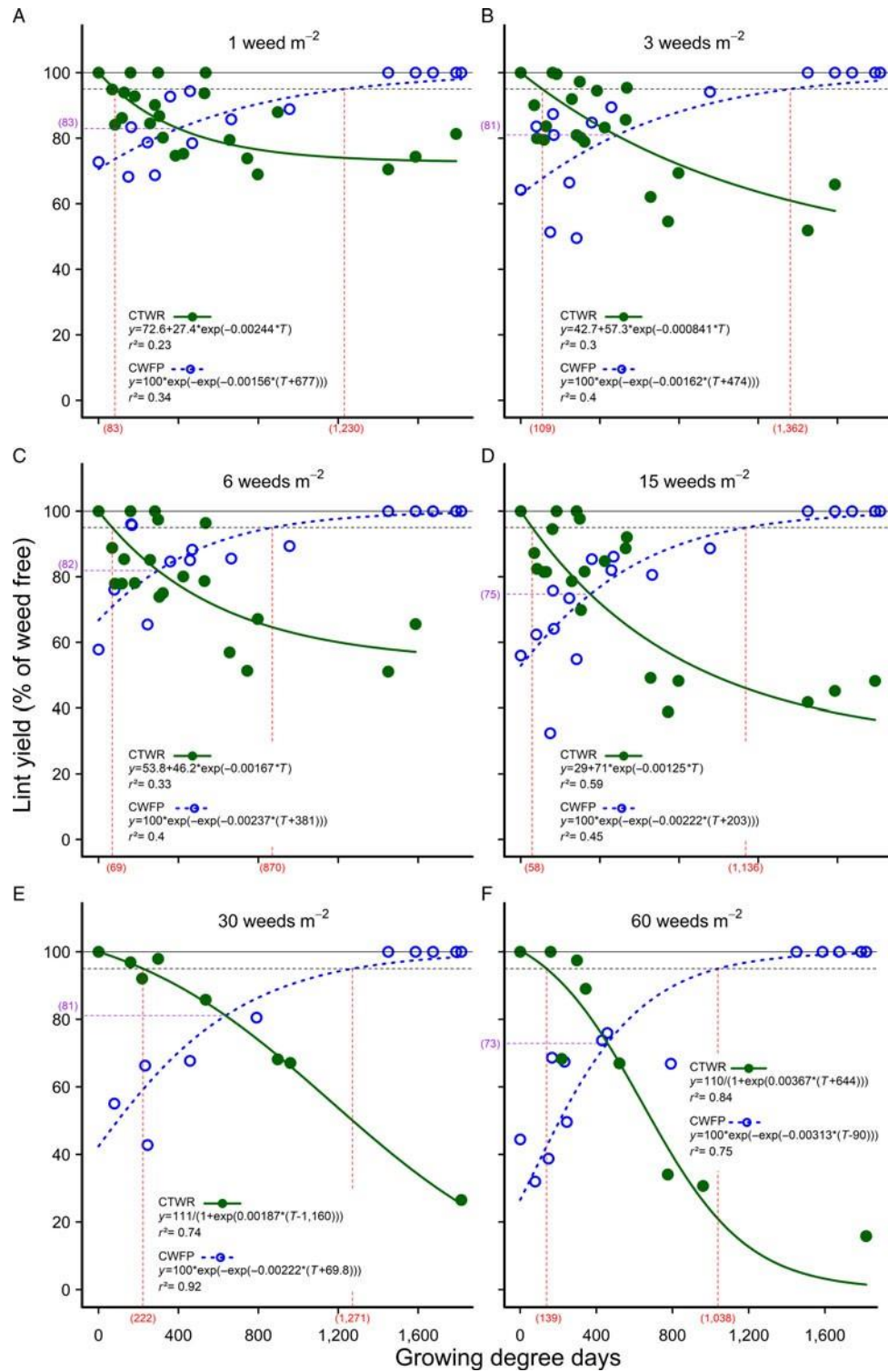


Figure 3. Relationships between the relative cotton-lint yield and mungbean interference durations (i.e., CTWR, indicated by green lines; CWFP, indicated by blue lines) for mungbean densities of (A) 1, (B) 3, (C) 6, (D) 15, (E) 30, and (F) 60 plants m⁻². Parameters of the functions are as follows: y is the relative lint yield; T is the cumulative degree days since planting. Data points are treatment means. Weed-free yields are indicated by horizontal solid lines and horizontal dashed lines indicate a 5% yield-reduction threshold. The intersection of the CTWR and CWFP lines with the yield-reduction threshold defines the critical period for weed control (CPWC). Dashed red lines and bracketed values show the limits of the derived CPWC curves. Dashed purple lines and bracketed values indicate the point of minimum yield loss. CTWR, critical time for weed removal; CWFP, critical weed-free period.

the level of weed competition experienced by the crop; thus, large increases in weed density caused only relatively small increases in the duration of the CPWC in their data. Ma et al. (2016), for

example, reported a large reduction in cotton yield from 1 velvetleaf plant m crop row⁻¹, but little additional effect from 9 weeds m⁻¹. No consistent increase in the duration of the CPWC with increasing

Table 1. The start and end of the CPWC using a 1% yield-loss threshold.

Weed density	CPWC start ^a	CPWC end
No. m ⁻²	GDD since planting	
1	15	2,227 ^b
3	21	2,370 ^b
6	13	1,556
15	11	1,872 ^b
30	51	2,006 ^b
60	42	1,559

^aAbbreviations: CPWC, critical period for weed control; GDD, growing degree days.

^bThese estimates of the end of the CPWC extend past full crop maturity.

weed density is apparent in our data, even though increasing weed density had a large impact on the maximum observed yield losses.

Dynamic Relationships for Cotton Lint Yield

Clearly, the cotton plants in this study were sensitive to competition from mungbean plants, with the duration of the CPWC extending to or past midseason for all weed densities, using the 5% yield loss-threshold (Figure 3). However, where the target weed is susceptible to glyphosate in a glyphosate-tolerant cotton crop, as is the case with most broadleaf weeds in the glyphosate-tolerant cotton crops commonly grown in Australia, a cost-based yield-loss threshold of less than 1% could be applied to the analysis on the basis of 2020 commodity prices. The 1% threshold extended the CPWC to full season for most weed densities (at or before crop emergence through to crop harvest for four of the six densities), but the issue in our data of an inconsistent trend in the CPWC remained (Table 1).

To address this issue of lack of consistent trend in the CPWC with increasing weed density, Charles et al. (2019b, 2019c) fit the relative lint yield data to extended Gompertz and logistic curves that included weed density as a covariate in the equations, allowing a dynamic CPWC to be calculated. Using this approach of including weed density as a covariate in the equations, the CPWC estimated by these curves for a 1% yield reduction threshold increased from 24 to 1,680 GDD for 1 mungbean plant m⁻², and 9 to 2,060 GDD (after harvest) for 60 mungbean plants m⁻² (Figure 4).

