10 years after Victoria's major Mexican feather grass incursion: Assessing eradication methods and germination behaviour

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Summary Mexican feather grass (*Nassella tenuissima* Trin. Barkworth) is a highly invasive weed, with a potential economic and environmental impact in Australia comparable to serrated tussock (*Nassella trichotoma* Nees Hack. ex Arechav.), a close relative and Weed of National Significance. In Victoria, *N. tenuissima* is declared as a State prohibited weed and is managed with the goal of eradication. In 2008, retailers sold approximately 4000 *N. tenuissima* plants throughout metropolitan Melbourne and elsewhere.

The decade-long response to this incursion provides an opportunity to assess both the management approach, and the species' reproductive behaviour in south-eastern Australia. Infestations are treated primarily by physical removal and then regularly monitored for re-emergence from seed germination. The management of over 400 infestations allows an assessment of the efficacy of this management approach towards eradication, with fewer than 1% of the infestations discovered from July 2008 to June 2010 having plants present in the 2016/2017 season. We also analysed incidences of seed germination to assess the risk of seedling emergence over time. For example, for infestations where seedlings have emerged, over 90% showed at least some germination occurring within the first two years of initial treatment, whereas over 5% showed germination occurring more than five years after initial treatment. This case demonstrates the importance of early intensive monitoring, as well as taking a long-term outlook in the design and execution of invasive plant eradication programs. It also highlights physical removal as a viable primary eradication method for *N. tenuissima* incursions of this type and scale.

Keywords *Nassella tenuissima,* biosecurity.

INTRODUCTION

A major pathway for species invasions is ornamental plants imported and cultivated for horticulture, such that the eradication of new invaders in their early stages can require management responses to be targeted towards private yards, parks and gardens. Estimates suggest most invasive plant species in countries like Australia (Virtue *et al.* 2004) and the United States of America (Reichard and White 2001) arrive via horticulture (e.g. nurseries, botanical gardens, seed trading). Gardens can therefore be a frontline in preventing the establishment of new invaders and their negative economic and environmental impacts, as is the case with Mexican feather grass (*N. tenuissima*) in south-eastern Australia.

Nassella tenuissima is native to the Americas, including Argentina, Chile, United States, and Mexico (Jacobs *et al.* 1998), and its appeal as an ornamental plant and broad climate tolerance have helped it become a successful invader. It is now an emerging invasive species in the Mediterranean region and South Africa (Brunel *et al.* 2010, Milton 2004). In Australia, the species' potential impact should it spread to agricultural regions is being recognised (McLaren *et al.* 1999). It shares a genus with two Weeds of National Significance, Chilean needle grass (*Nassella neesiana* (Trin. & Rupr.) Barkworth) and serrated tussock (*Nassella trichotoma*). Like *N. trichotoma*, it is also an unpalatable pastoral weed, and the two are challenging to distinguish in their vegetative state (Solarska *et al.* 2012). By some estimations, *N. tenuissima* has a greater potential invasive range than *N. trichotoma* (McLaren *et al.* 1999), highlighting the importance of managing this species for eradication.

In 2008, *N. tenuissima* was inadvertently sold by a number of retailers throughout Victoria and Australia. Investigations revealed that over 10,000 seeds may have been imported into Australia, with plants supplied to numerous retailers from January to May 2008. In Victoria, plants were traced using on-foot searches of over 150,000 front yards in suburban areas around these retails stores, voluntary product recalls, increased media coverage to promote self-reporting by the public, identification training for key stakeholders (e.g. Australia Post deliverers and utilities meter readers), and in 2009/2010, the use of credit card details to identify buyers. Over the 2008/2009 and 2009/2010 seasons, thousands of plants were recovered and almost 500 infestation sites, mostly in residential gardens on private property, were identified as requiring ongoing management. Most of these sites are still subject to monitoring. It is believed that over 2000 *N. tenuissima* plants sold during this period remain unaccounted for, although it is extremely rare for plants to be discovered outside of cultivation.

