Monitoring *Striga asiatica* (Orobanchaceae) seedbank for eradication success

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Summary To enable evaluation of eradication efforts for the parasitic plant *Striga asiatica* (L.) Kuntze, seed sachets were buried in perforated PVC canisters at 25 sites across the infested area in July 2016. The canister sites were selected based on the proximity to previous *S. asiatica* detections and cover the range of soil types and topographies associated within the eradication program treatment area. At each site the canisters received the same eradication treatments that were applied to the surrounding area. Site characteristics necessitated different combinations of false host (soybean), true host (corn), fumigant (dazomet), stimulant (ethylene) and herbicide treatments, to achieve a rapid decline in the soil seed bank.

The viability of retrieved seeds shows an accelerated decline (92.5 % to 6.5 % in four years) across all sites in response to the treatments irrespective of treatment combination used. The viability of seeds was found to vary across sites, burial depth and time. The information collected has provided valuable data for the eradication of *S. asiatica* in Australia and for evaluating the timing for release of paddocks from active treatment.

Keywords eradication, red witchweed, RWW, *Striga asiatica*, quantitative performance measures, persistent seedbank.

INTRODUCTION

Striga asiatica (red witchweed (RWW)) was first recorded in Australia in 2013 and by 2015 the Red Witchweed Eradication Program (RWWEP) was declared (Smith *et al*. 2019). RWW is an annual obligate root hemiparasite, dependent on attachment to a host plant to complete its lifecycle (Joel *et al*. 2013). Host plants for RWW include several important crop species such as rice (*Oryza sativa* L.), sugarcane (*Saccharum officinarum* L.), sorghum (*Sorgum bicolor* (L.) Moench) and corn (*Zea mays* L.) (Shaw *et al*. 1962). Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) have also been confirmed as host plants at the Ecosciences Precinct, Brisbane (J.S. Vitelli, pers com 2017). Individual RWW plants can produce up to 500,000 seeds (Shaw *et al*. 1962). The dust-like seeds are approximately 250 µm long and 120 µm wide and can remain viable in a soil seed bank for 14 years under field conditions (Bebawi *et al*. 1984). Soil cores collected from an infested site at Habana, prior to the commencement of the eradication program, indicated the presence of a soil seed bank of approximately 1,000,000 / m^2 (J.S. Vitelli, pers com 2015).

Successful eradication of an invasive plant is dependent on delimitation, containment and extirpation (Panetta and Lawes 2005). Progress towards achieving the goal of eradication success in a weed eradication program is usually evaluated based on the presence or absence of plants detected during surveillance (Panetta and Lawes 2007). An ongoing decline in area and the number of detected plants, in particular adult reproductive plants, can be used to evaluate the effectiveness of different control strategies used (Panetta and Lawes 2007). However, for species with a persistent seedbank or where control methods vary in efficacy, the use of more quantitative performance measures may be required. This is the case for RWW which not only has long lived seed but the control methods available also vary in effectiveness depending on the location of the seed in the soil profile.

The size of RWW seed make traditional methods for monitoring soil seedbank decline extremely difficult. The methodology required to collect soil samples and separate the seeds from the soil is complicated and time consuming. An alternative approach to collecting an extremely large number of soil samples would be to bury seed in retrievable canisters located throughout the infested area. Seed viability data can then be relayed back within the eradication program and management regimes adjusted accordingly. A shortcoming of this method is that there is a finite number of retrievable seeds at any one site. Despite the efforts to predict the likely timeframe to achieve successful eradication and cover the potential permutations of treatments used, there is a risk that the final retrieval may contain viable seeds. Without additional canisters available for continued monitoring, only predictions can be made based on the already collected results.