The lower limit of the dynamic CPWC using a 1% yield-loss threshold began before crop emergence and was in line with the findings for smelton in a low-yielding crop (2,560 kg seed cotton ha⁻¹) (Tingle et al. 2003) and the mimic weeds common sunflower (Charles et al. 2019c) and Japanese millet (Charles et al. 2019b) in high-yielding cotton. The lower limit of the CPWC was earlier than was reported for a range of other weeds, where the CPWC commenced up to 7 wk POST (Buchanan et al. 1977; Bukun 2004; Cardoso et al. 2011; Tursun et al. 2015, 2016), for crops ranging in yield from 2,000 to 8,000 kg seed cotton ha⁻¹ (Cardoso et al. 2011; Tursun et al. 2015). This difference is likely to be related to the timing of weed emergence, with the emergence of our mimic weed, with no seed dormancy, triggered by irrigation immediately after planting in our study. This contrasts with findings of other work, where naturally occurring weed populations were used and where weed germination was triggered by rainfall events that may not have occurred until days or weeks after crop planting. Webster et al. (2009) demonstrated the importance of cotton planting date, with early-planted cotton

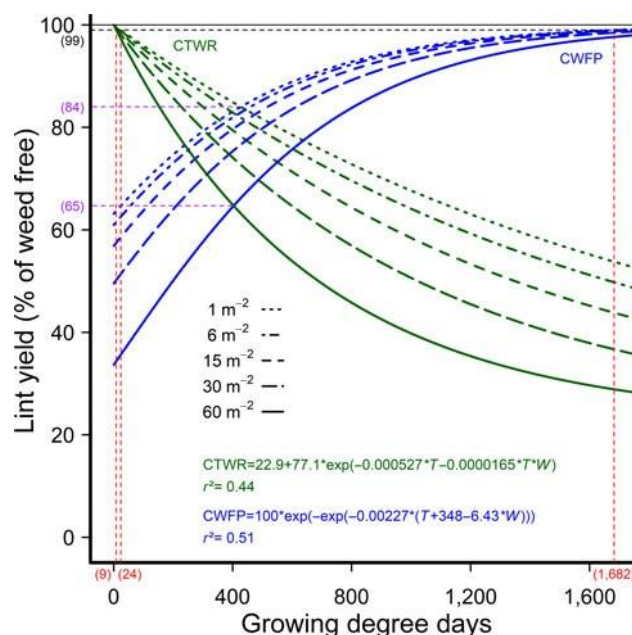


Figure 4. Dynamic relationships between the relative cotton-lint yield and mung-bean interference durations (i.e., CTWR, indicated by green lines; CWFP, indicated by blue lines using extended exponential (CTWR) and logistic (CWFP) functions including weed density as a covariate. Parameters of the models are as follows: T is the cumulative degree days since planting; W the weed density. The derived relationships for mungbean densities of 1, 6, 15, 30, and 60 plants m⁻² are presented as examples. The weed-free yield is indicated by the horizontal solid line and a 1% yield-reduction threshold by the horizontal dashed line. The intersection of the CTWR and CWFP lines with the yield-reduction threshold defines the critical period for weed control (CPWC). The limits of the CPWC for 1 and 60 mungbean plants m⁻² are shown by dashed red lines and bracketed values. The end of the CPWC for 60 mungbean plants m⁻² occurs at 2,060 growing degree days, beyond the limit of the figure. Dashed purple lines and bracketed values show the points of minimum yield loss for 1 and 60 mungbean plants m⁻².

more competitive with Benghal dayflower because of delayed emergence of this weed.

The upper limit of the dynamic CPWC of 1,682 GDD for 1 mungbean plant m⁻² corresponded to 161 d or more POST and occurred after the cotton was harvested in three of the five seasons in our experiments. This extended CPWC was longer than reported by most previous researchers at between 40 and 80 d after planting (Cardoso et al. 2011; Korres and Norsworthy 2015; Tingle et al. 2003; Tursun et al. 2015, 2016; Webster et al. 2009), but in line with the results of Bukun (2004), for a naturally occurring mixed-weed population.

The approach of Charles et al. (2019b, 2019c) to develop dynamic relationships, including weed density as a covariate in the equations, could also be applied to our height and biomass data (Figure 1). In each case, a dynamic model including weed density improved the fit of the data, as indicated by the AIC. These dynamic models allowed weed and crop height and biomass to be estimated for weed densities in the observed range of 1 to 60 mungbean plants m⁻² throughout the growing season (Figure 5).

In addition to weed density and the duration of weed competition, cotton yield has been related to weed biomass, with an inverse linear relationship resulting (Cortés et al. 2010; Scott et al. 2000; Smith et al. 1990). Over a range of weed species and types, Charles et al. (2019a) found that a combination of weed biomass and weed height better described the reductions in

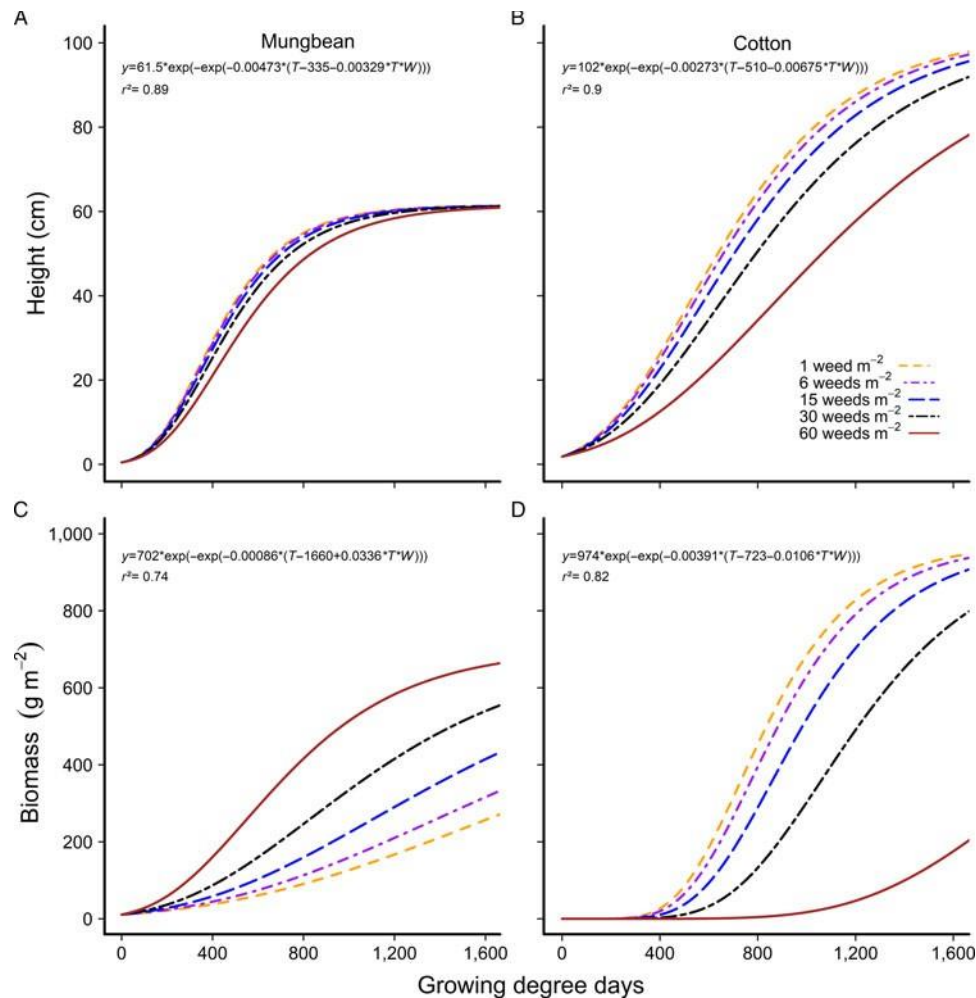


Figure 5. Dynamic relationships for (A) mungbean and (B) cotton height, and (C) mungbean and (D) cotton dry, aboveground biomass over time using extended exponential functions including weed density as a covariate. Parameters of the models are as follows: y is plant height or biomass; T is the cumulative degree days since planting; W is the weed density. The derived relationships for the mungbean densities of 1, 6, 15, 30, and 60 plants m^{-2} are shown as examples.

cotton-lint yield from weed competition than weed density. We tested combinations of the duration of weed competition, weed density, weed biomass, and weed height with our data and were unable to improve the fit of the relationship defining the CWF. However, a combination of the duration of weed competition and weed biomass gave a significant ($P < 0.05$) improvement in the fit of the CTWR curve (Figure 6) compared with the earlier relationship of the duration of weed competition and weed density (Figure 4). This improved relationship made little difference to the duration of the CPWC estimated from our data, which extended for the full growing season of the crop but lowered the point of minimum yield loss from a single weed control input to 39% with the highest weed pressure (Figure 6), compared with a 35% yield loss with the earlier model (Figure 4). Using weed biomass and height as the measures of weed competitiveness, it may be possible with these dynamic functions to develop multi-species competition models, as suggested by Charles et al. (2019a, 2019c).

