Soil seed bank under variegated thistle does not explain thistle dominance

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Abstract Variegated thistle *(Silybum marianum)* is a large, spiny annual that often forms dense monospecific communities on dry ridges and sunny hillslopes. The owner of a typical Poverty Bay hill-country farm with persistent variegated-thistle infestations reported that winter applications of herbicide were ineffectual in the long term as more variegated thistles simply recolonised the sprayed sites. An absence of preferred species, particularly perennial ryegrass *(Lolium perenne)* and legumes (*Lotus* and *Trifolium* spp.), in the soil seed bank under dense thistle populations may explain the persistence of these monospecific populations. To test this hypothesis, soil samples were collected from a dense and sparse variegated-thistle population in each of seven paddocks and incubated in a glasshouse. Emerged seedlings were identified and counted. The incubation was repeated three times. Total soil seed numbers were similar under both the dense and sparse populations with similar numbers of preferred legumes under both. However, there were significantly more perennial ryegrass seeds under the dense variegated-thistle populations compared with the sparse ones. Domination of thistles in densely infested patches was not due to lack of preferred species, or indeed other weed species, in the soil seed bank.

Keywords Variegated thistle, *Silybum marianum,* soil seed bank.

INTRODUCTION

Variegated thistle (*Silybum marianum* (L.) Gaetn.) is a robust annual or biennial thistle able to grow to a height of 2 m. It has large leaves and a tall flower stem with few spines on the upper part. The large, spiny flowers are solitary and contain seed that are too heavy to be carried far by their pappus (Dodd 1989). These characteristics enable variegated thistles to form dense, impenetrable stands that are self-perpetuating on the same location each year. On a typical Poverty Bay hill-country farm, winter applications of herbicide were reported by the farmer as ineffectual in the long term as the thistle simply recolonised the sprayed sites. The presence of adjacent sensitive crops limited the use of hormone-type herbicides to winter applications.

Persistent monospecific communities of variegated thistle might be explained by an absence of other competitive species, in particular perennial ryegrass *(Lolium perenne*) and legumes (*Trifolium* and *Lotus* spp.) in the soil seed bank enabling recolonisation of the same areas by variegated thistle year after year. If this is the case, then over-sowing these patches with useful pasture plant species may be a more durable control method than herbicide application. This paper reports on the testing of this hypothesis.

MATERIALS AND METHODS

An experiment was conducted by collecting soil samples on two occasions from paired sites in six paddocks (dense vs sparse thistle populations) on Wi Pere Station, Gisborne. These were incubated in the glasshouse and the emerged seedlings identified and counted.

Sample collection

The soil seedbank was sampled in a dense and a sparse variegated thistle patch in each of six north-facing steep uncultivatable paddocks on 6 December 2015. A patch of variegated thistle was categorised as dense or sparse based on a visual observation by the same operator for all patches. In a 'dense' patch, the variegated thistles formed a monoculture. In a 'sparse' patch, at least some grass was present amongst the variegated thistles. Each patch was at least 1 ha in size. All variegated thistle patches selected were on north-facing slopes and were either on ridge-tops (spreading down the hillside) or, in some cases, were mid-slope. At the time, variegated thistles were flowering and approximately 2 m in height. Within each variegated thistle patch, 22 random soil cores were taken, each 75 mm diameter by 50 mm deep, giving a total of about 4 kg of soil. The soil samples were bulked and returned to the laboratory for enumeration of the seed bank.

The procedure was repeated on 22 March 2016, after the variegated thistles had flowered and the seeds had dispersed.

Seed bank enumeration

The soil seed bank was measured using the repeat incubation method (Rahman et al. 1995). The soil core samples were weighed, broken up, and each was spread to about 20 mm depth in two 300 mm x 400 mm seed trays. The trays were laid out at random on the glasshouse bench and top-irrigated as required. Glasshouse air temperatures were maintained between 15°C (night) and 25°C (day). After 4–6 weeks, the emerged seedlings were identified, counted and removed, and the soil allowed to dry. Plants that could not be identified at this time were planted out into pots and grown on until they could be identified at least to genus level. When dry, the soil was remixed in the trays and irrigated for another incubation of seed. The soil samples collected on 6 December 2015 were subjected to three incubations and those collected on 22 March 2016 were subjected to four incubations.