If RWW was eradicated from Australia, it would be the first country in the world to achieve this. The United States of America has spent more than US$260 million over 66 years trying to eradicate RWW (Iverson *et al.* 2011), and in Africa it has been estimated that losses to farmers from *Striga* species amounts to US$7 billion annually (Berner *et al*. 1995). Eradication activities within the RWWEP center on regular surveillance for the presence of

RWW plants, optimization of control strategies that prevent the emergence and establishment of mature reproductive plants and treatments that target and accelerate the soil seedbank decline. A 100-point system has been implemented as the program moves from eradication to the release of previously infested areas from quarantine (Smith *et al*. 2019). All treated areas accumulate points according to both treatment activities and surveys employed. A total of 100 points is required for an area to become eligible for release from quarantine following the date of the last RWW detection (Smith *et al*. 2019).

This paper reports on the RWW soil seedbank viability over a 4-year eradication program across 25 sites known to be infested with RWW. Control efforts include the use of post-emergent herbicides, catch crops, trap crops and fumigants.

MATERIALS AND METHODS

An eradication monitoring study was established across 25 sites in July 2016. The sites were spread over eight infested properties near Habana (21°3'48"S, 149°4'21"E), Mackay, Queensland and transected the active eradication management zone of the RWWEP. The 25 sites varied in soil type and topography (Table 1). Soil types included friable non-cracking clay and clay loam (Dermosol), cracking clay (Vertosol) and sand or loam over sodic clay (Sodosol) (Holz and Shields 1985). All sites were located within zones where RWW had previously been detected. A total of 300 canisters (12 per location) each containing three RWW seed sachets placed at 10, 30 and 50 cm depth were used in this study. Five sites amenable to a soil fumigant application contained a fourth sachet placed on the soil surface.

All RWW seeds used in this experiment were collected from a nursery stock of plants grown within a QC2 quarantine facility at the Ecosciences Precinct, Brisbane between November 2013 to June 2014. Seed pods were stored at low humidity at 35°C until sufficient stock had been collected. Only seeds > 150 µm were used to maximize the germination viability (92.5 %) of the seed cohort. Approximately 100 seeds were measured by volume using a miniature scoop. The measured seeds were photographed for counting, then transferred into 40 mm x 65 mm, 62 µm aperture, sachets constructed from precision woven polyamide (nylon) mesh tubes (SAATIFIL PA 62/40 PW WH) that were sealed using self-adhesive nylon tape (PSP Spinnaker repair Tape). The sachets were installed into canisters on site in the field.

The canisters were 550 mm in length and constructed from 100 mm PVC pipe perforated with 10 mm holes to create 20 to 25 % open space. The seed sachets were placed at 10, 30 and 50 cm depths within the canisters and filled with 10 mm sieved soil collected from the study sites. Sixty canisters had an additional seed sachet placed at the soil surface.

Table 1: Soil type and land uses of each study location site.

Site	*P*	*T*	*Soil type* [a]	*Land use*
1	1	1	Dermosol	Cropping
2	1	1	Vertosol	Cropping
3	1	1	Dermosol	Cropping
4	2	3	Dermosol	Cropping
5	2	3	Dermosol	Grazing
6	2	3	Dermosol	Cropping
7	3	1	Dermosol	Cropping
8	4	1	Dermosol	Cropping
9	4	5	Sodosol	Cropping
10	4	1	Vertosol	Cropping
11	4	5	Vertosol	Cropping
12	5	1	Dermosol	Grazing
13	5	1	Dermosol	Grazing
14	5	5	Dermosol	Grazing
15	8	1	Vertosol	Cropping
16	8	3	Dermosol	Headland / Cropping
17	34	3	Dermosol	Cropping
18	34	3	Dermosol	Cropping
19	80	5	Vertosol	Cropping
20	80	3	Sodosol	Cropping
21[b]	2	3	Dermosol	Fenceline / Grazing
22[b]	2	3	Dermosol	Fenceline / Grazing
23[b]	4	1	Dermosol	Fenceline / Cropping
24[b]	4	5	Dermosol	Headland / Cropping
25[b]	5	5	Dermosol	Fenceline / Grazing

P = Property T = Topography

[a] Soil type and topography were determined from information from Holz and Shields (1985) held within the Queensland Soil and Land Information database. The topography rating reflects the undulation of the land from low (1) to severe (5). As undulation increases the crests and slopes are steeper and more complex, gullies are narrower and more frequent and farming land is more fragmented and less accessible.