We conclude that a high level of weed control must be maintained throughout the cropping season in high-yielding cotton where broadleaf weeds are present at densities of 1 or more

plants m^{-2} to ensure crop losses do not exceed the cost of weed control. Weeds present at lower densities will need to be controlled before they set seed, to protect lint quality, to avoid difficulties at harvest, and to manage herbicide resistance by greatly reducing the number of seeds in the weed seedbank over time (Korres and Norsworthy 2015; Thornby et al. 2013). To achieve this reduction in the weed seedbank, cotton growers will need to adopt a more integrated approach to weed control, replacing glyphosate with alternative control tools, especially where glyphosate-tolerant and -resistant weeds are present. These tools might include applications of residual herbicides such as diuron, fluometuron, metolachlor, pendimethalin, prometryn, and trifluralin, as well as POST applications of clethodim and haloxyfop. Dicamba and glufosinate could also be used POST on cotton varieties that include the resistance genes for these chemistries, and flumioxazin and paraquat may be used as in-crop shielded applications. In addition, cotton grower should be using spot spraying, interrow cultivation, and hand hoeing to ensure weed escapes are removed before they set seed. The differing costs of these alternatives to glyphosate will alter the value of the weed-control threshold and will need to be factored into the critical-period model.

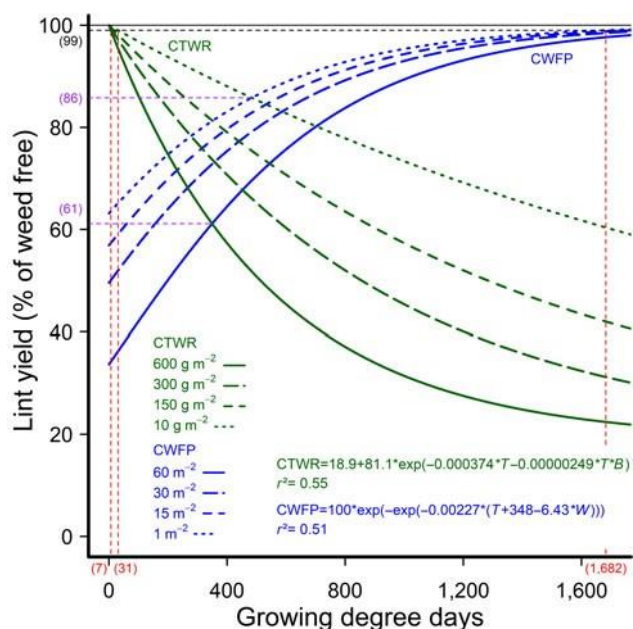


Figure 6. Dynamic relationships between the relative cotton-lint yield and mungbean interference durations (i.e., CTWR, indicated by green lines; CWFP, indicated by blue lines), using extended exponential (CTWR) and logistic (CWFP) functions including weed density (CWFP) and weed biomass (CTWR) as covariates. Parameters of the models are as follows: y is the relative lint yield; T is the cumulative degree days since planting; B is the weed biomass; W is the weed density. The derived relationships for mungbean biomass of 10, 150, 300, and 600 g m^{-2} are presented as examples for the CTWR relationship, and mungbean densities of 1, 15, 30, and 60 plants m^{-2} are presented as examples for the CWFP relationship. The weed-free yield is indicated by the horizontal solid line and a 1% yield-reduction threshold by the horizontal dashed line. Dashed red lines and bracketed values indicate the CPWC for 1 and 60 mungbean plants m^{-2} . Dashed purple lines and bracketed values show the points of minimum yield loss for a single weed-control input at 1 mungbean plant and 10 g biomass m^{-2} , and 60 mungbean plants and 600 $\text{g dry biomass m}^{-2}$.

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Developing a multispecies weed competition model for high-yielding cotton

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Abstract

Glyphosate-tolerant and glyphosate-resistant weeds are becoming increasingly problematic in cotton fields in Australia, necessitating a return from a glyphosate dominated system to a more integrated approach to weed management. The development of an integrated weed management system can be facilitated by identifying the critical period for weed control (CPWC), a model that enables cotton growers to optimize the timing of their weed control inputs. Using data from field studies conducted from 2003 to 2015, CPWC models using extended functions, including weed biomass in the relationships, were developed for the mimic weeds, common sunflower and Japanese millet, in high-yielding, fully irrigated cotton. A multispecies CPWC model was developed after combining these data sets with data for mungbean in irrigated cotton, using weed height and weed biomass as descriptors in the models. Comparison of observed and predicted relative cotton-lint yields from the multispecies CPWC model demonstrated that the model reasonably described the competition from these three very different mimic weeds, opening the possibility for cotton growers to use a multispecies CPWC model in their production systems.

Introduction

Weeds are ever-present pests of cotton production in Australia, with glyphosate-tolerant and glyphosate-resistant weeds becoming increasingly problematic over time because of overreliance on glyphosate in the farming system, combined with a reduction in the use of other weed control tactics (Charles et al. 2020a; Koetz 2019a; Werth et al. 2013). The increase in weed issues over the past decade has necessitated the return to a more integrated weed management (IWM) system on many cotton farms, with the increasing use of residual herbicides, interrow cultivation, and hand hoeing (Koetz 2019b). One of the concepts that could facilitate the adoption of an IWM system would be a weed control threshold, enabling cotton growers to optimize the timing of their weed control inputs (Knezevic and Datta 2015; Knezevic et al. 2002; Korres and Norsworthy 2015). A weed control threshold would help cotton growers balance the need to control weeds before they become problematic, against practical considerations such as the availability of equipment and labor and the costs of weed control, including the potential costs of crop damage and off-target herbicide movement. Weeds need to be controlled before they set seed and before weed competition increases to the level at which it results in yield reductions. However, weed control inputs need to be managed to minimize the number of inputs needed over the crop-growing season, and to reduce costs and negative production, and environmental effects (Taylor et al. 2004). A weed control threshold will help cotton growers balance those needs.

Pest control thresholds have been widely used in cotton production in Australia, starting with the introduction of SIRATAC, a pest threshold-based tool introduced in the 1980s for managing heavy infestations of insecticide-resistant helicoverpa (*Helicoverpa armigera* and *H. punctigera*; Hearn and Bange 2002). Since then, pest control thresholds have been adopted for all major insect and mite pests of cotton in Australia, with individual thresholds developed for each species or group of closely related species (Grundy 2019). The need for individual thresholds has been necessitated by the widely varying impacts of different insects. Thrips (*Thrips tabaci*, *Frankliniella schultzei*, *F. occidentalis*), for example, can cause unacceptable early-season damage to cotton, but at low numbers they can be beneficial to the crop later in the season because they are key predators of spider-mite (*Tetranychus urticae*) eggs, another major pest species (Grundy 2019). Spider mites are generally a later-season pest, with the threshold modified according to the expected length of the growing season for the differing cotton-growing regions. Hence, different thresholds are applied to thrips and mites because these pests impact the cotton crop in very different ways.