A standardised treatment method for *N. tenuissima* removal was developed and applied to infestation sites. Seed heads were first secured in a plastic bag to prevent accidental spread, and the tussock then chipped out, including the basal root ball (e.g. by mattock) to prevent regrowth. Entire plants were then bagged for removal. Seedlings were physically removed, or spot sprayed with herbicide (e.g. glyphosate). Seed germination suppressants (e.g. flupropanate 745 g L^{-1} as used in the Australian Capital Territory (ACT) response; (Connolly and Taylor 2016) were not advised due to risks identified with its widespread use in residential gardens. Sites are classified as 'active' or 'monitoring' at the end of each season, where active sites are those where target plants have been found in the current season, and monitoring sites had none found. Classifications determine the assessment schedule for the following season, with active sites assessed twice during the growth period covering both spring-summer and autumn germination periods. For monitoring sites, a single annual assessment is scheduled for the following season, and from 2012/2013, biennial assessments have been implemented for sites that have had a monitoring status for several years. Assessments only cease where there is proof of site extirpation (referred to here as eradication). This requires evidence that a seedbank does not exist (e.g. plants never seeded), or is unviable (e.g. based on a period of absence). We have currently set this period to be 10 years, although seed longevity is not known (Biosecurity Queensland 2016).

Here, we contribute to our ongoing review of this eradication program with analyses conducted to: (1) track the efficacy of treatment methods and adapt when necessary; and (2) determine patterns of seedling emergence to inform future management timelines and estimate the period of absence required to prove a seedbank is no longer at risk of germinating.

MATERIALS AND METHODS

Infestation sites included in this study are those recorded in Agriculture Victoria's State prohibited weed internal case management database ('BioWeb', based on the Microsoft SharePoint platform) with the date of first treatment/assessment between 1 July 2008 and 30 June 2010 (n = 488). Sites were excluded where infestations pre-date or were expressly not related to this incursion (n = 8), or where *N. tenuissima* was never confirmed to be present (n =). Sites at propagation or distribution centres for the original source businesses were also excluded (e.g. wholesalers, nurseries etc.; n = 4), as their treatment differed from other sites. The sites included in analysis (n = 474) are largely private residences where only a small number of plants were purchased (>75% had five or fewer plants).

Infestation characteristics and treatment efficacy At the initial and follow-up assessments of infestation sites, thorough searches of the property and surrounding areas were undertaken, with infestation data (e.g. number, age and location of seedlings) recorded. For this study, monitoring and treatment data for all *N. tenuissima* sites were extracted from BioWeb (as of 1 July 2017), and supplemented with operational records compiled during the initial response. Summary statistics were calculated based on this data at each site, describing overall characteristics of the infestations (e.g. proportion of sites that seeded, proportion of site with germinants, etc.).

Treatment efficacy was analysed qualitatively, by assessing the proportion of sites classified as 'active', 'monitoring' and 'eradicated' over time. This applied classifications determined at the end of each season from 2008/2009 to 2016/2017 based on each season's assessment data, e.g. active sites had *N. tenuissima* plants recorded in the current season, monitoring sites had not. Eradicated sites are largely those where seeding never occurred, or the seedbank is no longer considered a risk (e.g. where gardens have been built/concreted over).

Seed germination analysis Periodic assessment data allows the time between initial treatment and germination events to be analysed, as re-emergence of *N. tenuissima* plants after complete physical removal is assumed to occur solely by germination. Sites included in this analysis were restricted to those with a potentially viable seedbank, i.e. had seeded or were recorded as 'mature' (n = 369). To assess the likelihood that a site with a potential seedbank will begin to germinate after a period of monitoring, the time between each site's initial treatment and their first recorded re-emergence via germination was analysed. This seeks to inform future management of *N. tenuissima* sites with a potential seedbank, so that after a period with no germination detected, the risk of re-emergence may be considered low enough to confidently classify sites as ‘eradicated’. However, any analysis should be viewed and applied cautiously due to uncontrolled factors, e.g. the chance that seedlings were removed by landholders without our knowledge, and also varying weather conditions from 2008/2009–2016/2017 which are an inherent confound in assessing germination risk over time.

Time-to-event (survival) analysis was used to account for the right censoring of data given that germination could still occur in the future, an issue analogous to laboratory-based seed germination experiments (Ritz *et al*. 2013). As germination events were not recorded at exact times, a non-parametric cohort life-table method was used to estimate the survival function ($S(t)$) based on time intervals (McNair *et al.* 2012). In this analysis, $S(t)$ is the probability that a site will not have begun to germinate by time t (or equally, that its first germination event will occur at time $\geq t$). One-year time intervals are used up to the third year as minimum annual monitoring was implemented, and two-year time intervals are used thereafter, as the sites found to be active in the fifth ($n = 3$) and seventh ($n = 2$) years had been subject to biennial monitoring prior to seedlings being found. Input data for cohort life-tables is the initial total number of sites ($n = 369$), the number of sites that first germinated in each time interval, the number of sites ‘lost’ in each time interval (i.e. sites where germination was no longer discoverable, e.g. had been extirpated/eradicated). $S(t)$ estimates with standard deviations were calculated with R Statistical Package (v3.1.2. R Foundation for Statistical Computing, Vienna, Austria) using the life table function of the ‘KMsurv’ package (Klein and Moeschberger 1997). To characterise the probability of an infestation first re-emerging at a site over time, a non-linear regression (one-phase exponential decay) of $S(t)$ was applied. Regression analysis and figures were produced using GraphPad Prism (v7.04. GraphPad Software, La Jolla California).

