Smoking out the enemy: triggering agricultural weed seeds to germinate with karrikinolide

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Summary The smoke-derived chemical, karrikinolide (KAR_1), may be used to improve the efficiency of weed control efforts in agriculture by triggering weed seeds to germinate synchronously. Given that seed banks are complex mixes of species, we hypothesised that the germination response of weed seeds would depend on the dormancy state of the seeds, which would vary seasonally. To test this hypothesis, seeds of nine species were aged at three sites in Western Australia for up to 12 months. Samples were recovered intermittently and their germinability was assessed with and without KAR_1 and light. The marginal benefit of using KAR_1 to trigger germination varied throughout the year in accordance with the dormancy state of the seeds. We conclude that the efficacy of applying KAR_1 depends on how it interacts with the dormancy state of different species, and the best time to apply KAR_1 in a Mediterranean climate may be just prior to the start of the cropping season in autumn.

Keywords Dormancy, smoke, germination, karrikinolide, seed.

INTRODUCTION

Most agricultural weeds are annual species that are dispersed by seed. Seeds can persist in the soil between cropping seasons, forming a soil seed bank. This seed bank is complex, and consists of seeds of different species, genotypes, age and degrees of vigour and dormancy (Benech-Arnold *et al.* 2000). The consequence of soil seed banks being such complex mixes of seeds with different requirements for germination is that the seedlings do not emerge synchronously. Currently weed management in agriculture is cyclical, whereby rainfall is followed by the emergence of weed seedlings, which are controlled chemically, physically or culturally. This sequence is repeated following each rainfall event, until such time as weed control becomes physically or financially infeasible.

One way to improve the efficiency of weed management in agricultural systems would be to artificially synchronise the germination of weed seeds, as this would enable fewer and more strategic weed control efforts. A chemical known as karrikinolide, which was originally identified from smoke water, shows promise as a tool for synchronising the germination of weed seeds (Stevens *et al.* 2007, Chiwocha *et al.* 2009, Long *et al.* 2010). However, it is not known whether KAR_1 is equally efficient at stimulating all species at a given time, or whether there are seasonal and site-based factors that influence its efficacy.

Given the complex nature of soil seed banks, we reasoned that the marginal benefit of using KAR_1 to stimulate weed seeds to germinate synchronously will depend on the dormancy state of seeds, which will vary according to the species and time of year when KAR_1 is applied. This study aimed to assess the marginal benefit of applying KAR_1 to germinate seeds of nine agricultural weeds of the Western Australian wheat belt, both when the seeds were freshly dispersed, and in April, just prior to the start of the growing season.

MATERIALS AND METHODS

Plant material Seeds of *Arctotheca calendula* (L.) Levyns (capeweed), *Avena fatua* L. (wild oats), *Brassica tournefortii* Gouan (wild turnip), *Bromus diandrus* Roth (great brome), *Echium plantagineum* L. (Paterson's curse), *Lolium rigidum* Gaudin (annual ryegrass), *Raphanus raphanistrum* L. (wild radish), *Sisymbrium erysimoides* Desf. (smooth mustard) and *Sisymbrium orientale* L. (black Indian hedge mustard) were collected from the wheat belt zone of Western Australia in September to November 2008.

Seed burial experiment Seed burial trials were established at three sites in Western Australia in December 2008: Shenton Park (in Perth, S31°57', E115°48'), Northam (100 km inland from Perth, S31°45', E116°41') and Merredin (270 km inland from Perth, S31°30', E118°12'). All species were included in the Northam trial, whilst only four species were included at the Shenton Park and Merredin sites, namely *A. fatua*, *L. rigidum*, *R. raphanistrum* and *B. tournefortii*. Each site was divided into four blocks, and in each block ten bags of seeds for each species were buried at 1 cm depth. Seed bags were made of fibreglass fly-wire mesh and contained 500 seeds (*A. calendula*, *A. fatua*, *E. plantagineum*, *L. rigidum* and *R. raphanistrum*) or 1000 seeds (*B. tournefortii*,

S. erysimoides and *S. orientale*) per bag. Bags were uniquely labelled with numbered metal tags to facilitate identification and retrieval throughout the 2-year trial. For each species, one bag of seeds was retrieved from each block at each site in February, March, April, May, June, September and December 2009, with further retrievals planned for March, June and December 2010. In the case of *A. calendula*, fewer samples were available and retrieval times were assigned as March, May, September and December 2009 plus June 2010.

Germination testing Following retrieval, seed bags were opened and sub-samples of 25 seeds were germinated in Petri dishes on 1% w/v agar with and without 1 μM karrikinolide (KAR_1), at two temperature regimes (either 15°C constant and 20/10°C alternating, or 25°C constant and 35/20°C alternating, see Table 1), with and without 12 h alternating light. Dark treatments were imposed by wrapping Petri dishes in two layers of aluminium foil. Germination, defined as >2 mm radical protrusion, was scored 21 days after sowing.

Data analysis The marginal benefit of applying KAR_1 at each retrieval time was assessed by subtracting the without-KAR germination percentage from the with-KAR germination percentage for each lighting scenario, indicative of the responses of seeds on the soil surface (light) and buried seeds (dark). Data for blocks, temperatures and sites (where applicable) were pooled, such that each data point represents an assessment of 600 seeds for *A. fatua*, *L. rigidum*, *R. raphanistrum* and *B. tournefortii* and 200 seeds for the remaining species. An arbitrary threshold of 20% extra germination was applied to distinguish samples for which KAR_1 was beneficial.