A multispecies weed control threshold should, at least conceptually, be simpler to develop than generalized insect thresholds, because most weeds have similar competitive effects on a

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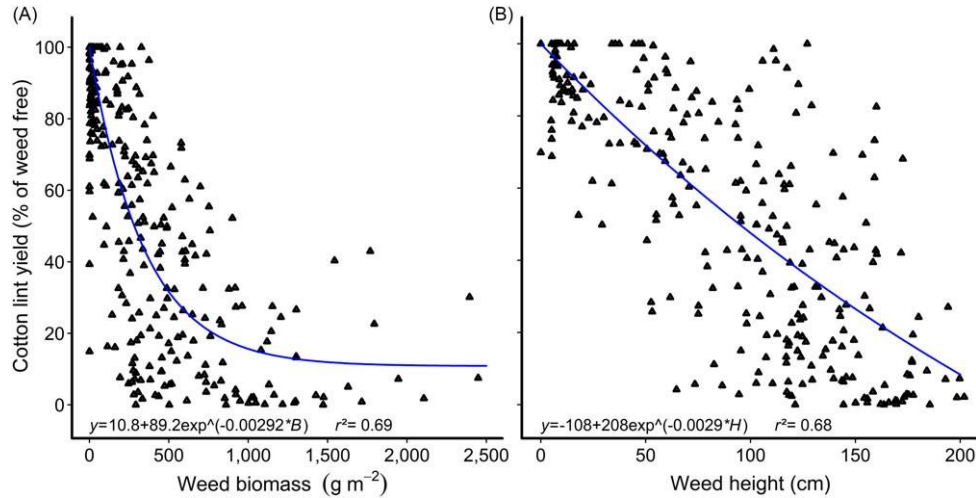


Figure 1. The influence of common sunflower (A) biomass, and (B) plant height on relative cotton-lint yield. Parameters of the models are as follows: y is the relative crop yield; B is the above-ground weed biomass; and H is the weed height. Data points for the relationships are treatment means.

crop, with the level of damage caused by plant competition most closely related to the time of weed emergence (relative to crop emergence) and duration of competition, weed density, and weed size (Askew and Wilcut 2001, 2002a, 2002b; Cortés et al. 2010; Fast et al. 2009; Korres and Norsworthy 2015; Ma et al. 2016; Scott et al. 2000; Webster et al. 2009). The impact of weed competition on a crop can also be affected by factors such as seasonal variation (Bukun 2004), soil moisture (Tingle et al. 2003; Vencill et al. 1993), soil fertility (Robinson 1976; Tursun et al. 2015), row spacing (Tursun et al. 2016), and crop health (Buchanan et al. 1977; Webster and Davis 2007). However, in fully irrigated cotton production in Australia, most of these factors are maintained as closely as possible to optimum, such that these factors should normally have little influence on the crop's response to weed competition. Hence, a generalized weed control threshold model for irrigated cotton in Australia might be possible if the model is able to account for the time of weed emergence, duration of weed growth, weed density, and weed size (Charles et al. 2019a).

Defining the critical period for weed control (CPWC) is an important step in developing an IWM program for a crop and a way to delineate a dynamic weed control threshold model (varying over the crop-growing season) that incorporates the effects of the time of weed emergence (relative to crop emergence) and the duration of weed growth on crop yield (Knezevic and Datta 2015). Previous competition studies have shown that other factors can also affect crop yield, such as weed density, weed height, and weed biomass (Askew and Wilcut 2001, 2002a, 2002b; Cortés et al. 2010; Fast et al. 2009; Scott et al. 2000), but these terms have not been included in CPWC models.

Historically, CPWC models have been site, season, species, and density specific (Charles et al. 2019b). Knezevic and Datta (2015) suggest using growing degree days (GDD) as the measure of time to reduce seasonal differences, and Charles et al. (2019b, 2020a, 2020b) reported that using GDD as the time descriptor overcame the effects of season on the CPWC relationship in fully irrigated cotton in Australian conditions. In an additional step, Charles et al. (2019b, 2020a, 2020b) included weed density as an extra term in the equations used to define the CPWC, and so were able to account for this factor in their CPWC models. In addition, Charles et al. (2020a) tested the possibility of using weed height or weed biomass (kilograms of above-ground dry matter per square meter) in place of weed density in

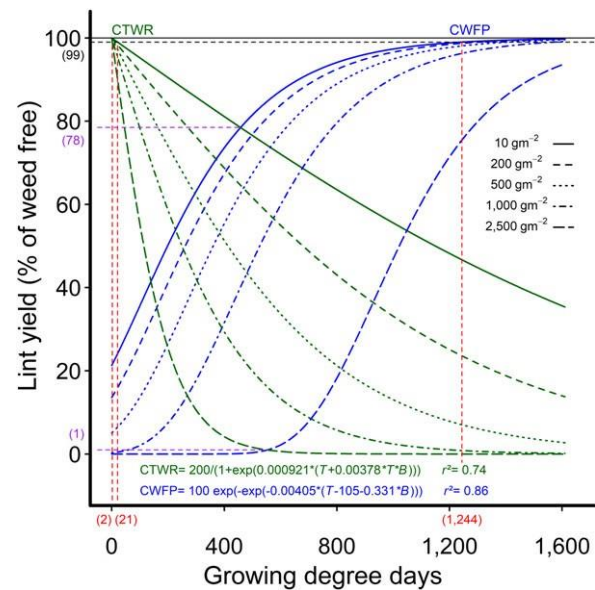


Figure 2. The critical period for weed control (CPWC) for common sunflower competing with cotton. The CPWC is defined by the intersection of the CTWR (green lines), and CWFP (blue lines), with a 1% yield-reduction threshold (horizontal dashed line). The derived relationships for common sunflower biomass of 10, 200, 500, 1,000, and 2,500 kg m^{-2} are presented as examples. Parameters of the curves are as follows: y is the relative lint yield; T is the cumulative degree days since planting; and B is the aboveground weed biomass. Data points for the relationships are treatment means. The horizontal solid line indicates the weed-free yield. The limits of the derived CPWC curves for 10 and 2,500 g m^{-2} are shown by the vertical dashed red lines and bracketed values. Points of minimum yield loss for 10 and 2,500 g m^{-2} are indicated by the dashed purple lines and bracketed values. CTWR, critical time for weed removal; CWFP, critical weed-free period.

their CPWC models and found that the critical time for weed removal (CTWR) relationship was improved for the mimic weed mungbean in fully irrigated cotton when weed biomass was used in the model. The substitution of weed biomass for weed density gave no improvement in the critical weed-free period (CWFP) relationship.

Thus, Charles et al. (2020a) were able to fulfil the theoretical requirement for a more generalized weed control threshold for irrigated cotton, incorporating the time of weed emergence, duration of competition, weed density and size into their extended CPWC

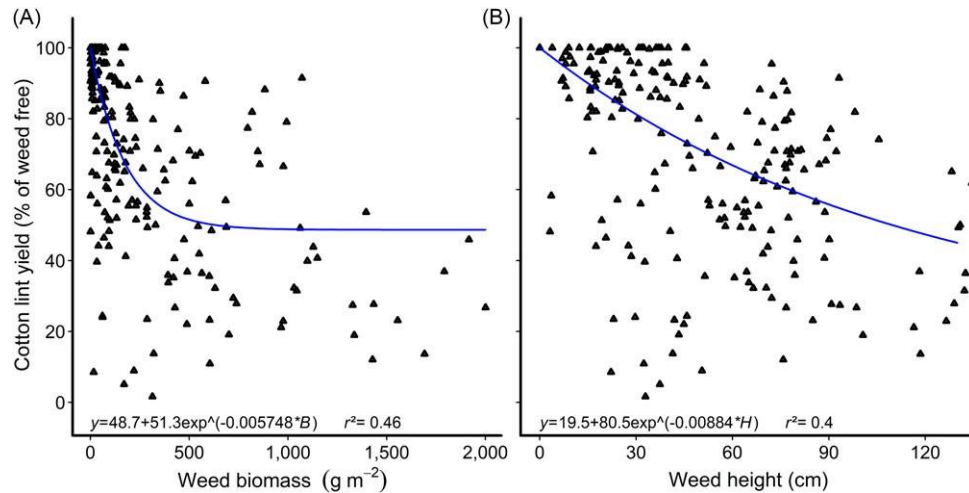


Figure 3. Reduction in cotton-lint yield with increasing Japanese millet (A) biomass and (B) height. Parameters of the models are as follows: y is the relative crop yield; B is the aboveground weed biomass; and H is the weed height. Data points for the relationships are treatment means.