Statistical analyses

For each sampling date, data from each incubation were pooled to give total seedlings emerged. These were normalised to seeds/kg of field moist soil and the paired data (Tables 1–4) evaluated with a t-test on the differences between Dense and Sparse using R (3.4.3). Data presented are the 95% confidence intervals for the mean paired difference.

Table 1 Pairwise comparison of soil seed bank (seed per kg soil) at six sites for variegated thistle, ryegrass and legumes under dense and sparse infestations of variegated thistle sampled on 6 December 2015. CI is the 95% confidence interval for the mean of the paired data.

	Variegated thistle		Ryegrass		All legumes		Total Seed	
Site	Dense	Sparse	Dense	Sparse	Dense	Sparse	Dense	Sparse
1	0	0	18	3	7	10	386	419
2	2	0	12	2	6	5	466	428
3	3	2	4	5	4	1	380	312
4	3	1	12	3	12	10	133	108
5	3	4	9	8	19	5	217	174
6	7	1	30	4	8	9	167	240
Total	**18**	**7**	**86**	**25**	**56**	**40**	**1748**	**1681**
%[1]	**1.1**	**0.4**	**4.9**	**1.5**	**3.2**	**2.4**	-	-
CI	-0.7–4.4		0.1–20.1		-3.6–9.1		-44.7–67.1	

[1] Percent of respective, Dense or Sparse, total seed found in the soil seed bank.

RESULTS

Forty-two different plant species were found in the samples collected on 6 December 2015 and 55 different species in the 22 March 2016 samples. Seven species were found only in the early samples but 20 different species were found in the second sampling that were not in the first. In total, 62 species were found (Appendix) but the majority were at populations of less than 100/kg soil. The species of legume most frequently found was birdsfoot trefoil (*Lotus corniculatus* L.) with small contributions of suckling clover (*Trifolium dubium* Sibth.), subterranean clover (*Trifolium subterraneum* L.) and white clover (*Trifolium repens* L.) (Tables 1 and 3).

Plant numbers for desirable species (ryegrass

Table 2 Pairwise comparison of soil seed bank (seeds per kg/soil) at six sites for all weeds, clammy goosefoot, foxglove and toad rush under dense and sparse infestations of variegated thistle sampled on 6 December 2015. CI is the 95% confidence interval for the mean of the paired data.

	All weeds		Clammy goosefoot		Foxglove		Toad rush	
Site	Dense	Sparse	Dense	Sparse	Dense	Sparse	Dense	Sparse
1	361	405	209	338	9	3	40	29
2	448	422	265	288	3	8	111	64
3	371	306	153	95	113	3	52	186
4	109	95	12	2	46	7	19	42
5	189	161	17	12	7	13	131	88
6	128	227	17	30	4	1	62	150
Total	**1606**	**1616**	**674**	**764**	**182**	**35**	**416**	**560**
%[1]	**91.9**	**96.2**	**38.6**	**45.5**	**10.4**	**2.1**	**23.8**	**33.3**
CI	-64.0–60.6		-80.1–50.2		-22.4–71.5		-101.3–53.2	

[1] Percent of respective, Dense or Sparse, total seed found in the soil seed bank.

Table 3 Pairwise comparison of soil seed bank (seeds per kg/soil) at six sites for variegated thistle, ryegrass and legumes under dense and sparse infestations of variegated thistle sampled on 22 March 2016. CI is the 95% confidence interval for the mean of the paired data.

	Variegated thistle		Ryegrass		All legumes		Total seed	
Site	Dense	Sparse	Dense	Sparse	Dense	Sparse	Dense	Sparse
1	0	3	0	6	21	21	264	536
2	3	2	3	0	30	54	319	278
3	2	9	2	1	4	59	365	370
4	10	1	1	0	64	62	261	430
5	2	26	1	9	51	195	555	2153
6	9	1	2	3	10	24	218	330
Total	**26**	**42**	**9**	**20**	**180**	**416**	**1983**	**4096**
%[1]	**1.3**	**1.0**	**0.5**	**0.5**	**9.1**	**10.2**	-	-
CI	-15.4–10.0		-6.4–2.8		-97.7–18.8		-1003–299	

[1] Percent of respective, Dense or Sparse, total seed found in the soil seed bank.

Table 4 Pairwise comparison of soil seed bank (seeds per kg/soil) at six sites for all weeds, clammy goosefoot, foxglove and toad rush under dense and sparse infestations of variegated thistle sampled on 22 March 2016. CI is the 95% confidence interval for the mean of the paired data.

