Herbicide resistant weeds in maize in New Zealand

A survey for herbicide resistant weeds in the Bay of Plenty and Waikato

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Abstract

Research on maize weeds in New Zealand is extensive, but few systematic efforts were made to catalogue weed species and determine their prevalence and abundance. Broadleaf weeds were early problems but became manageable when photosystem-II inhibitors and synthetic auxins were introduced in the 1950s. Annual grasses became the next problematic weeds, and newer herbicides were introduced in the 1970s. The first cases of resistance were reported in the 1980s, *Chenopodium album* L. and *Persicaria maculosa* Gray both evolved resistance atrazine after several years of selection. Perennial weeds *Oxalis latifolia* and *Calystegia sepium* ssp. *roseata* were identified as problem weeds. More herbicides were introduced. Atrazine-resistant *C. album* evolved additional resistance to dicamba in the eastern Waikato. The quarantine weed *Abutilon theophrasti* was unintentionally introduced to multiple maize farms. Most recently, the annual grass *Digitaria sanguinalis* evolved resistance to nicosulfuron in Bay of Plenty and Waikato maize. The most recent 'weeds survey' in maize is two decades old, in twenty Waikato, four Bay of Plenty and six Gisborne maize fields. Above-ground (spring seedlings) and below-ground (soil seedbank) weeds were recorded. Knowledge of current weed distribution and abundance is derived from multiple smaller studies, not a systematic study of maize weeds across a region.

Published studies of herbicide resistant weeds across the world are documented on a database. Estimates of weed species relative risks of evolving resistance were done in previous studies using those data. A list comprising thirty-nine common weeds associated with maize was generated from literature. Weeds were ranked for their risk of evolving herbicide resistance with a scoring protocol that accounts for the specific herbicides used in New Zealand maize. Seven weed species were classified as having a high risk of developing herbicide resistance: *Echinochloa crus-galli* (L.) P.Beauv., *Chenopodium album*, *Eleusine indica* (L.) Gaertn., *Xanthium strumarium* L., *Amaranthus powellii* S.Watson, *Solanum nigrum* L. and *Digitaria sanguinalis*. Seventeen species were classed as moderate risk and 15 were low risk. Herbicide classes associated with more resistant species were classed as high risk, these included acetohydroxy acid synthase (AHAS) inhibitors and photosystem-II inhibitors. Synthetic auxin herbicides had a moderate risk but only two herbicides in this class (dicamba and clopyralid) are registered for maize in New Zealand. All other herbicide mode-of-action groups used in maize were low risk. When accounting for herbicide groups used in maize, *E. crus-galli*, *E. indica*, *C. album*, *D. sanguinalis* and *A. powelli* were the five highest risk weeds.

Maize growers from two lists (Agribase and FAR) were called in random order for weed sampling. Thirty-six Waikato and sixteen Bay of Plenty maize fields were sampled prior to harvest, in late February-early March 2021. Weeds present were identified, and their percentage cover was estimated in two 133m transects. The first transect was in the 'headland', the area close to the field edge, and the second area in the centre of the maize field. Transect starting locations were randomized to minimize bias. Within each transect, six soil samples were taken at 33m intervals. Seeds were collected from individual plants throughout the field. As soon as possible, soil samples were taken back to the Ruakura glasshouse facility and germinated in trays. Seedlings were identified and enumerated, then soil was mixed; trays were assessed three times each. One-hundred and thirty-four plant species were observed in the fifty-two sampled sites in the field assessments and soil seedbanks. *Digitaria sanguinalis* (98%), *Chenopodium album* (85%) and *Persicaria*

spp. (71%) were the most widespread weeds, with *Setaria pumila*, *Sonchus oleraceus*, *Solanum nigrum*, *Erigeron* spp. and *Rumex obtusifolius* seen in more than half of the farms. *Digitaria sanguinalis*, *C. album*, *Persicaria* spp., *Cyperus rotundus*, *Echinochloa crus-galli* had the highest percentage cover estimates, also common were *Cynodon dactylon*, *Elytrigia repens*, *Paspalum distichum* and *Solanum nigrum*. Winter and spring weeds *Juncus bufonius*, *Poa annua* and *Stellaria media* occurred frequently in soil seedbanks. A principal component analysis was done for weed percentage cover and weed seedbank densities for the fifty-two farms. For the percentage cover PCA *C. album*, *C. rotundus*, *D. sanguinalis* and *Persicaria* spp. were important taxa, and for the seedbank density PCA *P. annua*, *J. bufonius*, *D. sanguinalis* and *S. media* were important drivers of farm-farm compositional differences. Perennial weeds are a major problem in the eastern Bay of Plenty. Weeds with a history of evolved resistance *C. album*, *P. maculosa* and *D. sanguinalis* are common in both regions.

Earlier studies showed that *Chenopodium album* L. evolved atrazine and dicamba resistance in Waikato farms, *Persicaria maculosa* L. in Waikato and *Digitaria sanguinalis* (L.) Scop. in the Bay of Plenty and Waikato farms. Apart from atrazine resistant *C. album* - presumed omnipresent in New Zealand maize - the prevalence of resistant weeds was unknown in maize. This study was the first systematic survey to estimate the prevalence of herbicide resistant weeds in Bay of Plenty and Waikato maize. Of the fifty-two farms visited, thirty-two, thirty-one and twenty-nine had *C. album*, *D. sanguinalis* and *Persicaria* spp. (*P. lapathifolia*, *P. maculosa*) seed collected and tested for herbicide resistance, respectively. *Persicaria* spp. seedlings did not survive any herbicide treatments, of atrazine (1500 g.ai.ha^{-1}), dicamba (600 g.ai.ha^{-1}), nicosulfuron (60 g.ai.ha^{-1}) and mesotrione (96 g.ai.ha^{-1}). *Chenopodium album* seedlings were treated with the same rates of the same herbicides as *Persicaria* spp., and no *C. album* samples survived dicamba, nicosulfuron or mesotrione, but samples from twenty-two farms survived atrazine with no visible damage. Farms with atrazine-resistant *C. album* populations had *C. album* higher seedbank densities than farms with susceptible populations. *Digitaria sanguinalis* seedlings were treated with nicosulfuron (60 g.ai.ha^{-1}). Ten farms had *D. sanguinalis* samples that survived, but three of those had low rates of survival. Even nicosulfuron resistant *D. sanguinalis* were stunted by the herbicide. A dose-response experiment to confirm and determine the level of resistance was set up, where plants from twelve farms were treated with 0, 15, 30, 60, 120, 240 g.ai.ha^{-1} nicosulfuron. Most resistant samples survived 60 g.ai.ha^{-1}, but two survived up to 240 g.ai.ha^{-1}. None of the six farms near the the first nicosulfuron resistant *D. sanguinalis* case had resistance, except for sites managed by the same contractor as the original site. Farms with nicosulfuron-resistant *D. sanguinalis* populations had significantly higher seedbank densities and percentage cover for *D. sanguinalis* compared to farms with susceptible populations. A total of twenty-four farms (46%) had a resistant weed, five of those had both nicosulfuron resistant *D. sanguinalis* and atrazine resistant *C. album*. Resistant *C. album* was detected across both regions, but was only in one eastern Bay of Plenty farm; resistant *D. sanguinalis* was sporadically distributed, but only detected in Waikato randomly sampled farms.

