NeoBiota 85: 167–195 (2023)
doi: 10.3897/neobiota.85.100825
https://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

A century of weed change in New Zealand's forage seed multiplication industry

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Academic editor: John Ross Wilson | Received 4 February 2023 | Accepted 30 May 2023 | Published 27 June 2023

Citation: Rubenstein JM, Hulme PE, Rolston MP, Stewart AV, Hampton JG (2023) A century of weed change in New Zealand's forage seed multiplication industry. NeoBiota 85: 167–195. https://doi.org/10.3897/neobiota.85.100825

Abstract
International seed trading provides a significant introductory pathway for weed seeds, and many globally established weeds originated as contaminants in agricultural seed lots. Management of these trade systems helps minimize agricultural losses and is an important means of preventing future biological incursions. Forage crop seed lots could be considered higher risk than seed lots of arable and vegetable crops, as they have been found to have a higher percentage of contaminated seed lots. Two of the most commonly used temperate forage crops worldwide are perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). New Zealand is one of the top producers of these crop seeds globally, and both species are commonly used in New Zealand pastures. Using historical and current seed lot analytical purity test results, we examined the frequency, identity and temporal changes of weed seeds found within agricultural seed lots of perennial ryegrass and white clover grown in New Zealand from 1912 to 2019. Overall, the percentage of contaminated forage seed lots decreased between approximately three to sixfold over the study period, indicating that herbicide availability, seed certification and improved crop management have been effective for weed control. However, we identified a handful of annual weed species that could become more problematic in the future, either because they showed an increasing presence trend in seed lots or were identified as the most common contaminants. In 2019, *Vulpia bromoides* was the most common contaminant in perennial ryegrass seed lots, and *Chenopodium album* was the most common in white clover seed lots. *Sherardia arvensis* and *Poa annua*, both significant species with an increasing presence trend, had the largest increases in perennial ryegrass seed lots over the study period. Conversely, *Rumex acetosella* had the largest presence decline for both crop species. There was a significant difference between the percentage of contaminant species that were grass weeds between study crops, where perennial ryegrass seed lots had

approximately four times more grass species than white clover. Considering New Zealand trades crop seed with approximately half of the world's countries and contributes substantially to the global supply of forage seed, our study provides a unique insight into changes of the weed spectrum throughout the seed for sowing system over the last century.

Keywords
Analytical purity, herbicides, *Lolium perenne*, perennial ryegrass, seed lots, *Trifolium repens*, weed seeds, white clover

Introduction

Nearly all aspects of forage seed production are highly managed, including the use of agrichemicals, fertilizers, grazing, irrigation, harvesting, as well as post-harvest management, including seed drying and seed cleaning technologies (Rowarth 1998; Rolston et al. 2006; McDonald and Copeland 2012). Even with the availability of these production advancements, weed seed contaminants within agricultural seed lots remain a global concern because of their negative environmental and economic impacts (Buddenhagen et al. 2021a; Rubenstein et al. 2021). For example, weed seed contaminants are responsible for the accidental introductions of non-native plant species (Lehan et al. 2013; Cossu et al. 2020). Agricultural seed lots are one of the primary culprits in this regard, as they provide a major contaminant pathway through which many globally naturalized weeds have originally entered new areas (Mack and Lonsdale 2001; Cuthbert 2013; Wilson et al. 2016; Gervilla et al. 2019). In addition, weed seed contaminants increase economic losses by substantially reducing crop yield (Oerke and Dehne 2004; Michael et al. 2010), and can cause a seed lot to be rejected from seed certification if minimum analytical (physical) purity requirements are not met, or if noxious weeds are present (Rowarth et al. 1995; Hampton 2015). If noxious weeds are detected in imported seed lots during the border inspection process, it is costly, as the contaminated seed lot must either be re-cleaned, re-exported or destroyed (United States Department of Agriculture 2019; Buddenhagen et al. 2022). Because of all these factors, the forage seed industry has made it a priority to keep contamination levels to a minimum, and seed cleaning remains the second largest cost in the production of common forage species such as white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*) (Brown et al. 1998; Hill et al. 1999).

This is especially true in New Zealand, which has strict biosecurity laws regarding the importation of agricultural seed and a long history of forage seed production dating back to the 1850s (Rowarth 1998; Rubenstein et al. 2021; Buddenhagen et al. 2022). Additionally, the absence of governmental subsidies within New Zealand's agricultural sector has placed additional pressure on growers to produce clean seed (Espig et al. 2022). Rubenstein et al. (2021) previously examined seed quality data for tens of thousands of agricultural seed lots entering New Zealand across approximately 1,400 crop seed species from more than 90 countries, and found that forage crops had a significantly higher percentage of contaminated seed lots when compared to other crops types, such as arable and vegetable species.

New Zealand is integral in the international trade of forage seed, as the world's largest exporter of white clover seed and the fourth largest exporter of ryegrass seed (Hampton et al. 2012; Observatory of Economic Complexity 2020a, b). New Zealand also trades crop seed with approximately half of the world's countries, and provides an additional production season for seed companies in the Northern Hemisphere during their winter months (Hampton et al. 2012; Ministry for Primary Industries 2020). Additionally, forage seed production is important domestically for New Zealand because pastoral farming for dairy, meat and wool is the primary land-use, and perennial ryegrass and white clover are the most common pasture (forage) species used (Kemp 1999; Ghanizadeh and Harrington 2019). These two crops are also important worldwide, as perennial ryegrass is one of the most commonly used grass species in temperate pastures (Thorogood 2003; Humphreys et al. 2010), and white clover is the legume species most commonly grown in association with temperate forage grasses (Laidlaw and Teuber 2021).

The first tangible international quality control efforts for forage seed lots were not implemented until the 1920s (Parsons 1985). Specifically, we refer to the introduction of what is now the OECD seed certification scheme, which for New Zealand began in 1929 and was implemented by the Department of Agriculture (now part of the Ministry for Primary Industries) (Hadfield 1929). This scheme served as a protection for the farming industry, to ensure agricultural seed lots met stringent genetic and analytical purity standards, and were also free of undesirable weeds and certain pathogens (Hampton 1994; Rowarth et al. 1995). Unlike most other countries, New Zealand has no seed law and involvement in this scheme has always been voluntary, although participation requires strict adherence to the rules and procedures (Hampton and Scott 1990; Melhuish 2008).

Prior to seed certification, weed seed contamination was one of the primary concerns for forage seed producers (Cockayne 1912b; Rowarth 1998), and it was common to have seed lot contamination levels that would far surpass today's minimum requirements (Parsons 1985; Stewart et al. 2022). Early in the 20th century, Levy (1917) conducted analytical purity tests on white clover seed lots and found that they contained anywhere from 25 to 70% contaminant seeds. In a similar study on ryegrass a decade later, Levy and Davies (1929) found that only one-third of seed lots being sold as perennial ryegrass were actually what was being advertised, with most being a mix of different ryegrass species. High contamination levels during this period can partly be attributed to the lack of quality controls when importing forage crop seed lots from abroad, which for New Zealand and other British colonies or dominions, primarily came from England (Mather et al. 1995; Stewart 2006). Although seed cleaning technologies still in use today (e.g. air screen cleaners and spiral separators) were already widely utilized prior to seed certification (Lonsdale 1911; Rolston et al. 2006), management options would have made quality control difficult, especially considering herbicides were not yet in use. For example, opportunist 'catch cropping' was a common practice within a mixed cropping system (Levy 1933), whereby when pasture production exceeded demand, grazing would stop in late spring and the resultant forage seed crop would be harvested and sold in the marketplace (McCaw 1990). The lack of crop rotation within this system also meant less cultivation, allowing weed seed banks to build up in the soil (Cardina et al. 1991). Prior to seed certification, it was also typical for perennial ryegrass and white clover being managed for seed production to both be sown in the same field with a cereal or brassica crop, whereby after the cereal crop was harvested, ryegrass would be harvested for seed in the following year and clover seed the year after (Rowarth 1998). Weed seeds aside, this practice made it extremely difficult to achieve high analytical purity for either forage seed crop.