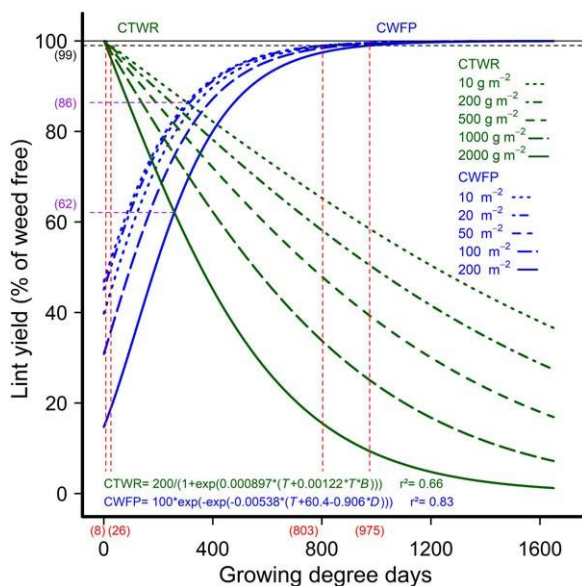


Figure 4. The critical period for weed control (CPWC) for Japanese millet competing with cotton. The CPWC is defined by the intersection of the critical time for weed removal (CTWR; green lines), and critical weed-free period (CWFP; blue lines), with a 1% yield-reduction threshold (horizontal dashed line). The derived relationships for Japanese millet biomass of 10, 200, 500, 1,000, and 2,000 kg m^{-2} are presented as examples for the CTWR relationship, and Japanese millet densities of 10, 20, 50, 100, and 200 plants m^{-2} are presented as examples of the CWFP relationship. Parameters of the curves are as follows: y is the relative lint yield; T is the cumulative degree days since planting; B is the aboveground weed biomass, and D is the weed density. Data points for the relationships are treatment means. The horizontal solid line indicates the weed-free yield. The limits of the derived CPWC curves for 10 and 2,000 g m^{-2} (CTWR), and 10 and 200 weeds m^{-2} (CWFP), are shown by the vertical dashed red lines and bracketed values. Points of minimum yield loss for 10 weeds and 10 g m^{-2} , and 200 weeds and 2,000 g m^{-2} , are indicated by the dashed purple lines and bracketed values.

model, because weed biomass (kg m^{-2}) includes components of weed size and weed density. The objective for this study was to determine whether this approach of including weed density and size in the CPWC model could be applied to other weeds with different morphologic characteristics, and in addition, whether this approach would allow a more generalized model to be developed that could be applied across a range of weed species and types.

Materials and Methods

Field studies were conducted at the Australian Cotton Research Institute, Narrabri (30.12°S, 149.36°E; elevation 201 m) on a heavy alluvial clay (fine, thermic, smectitic, Typic Haplustert) soil. Cotton crops were grown over six seasons from 2003 to 2016, in line with standard commercial practices using commercial cotton cultivars on raised hills, 1 m apart. Fields were fertilized with 180 kg N ha^{-1} , applied before planting, and were flood-irrigated as required.

Experimental Design

Within each season, the experiments used a randomized, complete block design with split plots and four replications; subplots were 4 rows (4 m) wide by 10 m in length. Main plots were times of weed planting and removal, and subplots were weed densities. The mimic weeds common sunflower, Japanese millet, and mungbean were individually planted with the crop or at predetermined periods after crop emergence, sown to achieve target densities. The times of weed planting and removal were measured in GDD since planting, defined as:

$$T = \sum \frac{(t_{min} + t_{max})}{2} - t_b \quad [1]$$

where t_{min} and t_{max} were the daily minimum and maximum air temperatures, respectively, and t_b was the base temperature of 15.5 C (Bukun 2004).

Weed planting and removal times were planned to occur at 150, 300, 450, 600, 750, and 900 GDD. Actual densities of established weeds, and times of planting and removal, were influenced by rainfall and irrigation events, with weed emergence delayed by inadequate soil moisture on some occasions. Not all target weed densities were achieved in all seasons.

Weed density and height were recorded at the time of weed removal and plants were weighed after drying at 70 C for at least 72 h in a forced-air oven. Cotton was mechanically harvested, and a single-saw gin was used to determine ginning percentage and lint yield. Additional details of the experiments are described in Charles et al. (2019b, 2020a, 2020b).

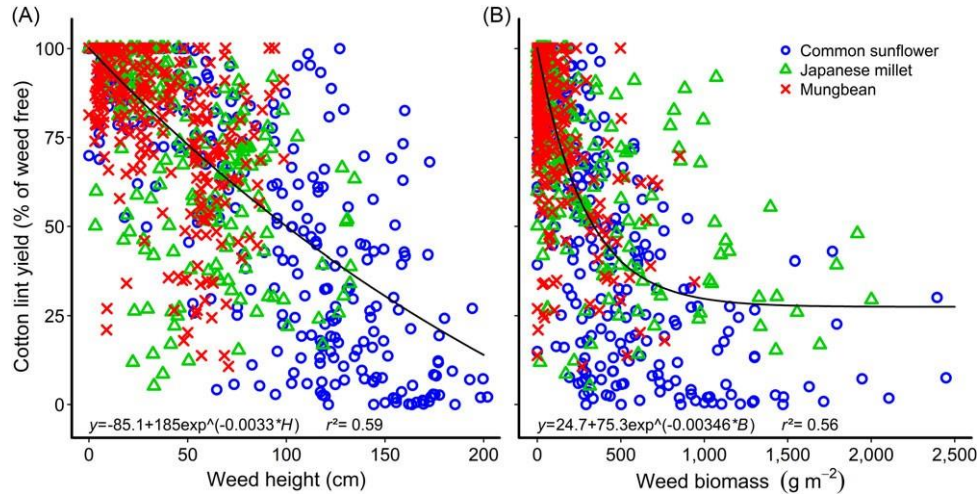


Figure 5. Cotton-lint yield as a function of (A) weed height and (B) weed biomass for the combined data sets from common sunflower, Japanese millet, and mungbean competition. Parameters of the models are as follows: y is the relative crop yield; B is the aboveground weed biomass; and H is the weed height. Data points for the relationships are treatment means.

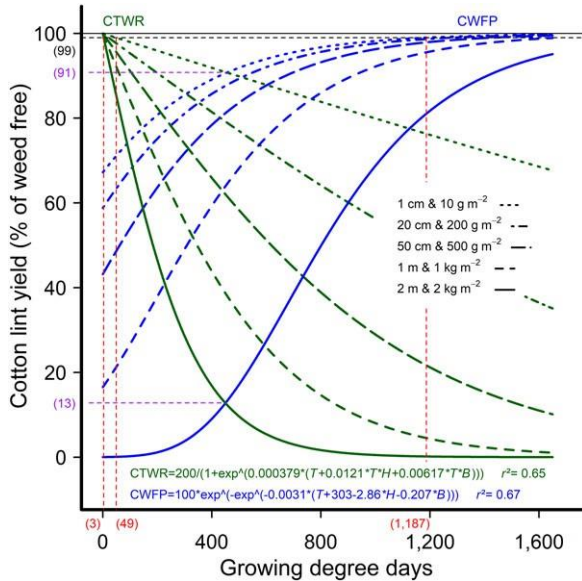


Figure 6. The critical period for weed control (CPWC) for cotton using a multispecies model. The CPWC is defined by the intersection of the critical time for weed removal (CTWR; green lines), and critical weed-free period (CWFP; blue lines), with a 1% yield-reduction threshold (horizontal dashed line). Parameters of the models are as follows: y is the relative lint yield; T is the cumulative degree days since planting; H is the weed height; and B is the aboveground weed biomass. The derived relationships for weed height and biomass of: 1 cm and 10 g m⁻²; 20 cm and 200 g m⁻²; 50 cm and 500 g m⁻²; 1 m and 1 kg m⁻²; and 2 m and 2 kg m⁻² are presented as examples. The horizontal solid line indicates the weed-free yield. The limits of the derived CPWC curves for weeds 1-cm tall and 10 g m⁻² biomass, and 2-m tall and 2 kg m⁻² are shown by dashed red lines and bracketed values. The points of minimum yield loss for weeds 1-cm tall and 10 g m⁻² biomass, and 2-m tall and 2 kg m⁻² are indicated by the dashed purple lines and bracketed values.