Results from the weed risk assessment show that more than half of species are at moderate-high risk of evolving herbicide resistance, but perennial weeds are less likely to evolve resistance. Commonly used photosystem-II inhibitors and AHAS inhibitors are high risk. The risk assessment may help maize growers avoid selecting for herbicide resistant weeds. The most observed weeds have either already evolved herbicide resistance (*D. sanguinalis*, *C. album*, *P. maculosa*) or were at high-risk of doing so (*E. crus-galli*). Perennial weeds are common in the eastern Bay of Plenty, especially *C. rotundus*. These weeds, and *C. dactylon*, *E. crus-galli*, *E. repens*, *P. distichum* and *S. nigrum* are abundant in fields. The most common weeds were tested for herbicide resistance, but only atrazine resistant *C. album* and nicosulfuron resistant *D. sanguinalis* were observed. Atrazine resistant *C. album* was common, in twenty-two farms (69% of tested farms, 42% of visited farms). No multiple-resistant *C. album* were detected. Nicosulfuron resistant *D. sanguinalis* was less common, in seven farms (23% of tested farms, 13% of visited farms). A similar proportion of resistant farms in Bay of Plenty-Waikato maize (46%) to Canterbury cereals (48%) was observed. Arable cropping is a repetitive environment that is highly selective for herbicide resistant, and tolerant, weeds. To prevent weeds from evolving herbicide resistance, integrated weed management practices are need. Chemical and non-chemical weed control strategies can reduce weed pressure and mitigate resistance evolution.

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Figure 0-1. The shore of Omaio, eastern Bay of Plenty.

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Chapter 1: Introduction and Literature Review

1.01 A weed is a weed is a weed.

Almost every extended work on weeds starts with discussion of definitions. In this context, the topic is on weeds of maize|kānga (*Zea mays* L.). Here, a weed is not necessarily an invasive plant, though some weeds can also be environmental weeds (*Sorghum halapense* (L.) Pers). It does not matter whether the plant is adventive or native, but native weeds are rare (pōhue *Calystegia sepium* ssp. *roseata* is an exception). Most naturalized plants in New Zealand were introduced by Europeans (Hulme 2020, Williams and Cameron 2006) and very few by Māori (Leach 2005). The concept of a weed is not the same for European settlers and indigenous Māori (Leach 2005); in Te Ao Māori, uncultivated plants are descendents of the atua Rongo-mā-Tāne and cultivated plants Haumia-tiketike (Phillips et al. 2016). In contemporary maize agriculture weeds are undesired plants that can cause yield loss and reduce farm profitability. Here, a weed is an unwanted plant that grows spontaneously in a maize crop.

1.02 Maize cropping in Aotearoa

1.02.1 History

Maize is believed to have first arrived in Aotearoa, at the Bay of Islands with the colonial explorer du Fresne in 1772 (King and Roa 2015). At the time, agriculture was dominated by indigenous Māori who cultivated plants brought into New Zealand kūmara, hue, taro, and plants native tī kōuka, aruhe, karaka (Best 1930, Leach 2005, Phillips et al. 2016). Kānga was adopted by many Māori, becoming a major crop, and was eaten in many ways (Bansal and Eagles 1985). Kānga wai (or kānga pirau) was invented, a product of the fermentation of maize soaked in a body of water for months (Rangiwai 2021). Kānga became an established crop in Northland (King and Roa 2015), Bay of Plenty, Gisborne and Hawkes Bay Māori communities (Yen 1959). After the Waikato wars in the 1860s land was taken by the Crown, and massive landscape transformation commenced, with lowland forests and wetlands converted into large scale pastureland sown with European grass species (Caradus et al. 2022, Nicholls 2002). Pastoral farming has been a significant part of the New Zealand export economy since (Caradus et al. 2022).

Most maize in the early 1900s was grown in Gisborne and Hawkes Bay (Bansal and Eagles 1985). Open-pollinated varieties were replaced by hybrids from the U.S.A. and by the 1960s, Wisconsin hybrids were dominant (Bansal and Eagles 1985). The development of cold tolerant and early-maturing cultivars expanded potential growing areas and the growing season of maize

(Eagles 1979, Wilson et al. 1994). Maize planting increased in Waikato in the 1960s due in part to agronomic advances (herbicides and new cultivars) but also as prices dropped for wool, meat and dairy products (Bansal and Eagles 1985). Highly developed ryegrass-clover (*Lolium-Trifolium*) pastures were predicted to hit upper limits of productivity, and mixed pasture and cropping farms were shown to be more efficient (Clark et al. 2001). Maize hybrids grown for silage became more common in the 1990s and are an important feed source for the dairy industry (Booker 2009, Clark et al. 2001, Dairy NZ 2017).

The area in cultivation for maize has gone from 3000 ha in 1961, 20000 ha in 1994 to 73000 ha in 2021, which is mostly attributed to the rise of silage maize production (AIMI 2021, Wilson et al. 1994). According to that 2020 estimate, 79% of maize hectarage is silage (AIMI 2021). Maize yields in New Zealand have continuously increased, due to advances in crop improvement and management (Morris et al. 2016). North Island regions Waikato, Bay of Plenty, Gisborne, Hawke's Bay and Manawatu-Whanganui are currently considered the most important maize-growing regions (Millner and Roskruge 2013). A significant amount of maize is grown in the Bay of Plenty and Waikato; recent estimates in these areas suggest that they account for up to 33% and 60% of the grain and silage production (respectively) in New Zealand (AIMI 2021, Stats NZ, 2021).

1.02.2 Agronomy

In the 1980s, a farmer survey that covered 40% of Waikato maize was done (Underwood 1985). Growers cultivated paddocks, with an initial cultivation (plough, rip, disc) followed by a seed bed preparation (disc & tyne, roller, power-harrow & roller); no growers used 'chemical tillage' (Underwood 1985). Since the 1980s new herbicides had been made available to allow for reduced tillage (Rahman 1985), but uptake has been limited (Ward and Siddique 2014). Maize was planted from October to November (Underwood 1985), but now maize can be planted from late September (Tsimba et al. 2013). Frost can kill maize planted too early, but maize planted too late will have lower yields than short season hybrids; weather risks determine the viability of short-season to long-season hybrids in particular areas (Tsimba et al. 2013). The average planting rate was was 83,700 plants per hectare, of which 94% established (Underwood 1985). Current maize plant populations recommended are on average 9000 plants per hectare higher for silage (85-115k.ha^{-1}) than grain maize (80-100kha^{-1}) (Corson Maize 2021, Pioneer 2022a, 2022b, VPMAXX 2022). Maize rows are planted at 75 cm spacing, but narrow row spacing is sometimes used (Stone et al. 2000).

Herbicides are applied before maize seedling emergence (pre-emergent herbicide), and after (post-emergent herbicide) (Rahman 1985). There are no genetically modified maize hybrids New Zealand; imidazolinone tolerant maize hybrids bred for resistance (not 'genetic modified') were trialed in New Zealand (James et al. 2001), but are not used currently. Fertilizers are applied during planting and/or side-dressed during the growing season (Underwood 1985). Insecticides are widely used, and treated seed is usually planted (Underwood 1985). Grain maize stubble is often grazed by cattle and left fallow over winter; winter weeds can be introduced by hay fed out in maize paddocks during winter (Underwood 1985). Silage maize is often cropped with a winter forage afterward, particularly Italian ryegrass (*Lolium multiflorum* Lam.) or cereal oats (*Avena sativa* (L.; Chakwizira et al. 2019). Before the next season begins, existing weeds and sown grasses are often terminated with glyphosate prior to cultivation (Rahman 1985).

Maize grown in cropland (continuously cropped), but it is also grown on dairy farms. It was estimated most silage maize in Waikato and Bay of Plenty are grown on dairy farms (63%, 64%), with the rest grown on cropland (36%, 37%; Booker 2009). Weeds, pests, diseases and soil structure damage reduce the quality and persistence of ryegrass-clover pastures, necessitating pasture renewal, a process where weed, pest and soil issues are addressed and new pasture seed is planted (Densley et al. 2011, Lane et al. 2009) Silage maize is often grown as a break crop as part of this process (Densley et al. 2001, 2011, Tozer et al. 2015a). It is estimated half of all maize silage hectarage planted by contractors was part of a pasture renewal sequence, the rest cropped continuously (Booker 2009).

1.03 Weed interference

Weeds cause maize yield losses through competition. Competition is a wide concept, and can involve competition for space, nutrients, water and light (Zimdahl 1980).