With the introduction of mechanization on farms in the 1930s (small tractors, self-propelled combines, header harvesters), it became more practical for New Zealand growers to produce their own forage seed than it was to import seed lots from abroad (Rolston et al. 2006). It was during this period that New Zealand elevated its international reputation for producing high quality pure seed, and the amount of forage seed they multiplied increased substantially (Hampton and Scott 1990; Rowarth et al. 1998b). During the 1940s, global advancements in seed cleaning technology helped decrease weed seed contamination. This included the widespread implementation of indent cylinders and gravity table separators (Hurst 1935; Bainer 1942; Gross and Perry 1945), which are still regularly used today in seed cleaning facilities, and have been found to significantly reduce seed lot contamination (Michael et al. 2010; McDonald and Copeland 2012). Furthermore, the introduction of herbicides and changes in soil pH management around this time drastically reduced weed seed contamination (Peterson 1967; Rowarth et al. 1998b; West and McBride 2005). Herbicides were particularly important, as they significantly increased crop yield and decreased human and mechanical resources required for weed control, and thus production costs were greatly reduced (Shaw 1964; Gianessi 2013).

Study aims

This study aims to determine the following in regards to perennial ryegrass and white clover seed grown in New Zealand, as they are two of the most commonly used forage species in temperate pasture systems worldwide: 1) how seed lot contamination has changed over the last century; 2) the most common contaminant species historically and recently; 3a) whether there is a significant relationship between time and presence of a given contaminant species; 3b) whether significant contaminant species have an increasing or decreasing presence trend over time, and; 4) whether there is a significant difference in the percentage of contaminant grass species in seed lots of perennial ryegrass and white clover.

We could find no previously published New Zealand or international studies on long-term trends in agriculture weeds found within forage seed crops. Considering New Zealand contributes substantially to the global supply of forage seed and trades crop seeds with approximately half of the world's countries, our study therefore provides a unique insight into changes of the weed spectrum not just in New Zealand, but throughout the forage seed for sowing system over the last century.

Methods

Data procurement

An extensive literature review was conducted to identify historical studies and datasets that reported analytical purity test results as they relate to plant contaminants within seed lots of perennial ryegrass and white clover grown in New Zealand. Both Rowarth (1989) and Rowarth et al. (1998a) were useful bibliographies for identifying forage seed production studies specific to New Zealand. We also found relevant papers using key search terms related to 'weed seed contamination' and 'analytical seed purity' through online resources such as Google Scholar, Google Books and the National Library of New Zealand website (www.natlib.govt.nz/collections). Hard copies of historical papers that were not available online were obtained from the archives at Lincoln University's library in Canterbury, New Zealand. We also contacted New Zealand seed testing laboratories, seed companies and governmental inspection agencies for in-house data and related grey literature. Sourcing historical purity test results proved challenging, but we obtained ten datasets from 1912–2009 that were suitable for analysis (Cockayne 1912a, c; Foy 1924, 1926; Johnston 1962; Rolston et al. 1985; Rowarth et al. 1990a, b, 1995; Hampton and Rolston 2021). For data from 2019, five major New Zealand seed companies provided our study with seed lot analytical purity test results, totaling 250 perennial ryegrass seed lots and 168 white clover seed lots. Both historical study data and the 2019 analytical purity tests noted any contaminant species found in seed lots on seed analysis certificates. Except for study data collected prior to the implementation of seed certification in 1929, our analysis focused on certified seed lots, and as such, they were sampled and tested in accordance with the rules of the International Seed Testing Association (hereafter referred to as **ISTA**) (International Seed Testing Association 2022). While ISTA rules regarding maximum seed lot size have changed over time for grass crops (increased from 5,000 kg to 10,000 kg), the protocols for analytical purity testing of forage species have remained mostly unchanged since ISTA's inception. New Zealand has been an ISTA member since 1925 (Rowarth 1998), and although study data prior to this date did not utilize ISTA rules when sampling and testing seed lots, they would have utilized similar methodologies that were already in place at their national seed testing laboratory, which began providing growers with seed lot analytical purity results in 1909 (Johnson 1984; Melhuish 2008). Because of all of these aforementioned factors, both recent and historical analytical purity test results should be considered comparable for analysis.

Data preparation

Data fields from all seed lot analytical purity tests used for analysis included: sampling year, total samples tested, contaminant species and the percentage of seed lots with the contaminant, which we defined as the number of seed lots where the contaminant was present for a given year divided by total seed lots tested for the same year. Plant names were standardized using taxonomy listed in the International Plant Name Index (International

Plant Names Index 2021) (www.ipni.org). Historic studies primarily reported contaminants to the species level, but in some cases they were only reported to the genus level (e.g. *Cerastium* sp.). This can occur when contaminants within the same genus are difficult to distinguish morphologically (James et al. 2014; Rubenstein et al. 2021). Because the number of seeds of each contaminant found in a seed lot was not recorded, we only considered absence/presence of a contaminant species and not its abundance (Rubenstein et al. 2021). We implemented a threshold that eliminated any datasets from the analysis that sampled fewer than 25 seed lots, so that rare contaminant species did not skew data by appearing to be more prevalent than they were. This data filtering resulted in eleven years of datasets for perennial ryegrass (mean of 922 seed lots per year) and seven years for white clover (mean of 563 seed lots per year) being retained for analysis.

Both Cockayne (1912a, c) and the recent 2019 analytical purity tests reported all contaminant species present, even if they were only found in one seed lot. The remaining datasets reported any contaminant species present as long as they surpassed a minimum threshold based on the percentage of seed lots in which they were detected, which for white clover was a mean minimum value of 0.48%, and for perennial ryegrass was 0.56%. Because datasets analyzed reported either all contaminant species present or any contaminants that were present in approximately 0.5% or more of seed lots, we assigned a 0% presence to any non-reported contaminant species for a given sampling year, as long as they were noted in other years for that respective crop. However, the white clover dataset from 1923 only reported six common and/or problematic contaminant species, even though the author noted that 62 species were present (Foy 1924). In addition, the perennial ryegrass datasets from 2003 and 2009 only reported eight common and/or problematic contaminant species (Hampton and Rolston 2021). Because of these factors, zero values were not used for non-reported species for the 1923 white clover and 2003/2009 perennial ryegrass datasets, and instead non-reported species were categorized as 'no data' for these years. All contaminant species reported in perennial ryegrass and white clover seed lots are presented in Suppl. material 1. The tables also indicate the percentage of seed lots in which the contaminant species were present, as well as whether they were classified as a grass or non-grass.

Up until approximately the late 1990s, New Zealand used a four component seed lot analytical purity reporting system for domestic seed analysis certificates (Cousins 2022), which included: pure seed, other crop seeds, weed seeds and inert matter. This differed from the international system, which moved to a three component system in 1974, after an ISTA rule change merged 'weed' and 'other crop' into the 'other seed' component (Jensen 2009). New Zealand's four component reporting system for domestic seed lots meant that common contaminants considered 'other crop' in white clover and perennial ryegrass seed lots were not included in study results from the 1930s to the late 1990s, since they would have not been listed under the 'weed' component (Dingwall 1969). Because of this reporting variability, the following crop species that were noted as contaminants pre-seed certification and/or post 2000, were excluded from analysis: *Agrostis* sp., *Cynosurus cristatus*, *Dactylis glomerata*, *Lolium multiflorum*, *Lolium perenne*, *Lotus corniculatus*, *Lotus pedunculatus*, *Medicago sativa*, *Phleum pratense*, *Poa pratensis*, *Trifolium pratense*.