	All weeds		Clammy goosefoot		Foxglove		Toad rush	
Site	Dense	Sparse	Dense	Sparse	Dense	Sparse	Dense	Sparse
1	242	509	114	19	0	61	63	325
2	286	224	71	66	10	17	77	93
3	359	309	50	43	129	23	92	120
4	196	368	8	38	38	2	93	200
5	503	1948	28	321	3	185	383	873
6	207	302	46	123	5	7	56	78
Total	**1794**	**3660**	**318**	**610**	**184**	**295**	**764**	**1688**
%[1]	**90.5**	**89.4**	**16.0**	**14.9**	**9.3**	**7.2**	**38.5**	**41.2**
CI	-909–287		-188–90.4		-121–84.0		-353–44.8	

[1] Percent of respective, Dense or Sparse, total seed found in the soil seed bank.

and all legumes), variegated thistle, all weeds and the individual weeds that occurred in highest numbers i.e. clammy goosefoot (*Dysphania pumilio* (R.Br.) Mosyakin & Clemants), foxglove (*Digitalis purpurea* L.) and toad rush (*Juncus bufonius* L.) are presented in Tables 1–4. There were no significant differences ($P<0.005$) between dense and sparse populations for any of the above species in either sampling except for ryegrass in the first sampling. Here, there were significantly more ryegrass seedlings in the dense variegated thistle stands than in the sparse stands.

DISCUSSION

The hypothesis that self-perpetuating variegated thistle patches were due to an absence of other species in the soil seed bank was not proven. In fact, the only significant difference in the presence of other species was observed for ryegrass in the first sampling; significantly more ryegrass was found under the dense than sparse infestations, the opposite of our hypothesis (Table 1). Likely reasons for this may have been the location of the patches in the paddocks or the dense patches protecting the few ryegrass seed heads from being grazed. Dense patches are often located on the tops of ridges, which are areas where stock congregate meaning these areas are usually higher in fertility and flatter in topography than patches on the mid-hillslopes. Thus, the dense patches may have been in areas that were more conducive to perennial ryegrass growth and if ryegrass plants survive the competition then their seed heads are protected from grazing by the large variegated thistle plants. In selecting the paired plots care was taken to match their aspect and the success of this is reflected in the similarity of the weed species and seed numbers, particularly for the three most abundant weeds clammy goosefoot, foxglove and toad rush.

However, the quantity of seeds of ryegrass was very low compared to the number of weed seeds, most likely due to low levels of dormancy and persistence in the soil for perennial ryegrass (Howe & Chancellor 1983). The low quantity of perennial ryegrass seeds found in the seed bank in spring is consistent with a recent Waikato study of grazed perennial ryegrass pastures, where the emergence of perennial ryegrass from the seed bank from soil sampled in spring was also low (Tozer et al. 2010). The number of legume seeds (Tables 1 and 3) was rather high under both the dense and sparse infestations, particularly at the second sampling. This was encouraging because given the opportunity they could recolonise the

variegated thistle sites, but as their numbers were similar under both dense and sparse infestations, the absence of dominant recolonisation must be due to other factors.

Despite the very high number of other weed seeds in the seed bank, variegated thistle was able to dominate the areas where present and form dense thickets. Here, we suggest it is the very large mass of variegated thistle seed (1.91 g/100 seed, Groves & Kaye 1989; c.f. 0.32-0.50 g/100 seed for perennial ryegrass, Hampton 1986, and 0.058-0.060 g/100 seed for white clover, Clifford 1985) that allows it to dominate all other species present. Variegated thistle was no quicker than other species to emerge in the incubation trays but it grew very rapidly after emergence. Plants spread horizontally and reached 200 mm in diameter in just 3–4 weeks, which is consistent with the heavy seed. Thus, the abilities of variegated thistle plants not only to grow rapidly, but also to cover ground and shade out other species are likely to be key reasons why it forms dense impenetrable thickets rather than the composition of the seed bank per se (Chambreau & MacLaren 2007). The heavy seeds also disperse within the vicinity of the parent plants, increasing opportunities for seedling establishment and the crowding out of other pasture species (Roche 1991). These attributes of variegated thistle, and its sensitivity to light deprivation during establishment (Young et al. 1978; Montemurro et al. 2007) mean that there was likely a lack of ground cover in autumn to inhibit the germination of variegated thistle in the sites with dense infestation whereas there was more ground cover present on the sparse sites. Under the dense infestation, 80 perennial ryegrass seeds/kg roughly equals 200 kg seed/ha or 10 times the recommended sowing rate. Therefore, it appears that it is not a lack of seed in the soil seed bank but rather this seed does not germinate at a time that enables it to shade out the variegated thistle. Finding a method to maintain, or establish, dense ground cover in late summer and autumn to inhibit variegated thistle growth during their seedling stage needs to be explored.