Weeds can produce allelochemicals, to reduce competitor plant growth, inhibit seed germination (Qasem and Foy 2001); the maize herbicide mesotrione was developed from allelochemicals of *Melaleuca citrina* (James et al. 2006a). Weeds can effect maize growth by inducing a 'shade-avoidance' response, which affects resource allocation, growth and yield (Page et al. 2009, Rajcan and Swanton 2001). Concepts of weed competition, allelopathy and other effects that interfere with crop growth are referred to as 'weed interference'.

Weeds can contribute to decreased crop quality as well as yield. Most maize grown is silage (AIMI 2021, Booker 2009); silage maize is harvested as whole plants, chopped and ensiled. Weeds present in the paddock at high densities will become silage and can reduce feed quality (Pilipavičius et al. 2003). Several maize weeds like *Conium maculatum*, *Datura stramonium*, *Persicaria* spp., *Phytolacca octandra*, *Solanum* spp., can be poisonous to livestock (Connor 1977, Puschner et al. 2006).

It is estimated weeds in maize have the potential to cause 40% yield losses globally (Oerke 2006). Trials of weed interference in maize summarized by Zimdahl (1980) cite a wide range of losses, from 8-25% caused by *Setaria* spp., *C. album* 15-22% and *Amaranthus* spp. 15-36%. Yield losses from weed interference can range from 30-70% in maize in New Zealand (James et al. 2000). Maize is most vulnerable in the early season, before it establishes a canopy (Page et al. 2012). The timing can vary for the maize 'critical period' (4-10 weeks after planting), where weeds have the highest potential to cause yield loss and where post-emergent weed control is most effective (Hall et al. 1992, James et al. 2000 p. 2, 2006b, 2007). This period is believed to be related to when weeds attain complete ground cover (James et al. 2000).

1.04 Herbicide resistance

1.04.1 Herbicides

Weeds were first controlled with 'blood, sweat and tears', then machinery, then chemical herbicides (Zimdahl 2007). Herbicides act on specific pathways of plant metabolism, growth and photosynthesis (Duke 1990), to stunt growth or cause death to weeds.

1.04.2 Resistance history

In 1957, the first weed was confirmed to have evolved herbicide resistance: *Daucus carota* L. to 2,4-D in Canadian roadsides (Whitehead and Switzer 1963). The same year, *Commelina diffusa* in Hawaiian sugarcane became resistant to 2,4-D (Hanson 1962). The next reported case was more than ten years later, in a plant nursery in Washington (Heap 2022, Ryan 1970). Reports of resistance grew, to 41 in 1980, 191 in 1995 and now 511 unique cases (Heap 2022, Shaner 2014). Unique cases are defined as species × site of action, but the total amount of non-unique cases was 73 in 1980, 454 in 1995 and is currently 1626 (Heap 2022). The first case of resistance in maize was *Amaranthus hybridus,* identified as resistant to atrazine in Maryland, United States in 1974 (Ritter et al. 1985). Weeds continued to evolve resistance to triazines in maize, with the first case in New Zealand in 1979 (Rahman et al. 1983c). Photosystem-II inhibitor resistance continued to evolve in maize weed populations across the world, with 125 cases in 1991 (Heap 2022), the year the first case of resistance to other herbicide groups in maize was reported, with *Setaria faberi* in Wisconsin, United States resistant to ACCase-inhibitors (Stoltenberg and Wiederholt 1995). Then, in 1993, AHAS-inhibitor resistant *Amaranthus* spp. were observed (Horak and Peterson 1995). Two years later, two Illinois weeds became the first maize weeds to evolve multiple resistance, *Amaranthus tuberculatus* and *Kochia scoparia*, both to AHAS-inhibitors and photosystem-II inhibitors (Foes et al. 1998, 1999). At-least sixteen weeds have evolved multiple herbicide resistance in maize: *Amaranthus* spp. (*A. hybridus*, *A. palmerii*, *A. powelli*, *A. retroflexus*, *A. tuberculatus*), *Ambrosia* spp. (*A. artemisifolia*, *A.*

trifida), *Bidens* spp. (*B. pilosa, B. subalternans*), *Carduus acanthoides*, *Chenopodium album*, *Eleusine indica*, *Erigeron* spp. (*E. bonariensis, E. sumatrensis*), *Euphorbia heterophylla*, and *Lolium multiflorum* (Heap 2022). New resistance continued to accumulate, and now there are weeds with resistance to up to five herbicide mode-of-action groups (Kumar et al. 2019), and there are 326 cases of herbicide resistance in maize (Heap 2022). Resistance cases are increasing, but more concerning is the rise of multiple resistant weeds.

In the past, herbicides were grouped by their chemical groups. Atrazine is a triazine herbicide, alachlor a chloroacetamide and dicamba a hormonal herbicide (Rahman 1985). In the 1990s, weed scientists classified herbicides by their site of action (Retzinger and Mallory-Smith 1997); atrazine was classified as a photosystem-II inhibitor (group 5/C_1), dicamba as a synthetic auxin (4/O) and alachlor had an unknown site of action but was classified as a group 15/K_3 herbicide with other chloroacetamides (Retzinger and Mallory-Smith 1997).

To become 'resistant' a weed must first be 'susceptible'. There is a lot of variation in natural tolerance to herbicides between weed species and within weed populations. Weeds with naturally tolerance to herbicides are considered to be 'tolerant', this contrasts with weeds historically susceptible to herbicides that have evolved resistance. In cropping fields, selection for naturally tolerant weeds and herbicide resistant weeds simultaneously occurs. For example, in Waikato maize crops, grass weeds like *Digitaria sanguinalis* and *Panicum dichotomiflorum* that have some natural tolerance to atrazine became common with the widespread usage of atrazine, at the same time resistant populations of *Chenopodium album* and *Persicaria maculosa* became immune to the herbicide (Rahman 1985, Rahman et al. 1983a, Rahman and Patterson 1987). Two broad kinds of resistance exist: target site and non-target site resistance (Délye et al. 2013b, Ghanizadeh and Harrington 2017a, Murphy and Tranel 2019).

Mutations that confer herbicide resistance can be within a populations standing variation, or arise *de novo* through mutation (Kreiner et al. 2018). It is unknown what the relative contributions of each are to herbicide resistance. Standing variation that would confer herbicide resistance was present in *Alopecurus myusoroides* specimens collected before herbicides were invented (Délye et al. 2013a). Studies investigating the standing variation of herbicide resistance of *Lolium rigidum* found rates of resistance to AHAS-inhibitors between 2.2×10^{-5} and 1.2×10^{-4} (Preston and Powles 2002). *De novo* mutation rates for herbicide resistance were estimated to be lower than 1.4×10^{-8} for *Amaranthus hypochondriacus* to AHAS-inhibitor herbicides (Casale et al. 2019); for Arabidopsis thaliana, the rate of mutation was estimated to be 3.2×10^{-5} for AHAS-inhibitor herbicides (Jander et al. 2003).

1.04.3 Mechanisms of resistance

Target-site resistance is where a mutation incurs reduced herbicide efficacy in the target enzyme of a herbicide (Murphy and Tranel 2019). In the case of atrazine resistance, a single nucleotide mutations in *psbA* can confer an altered D1 protein, reducing atrazine binding affinity (Hirschberg et al. 1984). The herbicide is less effective in preventing enzyme function, and the plant will have increased survival toward the herbicide. Often target site mutations are believed to have a fitness cost associated, but this is not always true (Murphy and Tranel 2019). A similar mechanism where the target site is not mutated, but is overexpressed can confer resistance (Délye et al. 2013b, Ghanizadeh and Harrington 2017a); in that case, herbicides are unable to inhibit increased numbers of the target site sufficiently to cause plant death.

Non-target site resistance can be conferred by a variety of mechanisms; reduced penetrance, compartmentalization, metabolism, root exudation (Délye et al. 2013b, Gaines et al. 2020, Ghanizadeh and Harrington 2017a). An *Amaranthus palmeri* population in Kansas has evolved metabolic resistance to atrazine, in that population, atrazine is conjugated with glutathione by the enzyme glutathione-S-transferase at 24× the rate of a local susceptible population (Nakka et al. 2017). Transgenerational epigenetic inheritance of herbicide resistance may be another mechanism (Markus et al. 2018). Plants can be viewed as holobionts, with microbial and fungal endophytes assisting metabolism, stress responses and reproduction (Caradus and Johnson 2020, Cheplick 2018, Roughgarden et al. 2018); microbial or fungal endophytes have potential to be involved in weed herbicide resistance (Tétard-Jones and Edwards 2016, Vila-Aiub et al. 2003).