Statistical analysis

The annual mean percentage of contaminated seed lots was determined by averaging all of the individual percentages of seed lots where each contaminant species was present (including zero values) in a given sampling year. White clover data from 1923 and perennial ryegrass data from 2003 and 2009 were excluded from this portion of the analysis because only six to eight common weed species were reported for these years. The most common forage crop weeds were the contaminant species with the overall top five highest values with regards to the mean percentage of seed lots they were reported in. To determine whether there was a significant relationship between time and presence of a given contaminant species, Kendal's Tau-b correlation tests (two-tailed) were conducted using the percentage of seed lots containing a contaminant and the corresponding sampling year as variables. Kendall's Tau-b was used because it is well-suited for small sample sizes and non-monotonic data with ties (Costner 1965). Ties occur when the percentage of seed lots containing a given contaminant species is the same for multiple sampling years. For this portion of the analysis, to reduce the undue influence of interpolated data (assigned stand-in zero values) on our results, we only included contaminant species that were recorded as present/absent in at least one-third of sampling years for a given crop. We also compared percentage of seed lots containing a contaminant species and the corresponding sampling year on a scatter plot to determine whether they had a linear or non-linear relationship. To see if there was a significant relationship between the number of contaminant species reported and the total number of seed lots tested (Table 1), we conducted a Pearson's correlation statistical test. Perennial ryegrass data from 2003 and 2009 were excluded from the Pearson's test. Results from this correlation statistical test were non-significant for both perennial ryegrass and white clover.

A Wilcoxon signed-rank test was used to compare the percentage of contaminant grass species in seed lots of perennial ryegrass and white clover, for coinciding study years. IBM's SPSS software was used for statistical analysis and visualizations throughout this study (George and Mallery 2019).

Data resources

Data related to contaminant species found within seed lots of perennial ryegrass and white clover are provided in the subsequent Tables and Supporting Information of this article.

Results

Between recent and historical data, there were 79 contaminant species (63 genera) reported in seed lots of perennial ryegrass and 90 species (68 genera) in seed lots of white clover, of which 46 species overlapped in both crops (Suppl. material 1). Over the study period, the mean percentage of contaminated seed lots declined in both study crops (Fig. 1). Accounting for all contaminant species for each sampling year, the

Table 1. Number of seed lots tested and number of contaminant species reported for perennial ryegrass and white clover. Note that datasets from 2003 and 2009 only reported eight common and/or problematic species.

Sampling year	Perennial ryegrass seed lots tested	Number of contaminant species reported in perennial ryegrass seed lots	White clover seed lots tested	Number of contaminant species reported in white clover seed lots
1912	52	31	27	78
1923	1537	39	325	62
1925	1178	24	237	29
1962	100	16	No Data	No Data
1984	1445	40	No Data	No Data
1989	1289	33	537	19
1993	2537	33	1715	19
1994	1563	33	934	19
2003	100	8	No Data	No Data
2009	100	8	No Data	No Data
2019	245	49	168	45

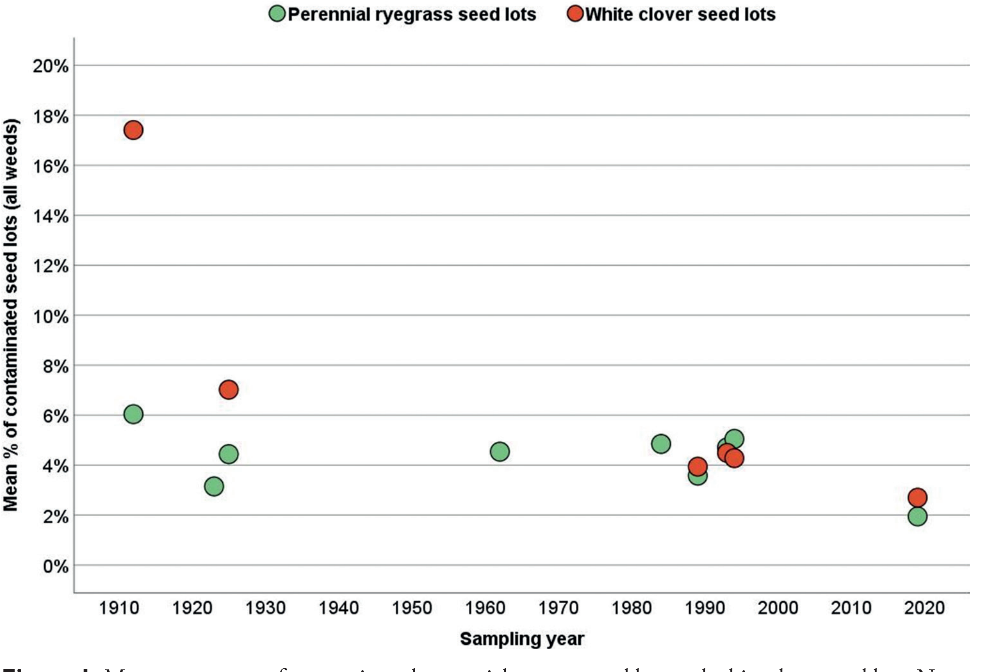

Figure 1. Mean percentage of contaminated perennial ryegrass seed lots and white clover seed lots. Note: Y-axis only goes up to 20%.

overall mean percentage of contaminated perennial ryegrass seed lots was 4.3% and for white clover seed lots it was 6.6%. For both crops, the earliest sampling year (1912) had the highest mean percentage of contaminated seed lots and the most recent sampling year (2019) had the lowest mean percentage. For perennial ryegrass, this value was three times larger in 1912 than in 2019, and in white clover it was 6.4 times larger in 1912 than 2019. While the mean percentage of contaminated white clover seed lots in 1912 was nearly three times larger than perennial ryegrass, by 2019 both crop species had similar values at 2.7% and 2.0%, respectively (Fig. 1).

Overall, *Bromus hordeaceus* was the most common contaminant in perennial ryegrass, where it was present in 68.4% of all seed lots (Fig. 2). It was also one of the top five most common contaminant species in perennial ryegrass for every sampling year. When compared to the results for an individual species, such as *Bromus hordeaceus*, the overall mean percentage of contaminated seed lots for all contaminant species (Fig. 1) was relatively low. This was partly due to the fact that the mean percentage