[b] Sites with surface seed sachets included.

Canisters were buried in July 2016 and capped with a Mozzie Stoppa™ Original Screen at the soil surface to prevent loss of seed sachets. Eradication treatments commenced immediately.

At each site the canisters received the same eradication treatments that were applied during the management regime. Treatment selection included soybean (*Glycine max* (L.) Merr.) sown at 50 t ha^{-1} as a false host crop; corn (*Zea mays* L.) sown at 30 t ha^{-1} as a sentinel true host crop; ethylene gas was used as a stimulant and injected into the soil using a tractor mounted with a custom built injection system pulling a tyne through the soil and releasing ethylene at a rate of 2.0 kg ha^{-1} (1675 L ha^{-1}) applied at a depth of 15 to 30 cm and the fumigant dazomet (Basamid®) applied at 330 kg a.i. ha^{-1} to bare ground along headlands and fence lines. A range of herbicides (glyphosate, haloxyfop, imazapic and halsulfuron) were also applied to maintain weed control and minimise the presence of unwanted host plants. The combination and frequency of treatment application varied both over time and between sites.

Sampling occurred on eight occasions between November 2016 and November 2020. At each sampling event only 4 to 13 sites were randomly selected for canister exhumation with a maximum of six canisters removed at any one site. The remaining six canisters were left in-situ until the treated areas had reached the desired 100 points, as determined by the RWW program point system (Smith *et al*. 2019). At which point three canisters would be removed at these sites and RWW seed viability tested to help determine if these areas can progress from active eradication treatment to a monitoring phase.

All retrieved sachets were stored at low humidity at 35°C until processed. At processing, each sachet was washed to remove exterior dirt. The contents of the sachet were then flushed with RO water onto a Whatman® number 1 filter paper. The opened sachets were checked under a microscope for remaining seeds and seed coats. All seeds and seed coats were counted and intact seeds tested for J Ecol. viability. Viability testing was performed using 1 % 2,3,5-triphenyl tetrazolium chloride (TTC) as per Moore (1976). Seeds were immersed in 80 µl TTC and stored in the dark at 35°C for seven to ten days. Embryos turning red to pink in colour were considered viable.

RESULTS

The treatments applied during the 4-year period significantly reduced the seed viability across all sites. The viability of seeds started at 92.5 % at the time of field burial and declined to 3.5, 8.9 and 7.2 % at 10, 30 and 50 cm depths respectively by 2020 (Figure 1). No surface sachets were present in canisters retrieved in 2020.

Differences in the combination of eradication treatments applied, seed sachet burial depth and individual site conditions contributed to the variability observed for the seed viability decline. For example, in 2016, the viability of surface seed declined to 8.8 % after a single application of dazomet, while in the same canisters, buried seed viability was 19.5 %, 38.2 % and 46 % at 10, 30 and 50 cm. The viability of seeds collected at the same retrieval event from sites not treated with dazomet was 47.7 %, 49.8 % and 50.1 % at 10, 30 and 50 cm.

Viability differed across seed burial depth. The viability of seeds from sites 13 and 20 decreased more rapidly at 10 cm (39.8 %) than 50 cm (55.8 %), whereas the seed viability from sites 2, 11 and 19 was lowest at 50 cm (20.8 %) and remained higher at 10 cm (48.5 %).

Irrespective of burial depth, seed buried at site 10 declined most rapidly, dropping to 8.8 % viability in 2017. Seeds at site 5 retained the highest viability at 56.7 %, 40 % and 52.8 % at 10, 30 and 50 cm in 2018.