1.05 A history of maize weed research

There is a long history of research on maize weeds in New Zealand, particularly in Waikato and Gisborne. As mentioned, most maize was grown in Gisborne and Hawkes Bay before the 1960s. In the 1940s, some of the earliest herbicides (the synthetic auxins MCPA and 2,4-D) were introduced to agriculture (Kudsk and Streibig 2003). *Chenopodium album, Amaranthus powelli* (recorded as *Amaranthus retroflexus)*, *Solanum nigrum, Echinochloa crus-galli* and *Nicandra physalodes* were major weeds in 1950s Gisborne maize (McKee 1955). Widespread 2,4-D usage was able to remove many broadleaf weeds but not grass weeds (Rahman 1985, Woon 1970). Triazine herbicides became available in the 1950s (Kudsk and Streibig 2003), and weeds in 1960s Waikato maize were being controlled by atrazine. *Amaranthus powelli, Chenopodium album* and *Persicaria maculosa* were common broadleaf weeds and annual grasses *Digitaria sanguinalis, Panicum capillare* and *Panicum dichotomiflorum* were beginning to become common (Patterson 1961, Thompson 1962). Grass weeds continued to be problematic in the 1970s particularly as atrazine was not effective on *D. sanguinalis,* *Panicum* spp. and *Setaria pumila* thought it did control broadleaf weeds and *E. crus-galli* (Patterson 1971, Woon 1971). Substituted urea, thiocarbamates, chloroacetamide and dinitroaniline herbicides were developed to control the grass weeds (Capper 1975, Cumberland et al. 1970, Kitchener 1971, Rahman 1985, Rahman and James 1992, Rowe et al. 1976, Sumich 1963). Common Waikato grass weeds were *Digitaria sanguinalis*, *Panicum dichotomiflorum* and *Echinochloa crus-galli* (Rahman et al. 1983b).

Glyphosate became used to control weeds before planting maize (Bishop and Field 1987, Rahman 1985, Rahman et al. 1983b). Waikato maize hectarage grew. Overusage of the thiocarbamate EPTC + antidote appeared to select for microbial resistance, as the herbicide dissipated from soils with histories of use (Rahman and James 1983). Then, the first cases of resistant weeds emerged. *Chenopodium album* and *Persicaria maculosa* populations evolved herbicide resistance toward atrazine (Rahman et al. 1983a, Rahman and Patterson 1987). Resistant *Chenopodium album* could survive up to 60× more atrazine than susceptible populations (Rahman et al. 1983c), and resistant *Persicaria maculosa* up-to 10× more than susceptible populations (Rahman and Patterson 1987). Resistant *Chenopodium album* was believed to be in almost all maize crops, but resistant *P. maculosa* was not thought to have spread widely (Rahman 1990 p. 199). Other herbicides were available, and effective on those resistant weeds, like the synthetic auxin dicamba.

A list of the most common maize weeds was published in 1985 (Rahman 1985). The sulfonylurea nicosulfuron started to be used in 1990s to control some annual and perennial grass weeds as well as broadleaf weeds (James et al. 2006b, James and Rahman 1997, Rahman and James 1993a). Seedbank studies in 1997 at 15 sites, and again in 1999-2002 at 30 sites recorded the following weeds as common: *C. album, P. maculosa, S. nigrum, D. sanguinalis, P. dichotomiflorum* (Rahman et al. 1997, 2006). New herbicides entered the local market in the 2000s, a triketone herbicide mesotrione, a pyrazol herbicide topramezone and a pyrimidinedione herbicide saflufenacil (James et al. 2006a, Rahman et al. 2014a, Trolove et al. 2011). A trial showed evidence that atrazine-resistant *C. album* was present in the Bay of Plenty and Manawatu (James et al. 2007).

Chenopodium album populations in eastern Waikato evolved resistance to dicamba in the 2000s (James et al. 2005). The original site had had dicamba applied for twenty years to treat atrazine resistant *C. album*; at the time it was estimated 100 hectares were affected (James et al. 2005). *Setaria pumila* rapidly grew in distribution (Lamoureaux 2014). The quarantine weed *Abutilon theophrasti* was unintentionally introduced to several maize fields (James and Cooper 2012). Soils with a histories of repeated use of atrazine were shown to have increased degradation of atrazine (James et al. 2010c). The weeds *Oxalis latifolia* (Waikato), *Panicum milleaceum* (Gisborne), *Xanthium* *strumarium* (Bay of Plenty, Waikato) and *Cyperus rotundus* (Bay of Plenty) were identified as problem weeds more recently (James et al. 2010b, 2016, James and Cooper 2012, Rahman et al. 1998b, 2002). The most recent major weed problem in maize is the evolution of nicosulfuron resistance in some *D. sanguinalis* populations in Bay of Plenty and Waikato (Buddenhagen et al. 2021a, Harrington and James, T.K. 2022, Hutching 2017). The resistant population first identified was 2× more resistant than a susceptible population, surviving the label rate (60g.ai.ha^{-1}) (Harrington and James, T.K. 2022).

1.06 Herbicide resistance in New Zealand

The first weed to evolve herbicide resistance in New Zealand was *C. album* to atrazine in Waikato maize (Ghanizadeh and Harrington 2019b, Rahman et al. 1983c). Since then, another three cases of herbicide resistance have occurred in maize: *P. maculosa* to atrazine in Waikato, *C. album* to dicamba in Waikato and *Digitaria sanguinalis* to nicosulfuron in Bay of Plenty and Waikato (Buddenhagen et al. 2021a, James et al. 2005, Rahman and Patterson 1987). Twenty-five cases of resistance had occurred between 1983 and 2019: two in turf, six in horticultural crops, five in pasture and nine in other arable crops (Ghanizadeh and Harrington 2019b). Here, a 'case' is evolution of resistance for one mode-of-action for one species; for instance there are two *C. album* 'cases'.

Two thistles evolved herbicide resistance in pastures to synthetic auxins: *Carduus nutans* in Hawkes Bay and Waikato (Harrington 1989, Harrington and Popay 1987) and *Carduus pycnocephalus* in Hawkes Bay (Harrington and Hewage 1997). The buttercup *Ranunculus acris* evolved synthetic auxin resistance in Golden Bay and Canterbury (Bourdôt and Hurrell 1988), then additional resistance to AHAS-inhibitors in Golden Bay (Lusk et al. 2015). Chilean needle grass (*Nassella neesiana*) in the Hawkes Bay evolved resistance to dalapon (Hartley 1994). Two nightshades (*Solanum americanum*, *Solanum nigrum*) evolved resistance to paraquat in Northland kūmara crops (Lewthwaite and Triggs 2009). Ryegrasses (*Lolium multiflorum* and *Lolium perenne*) in Marlborough and Nelson vineyards became resistant to glyphosate (Ghanizadeh et al. 2013) and cross-resistance to glufosinate and amitrole (Ghanizadeh et al. 2015a). Ryegrasses also evolved resistance in Canterbury cereals to ACCase-inhibitors and AHAS-inhibitors (Gunnarsson et al. 2017). *Avena fatua* became resistant to ACCase-inhibitors in Canterbury cereals (Harrington and Chynoweth 2014), *Stellaria media* to AHAS-inhibitors in Southland cereals (Seefeldt et al. 2001) and *Solanum nigrum* to triazines in Manawatu peas (Harrington et al. 2001). Two resistant weeds have been found at golf courses, synthetic auxin resistant *Soliva sessilis* in Auckland (Ghanizadeh et al.

2021, Harrington et al. 2001), and haloxyfop resistant *Poa annua* in Taranaki (Ghanizadeh et al. 2020).