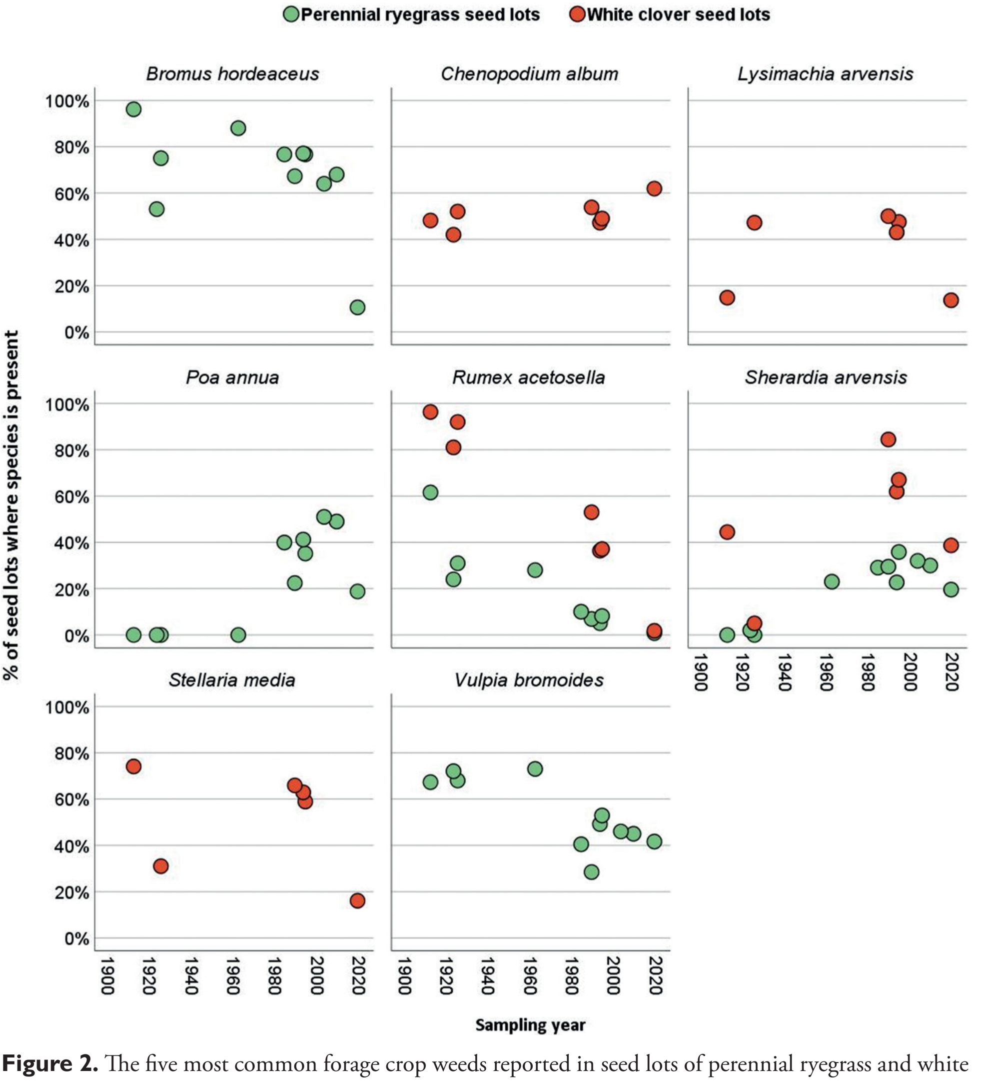

Figure 2. The five most common forage crop weeds reported in seed lots of perennial ryegrass and white clover. Values based on the percentage of seed lots with contaminant. Contaminants are listed in alphabetical order.

value for all contaminant species was calculated to include zero values for any species that were not reported in a given year, provided that they were reported in other sampling years. For 2019, which was the most recent sampling year, *Vulpia bromoides* was the most common contaminant in perennial ryegrass (present in 41.6% of seed lots).

Rumex acetosella was the most common contaminant in white clover, where it was present in 56.8% of seed lots. It was also one of the top five most common contaminant species in white clover for every sampling year except 2019, when its presence declined to 1.8%. *Chenopodium album* was the most common contaminant in white clover in 2019 (present in 61.9% of seed lots). Of the most common weeds in perennial ryegrass and white clover, *Rumex acetosella* and *Sherardia arvensis* were the only species that occurred as a top five contaminant in both crops. These contaminants also had similar temporal trends in both crops, with *Rumex acetosella* reaching its highest presence in 1912, while *Sherardia arvensis* reached its peak in the late 1980s/early 1990s. The overall mean percentage of seed lots where these contaminants were present was approximately three times larger in white clover than perennial ryegrass.

Significant contaminant species with an increasing presence trend

The Kendall rank correlation (time versus percentage of seed lots where contaminant was present) identified eight significant species in perennial ryegrass that showed an increasing trend over time regarding the percentage of seed lots where they were present (Table 2). *Phalaris minor* had the strongest positive correlation between variables, followed by *Polygonum aviculare*. No significant contaminant species in white clover were found to have an increasing trend over time.

For perennial ryegrass, *Sherardia arvensis* had the largest increase over the study period in the percentage of seed lots where present (+20%), followed by *Poa annua* (+19%) (Fig. 3). Excepting *Sherardia arvensis*, none of the significant increasing contaminant species were recorded in seed lots of perennial ryegrass until the 1980s and 1990s. For all the significant contaminant species except *Anthemis arvensis*, *Phalaris minor* and *Polygonum aviculare*, the relationship between time and the percentage of seed lots where present was non-linear. The three aforementioned species all had weak linear relationships, with tailing off in later years.

Table 2. Significant contaminant species with an increasing presence trend in perennial ryegrass seed lots. Contaminants are listed based on descending Kendall rank correlation values.

Contaminant	Common name in New Zealand	Kendall rank correlation coefficient	P-value
Phalaris minor	Lesser canary grass	0.85	0.002
Polygonum aviculare	Wireweed	0.67	0.016
Anthemis arvensis	Corn chamomile	0.62	0.033
Galium aparine	Cleavers	0.62	0.033
Veronica sp.	Speedwell	0.62	0.033
Poa annua	Annual poa	0.56	0.020
Lapsana communis	Nipple wort	0.55	0.049
Sherardia arvensis	Field madder	0.48	0.042

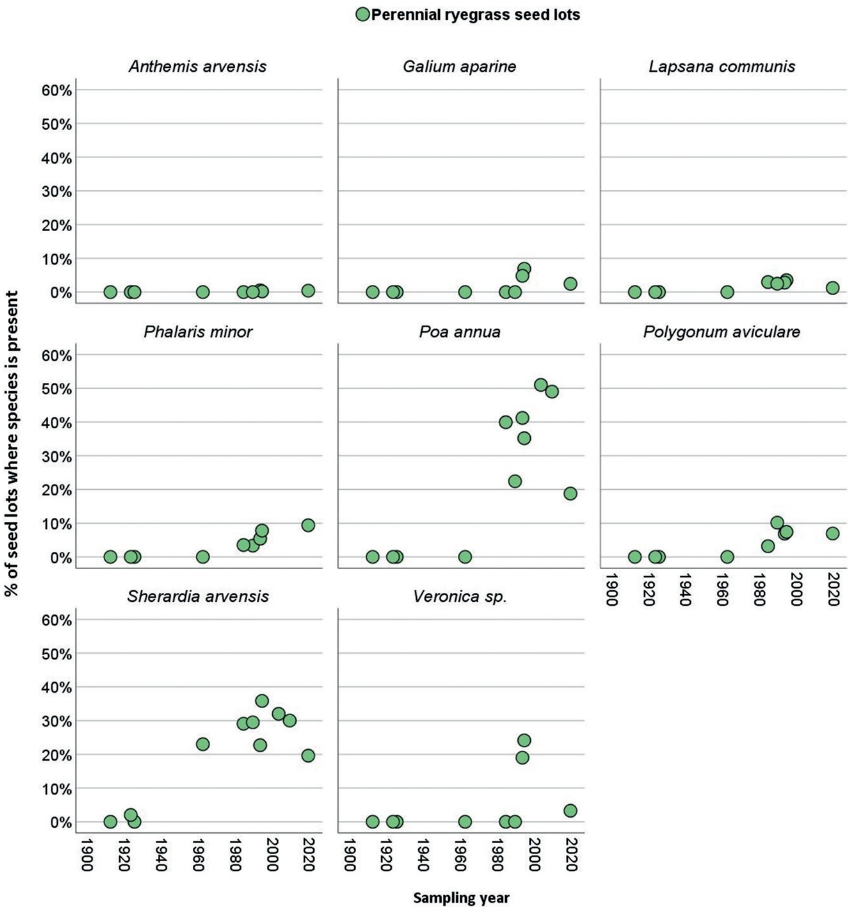

Figure 3. Significant contaminant species with an increasing presence trend in perennial ryegrass seed lots. Contaminants are listed in alphabetical order. Note: Y-axis only goes up to 60%.

Significant contaminant species with a decreasing presence trend

The Kendall rank correlation identified five significant contaminant species in perennial ryegrass and six significant contaminant species in white clover that showed a decreasing trend over time in regards to the percentage of seed lots where they were present (Table 3). Of the significant species with a decreasing presence trend in perennial ryegrass, *Plantago lanceolata* and *Rumex acetosella* had the strongest negative correlation between variables. Of the species with a decreasing trend in white clover, *Cerastium* sp. had the strongest negative correlation.