RESULTS

Infestation characteristics and treatment efficacy

Greater than three quarters of sites (77.85%) produced or were very likely to have produced seed at first inspection (Table 1). Germination was common, with over a third (37.97%) recording seedlings after first treatment. Secondary seeding was rare, with <5% of sites recording seeding plants after initial treatment, and the mean number of germination events recorded was less than two in sites where germination occurred (Table 1).

The progress of sites from active to monitoring then eradication shows the number of active infestations peaked in 2009/2010, coinciding with the influx of new cases stemming from tracing investigations in that year (Figure 1). Active cases have since rapidly and continuously declined, resulting in <1% of cases active at the end of 2016/2017. Meanwhile, the number of sites proven to be eradicated has steadily increased (Figure 1).

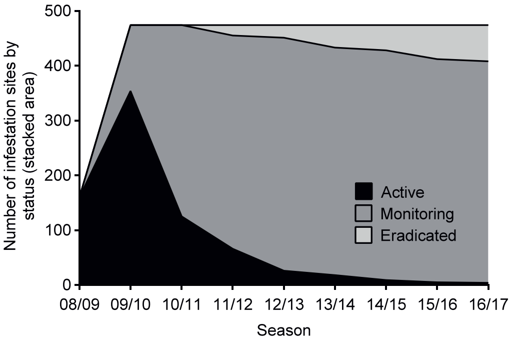

Figure 1. Infestation status across all sites as of the end of each season, 2008/2009–2016/2017.

Table 1. Site and germination summary statistics.

Number of sites	**474**
Seeding status of sites at first treatment	369 (77.85%) recorded as seeded or as ‘mature’. 39 (8.23%) recorded as having not seeded. 66 (13.92%) status unknown.
Sites with seed germination after first treatment	180 (37.97%) sites recorded germinants found on follow-up inspections.
Sites with secondary seeding	15 (3.16%) sites recorded seed being set at follow-up inspections.
Average number of germination events recorded per site	0.861 (s.d. 1.469) at all sites. 1.958 (s.d. 1.031) at sites where post treatment germination occurred.

Seed germination analysis At infestations where germination has occurred, most (132 sites) had seedlings recorded within one year of initial treatment, however, two sites had their first germination event recorded 5–7 years after treatment (n = 2, Figure 2a). Furthermore, at 15 sites seedlings have been found more than five years after initial treatment (data not shown), suggesting seedbanks can remain viable for an extended period. Similarly, of the seeded/mature sites that did germinate, the majority did so within the first year after treatment, and more than 90% showed germination within two years (Figure 2b).

Non-linear regression ($R^2 = 0.9997$, Figure 3) was applied to the survivor function estimates (Table 2), giving a decay constant $K = 1.342$ (95% CI: 1.256, 1.437), and a lower limit of 0.5191 (95% CI: 0.5129 to 0.5252). In this study, the lower limit may be interpreted as the probability that a site will never germinate. Applying this K value to the program's current eradication period of 10 year continuous absence, corresponds to a <0.0001% chance that a site with a potential seedbank will first germinate more than 10 years after treatment. However, there is potential that germination has not yet occurred due to environmental factors, e.g. weather conditions or other local factors, that this kind of estimation must be considered with caution.

Table 2. Survivor function estimates for first recorded germination at seeded/mature infestations, based yearly time intervals since first treatment.