A survey of cereal farms found another four cases in grass weeds (*Avena fatua* to pyroxsulam, *Bromus catharticus* to pyroxsulam, *Phalaris minor* to iodosulfuron and clodinafop) and two cases for broadleaf weeds (*Sonchus asper* to iodosulfuron, *Sonchus oleraceus* to iodosulfuron; (Buddenhagen et al. 2021a). Four more cases were confirmed for samples sent by agronomists and farmers suspected of resistance: *Poa annua* to iodosulfuron in Southland cereals, *Lolium multiflorum* to glyphosate in Canterbury barley and *Stellaria media* to flumetsulam in Waikato and Canterbury ryegrass pastures (Buddenhagen et al. 2021a). To date, this brings the total to thirty-five 'cases', but more weeds suspected of resistance are being investigated and field surveys of maize, cereals and vineyards are being done.

The extent of farms affected by herbicide resistance varies. Some herbicide resistant weeds are widespread, like *C. album* in maize (Rahman 1990), and *Lolium* spp. in cereals and vineyards (Buddenhagen et al. 2021a, Ghanizadeh et al. 2015a). Others, like *Persicaria maculosa* in maize are believed to not have spread far (Rahman 1990). Newer cases like *Poa annua* in Southland and *Digitaria sanguinalis* in Waikato (Buddenhagen et al. 2021a), have not had their prevalence studied yet, and may or may not be already widespread.

1.07 Herbicide resistance in maize weeds in New Zealand

Chenopodium album, *Persicaria maculosa* and *Digitaria sanguinalis* have evolved resistance in maize.

Chenopodium album initially was detected with atrazine resistance in farms between Otorohanga-Ohaupo in southern Waikato (Rahman et al. 1983c). This first case of resistance in New Zealand arose from years of repeated atrazine usage, which selected for *C. album* able to tolerate 60× higher doses of atrazine than susceptible populations. Atrazine-dicamba resistant *C. album* are cross-resistant to other triazines, but not bromoxynil (Rahman et al. 2014b), which is in a different sub-group of photosystem-II inhibitor herbicides (HRAC 2022). Atrazine-resistant *C. album* had reduced fitness compared to susceptible populations (Rahman 1990). It is not known what the mechanism for atrazine resistance in these populations is, but it is believed that a mutation of the target site gene (*psb*A) is responsible (Ghanizadeh and Harrington 2019b). The *psbA* gene is encoded in the chloroplast, so target site resistance to atrazine is maternally inherited (Murphy and Tranel 2019). The specific mutation Ser-264-Gly is believed to be the one present in atrazine resistant *C. album* as it is the most common, and known to have fitness costs, as observed (Ghanizadeh and Harrington 2019a, 2019b). It is believed that atrazine-resistant *C. album* is present in almost all maize fields in New Zealand (Harrington and James 2005, James et al. 2005), though there is limited published evidence of this outside of Waikato (James et al. 2007).

In 2003, atrazine-resistant *C. album* populations near Morrinsville, Waikato evolved dicamba resistance (James et al. 2005). Dicamba-resistant *C. album* are 19× more resistant than susceptible populations (Ghanizadeh et al. 2015b). Those atrazine-dicamba multiple resistant *C. album* were morphologically distinct, with lighter-coloured and smoother-margined leaves (James et al. 2005). Those populations were also resistant to atrazine, and later studies showed that there was cross-resistance to pyridine carboxylic acid synthetic auxins (clopyralid, aminopyralid) but not phenoxy acid synthetic auxins (2,4-D, mecoprop; Ghanizadeh and Harrington 2017b, Rahman et al. 2014b). A significant fitness cost was imposed on the plants because of their evolved multiple resistance (Ghanizadeh and Harrington 2019a). Dicamba resistance in *C. album* populations was investigated for non-target site mechanisms, but none were confirmed (Ghanizadeh et al. 2018). Target site resistance had not been reported for weeds resistant to synthetic auxins before 2018, when a *Kochia scoparia* population was confirmed to have a Gly-73-Asn mutation in the *KsIAA16* gene (LeClere et al. 2018). Dicamba resistant *C. album* is currently only known to be present in eastern Waikato farms.

Persicaria maculosa evolved atrazine resistance in a farm in Te Kowhai, Waikato in the 1980s (Rahman and Patterson 1987). The population could survive 10× the dosage of atrazine compared to a susceptible population. No further research came out on this biotype, and it was assumed to not have spread far (Harrington and James 2005, Rahman 1990). Unrelated, *Persicaria maculosa* populations in Southland cereals are believed to have evolved resistance to AHAS inhibitors (FAR 2018). The prevalence of atrazine resistant *Persicaria maculosa* is unknown.

Digitaria sanginalis first was reported resistant to nicosulfuron in 2017 (Harrington and James, T.K. 2022, Hutching 2017). This resistant biotype was collected from the western Bay of Plenty, in a paddock cropped annually for six years using nicosulfuron (Hutching 2017) Studies revealed this biotype was 2× resistant compared to a susceptible population (Harrington and James, T.K. 2022). Two years after, two farms in the eastern Waikato submitted *D. sanguinalis* samples for herbicide resistance testing, which was confirmed true (Buddenhagen et al. 2021a). The prevalence of nicosulfuron resistant *D. sanguinalis* is unknown.

1.08 Risk of herbicide resistance

Weeds that have evolved resistance in maize in New Zealand have also evolved resistance in other parts of the world. A global database of herbicide resistant weeds provides insights onto the frequency of evolved resistance in weeds (Heap 2014 p. 2014). The repetitive evolution of herbicide resistance has not been equal, with some weeds evolving herbicide resistance more than others (Heap 2014, Holt et al. 2013). Particular traits are believed to make plants weedy, 'Baker's ideal weed' would have seed dormancy, high seed longevity, unspecialized pollination, the ability to self-pollinate and high seed output (Baker 1965). Trait-based weed assessments have become commonplace when assessing plants for introduction, or environmental weeds for risk (Gordon et al. 2016, Hulme 2012). Similarly, certain traits are believed to increase the likelihood of weeds evolving herbicide resistance: weeds with higher 'mutational target size' (genetic material able to confer resistance when altered by mutation) are believed likely to evolve resistance more often (Kreiner et al. 2018). Possibly the largest factor of a particular weeds risk of evolving resistance is it's abundance; the chance of a resistance conferring mutation (*de novo* or standing variation) being selected for increases with population size (Délye et al. 2013b).

Chenopodium album has fifty-two confirmed resistance cases, *Persicaria maculosa* has five and *Digitaria sanguinalis* has thirteen (Heap 2022). There are forty-five documented cases of *Chenopodium album* with photosystem-II inhibitor resistance, six with AHAS-inhibitor resistance and one with dicamba resistance (that being the one in Waikato maize; Heap 2021). *Digitaria sanguinalis* has three cases of atrazine resistance (although atrazine rates in New Zealand do not control *D. sanguinalis*), seven cases of ACC-ase inhibitor resistance, three cases of AHAS inhibitor resistance. *Persicaria maculosa* has evolved atrazine resistance four times, in the 1980s in New Zealand maize, French maize, Czech railways, and in the 2000s in US blueberries (Barralis et al. 1979, Heap 2022, Mikulka and Chodová 2000, Rahman and Patterson 1987). It has evolved AHAS-inhibitor resistance in Norwegian cereals (Heap 2022). The related species *Persicaria lapathifolia* has evolved atrazine resistance in French, German and Spanish maize and Czech railways (Barralis et al. 1979, Deprado et al. 1995, Heap 2022, Mikulka et al. 1988, Mikulka and Chodová 2000). It also has evolved AHAS-inhibitor resistance in Canada (Heap 2022). Other common weeds in maize *Echinochloa crus-galli* (51), *Panicum dichotomiflorum* (1) and *Solanum nigrum* (14) have evolved resistance elsewhere, but not in New Zealand (Heap 2022).