Rumex acetosella had the largest decline in the percentage of seed lots where present for both crop species, decreasing approximately 61% in perennial ryegrass seed lots and 95% in white clover (Fig. 4). In perennial ryegrass, *Hypochaeris radicata* had the second largest presence decrease in seed lots (-44%), and in white clover *Plantago lanceolata* had the second largest presence decrease in seed lots (-88%). *Rumex acetosella* and

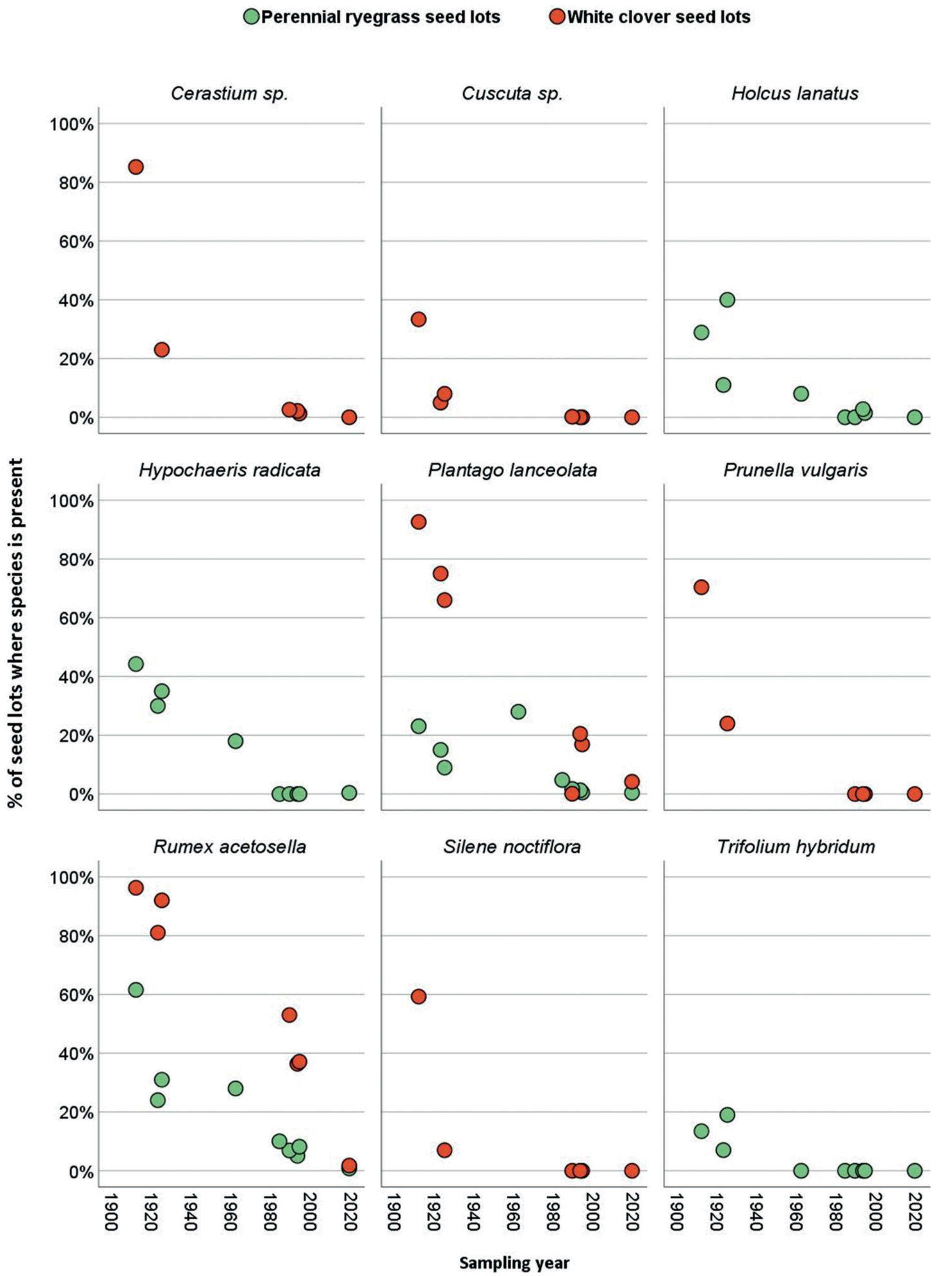

Figure 4. Significant contaminant species with a decreasing presence trend in perennial ryegrass and white clover seed lots. Contaminants are listed in alphabetical order.

Plantago lanceolata were the only significant weeds that overlapped in both crops. For all of the significant contaminant species in perennial ryegrass, the percentage of seed lots where they were present had dropped below 1% by 2019, with most having already reached this level by 1994. For all the significant species in white clover, the percentage

Table 3. Significant contaminant species with a decreasing presence trend in seed lots. Contaminants are listed based on ascending Kendall rank correlation values.

Contaminant	Common name in New Zealand	Kendall rank correlation coefficient	P-value	Crop species with decreasing presence trend
Cerastium sp.	Mouse-ear chickweed	-1.00	0.000	White clover
Plantago lanceolata	Narrow-leaved plantain	-0.83	0.002	Perennial ryegrass
Cuscuta sp.	Dodder	-0.82	0.012	White clover
Rumex acetosella	Sheep's sorrel	-0.81	0.011	White clover
Prunella vulgaris	Selfheal	-0.78	0.042	White clover
Rumex acetosella	Sheep's sorrel	-0.78	0.004	Perennial ryegrass
Silene noctiflora	Night-flowering catchfly	-0.78	0.042	White clover
Plantago lanceolata	Narrow-leaved plantain	-0.71	0.024	White clover
Trifolium hybridum	Alsike clover	-0.62	0.033	Perennial ryegrass
Holcus lanatus	Yorkshire fog	-0.61	0.025	Perennial ryegrass
Hypochaeris radicata	Catsear	-0.61	0.028	Perennial ryegrass

of seed lots where they were present ranged from 0 to 4% by 2019, with more than half already having dropped to 0% from the 1980s onwards. *Prunella vulgaris* was the only significant contaminant species that was not reported again after the implementation of seed certification in the 1920s. It is worth noting that for some significant contaminant species, the relationship between time and the percentage of seed lots where present was non-linear. This was the case for *Plantago lanceolata* in perennial ryegrass, where its presence increased in seed lots until the 1960s and then decreased thereafter.

Grass species contaminants

Of the contaminant species reported from perennial ryegrass and white clover seed lots, 22.0% were grass species (Suppl. material 1). There was a significant difference between the percentage of contaminant species that were grass weeds in seed lots of perennial ryegrass and white clover (p-value of 0.027 from Wilcoxon signed-rank test). The overall mean percentage of contaminant species that were grass weeds in seed lots of perennial ryegrass was 28.6%, which was 3.9 times larger than the mean percentage in seed lots of white clover (Fig. 5). Compared to the sampling years from the 1990s, in which the percentage of grass contaminant species in seed lots of perennial ryegrass was approximately 36%, this value had decreased to around 14% by 2019. The percentage of grass contaminant species in seed lots of white clover was between approximately 10 to 11% in earlier sampling years, but decreased by nearly half by 1989.

Discussion

Herbicide use

In comparison to other weed management tools, the introduction of herbicides in the 1940s had the largest impact on decreasing seed lot contamination (Shaner 2014). The first herbicides, such as 2.4-D, were effective in controlling broadleaf weeds in grass crops

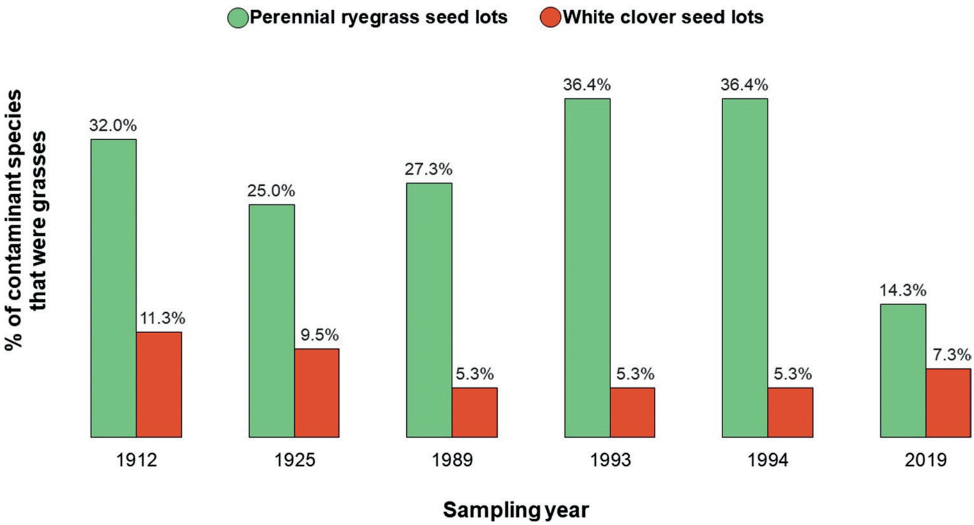

Figure 5. Comparison in the percentage of contaminant species that were grasses between perennial ryegrass and white clover seed lots.