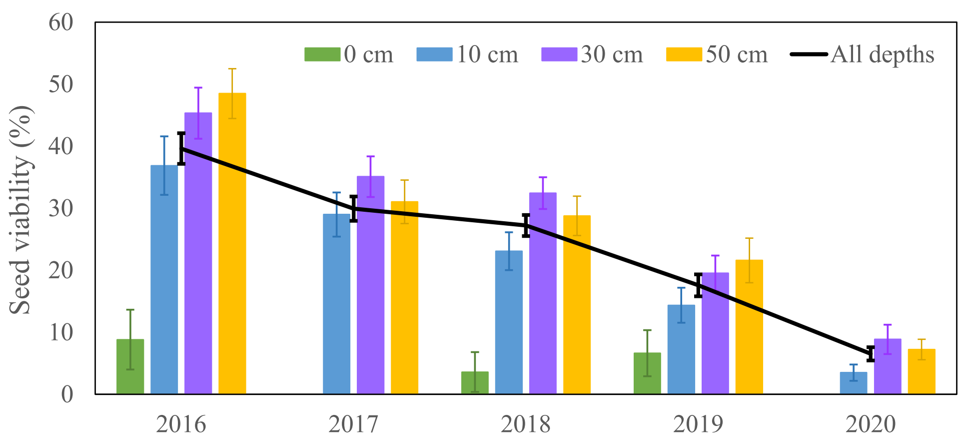

Figure 1. Effect of ongoing paddock applied treatments on seed viability at different seed burial depth. Each sample is the mean seed viability from all seed sachets retrieved during the year (standard error bars shown).

DISCUSSION

Seed viability declined with time as the number, frequency and range of treatments were applied across all 25 sites. Despite seed viability declining to 6.5 % viability across all sites within 4 years, this value could potentially equate to 65,000 seeds / m^2 remaining in core RWW infested areas.

Differences in soil type and topography appear to strongly influence seed longevity. Heavy clay soil combined with prolonged wet conditions are likely to have caused the accelerated seed bank decline (20.8 %) observed at 50 cm depth for sites 2, 11 and 19. Whereas at site 5 the highest retained viability (49.8 %) across all depths was likely attributed to its location on the side of an eastern facing, gently sloping paddock, with good drainage and friable clay loam soil.

The current data indicates that an accelerated seedbank decline of *Striga asiatica* in Australia could be achievable within five to eight years compared with the expected timeframe of 14^+ years for natural attrition alone. Successful extirpation of the seed bank depends on preventing seed recruitment. This is especially true for short lived annual species such as *S. asiatica* which can reach reproductive maturity within two to four weeks following emergence. If mature plants are detected, the timely intervention of treatments to prevent incorporation of new seeds into the soil profile is essential. Fortunately, for the RWWEP a relatively straightforward method is available for treating newly emerged RWW plants that is both effective at destroying the plant and any seed present on the soil surface. In these instances, the immediate area (2 m x 2 m) is incinerated and then followed with a tarped dazomet treatment. The scheduled frequency of surveillance of every 5–10 days undertaken within the Eradication Program (Smith *et al*. 2019) also minimises the possibility that seeds have time to reach maturity.

Determining when an area is free of RWW based on the number of treatments applied can be extremely challenging as evident in the USA where an eradication program in North and South Carolina was established in 1956. Despite its success in reducing the quarantined area from 175,000 ha to approximately 445 ha on the Carolina Coastal Plain the eradication program is still active after 66 years (Iverson *et al.* 2011). Currently, treatments and surveillance activities in the US determine an annual hand back of approximately 40 ha to land holders but there is also a return of a similar area back into the program as RWW is subsequently detected in released areas. It is estimated that it will take an additional 10–15 years to fully eradicate RWW from the USA (R.G. Westbrooks, pers com 2019).

The eradication monitoring data collected in this study has been critical for assessing progress towards the goal of eradication of RWW in Australia. Insights gained from monitoring seed bank decline has aided the decision for timely hand back of paddocks based on evidence of a declining seed bank. However, caution also needs to be exercised that the intrinsic variability of each site may not always lend itself to a hand back based solely on a scoring system reliant on the accumulation of treatments and surveys.

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