Between these weeds, most cases are in photosystem-II inhibitors. These are all globally distributed weeds of maize, so it is logical that they would have repeatedly evolved resistance to one of the most widely used maize herbicides. Atrazine itself has the highest number of resistant weeds (Heap 2022). The herbicide groups with the most species resistant are AHAS-inhibitors and photosystem-II inhibitors (Heap 2014, 2022). Knowledge from this global database (Heap 2022), is used to assign risk to weeds and herbicides for their risk of herbicide resistance evolving in European risk assessments (EPPO 2015, Moss et al. 2019). These risk assessments considered the risk of a herbicide mode of action, risk of a weed species and risk of management style (EPPO 2015, Moss et al. 2019). Species 'inherent' risk was based on the number of times that a weed had evolved resistance, but was believed to be explained by the biology and genetics of weeds (Moss et al. 2019).

A risk assessment of weeds in cereal crops in Canterbury was done by adapting this method to score a list weeds present (Ngow et al. 2020). That study showed *Poa annua, Lolium* spp., *Erigeron* spp., *Raphanus raphanistrum, Avena* spp., *D. sanguinalis* and *Stellaria media* to be the ten highest risk weeds (Ngow et al. 2020). A recent herbicide resistance survey in Canterbury cereals revealed herbicide resistance to be common for *Lolium* spp. and *Avena fatua* (Buddenhagen et al. 2021a). Other weeds *Bromus catharticus, Phalaris minor* and *Sonchus* spp. ranked moderate-high risk (Ngow et al. 2020), and were found to be herbicide resistant (Buddenhagen et al. 2021a). The most frequent herbicide group with weeds detected resistant to were AHAS-inhibitors (Buddenhagen et al. 2021a), which was assessed to be high risk (Ngow et al. 2020). A similar risk assessment could be done for maize weeds.

1.09 Surveying for resistance

Herbicide resistance surveys show the extent of resistant weeds. Prevention of the spread of resistant weeds is only possible when it is known where the resistant weed is. While there had been many herbicide resistance studies, no randomized survey had been done before (Buddenhagen et al. 2021a). Now, there are surveys in maize (this study), vineyards and cereals.

Past research in maize weed resistance investigates the issues of identifying the level of resistance (James et al. 2005, Rahman et al. 1983a, Rahman and Patterson 1987), effective alternatives (Rahman et al. 1983c, 2014b), (often) the mechanism of resistance (Ghanizadeh et al. 2018), but not the distribution of resistance. While past studies have investigated farms local to initial cases of resistance (James et al. 2005, Rahman et al. 2008), there has been no systematic survey for resistance. For *C. album*, it is believed atrazine resistant populations were in almost all maize farms in New Zealand (James et al. 2005). Studies of dicamba resistant *C. album* were done in a small amount of farms around Matamata (Ghanizadeh and Harrington 2017b, James et al. 2005, Rahman et al. 2008, 2014b), but the extent of this biotype is unknown.

In Western Australia, a 1998 survey of arable fields found widespread resistance of the grass weed *Lolium rigidum* Gaud. toward acetyl-CoA-carboxylase (46%) and acetohydroxyacid synthase inhibitor herbicides (64%) (Llewellyn and Powles 2001). This increased in the next survey, *L. rigidum* resistant to ACCase (68%), AHAS inhibitor herbicides (88%) and <1% of farms with glyphosate-resistant *L. rigidum* (Owen et al. 2007). A 2010 survey revealed cosmopolitan resistance to ACCase (96%) and AHAS (98%) inhibitor herbicides, but also widespread resistance to trufluralin (27%) and to clethodim (65%), which is in a different subgroup to other ACCase herbicides (Owen et al. 2014). In the 2010 survey glyphosate resistance was rare (7% of farms). A 2015 survey has shown no major changes, with widespread resistance to ACCase (96%) and AHAS inhibitor herbicides (99%), trifluralin resistance (30%) and uncommon glyphosate resistance (8%), though it appears some populations are becoming resistant to atrazine (2%) and prosulfucarb + s-metalachlor (11%) (GRDC 2020). It is clear through this repeated monitoring, that herbicide resistance has become increasingly widespread, and multiple groups of herbicides are now no longer effective on *L. rigidum* in Western Australia. A similar effort to this monitoring of herbicide resistance in Western Australia could be applied to crops in New Zealand with a high risk of having herbicide resistant weeds.

A study of the costs of surveying New Zealand for herbicide resistant weeds was published to optimize the future herbicide resistance surveys (Buddenhagen et al. 2019). Owing to their intensive use of herbicides, arable (including maize) farms are some of the highest risk land use types for developing herbicide resistant weeds. Vineyards have relied on glyphosate to control weeds, which has resulted in glyphosate resistant *Lolium* spp. (Ghanizadeh et al. 2016). Glufosinate and ACCase inhibitors haloxyfop and clethodim are used on resistant *Lolium* spp. in those vineyards. Fruit orchards use glyphosate, amitrole, glufosinate but also residual herbicides especially triazines (Harrington et al. 1992). Both vineyards and fruit orchards should have a high likelihood of containing herbicide resistant weeds. Dairy farms utilize synthetic auxins and AHAS-inhibitors to control broadleaf weeds, which have become resistant, similarly, thistles in sheep and beef farms have developed resistance (Ghanizadeh and Harrington 2019c). The land areas of those pastoral farms are often immense and are not feasible to sample intensively. Vegetable farms are not believed as likely to have resistant weeds. Herbicides are used in other non-agricultural settings such as turf, roadsides, railways and waste areas, where resistant weeds could be present. Due to the large number of arable farms, Canterbury and Waikato are the most important regions to survey for arable weed resistance (Buddenhagen et al. 2019).

Recently Canterbury cereal farms were surveyed for herbicide resistance (Buddenhagen et al. 2021a). Widespread resistance for *Lolium* spp. toward ACCase (40%) and AHAS (23%) inhibitor herbicides were detected. Other weeds were found with resistance in that survey, including *Avena fatua* L. (ACCase, AHAS), *Bromus catharticus* Vahl (AHAS), *Phalaris minor* Retz. (ACCase, AHAS) and *Sonchus* spp. (AHAS). Samples sent from agronomists and growers suspected of resistance were included in that study and are occasionally being sent to the Ruakura facility for testing. Several nicosulfuron resistant *Digitaria sanguinalis* had been sent in from eastern Waikato maize farms (Buddenhagen et al. 2021a), warranting further research into how widespread resistant *D. sanguinalis* is.

1.10 Thesis objectives

In this study the objectives were to:

- Determine which weeds of maize in New Zealand are most likely to develop resistance.
- Carry out weed surveys and seed bank studies in randomly selected farms in two major maize producing regions (Waikato and Bay of Plenty) to determine which weeds are important for maize growers.
- Determine the regional prevalence of herbicide resistant weeds on maize farms in those regions by testing seed collected from weeds that have survived control measures.

These objectives were addressed in Chapter 2: A herbicide risk assessment for weeds in maize in New Zealand, Chapter 3: Weeds in maize in Bay of Plenty and Waikato and Chapter 4: Prevalence of herbicide resistant weeds in maize in Bay of Plenty and Waikato. Chapter 5 discusses the implications of the results of the previous chapters.