such as perennial ryegrass (Gianessi and Reigner 2007). This included contaminants like *Hypochaeris radicata* (DiTomaso et al. 2013), a broadleaf weed which we identified as significant and had the second largest presence decrease in perennial ryegrass seed lots over the study period (-44%), declining by nearly half between the 1930s and 1950s. *Plantago lanceolata*, a significant broadleaf weed which had the second largest presence decrease in white clover seed lots over the study period (-88%), was also effectively controlled by 2.4-D (DiTomaso et al. 2013). However, when 2.4-D was used to remove *Plantago lanceolata* from white clover, in order to avoid destroying the seed crop, it was necessary to have long fallow periods so that the weed could germinate and the herbicide could be applied prior to sowing. This practice declined after the broadleaf herbicide 2.4-DB was first introduced in the mid-1950s in New Zealand (Matthews 1955), which unlike 2.4-D, did not damage white clover (Peters and Davis 1960). Both of these herbicides helped reduce the presence of *Plantago lanceolata* in white clover seed lots (Novachem agrichemical manual 2022), and the species went from being present in 66% of seed lots in the 1920s, to approximately 17% by the late 1980s, and finally 4% in 2019.

Up until the 1960s, herbicide chemistry had primarily focused on broadleaf weeds, and as such, early herbicides were mostly effective in perennial ryegrass but not white clover seed crops. However, the widespread introduction of grass herbicides in the early 1960s, such as paraquat (Gianessi and Reigner 2007; Ministry for Primary Industries 2022), made chemical control of grass weeds possible in white clover (Novachem agrichemical manual 2022). Although no grass weed species were identified as significant in white clover seed lots, there were common grass crop species reported in white clover, like *Poa pratensis*, which was present in 44% of white clover seed lots in 1912 (Cockayne 1912c). However, we excluded 'other crop' species from our analysis, since

New Zealand continued to report them separately from 'weeds' on domestic seed analysis certificates from the 1930s until the 2000s (Cousins 2022). That being said, white clover contaminants such as *Poa pratensis* are effectively controlled by paraquat (Klingman and Murray 1976). Aside from paraquat, the introduction of glyphosate in New Zealand in the mid-1970s also helped control *Poa pratensis* and a wide array of other broadleaf and grass contaminant species (DiTomaso et al. 2013; Thompson and Chauhan 2022). In the production of white clover, glyphosate reduced the need for long fallow periods and allowed for stale seedbeds, which is a practice that promotes the germination of weeds prior to sowing the crop seed, after which glyphosate is applied to kill the weeds before crop emergence (Caldwell and Mohler 2001). In addition, generic versions of glyphosate became available in New Zealand in the early 1990s, which led to an increase in its use on a broad variety of weeds (Rolston et al. 2006).

During the 1970s, selective grass herbicides that were safe for use in ryegrass crops were introduced in New Zealand. These included ethofumesate (Rolston et al. 2006; Ministry for Primary Industries 2022), which controlled a wide range of grass weeds in perennial ryegrass, including *Vulpia bromoides* and *Bromus hordeaceus* (Foundation for Arable Research 2016, 2021), which we identified as the two most common contaminants in perennial ryegrass seed lots. Although *Vulpia bromoides* has remained a common contaminant in perennial ryegrass throughout the study period, its seed lot presence decreased by approximately 30% during the period when ethomfumesate was introduced. Also during the 1970s, selective herbicides which target specific broadleaf weed species, such as clopyralid (formally known as 3.6-DPA), were introduced in New Zealand (Ministry for Primary Industries 2022). Clopyralid effectively controlled weeds like *Rumex acetosella* in perennial ryegrass (Appleby 2005; DiTomaso et al. 2013). We identified *Rumex acetosella* as a significant contaminant species with a decreasing presence trend in perennial ryegrass seed lots, with declines of nearly threefold in the early 1980s, shortly after clopyralid was introduced. Around this period, *Rumex acetosella* was also found to have decreased in forage crop seed lots in other parts of the world, such as the United States' Pacific Northwest (Alderman et al. 2011). We also noted that this species had a decreasing presence trend in white clover seed lots, declining by 95% over the study period. These decreases can partially be attributed to the addition of agricultural lime to soils in the 1950s in New Zealand, which made them less acidic (Rowarth 1998). Although clover species tend to be sensitive to acidic soils (Caddel et al. 2004), *Rumex acetosella* prefers them and is less competitive as pH increases (Stopps et al. 2011). Additionally, the availability of glyphosate in the 1970s and the introduction of flumetsulam in the mid-1990s (Rolston and Archie 1997), a selective broadleaf herbicide that is effective on *Rumex* spp. seedlings in legume crops, would have further reduced the presence of *Rumex acetosella* in white clover seed lots (DiTomaso et al. 2013; Novachem agrichemical manual 2022). Prior to the introduction of flumetsulam white clover producers were still limited to a few common herbicides to control broadleaf weeds (Rolston and Archie 1997).

Also released in the mid-1990s was the diflufenican-based herbicide Jaguar (Ministry for Primary Industries 2022), which killed a wide array of broadleaf weeds in perennial ryegrass and white clover, including weeds that other herbicides could not effectively control, such as *Galium aparine* and *Veronica* spp. (Rolston and Archie 1997). While we identified these contaminant species as significant with an increasing presence trend in perennial ryegrass seed lots, their relationship between time and the percentage of seed lots where present was non-linear, and they decreased approximately three to sevenfold after Jaguar was released in New Zealand. Aside from these contaminant species, diflufenican is also used to control *Cerastium* sp. and *Silene noctiflora* (Novachem agrichemical manual 2022), both of which were significant contaminant species that had a decreasing presence trend in white clover seed lots.

While herbicides have reduced weed seed contamination in seed lots in New Zealand, herbicide resistant weed species have increasingly been reported, the majority in forage and arable crops (Ghanizadeh and Harrington 2021). Contaminant species such as *Chenopodium album*, *Phalaris minor*, *Poa annua* and *Stellaria media*, all of which our study identified as either being a significant species with an increasing presence trend or being the most common weeds, are already reported as herbicide resistant in New Zealand. *Chenopodium album*, which we identified as one of the most common contaminants in white clover seed lots, was the first weed in New Zealand to be reported as having herbicide resistance, after Group 5 resistant biotypes were detected in arable crops in 1979 (Rahman et al. 1983). In 2005 it was found to have also developed resistance to Group 4 herbicides in arable crops, including clopyralid (James et al. 2005; Heap 2020). In 1995, *Stellaria media*, which we identified as one of the most common contaminants in white clover seed lots, was reported to have developed resistance to Group 2 herbicides in cereal crops (Seefeldt et al. 2001). In 2013, *Poa annua* (significant with an increasing presence trend in perennial ryegrass) with resistance to Group 1 herbicides was reported in turf grass in New Zealand (Mitchell and Hannan 2014; Ghanizadeh and Harrington 2021). Internationally, *Poa annua* has been found to be resistant to glyphosate, along with nearly forty other species, including perennial ryegrass itself (Heap and Duke 2018). Most recently, in 2019, the first known New Zealand case of herbicide resistant *Phalaris minor* (significant with an increasing presence trend in perennial ryegrass) was discovered on arable farms in Canterbury (Buddenhagen et al. 2021b). Considering this was the first report of Group 1 herbicide resistance for this species, it could signal a future upsurge in the number of cases (Hulme 2022). This weed is also common outside of New Zealand, and recently (alongside other *Phalaris* spp.) was reported as one of the top three most common contaminants in seed lots of forage and arable crops throughout the Mediterranean (Gervilla et al. 2019; Cossu et al. 2020).