Statistical Analysis

The data sets used by Charles et al. (2019b, 2020a, 2020b) were analyzed using R statistical software, version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) with a significance level of $P < 0.05$. Regression analysis was used to test the relationships between the relative cotton-lint yield (i.e., lint yield relative to the weed-free control in each season); and weed density, biomass,

and height, using the coefficient of determination (r^2) to assess the fit of each model. Data were fit to Gompertz, logistic and exponential functions as described by Charles et al. (2019b, 2020b), with the exponential function substituted for the logistic function where the shape of the curve did not allow the logistic function to be fit, or where the exponential improved the fit of the data, as indicated by the Akaike information criterion (AIC). Weed density, height, and biomass were then fit to the CPWC relationships described by Charles et al. (2019b, 2020b) using the AIC to determine the model with the best fit. The CTWR models were constrained to 100% relative yield where no weeds were present.

Data sets from all three mimic weeds, common sunflower (Charles et al. 2019b), Japanese millet (Charles et al. 2020b), and mungbean (Charles et al. 2020a) were combined in the present study to test whether a multispecies CPWC model could be fit to the combined data set. Linear regression was used to test the association between relative cotton-lint yield and experimental year; weed species, density, biomass, and height; time of emergence; and time of removal. The data were combined over years because experimental year was not a significant factor in the regression. The combined data set was used to develop new multispecies CTWR and CWFP models. The fit of these multispecies models was tested over the three weed species by using the functions to generate predicted lint-yield reductions for each species and contrasting the predicted to the observed yield reductions. A simple linear model was fit to this contrast of predicted and observed yield reductions for each species and 95% confidence intervals of the lines were generated. The fitted lines for each species were compared with a line generated for the combined data set and the overlap of the confidence intervals was examined.

Results and Discussion

Developing Dynamic Models for the Mimic Weed Common Sunflower

A dynamic relationship was developed by Charles et al. (2019b) to define the CPWC for common sunflower, a large mimic weed, in high-yielding cotton, using weed density and the duration of weed competition as descriptive elements in the models. However, more

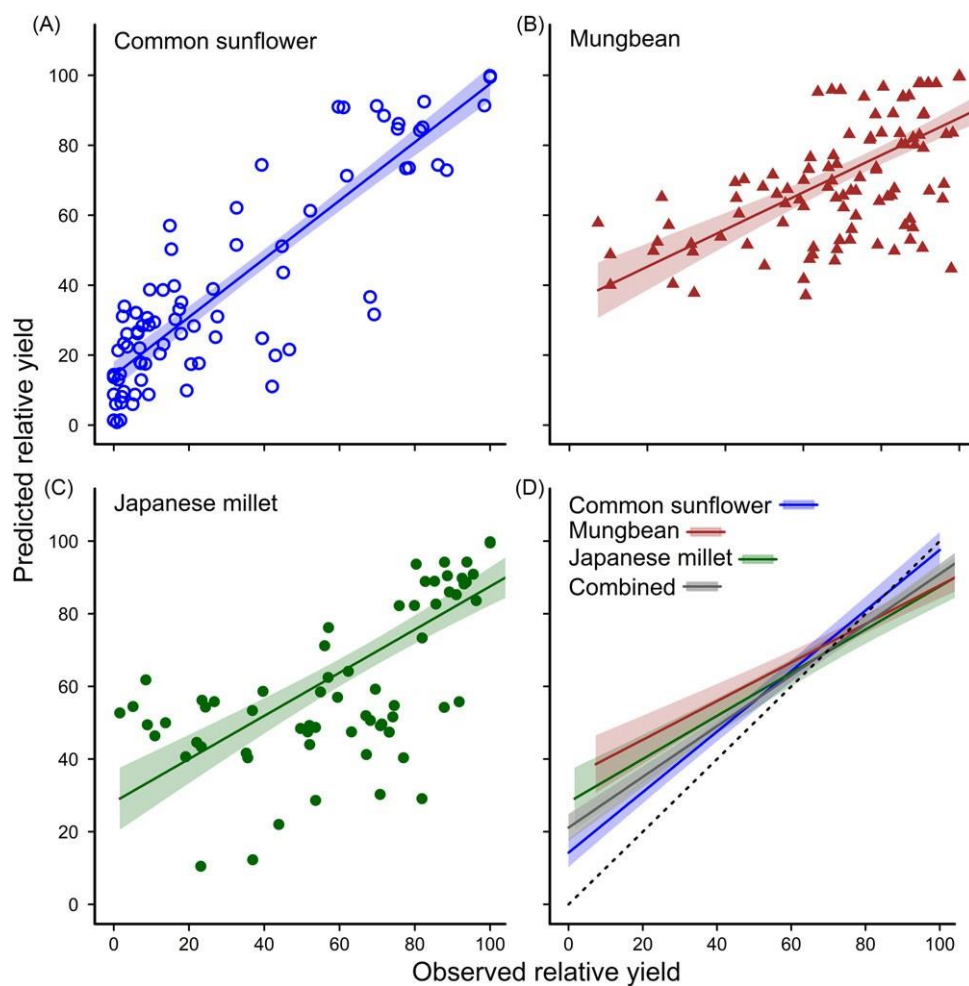


Figure 7. Estimated linear relationships and 95% confidence intervals for observed and predicted relative crop yield using the multispecies critical weed-free period model for (A) common sunflower, (B) mungbean, and (C) Japanese millet. The relationship (D) for the combined data set and each species is shown against a 1:1 line. Data points for the relationships are treatment means.

recent research using mungbean, a smaller broadleaf mimic weed, found that the relationships could be improved by substituting weed biomass (kg m^{-2}) for weed density (plants m^{-2} ; Charles et al. 2020a). Charles et al. (2020a) also tested weed height and combinations of weed density, height, and biomass as factors, but found the best fit with weed biomass. Weed biomass and weed height had previously been shown to be correlated to cotton-lint yield (Askew and Wilcut 2001, 2002a, 2002b; Charles et al. 2019a; Cortés et al. 2010; Fast et al. 2009).

Using the data published by Charles et al. (2019b), we tested the relationships between relative cotton-lint yield and weed (common sunflower) biomass, height, and density. There were strong associations between the relative lint yield and weed biomass ($r^2 = 0.69$), and height ($r^2 = 0.68$), with weed biomass being the stronger relationship (Figure 1), but no apparent relationship with weed density ($r^2 = 0.02$).

We tested the fit of weed density, height, and biomass on the dynamic relationships defining the CPWC for common sunflower using the data published by Charles et al. (2019b) and found the fit of both the CTWR and CWFPP relationships was most improved by substituting weed biomass for weed density (Figure 2). The CPWC defined by these curves extended from 21 GDD to 1,244 GDD for 10 g weed m^{-2} when a 1% weed control threshold was applied, and for the full

season (2 GDD to picking) with $2.5 \text{ kg weed m}^{-2}$, the maximum dry aboveground biomass of common sunflower reported by Charles et al. (2019b). Our model shows that the presence of 1.09 kg m^{-2} or more of weeds at any stage during the season (4 GDD to 1,600 GDD) reduced the cotton-lint yield by more than the 1% threshold.