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APPENDIX

Complete list of 62 species found in the soil seed bank from both sampling dates:
allseed (*Polycarpon tetraphyllum* L.),
amaranthus (*Amaranthus* sp. L.),
annual poa (*Poa annua* L.),
Australian fireweed (*Senecio bipinnatisectus* Belcher),
birdsfoot trefoil (*Lotus corniculatus* L),
broad-leaved dock (*Rumex obtusifolius* L.),
broad-leaved fleabane (*Erigeron sumatrensis* Retz.),
broad-leaved plantain (*Plantago major* L.),
browntop (*Agrostis capillaris* L.),
Californian thistle (*Cirsium arvense* (L.) Scop.),
catchfly (*Silene gallica* L.),
catsear (*Hypochaeris radicata* L.),
chickweed (*Stellaria media* (L.) Vill.),
clammy goosefoot (*Dysphania pumilio* (R.Br.) Mosyakin & Clemants),
creeping buttercup (*Ranunculus repens* L.),
dandelion (*Taraxacum officinale* F.H.Wigg.),
dove's foot (*Geranium molle* L.),
foxglove (*Digitalis purpurea* L.),
hawkbit (*Leontodon saxatilis* Lam.),
hawksbeard (*Crepis capillaris* (L.) Wallr.),
horned oxalis (*Oxalis corniculata* L.),
hydrocotyle (*Hydrocotyle moschata* G.Forst.),
hyssop loosestrife (*Lythrum hyssopifolia* L.),
Indian doab (*Cynodon dactylon* (L.) Pers.),
inkweed (*Phytolacca octandra* L.),
jointed rush (*Juncus articulatus* L.),
kōpata (*Pelargonium inodorum* Willd.),
mouse-ear chickweed (*Cerastium glomeratum* Thuill.),
narrow-leaved plantain (*Plantago lanceolata* L.),
nettle (*Urtica urens* L.),
pale flax (*Linum bienne* Mill.),
parsley piert (*Aphanes inexpectata* W.Lippert),
paspalum (*Paspalum dilatatum* Poir.),
penny royal (*Mentha pulegium* L.),
perennial ryegrass (*Lolium perenne* L.),
prickly sow thistle (*Sonchus asper* (L.) Hill),
purple cudweed (*Gamochaeta coarctata* (Willd.) Kerg.),
purslane (*Portulaca oleracea* L.),
ratstail (*Sporobolus africanus* (Poir.) A.Robyns & Tournay),
red fescue (*Festuca rubra* L.),
rice grass (*Microlaena stipoides* (Labill.) R.Br.),
rough bristle grass (*Setaria verticillata* (L.) P.Beauv.),
scarlet pimpernel (*Lysimachia arvensis* (L.) U.Manns & Anderb.),
Scotch thistle (*Cirsium vulgare* (Savi) Ten.),
sedge (*Cyperus* sp. L.),
sheep's sorrel (*Rumex acetosella* L.),
small-flowered mallow (*Malva parviflora* L.),
smooth witchgrass (*Panicum dichotomiflorum* Michx.),
soft brome (*Bromus hordeaceus* L.),
scrambling speedwell (*Veronica persica* Poir.),
subterranean clover (*Trifolium subterraneum* L.),
suckling clover (*Trifolium dubium* Sibth.),
summer grass (*Digitaria sanguinalis* (L.) Scop.),
sweet vernal (*Anthoxanthum odoratum* L.),
tall willow-herb (*Epilobium ciliatum* Raf.),
toad rush (*Juncus bufonius* L.),
trailing St John's wort (*Hypericum humifusum* L.),
twin cress (*Lepidium didymum* L.),
variegated thistle (*Silybum marianum* (L.) Gaertn),
velvety nightshade (*Solanum chenopodioides* Lam.),
white clover (*Trifolium repens* L.),
Yorkshire fog (*Holcus lanatus* L.).