Significant contaminant species with an increasing presence trend

Of the eight significant species that had an increasing presence trend over time in perennial ryegrass seed lots, both *Sherardia arvensis* (+20%) and *Poa annua* (+19%) had the biggest increase over the study period, indicating that these species could become more problematic in the future. Their presence increases can partially be explained by the end of the opportunist 'catch cropping' system (focused on livestock production) in the late 1980s, before New Zealand's forage seed multiplication industry finished transitioning into an annual arable system with five to ten-year crop rotations that incorporated forage species like perennial ryegrass and white clover (Rolston et al. 1990; Rowarth 1998; Buddenhagen et al. 2021b). This transition led to a shift from perennial weeds, including those significant species with a decreasing presence trend like *Hypochaeris radicata* and *Plantago lanceolata*, to annual weeds that are common in arable crops, such as *Poa annua* and *Sherardia arvensis*. While the majority of the significant contaminant species that had a decreasing presence trend were not annuals, all of the significant species with an increasing presence trend were annuals (Breitwieser et al. 2010–2022). This includes *Polygonum aviculare*, a weed which Rubenstein et al. (2021) previously identified as the most common contaminant species in ryegrass seed lots imported into New Zealand. While a transition to specialized arable crop rotations has changed the weed spectrum, one of the major benefits is that it allows for future planning regarding weed control. Growers will often select an arable crop that can tolerate herbicides used to control weeds in forage seed crops that will be grown later in the rotation. Also, increased crop rotation results in more soil cultivation, which encourages the germination of weed seeds, hence giving more opportunity to control them.

Another reason for changing weed spectrums can be attributed to a greater availability of irrigation that began in the 1980s in New Zealand's major seed production region, Canterbury (Hampton et al. 2012). Canterbury has the most amount of irrigated land in New Zealand (primarily used for pasture), and has had threefold increases in the total area of irrigated land between 1965 and 1985, before nearly doubling again by the mid-1990s (Lincoln Environmental 2000; Stats NZ 2021). The amount of irrigated land in Canterbury has continued to rapidly increase since the 1990s (Brown 2016), and it is estimated that more than 70% of seed crops are grown here using irrigation (Rolston et al. 2006). Significant species with an increasing presence trend in perennial ryegrass seed lots, like *Poa annua* and *Phalaris minor*, prefer damper environments and became more competitive under increased irrigation. Prior to increased irrigation use in Canterbury, it was drier here than in other areas in New Zealand where forage seed had historically been multiplied (National Institute of Water and Atmospheric Research 2012), such as Banks Peninsula, Southland and Manawatu (Rolston et al. 2006; Stewart et al. 2022). After the seed industry became centered in Canterbury in the 1980s, wetland weeds that were once prevalent in the aforementioned areas would have been outcompeted in drier environments. This included *Holcus lanatus* (decreasing presence trend in perennial ryegrass), a significant contaminant species that prefers moist conditions (DiTomaso et al. 2013).

Although there were eight significant contaminant species in perennial ryegrass that showed an increasing trend in seed lots, no increasing contaminant species were identified as significant in white clover. This can partially be explained by the fewer degrees of freedom used in Kendall rank correlation tests for the white clover analysis. Unfortunately, there were fewer analytical purity studies published on seed lots of white clover when compared to perennial ryegrass, resulting in about one-third fewer years' worth of available study data. Even though it was not identified as significant in our study, *Chenopodium album* is a weed of particular concern in the production of forage and arable crops (Deo et al. 1993; Rowarth et al. 1995). Our study found that *Chenopodium album* was the most common contaminant in white clover seed lots in 2019, where it was present in 61.9% of seed lots. Compared to other contaminant species in white clover, it also had the largest increase over the study period in the percentage of seed lots where it was present (+14%). Aside from already being known to be herbicide resistant in New Zealand, Rubenstein et al. (2021) identified *Chenopodium album* as the most common contaminant species reported in agricultural seed lots from over 1,400 crop species imported into New Zealand. Similarly, Ikeda et al. (2022) identified it as one of the most common contaminants in imported arable seed lots entering Japan, and Singh et al. (2010) noted it was the most common contaminant in imported red clover (*Trifolium pratense*) seed lots entering India. *Chenopodium album*'s commonality in forage seed lots can partially be explained by its ability to adapt to a wide array of environmental conditions (Williams 1963). *Chenopodium album* tends to germinate and emerge later than the white clover seed crop, thus avoiding post-emergence herbicide applications, at which point it can set seed from small late emerged plants (Rubenstein et al. 2021).

Trifolium glomeratum had the second largest increase over the study period in the percentage of white clover seed lots it was present in (+13%). For white clover crops, other *Trifolium* spp. are often the most common contaminant species reported in their seed lots (Rubenstein et al. 2021). This is largely due to the contaminant species and crop being in the same genus, which means that chemical control options are limited, since most herbicides would also destroy the crop. An exception to this is the herbicide flumetsulam, which is successful in controlling certain *Trifolium* spp. in white clover; however it is ineffective in controlling *Trifolium glomeratum* (Kelly 2022). Additionally, the similarity in shape and size of *Trifolium glomeratum* seeds and white clover seeds makes separation of the two difficult during cleaning (James et al. 2012). This contaminant species is also an annual, and has likely become more common as Canterbury transitioned into an annual arable system.

Most common weeds

In perennial ryegrass seed lots, *Bromus hordeaceus* was the most common contaminant species over the study period, and it was one of the top five most common weeds for each individual sampling year. This contaminant species is an annual grass and is common in a variety of forage crops (Dastgheib and Poole 2010). Because of the damage to *Bromus hordeaceus* seed which occurs as a result of threshing, it is one of the most difficult contaminants to remove from perennial ryegrass (Saxby 1941). When threshing removes the awns or wings, *Bromus hordeaceus* seeds are almost indistinguishable from perennial ryegrass seed in both shape and size, and are therefore extremely difficult to remove during cleaning (Saxby 1941; Rolston et al. 1985). Also, since this contaminant is a grass species, options for control by herbicides in perennial ryegrass seed crops are limited. *Bromus hordeaceus* is also prevalent in arable crops (Dastgheib and Poole 2010), and is notoriously difficult to control in wheat and barley (Rolston et al. 2003; Michael et al. 2010). As it became more common to rotate perennial ryegrass with arable crops in Canterbury, this weed would have built up seed banks during the rotation (Rolston et al. 2003; Kelly 2022). Historically, an effective control measure for *Bromus hordeaceus* and other weeds of grass seed crops involved burning stubble after the harvest (Dastgheib and Poole 2010). However, because of environmental concerns over prescribed burns, it is now more common to either graze or bale stubble, which unlike burning, has no impact on weed seeds on the soil surface.

In white clover seed lots, we identified *Rumex acetosella* as the most common contaminant species for all years combined, and in perennial ryegrass seed lots it was one of the top five most common weeds. Aside from being one of the most common contaminant species in both crops, it was also identified as a significant species with a decreasing presence trend in both crops, with presence levels dropping below 2% by 2019. This is worth noting, because while *Rumex acetosella* was very common in both crops, this does not preclude the weed from steadily decreasing over time. In fact, *Rumex acetosella* had the largest decline in both crops of any of the significant contaminant species, based on the percentage of seed lots where present. Because of this, additional recent sampling years would be useful to determine if this trend continues after 2019.