RESULTS

KAR_1 effectively triggered the germination of freshly collected seeds of four of the nine species tested, whilst it enhanced the germination response for seeds of a further two species following burial in the field for 4 months (Table 1). The marginal benefit of using KAR_1 to germinate seeds of *B. tournefortii* and *R. raphanistrum* was consistently ≥20% irrespective of lighting conditions or when the seeds were tested. KAR_1 was also beneficial for fresh and aged seeds of *A. fatua*, but only when seeds were exposed to light. In the case of three species, *L. rigidum*, *B. diandrus* and *E. plantagineum*, KAR_1 did not significantly enhance germination under any of the tested conditions, neither when the seeds were fresh nor following any period of burial up to 6 months (data not shown).

The marginal benefit of KAR_1 was transient for three species that had highly dormant seeds when fresh. More seeds of *S. erysimoides* germinated with KAR_1 in alternating light conditions when the seeds were fresh, but by April KAR_1 was only beneficial in the dark. For *S. orientale*, KAR_1 triggered germination of seeds in darkness following 4 months of burial, whereas it was not beneficial under either lighting regime when the seeds were fresh. Similarly for *A. calendula*, seeds could not be triggered to germinate with KAR_1 when fresh, but the seeds became responsive to KAR_1 by April.

DISCUSSION

Our hypothesis that the response of seeds to KAR_1 depends on the dormancy state of the seeds and the season was partially accepted, as KAR_1 transiently stimulated germination of three of the nine species tested. Seeds of *S. erysimoides*, *S. orientale* and *A. calendula* were all physiologically dormant when fresh, and their response to KAR_1 was enhanced following burial for 4 months. However, in the case of the remaining species (*A. fatua*, *B. tournefortii* and *R. raphanistrum*) or non-beneficial (*L. rigidum*, *B. diandrus* and *E. plantagineum*), KAR_1 consistently enhanced the germination responses, with no dependence on seasonality or dormancy state.

Seasonal responsiveness to smoke signals has been reported for numerous Australian native species that have evolved in fire-prone environments (Baker *et al.* 2005a, b, Commander *et al.* 2009). As in these studies, natural dormancy cycling underpinned the response of seeds to KAR1 in our study. Many annual weed species in agricultural systems in Mediterranean climates are dormant when dispersed in late spring and early summer. Seeds then experience an extended period of dry after-ripening in the soil over summer before the rains, and cropping season, begin in autumn. Our results suggest that KAR1 might be most effective at triggering synchronous germination if applied just prior to the autumn rains, when seeds are less dormant and more sensitive to KAR1.

A further interesting implication of this study is that the response of seeds to KAR_1 can vary according to the presence or absence of light. In the case of *A. fatua*, KAR_1 was only beneficial when applied to seeds experiencing 12 hourly alternating light, which suggests that KAR_1 may only prove advantageous for seeds on the soil surface. In contrast, *S. orientale* and *S. erysimoides* were positively triggered to germinate with KAR_1 in the dark following burial, suggesting that KAR_1 may be most beneficial for triggering the germination of buried seeds. A dependence for light in determining the KAR_1 response of seeds was recently reported for the model species *Arabidopsis thaliana*

Table 1. The marginal benefit of applying karrikinolide (KAR_1) to enhance the germination of weed seeds. Seeds were germinated in the laboratory using 1 µM KAR_1, following collection and again following burial for 4 months at three sites in Western Australia. Species were tested at two temperature conditions, one constant and one 12 h alternating temperature (indicated by superscripts: [1] 15°C constant and 20/10°C alternating, [2] 25°C constant and 35/20°C alternating), but here, results for the constant and alternating temperatures have been pooled for each species. ✓ = ≥20% extra germination with 1 µM KAR_1, × = <20% extra germination with 1 µM KAR_1. n = 600 seeds for *A. fatua*, *L. rigidum*, *R. raphanistrum* and *B. tournefortii* and 200 seeds for the remaining species.

	December 2008 (fresh seeds)		April 2009 (4 months in field)	
Species	Light	Dark	Light	Dark
Brassicaceae				
Brassica tournefortii[1]	✓	✓	✓	✓
Raphanus raphanistrum[1]	✓	✓	✓	✓
Sisymbrium erysimoides[2]	✓	×	×	✓
Sisymbrium orientale[2]	×	×	×	✓
Poaceae				
Bromus diandrus[1]	×	×	×	×
Lolium rigidum[1]	×	×	×	×
Avena fatua[1]	✓	×	✓	×
Boraginaceae				
Echium plantagineum[2]	×	×	×	×
Asteraceae				
Arctotheca calendula[2]	×	×	✓	✓

(Nelson *et al.* 2009), and genetic studies continue to investigate the underlying mechanisms.

Considering the botanical families represented in this study, it appears that the mustard family (Brassicaceae) may be naturally responsive to KAR_1 and a worthy target for follow-up studies. Brassica weeds are particularly problematic in agriculture because they are closely related to major broad-acre crops, such as canola, and to horticultural crops, such as broccoli and cauliflower (Cheam *et al.* 2008). Thus, using KAR_1 to deplete the soil seed bank of weed seeds prior to planting the crop could significantly enhance the efficiency and productivity of these systems.

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