Preface to Chapter 2

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A herbicide resistance risk assessment for weeds in maize in New Zealand

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Abstract Despite an extensive history of research into herbicide resistance in New Zealand maize, some aspects remain understudied. Herbicide resistance was first detected in New Zealand in the 1980s in maize crops, with atrazine resistance in *Chenopodium album* L. and *Persicaria maculosa* Gray. Since then, *Chenopodium album* has also developed resistance to dicamba, and in the last five years *Digitaria sanguinalis* (L.) Scop. populations have been reported to be resistant to nicosulfuron. Here we estimate the risk of herbicide resistance arising in 39 common maize weeds. A list of weeds associated with maize was generated, omitting uncommon weeds and those that grow outside of the maize growing season. Weeds were ranked for their risk of evolving herbicide resistance with a scoring protocol that accounts for the specific herbicides used in New Zealand maize. Seven weed species were classified as having a high risk of developing herbicide resistance: *Echinochloa crus-galli* (L.) P.Beauv., *Chenopodium album*, *Eleusine indica* (L.) Gaertn., *Xanthium strumarium* L., *Amaranthus powellii* S.Watson, *Solanum nigrum* L. and *Digitaria sanguinalis.* Seventeen species were classed as moderate risk, and 15 were low risk. Herbicide classes associated with more resistant species were classed as high risk,these included acetohydroxy acid synthase inhibitors and photosystem-II inhibitors. Synthetic auxins had a moderate risk but only two herbicides in this class (dicamba and clopyralid) are registered for maize in New Zealand. Other herbicide mode-of-action groups used in maize were low risk. We recommend outreach to farmers regarding weed-control strategies that prevent high-risk species from developing resistance. High-risk herbicide groups should be monitored for losses of efficacy. Resistance surveys should focus on these species and herbicides.

Keywords *Zea mays*, silage, agricultural chemicals, summer weeds

INTRODUCTION

Maize (*Zea mays* L.) is a major crop in New Zealand, principally grown for livestock feed as silage or grain (Booker 2009). In 2018, sales of grain and silage maize were estimated to be $267 million (Robertson & Hurren 2019). Approximately 58,000 hectares produced 1.2 million tonnes of dry matter of silage, and 15,000 hectares of maize were harvested to produce 180 thousand tonnes of grain in 2020 (AIMI 2020). Maize is grown primarily in the warmer parts of New Zealand, with most of the production in Waikato. Yields have steadily increased over the last 25 years (Morris et al. 2016) and are among the highest in the world (FAO 2021). In maize, weeds are estimated to have the potential to cause yield losses up to 40%, which is more than the sum of the potential yield loss caused by pests and diseases (Oerke 2006); thus, weed control offers the most cost-effective plant-protection measure. Early-season weed control is critical for the establishment and growth of young plants (Page et al. 2012). To control weeds, a standard recommended herbicide programme (see: FAR 2020) would commonly use a pre-emergent residual herbicide (e.g., acetochlor, pendimethalin, saflufenacil, atrazine) followed by a post-emergent herbicide (e.g., nicosulfuron, topramezone, mesotrione, atrazine). Details of registered herbicides are provided in Table 1. Two synthetic auxin herbicides dicamba and clopyralid are used specifically for broadleaf weeds. Glyphosate was not classed as a maize herbicide in our study, as glyphosate-tolerant maize cultivars are not grown in New Zealand. In silage maize, there is usually a winter rotation (e.g., sowing of *Lolium multiflorum* Lam. or *Avena sativa* L.) that needs to be terminated with glyphosate prior to planting. Following a grain-maize crop, land will usually be left fallow over winter, but weeds are usually controlled similarly with an application of glyphosate pre-planting. Soil is typically cultivated pre-planting but strip-till and no-till techniques are increasingly being adopted. Herbicides are the most widely used method of weed control, but some are becoming less effective with the evolution of herbicide-resistant weeds.

Resistance-conferring mutations arise rarely and selection occurs instantaneously on sprayed weeds; with successive generations and treatments plants with resistance-conferring mutations preferentially reproduce

Table 1 Herbicides registered for use in maize crops in New Zealand, adapted from Harrington and James (2020). Herbicide modes of action are grouped by their Herbicide Resistance Action Committee (HRAC) classification.

HRAC Group	Mode of Action	Chemical group	Active ingredient	Application
2	AHAS Inhibitor	triazolopyrimidine	nicosulfuron	post
		sulfonylurea	halosulfuron	post
			flumetsulam	post
5	PS-II inhibitor	triazine	atrazine	pre/post
			cyanazine	pre/post
			terbuthylazine	pre/post
5		triazinone	metribuzin	pre/post
5		substituted urea	linuron	pre/post
5		nitrile	bromoxynil	post
14	PPO inhibitor	pyrimidinedione	saflufenacil	pre
27	HPPD inhibitor	triketone	mesotrione	pre/post
		pyrazole	topramezone	post
3	microtubule assembly inhibitor	dinitroaniline	pendimethalin	pre
15	VLCFA synthesis inhibitor	chloroacetamide	acetochlor	pre
			alachlor	pre
			dimethenamid-P	pre
			s-metolachlor	pre
			propachlor	pre
4	synthetic auxin	benzoic	dicamba	post
4		pyridine	clopyralid	post

to become increasingly dominant in the population (Délye et al. 2013). Globally there are 61 herbicide resistant weed species in maize with 323 unique cases (species × mode-of-action; Heap 2021); meaning that 20% of all herbicide resistance cases have been documented in maize. Three species have developed resistance in New Zealand maize: *Persicaria maculosa* Gray, *Chenopodium album* L. and *Digitaria sanguinalis* (L.) Scop. (Ghanizadeh & Harrington 2019; Buddenhagen et al. 2020, 2021). Atrazine-resistant *Chenopodium album* was the first case of herbicide resistance detected in New Zealand (Rahman et al. 1983). *Persicaria maculosa* was next reported resistant to atrazine (Rahman & Patterson 1987). Triazine-resistant *Chenopodium album* populations in Waikato later also evolved resistance to dicamba (James et al. 2005). Reports of nicosulfuron-resistant *Digitaria sanguinalis* have been confirmed recently in Waikato and Bay of Plenty (Buddenhagen et al. 2021). Other weed species present in maize have become resistant in other crops, for example *Solanum nigrum* L. in peas in Manawatu (Harrington et al. 2001). Despite a long history of research, there has not been a systematic herbicide-resistance survey in maize, so the prevalence of resistant weeds in New Zealand maize farms is not well-known. Certain weed species appear to evolve resistance more often than others (Holt et al. 2013), and within some herbicide modes of action than others (Heap 2014). Many weed species present in maize crops in New Zealand are well represented in a global database of herbicide-resistant weeds (Heap 2021). We use these data and a previously developed protocol (Ngow et al. 2020) to estimate the risk that maize weeds in New Zealand will evolve herbicide resistance.

MATERIALS AND METHODS

Species list

A list of weed species was constructed, based primarily on an earlier list compiled by Rahman (1985), and cross validated against more recent sources (e.g., (James et al. 2006, 2015; Rahman et al. 2006) including grey literature (e.g., Foundation for Arable Research, 2013). Only weeds growing primarily in the summer season were included, and cool-season weeds like C_3 grasses (e.g., *Bromus catharticus* Vahl and *Lolium* spp.), and primarily winter- and spring-active broadleaf species (e.g., *Stellaria media* (L.) Vill., *Veronica* spp. and *Lepidium didymum* L.) were excluded. Also excluded are uncommon weeds (e.g., *Amaranthus viridis* L.) and "first-year-out-of-pasture" weeds (e.g., *Ranunculus acris* L.) but the high priority regulated weeds *Abutilon theophrasti* Medik. and *Xanthium strumarium* L. are included. Nomenclature follows the Flora of New Zealand Online (Breitwieser et al. 2010), and authorities and scientific names are listed in Table 2.

Table 2. List of 39 species identified as being occurring in New Zealand maize crops.