Seed certification and policy changes

After the implementation of seed certification in 1929, New Zealand farmers could be assured that forage seed lots met strict genetic and analytical purity standards (Hampton 1994; Rowarth et al. 1995). In order to meet certification standards, growers had to comply with field history and isolation distance requirements. Field history requirements meant that minimum time intervals between cultivation had to be observed when growing different species (or cultivars of the same species) in the same field (Eaden 1979). Isolation distance requirements stated that cross pollinating species must be grown apart at a set minimum distance from one another (Eaden 1979). These certification requirements led to producers taking more care when growing forage crops, such as Italian ryegrass (*Lolium multiflorum*) and red clover, in the same farm as perennial ryegrass and white clover. Although we excluded crop seed species as contaminants from our analysis, it is worth noting that prior to seed certification Italian ryegrass was present in approximately 44 to 74% of perennial ryegrass seed lots, and red clover was present in approximately 52 to 68% of white clover seed lots (Cockayne 1912a, c; Foy 1924, 1926). However, in 2019, Italian ryegrass was not reported in any perennial ryegrass seed lots and red clover was present in less than 7% of white clover seed lots.

Cuscuta sp. is a broadleaf contaminant which we identified as a significant species with a decreasing presence trend in white clover seed lots, after declining from approximately 15% of seed lots prior to the 1930s to 0% from the 1980s onwards. Aside from herbicide use, this decrease can also be explained by New Zealand's industry-led policy changes in the 1980s. Along with thirteen other species, it was dubbed an 'undesirable weed' by what is now the New Zealand Grain & Seed Trade Association (Young 1984). If an undesirable weed was detected in a seed lot, it would substantially lower its selling price, and therefore farmers prioritized control of these species. Additionally, there was an increase in the production of New Zealand-bred white clover seed starting in the 1930s, with the creation of cultivars such as Huia (Caradus et al. 1995). The success of New Zealand's breeding program reduced the need for importing white clover seed lots from abroad, and the percentage of imported seed lots containing *Cuscuta* sp., and other internationally common contaminants, likely decreased. This is to be expected, since historically the weed species commonly found in the pastures of New Zealand's major trading partners (e.g. England) would be similar to those found in New Zealand (Allen 1955).

Seed cleaning technology

Seed cleaning technology has had a major impact on reducing seed lot contamination and the machinery used prior to seed certification (air screen cleaners and spiral separator), as well as those developed later (indent cylinders and gravity table separators), are still regularly used today (McDonald and Copeland 2012). The difficulty in separating the crop seed and contaminant species in the cleaning process is largely dependent on the similarity in shape, size and texture of the seeds. For example, significant species with a decreasing presence trend in perennial ryegrass seed lots, such as *Holcus lanatus* and *Rumex acetosella*, which are different in shape and size to perennial ryegrass seed (James et al. 2012), are easy to clean out of perennial ryegrass seed lots (Hartley 1969). The same is true for *Cerastium* sp. (significant species with a decreasing presence trend in white clover) in white clover seed lots (Hartley 1969). Conversely, *Chenopodium album* seed is a similar shape and size to white clover seed, which makes it difficult to clean out of these seed lots (Hartley 1969).

Grass contaminants

Overall, the mean percentage of contaminant species that were grasses was approximately four times larger in seed lots of perennial ryegrass than white clover. As was previously discussed regarding *Trifolium* spp., this difference in values between study crops is to be expected since it is harder to clean or use chemical controls when a grass contaminant is present in a grass crop, especially since non-selective herbicides can be damaging to a wide array of genera within the same family. In perennial ryegrass seed lots, the percentage of species that were grasses decreased 2.5 times from the 1990s to 2019. This decline can partially be explained by the increased use of ethofumesate, an herbicide which controls a wide range of grass weeds in perennial ryegrass. It decreased in price in New Zealand in the late 1990s when generic versions become available (Ministry for Primary Industries 2022), making it more economical for use in grass seed crops.

Conclusion

Rubenstein et al. (2021) found that forage crops had the largest percentage of contaminated seed lots when compared to other crop seed types, such as vegetable and arable crop species. Additionally, perennial ryegrass and white clover are two of the most widely utilized forage species in pasture systems around the world, and as such, related studies identifying weed trends within them should be prioritized. Taking all of this into account, it is surprising that our study was the first to examine long-term weed seed contamination trends within these crops. However, this is somewhat to be expected, considering that past analytical purity studies that looked at even one or two years of data were rare, and that current data held by seed companies or quality assurance laboratories are commercially sensitive and primarily unavailable to researchers. Furthermore, it is difficult to make comparisons of historical data collected over various decades, as it could be assumed that analytical purity sampling protocols have changed over time. However, our analysis focused on certified seed lots that were sampled and tested in accordance with the rules of ISTA, of which New Zealand has been a member since the 1920s. Other than an increase in the maximum seed lot size for grass crops, the methods for analytical purity testing of forage species have changed little since ISTA's inception. While study data collected prior to the implementation of seed certification did not follow ISTA rules, they employed similar techniques that were already in place at the national seed testing laboratory that began providing New Zealand growers with analytical purity results in 1909. Therefore, recent and historical results are comparable for analysis, taking into account the aforementioned factors.

Although we identified several annual weed species that could become more problematic in the future because they showed an increasing presence trend in seed lots or were identified as the most common contaminants, overall the percentage of contaminated forage seed lots has decreased between approximately three and sixfold since the early part of the 20th century. This indicates that herbicide availability, seed certification and improved crop management have been effective for weed control. However, while the percentage of contaminated seed lots has decreased, unless control measures are maintained, contamination levels can quickly increase. Additionally, weed seeds still remain a concern given the rise in reports of herbicide resistant weeds, a reduction in herbicide availability from a lack of new chemistry, decreases in herbicide use amidst regulatory bans, and the risk of introducing non-native species (Rowarth 1998; Kudsk and Streibig 2003; Hulme 2005, 2022). An alternative approach to address weed seed contamination would be to incorporate recent seed cleaning technology into forage seed production. For example, seed color sorters are an effective commercial cleaning technology commonly used in high-value vegetable seed production, but have not been considered economically practical for forage seeds because of their relatively low market value per kilogram (Rubenstein et al. 2021). However, recent improvements and cost reductions to color sorters have made their use more feasible for cleaning other relatively lower market value crop seeds, such as cereals (Pearson 2010).

Because of New Zealand's long history of forage seed production, large number of international trading partners, and its role as a primary seed producer of common forage crops, our study is in a unique position to identify weed trends occurring globally throughout the forage seed industry. However, we were only able to identify trends based on the absence/presence of a contaminant species, since previous studies did not report the number of weed seeds. This type of information would be useful for future studies investigating propagule pressure. Considering the lack of historic or current analytical purity data, we believe there is a need for the development and maintenance of a national (or multi-national) seed lot analytical purity database. This could be managed by individual country's ISTA accredited seed testing laboratories, who could digitize the information from seed lot analysis certificates already being provided to seed companies.

Acknowledgments

The authors would like to thank the following seed companies for providing data: Barenbrug, Cropmark Seeds Limited, Luisetti Seeds, PGG Wrightson Seeds Ltd. and Seed Force Ltd. We would also like to thank the following people for their assistance: Jennifer Bufford from Manaaki Whenua – Landcare Research, Murray Kelly from PGG Wrightson Seeds Ltd., Trevor James from AgResearch Ltd., Kerry Harrington from Massey University, Will Godsoe from Lincoln University, Blair Galvin from University of Canterbury and Jennifer McCulloch from the Foundation for Arable Research.

Funding was provided by the Seed Industry Research Centre (www.sirc.co.nz), a partnership between research providers and major New Zealand seed companies. Funding was also provided by Plant and Food Research Ltd. via the Better Border Biosecurity research collaboration (www.b3nz.org.nz). This publication has been financed by the Lincoln University Open Access Fund.

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Supplementary material 1

Supporting information

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Data type: Weed seed occurrences (word document)

Explanation note: table S1. Contaminant species reported in perennial ryegrass seed lots and corresponding percentage of seed lots where present. table S2. Contaminant species reported in white clover seed lots and corresponding percentage of seed lots where present.

Link: https://doi.org/10.3897/neobiota.85.100825.suppl1