Time interval (years)	Number of sites recording first germination	*S(t)*, as of the start of the interval (standard deviation)
0–1	132	1.000 (0.000)
1–2	31	0.642 (0.025)
2–3	9	0.556 (0.026)
3–5	4	0.531 (0.026)
5–7	2	0.520 (0.026)
>7	0	0.514 (0.026)

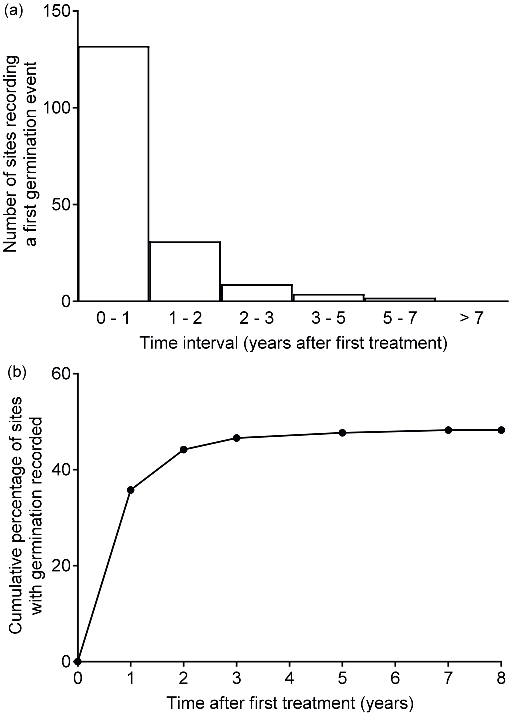

Figure 2. (a) Number of sites where germination first occurred within the time interval, taken from the time of initial treatment; and (b) the cumulative percentage of sites displaying germination since the first treatment (seeded/mature sites only).

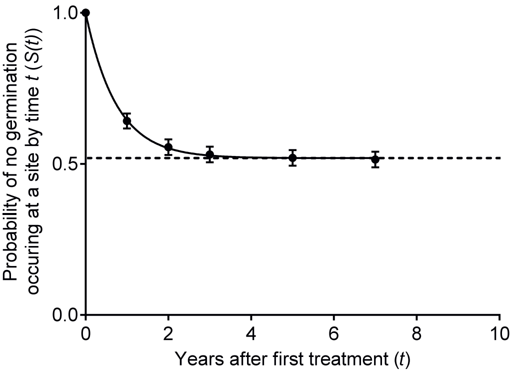

Figure 3. *S(t)* is the probability that a site will not have recorded germination by time *t*. The solid curve is a non-linear regression fit to *S(t)* estimates. The dotted line is its lower limit ($S(\infty) = 0.5191$).

DISCUSSION

The capacity of *N. tenuissima* to readily reproduce in Victoria is shown by most infestations having seeded when found, and seedlings later being found at around half of those sites. As the incursion was discovered fairly quickly and the data analysed here is that of sites found within two years of its discovery, much of the seed had a relatively short window to develop, and the seedbank only a short time to accumulate. While this study is not representative of infestations that have developed a larger seedbank over many years, it does show that a substantial proportion of these infestations rapidly developed viable seedbanks within a short period.

Nassella tenuissima does not appear to have an extensive history of invasiveness overseas, although it has proven difficult to eradicate after escaping from cultivation in California and New Zealand (Jacobs *et al.* 1998). It is also problematic in South Africa (Milton 2004). Predictions for its potential distribution in Australia range from 14.1 to over 100 million ha, including large areas of Victoria (McLaren *et al.* 1999, Biosecurity Queensland 2016). The capacity of *N. tenuissima* to reproduce in Victoria shown here highlights the real threat this species poses.

The physical removal treatment method used by Agriculture Victoria to destroy mature *N. tenuissima* plants has been effective, with few incidences of secondary germination and a clear trend towards eradication of sites. Nonetheless, many plants from this incursion remain unaccounted for, so effective management of this incursion also requires ongoing detection of undiscovered plants. The physical similarities to *N. trichotoma* are a barrier to this, and work improving identification (e.g. Solarska *et al.* 2012) and weed identification training (e.g. Agriculture Victoria's Weed Spotter program) are essential to preventing the establishment of *N. tenuissima* in Victoria.

A major issue for the future of the *N. tenuissima* program is the uncertainty surrounding seedbank longevity. With a lack of seed longevity data, this study can be valuable for informing management decisions despite the limitations of this dataset. Our results are consistent with the ACT experience (Conolly and Taylor 2016), showing infestations can remain active via germination for 7+ years and suggesting seed longevity is at least seven years.

Applying time-to-event (survival) analysis to the time between initial treatment and the first recorded germination event at sites, we could characterise the risk of infestations re-emerging following treatment. This showed the risk of re-emergence is particularly high in the first two years after removal, and that the risk of re-emergence after a prolonged absence (e.g. 10 years) is low. Were more specific data consistently collected over the course of the project (e.g. exact numbers of seedlings at each assessment and the presence/number of seedlings at initial inspections), predictions could be further refined. While our results are somewhat narrow in application, this shows how project data can be used to inform future planning in biosecurity programs.

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