This model, based on weed biomass, is both a statistical and practical improvement over the original CPWC model based on weed density (Charles et al. 2019b) because it includes a measure of weed size (biomass), allowing for variation in the size and the growth rate of the weeds, and allowing for the possibility that the model might be more widely applied to a range of weed species and types, with similar morphologic traits. This possibility of developing a multispecies competition model was proposed by Charles et al. (2019a), who developed a simple model relating cotton-lint yield loss to a combination of weed height and weed biomass for three very different mimic weed species: common sunflower; mungbean; and Japanese millet.

Developing Dynamic Models for the Mimic Grass Weed Japanese Millet

A dynamic relationship defining the critical period for weed control for Japanese millet, a mimic grass weed, in high-yielding cotton

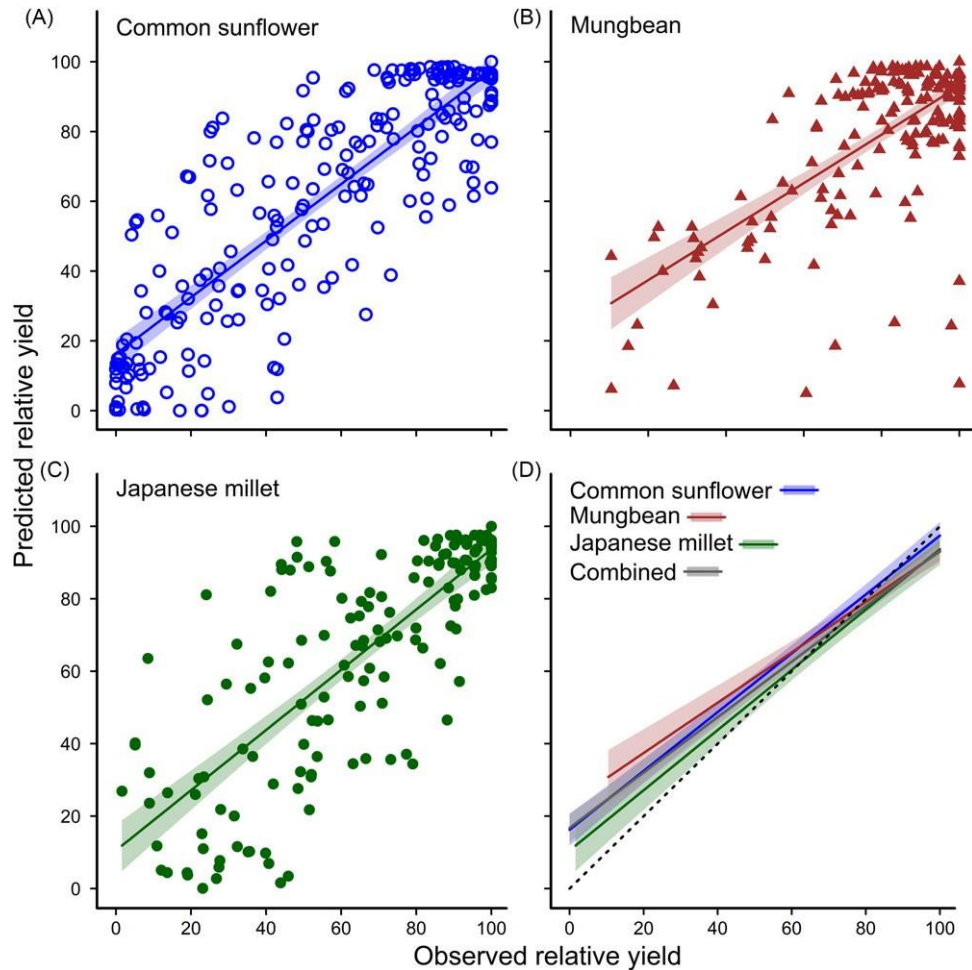


Figure 8. Estimated linear relationships and 95% confidence intervals for observed and predicted relative crop yield using the multispecies critical time for weed removal model for (A) common sunflower, (B) mungbean, and (C) Japanese millet. The relationship (D) for the combined data set and each species is shown against a 1:1 line. Data points for the relationships are treatment means.

was developed by Charles et al. (2020b) using weed density and the duration of weed competition as descriptors in the models. Using these data, we tested the relationships between the relative lint yield and weed biomass, height, and density, with the data averaged over the remaining factors. The results mirrored the earlier findings with common sunflower, with lint yield associated with weed biomass ($r^2 = 0.46$) and weed height ($r^2 = 0.4$; Figure 3), but poorly related to weed density ($r^2 = 0.04$).

We tested the fit of weed density, biomass, and height on the CPWC relationships for Japanese millet published by Charles et al. (2020b) and found an improvement in the fit of the CTWR when weed biomass was substituted for weed density, but no improvement in the CWF model with any combination of weed density, biomass, or height (Figure 4). Charles et al. (2020a) similarly reported that the CTWR relationship for mungbean was improved when weed biomass was substituted for weed density in the relationship, but that the CWF model was not improved by substituting either weed biomass or weed height into the relationship. The CPWC defined for Japanese millet using the new CTWR curve begins earlier in the season than the original CPWC (Charles et al. 2020b), beginning at 26 GDD with 10 g weed m^{-2} and 8 GDD with 2 kg weed m^{-2} . There was no substantial change in the points of minimum yield loss with the new model.

Developing Dynamic Models Using the Combined Data Sets for Three Mimic Weeds

To test the possibility of developing a multispecies competition model for irrigated cotton, we combined the data sets for the mimic weeds common sunflower (Charles et al. 2019b), Japanese millet (Charles et al. 2020b), and mungbean (Charles et al. 2020a). We tested the associations between relative cotton-lint yield and experimental year; and weed species, density, biomass, height, time of emergence, and time of removal. Experimental year was not a significant factor in the regression, but all other factors were significantly associated with lint yield. Weed density and weed species, however, were only weakly related to relative yield ($r^2 = 0.003$ and $r^2 = 0.04$, respectively).

The relative lint yield of cotton was most strongly associated with weed height ($r^2 = 0.59$) and weed biomass ($r^2 = 0.38$), with the times of weed emergence and weed removal less strongly related, $r^2 = 0.09$ and $r^2 = 0.15$, respectively. The relationship improved when the times of weed emergence and weed removal were both included in the regression ($r^2 = 0.33$). The correlations of lint yield with weed height and biomass, and the times of weed emergence and removal, were further improved by using exponential (weed height and biomass; Figure 5), and Gompertz functions (times of weed emergence and removal).

Using the combined data set, we developed new CTWR and CWFPP relationships with weed density, biomass, and height as factors separately and in combination. The best fit for both relationships occurred when weed height and biomass were included as factors by developing a multispecies CPWC model (Figure 6) in line with the findings of Charles et al. (2019a) who also related relative lint yield to weed height and biomass. The CPWC derived from the multispecies models extended from 49 to 1,187 GDD for weeds 1 cm tall and weighing 10 g m⁻², and for the full season (3 GDD to picking) with weeds that were 2 m tall and 2 kg m⁻² biomass. The presence of weeds that were 85 cm tall and of 850 g m⁻² biomass or more reduced the cotton-lint yield at any stage in the season (to 1,600 GDD) by more than the 1% yield-loss threshold. The reduction in cotton-lint yield below the 1% yield-loss threshold was similarly caused by weeds of any height (1 cm or more) where weed biomass exceeded 2,010 g m⁻², or any biomass (10 g m⁻² or more) where weed height exceeded 145 cm.

We tested the fit of the CWFPP model by comparing observed and predicted yield reductions for each species against the combined data set. The 95% confidence intervals for common sunflower and Japanese millet overlap the confidence interval for the combined data set throughout its length, indicating that the multispecies model reasonably predicts the yield loss of these two very different mimic weeds: a large broadleaf weed and a much smaller grass weed (Figure 7). The confidence interval for the combined data set overlaps the confidence interval for mungbean for relative yields of 40% or more. The multispecies model overestimates the yield loss from competition from mungbean below 40% relative yield, although the difference between the two confidence intervals was very small, and increased to 1% of relative yield at the lowest observed yield of 7%. We also note that the multispecies CWFPP model underestimates all yield losses below 71% relative yield (the relationship is above the 1:1 line) and overestimates yield losses at higher yields. This inaccuracy appears to be caused by the nature of the model used. Future work should explore alternative mathematical relationships to correct this issue with our model. Nevertheless, we contend that the multispecies CWFPP model reasonably represents the competition relationships of these very different mimic weeds.

We similarly tested the fit of the CTWR model by comparing observed and predicted yield reductions for each species against the combined data set. The 95% confidence interval for the combined data set overlapped the confidence intervals for all three mimic weeds throughout their length, indicating that the multispecies model reasonably predicted the yield loss of these weeds (Figure 8). Again, we note that the multispecies CTWR model underestimates yield losses below 68% relative yield (the relationship is above the 1:1 line) and overestimates yield losses at higher yields. Correcting this inaccuracy should be an aim of future work.

Further Development of a Multispecies Model

A multispecies CPWC model will be a valuable tool for developing IWM systems in irrigated cotton, enabling Australian cotton growers to optimize their weed control inputs (Knezevic and Datta 2015). The multispecies model developed in this study was generated from data from three very dissimilar mimic weed types and so should be applicable to many of the weed species commonly found in cotton in Australia (Charles et al. 2019a, 2020a; Werth et al. 2013). In addition, the model uses growing degree days as the unit of time and weed biomass and height as measures of weed size (Knezevic and Datta 2015). We expect our model, which

includes all these factors, will be widely applicable to cotton production in Australia, and we will be testing this expectation in future studies.

Nevertheless, the model has been developed using artificial “mimic” weeds, sown in single rows offset from the crop row, in a single location, and has not been tested against prostrate weeds, naturally occurring weed populations, staggered germinations, or within the more typical cotton production system of successive weed germinations and multiple control events. Future work should test the multispecies CPWC model against naturally occurring weeds in a more typical production system, and in other cotton-producing areas in Australia, to ensure the model is applicable to the real situation of weeds and cotton over the diversity of the production area.

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SUMMARY and CONCLUSION

The cotton cropping system has been described as one of the few intensive, broad-acre cropping systems in Australia. Cotton is typically a highly-profitable crop, but requires high levels of inputs and management to produce the high yields regularly achieved when water is not a limiting input.

Weed management has been a challenge for the modern Australian cotton industry since its inception, and remains a challenge. Over time the cotton industry developed a weed management system which included multiple applications of residual herbicides pre- and/or at-planting, combined with inter-crop cultivation, post-emergence herbicides and hand-hoeing. This system adequately managed most weeds, but was expensive both in terms of the cost of inputs, and the cost of damage to the crop and the environment. Substantial losses of cotton seedlings could occur when crops established in cold and wet conditions, due to poor crop tolerance to residual herbicides under these conditions. Some damage was also commonly seen later in the season when cotton roots encountered residual herbicides or were pruned by cultivators.

The introduction of cotton varieties including the glyphosate tolerance (Roundup Ready®) trait, and later the Roundup Ready Flex trait, enabled cotton growers to achieve high levels of in-crop weed control with less reliance on the use of residual herbicides, and went hand-in-hand with increasing crop yields. Over time, however, a steady increase in the numbers of glyphosate tolerant and glyphosate resistant weeds in many fields has challenged this system, necessitating a return to a more-integrated approach to weed management in cotton, requiring a wider variety of inputs and a higher level of management to coordinate and optimise the outcomes from these inputs.

Pest management thresholds have become important tools for cotton growers, enabling them to coordinate strategic inputs to maintain a wide variety of pests at or below acceptable levels. Likewise, a multi-species weed control threshold model would assist cotton growers to coordinate and optimise weed management inputs, with the ultimate aim of achieving even higher crop yields, while reducing the impact of weeds and herbicide resistance on the cotton industry.

The aim of this research was to develop a multi-species weed control threshold model for weeds in irrigated cotton. This aim was achieved by applying the critical period for weed control concept, using three differing mimic weeds (sunflower, a large, broadleaf plant; Japanese millet, a grass; and mungbean, a medium-sized, broadleaf plant), competing with

fully irrigated, high-yielding cotton. The traditional model approach applied to the critical period concept, using logistic and Gompertz equations, was successfully extended, integrating aspects of weed size and density into the models, and ultimately resulting in a multi-species weed threshold model for cotton. Statistical analysis of the model's output confirmed that the multi-species model was reasonably able to describe the competition from these differing three mimic weeds, leading to the conclusion that the model could be able to describe the competition from the range of weeds that occur in the cotton production system.

This multi-species weed competition model will be a valuable tool for facilitating the application of improved weed management systems in irrigated cotton, enabling Australian cotton growers to optimise their weed control inputs. Nevertheless, the model could be further refined. The model has been developed using artificial "mimic" weeds, sown in single rows off-set from the crop row, and has not been tested against prostrate weeds, naturally occurring mixed weed populations, staggered germinations, or within the more typical cotton production system of successive weed germinations and multiple control events. Future work should test the multi-species CPWC model against naturally-occurring weeds in a more typical production system to ensure the model is applicable to the real situation of weeds and cotton.

The model might be further refined by also considering: the competitiveness of the cotton crop, using the range of commercially available cotton varieties and possible interactions with the use of cotton growth regulators; cotton planting configurations, from ultra-narrow row cotton, normally with 6 rows on a 2-metre wide bed, to cotton on 1.5 metre row spacing (1-metre spacing remains the industry standard practice in irrigated cotton); and the effect of climatic factors, with cotton now commercially grown in Australia from the far-north of Queensland, the Northern Territory and Western Australia, to the far-south of Victoria. Differing irrigation systems may also affect the rate of crop and weed growth.

In addition, the model could be used to predict yield losses when weed control inputs are delayed, but to do this would require a knowledge of the typical growth rates of the weeds present in the field. This information is not currently available for most of the weeds commonly found in cotton. Future work should include determining the growth rates of the weeds most commonly found in Australian fields.

Finally, application of the model assumes that weeds are uniformly distributed across a field, and equally, that the weed height and biomass determined from a sample of a field is representative of the whole field. This issue of the accuracy of pest sampling is common to all pest threshold models used by the cotton industry and can be lessened by repeated sampling

and the skills of the persons undertaking the sampling. Nevertheless, weeds patchiness is common in cotton, with weeds frequently not evenly distributed throughout a field, challenging the use of limited sampling to characterise a field and determine the weed pressure status of the field. Ideally, the practical application of the weed control threshold model might be improved if remote sensing could be used to reduce the vagaries of in-field sampling. Remote sensing might be used to better characterise a field, enabling sampled spots to be related to the whole field, or it might be possible to estimate weed height and biomass directly with remote sensing, allowing a whole field to be assessed in a short period of time.

Notwithstanding these limitations, the findings from these studies represent a quantum step in defining a multi-species weed control threshold model for irrigated cotton and will contribute to the continuing sustainability and profitability of the Australian cotton industry. Further, our approach should be applicable to other intensively farmed, fully irrigated crops, where crop yields are not highly influenced by environmental variability, such as many of the horticultural crops. Future investigations could include some of these other intensively grown crops.

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