No.	Species	Common Name[1]	Family	Growth Form	Citation
1	*Abutilon theophrasti* Medik.	velvetleaf	Malvaceae	annual forb	(James & Pene 2018)
2	*Amaranthus blitum* ssp *oleraceus* (L.) Costea	purple amaranth	Amaranthaceae	annual forb	Author observation
3	*Amaranthus powellii* S.Watson	redroot	Amaranthaceae	annual forb	(Rahman 1985)
4	*Bidens frondosa* L.	beggars' ticks	Asteraceae	annual forb	(James et al. 2015)
5	*Calystegia sepium* ssp *roseata* Brummitt	pink bindweed	Convulvulaceae	perennial vine	(James et al. 2006)
6	*Calystegia silvatica* ssp *disjuncta* Brummitt	great bindweed	Convulvulaceae	perennial vine	(Rahman 1985)
7	*Chenopodium album* L.	fathen	Amaranthaceae	annual forb	(Rahman 1985)
8	*Cirsium arvense* (L.) Scop.	California thistle	Asteraceae	perennial forb	(Rahman 1985)
9	*Conium maculatum* L.	hemlock	Apiaceae	annual/biennual forb	(James et al. 2007)
10	*Cynodon dactylon* (L.) Pers.	Indian doab	Poaceae	perennial graminoid	(Rahman 1985)
11	*Cyperus rotundus* L.	purple nutsedge	Cyperaceae	perennial graminoid	(Rahman et al. 1998)
12	*Datura stramonium* L.	thorn apple	Solanaceae	annual forb	(Rahman 1985)
13	*Digitaria sanguinalis* (L.) Scop.	summer grass	Poaceae	annual graminoid	(Rahman 1985)
14	*Echinochloa crus-galli* (L.) P.Beauv.	barnyard grass	Poaceae	annual graminoid	(Rahman 1985)
15	*Eleusine indica* (L.) Gaertn.	crowfoot grass	Poaceae	annual graminoid	(Rahman 1985)
16	*Elytrigia repens* (L.) Nevski	couch/twitch	Poaceae	perennial graminoid	(Rahman 1985)
17	*Fallopia convolvulus* (L.) Á.Löve	cornbind	Polygonaceae	annual vine	(FAR, 2013)
18	*Galinsoga parviflora* Cav.	galinsoga	Asteraceae	annual forb	Author observation
19	*Malva parviflora* L.	small-flowered mallow	Malvaceae	annual forb	(Rahman et al. 2006)
20	*Modiola carioliana* (L.) G.Don	creeping mallow	Malvaceae	annual/perennial forb	Author observation
21	*Nicandra physalodes* (L.) Gaertn.	apple of Peru	Solanaceae	annual forb	(James et al. 2007)
22	*Oxalis latifolia* Kunth[2]	fishtail oxalis and pink shamrock[2]	Oxalidaceae	perennial forb	(FAR, 2013)
23	*Panicum capillare* L.	witchgrass	Poaceae	annual graminoid	(FAR, 2013)
24	*Panicum dichotomiflorum* Michx.	smooth witchgrass	Poaceae	annual graminoid	(Rahman 1985)
25	*Panicum miliaceum* L.	broomcorn millet	Poaceae	annual graminoid	(James et al. 2010)
26	*Paspalum distichum* L.	Mercer grass	Poaceae	perennial graminoid	(Rahman 1985)
27	*Persicaria hydropiper* (L.) Spach	waterpepper	Polygonaceae	annual forb	(Rahman 1985)
28	*Persicaria lapathifolia* (L.) Gray	pale willow weed	Polygonaceae	annual forb	Author observation
29	*Persicaria maculosa* Gray	willow weed	Polygonaceae	annual forb	(Rahman 1985)
30	*Polygonum aviculare* L.	wireweed	Polygonaceae	annual forb	(Rahman et al. 2004)
31	*Portulaca oleracea* L.	purslane	Portulacaceae	annual forb	(Rahman et al. 2004)
32	*Rumex obtusifolius* L.	broadleaf dock	Polygonaceae	perennial forb	(Rahman 1985)
33	*Rumex pulcher* L.	fiddle dock	Polygonaceae	perennial forb	Author observation
34	*Setaria pumila* (Poir.) Roem. & Schult.	yellow bristle grass	Poaceae	annual graminoid	(James et al. 2015)
35	*Setaria verticillata* (L.) P.Beauv.	rough bristle grass	Poaceae	annual graminoid	(Rahman 1985)
36	*Solanum americanum* Mill.	small-flowered nightshade	Solanaceae	annual forb	Author observation
37	*Solanum nigrum* L.	black nightshade	Solanaceae	annual forb	(Rahman 1985)
38	*Xanthium spinosum* L.	Bathurst bur	Asteraceae	annual forb	Author observation
39	*Xanthium strumarium* L.	Noogoora bur	Asteraceae	annual forb	(Rahman 1985)

[1] Common names obtained from Nicol 1997.

[2] The common name "pink shamrock" is associated with the species *Oxalis vallicola* (Rose) R.Knuth and is listed as a synonym of *Oxalis latifolia* Kunth in the *Flora of New Zealand Online*, however the name *Oxalis vallicola* is used in some sources, e.g. https://www.massey.ac.nz/massey/learning/colleges/college-of-sciences/clinics-and-services/weeds-database/pink-shamrock.cfm

Ranking herbicide groups and species by resistance cases

The method of ranking herbicide groups and weed species has been described previously (Ngow et al. 2020), and is adapted from previous herbicide resistance risk assessment tools (EPPO, 2015; Moss et al. 2019). The analyses rely on data from the International Survey for Herbicide Resistant Weeds (Heap 2021) and the Herbicide Resistance Action Committee system (HRAC 2021) for classifying modes of action for individual herbicide active ingredients. The method accounts for not only the level of risk for each mode-of-action based on the number of species with resistance worldwide but also for weed species based on the number of documented cases of evolved resistance in each weed species. The herbicide mode-of-action risk scoring system and the risk scores were the same as those of Ngow et al. (2020). The species-risk scores were determined using the number of resistance cases from the International Survey of Herbicide Resistant Weeds (Heap 2021); here, cases were defined as a record of herbicide resistance for a weed species to any mode of action. Risk scores were designated as ≥10 cases for high risk (score = 3), < 10 for moderate (score = 2) and no cases recorded as low risk (score = 1).

As an example, there are 13 cases of herbicide resistance for *Digitaria sanguinalis* across three modes of action, for a high species risk score of 3 (Table 3). The herbicide-risk scores for HRAC herbicide groups in which *Digitaria sanguinalis* has evolved resistance were then examined. There are cases in two herbicide modes of action used in maize, acetohydroxyacid synthase inhibitors (HRAC group 2; AHAS) and photosystem-II inhibitors (HRAC group 3; PS-II), each with >9% of all resistance cases so the risk score here is also 3. There are also seven cases of *Digitaria sanguinalis* becoming resistant to acetyl-coenzyme-A carboxylase inhibitors (HRAC group 1; ACCase) – a herbicide group that presents a high risk of resistance. However, that herbicide group is not used in maize so it was omitted from the maize total. The two different herbicide risk scores were added, then multiplied by the species "inherent" risk to obtain a total species risk score ((3+3) × 3 = 18).

This total risk score incorporates the mode-of-action risk score for herbicides used in New Zealand maize (Table 1). The number of cases of herbicide resistance for each species that has at least one resistance case by mode of action is provided in Table 3. Of the total risk score, the risk component attributable to maize is shown as an orange bar based on those herbicides used in maize in New Zealand while the component of risk for herbicides not normally used in maize fields is shown as a black bar (Fig 1). Graphs were generated in ggplot2 and tidyverse, both packages in the R statistical platform (Wickham 2016; Wickham et al. 2019; R Core Team 2020).

RESULTS

We identified 39 weed species associated with maize in New Zealand (Table 2). Seven species were classified as high-risk (total cases > 10), in order of cases: *Echinochloa crus-galli* (L.) P.Beauv. (11 in maize and 63 total), *Chenopodium album* (36, 52), *Eleusine indica* (L.) Gaertn. (8, 44), *Xanthium strumarium* (1, 20), *Amaranthus powellii* S.Watson (6, 16), *Solanum nigrum* (8, 14) and *Digitaria sanguinalis* (3, 13) (Table 3). There are 17 moderate-risk species (<10 total cases > 0) including two *Panicum* spp., three *Persicaria* spp. and two *Setaria* spp. No cases of resistance were found for 15 species so these were classified as low risk. These species are: *Conium maculatum* L., *Bidens frondosa* L., *Xanthium spinosum* L., *Calystegia sepium* ssp *roseata* Brummitt, *Calystegia silvatica* ssp *disjuncta* Brummitt, *Cyperus rotundus* L., *Malva parviflora* L., *Oxalis latifolia* Kunth, *Panicum miliaceum* L., *Cynodon dactylon* (L.) Pers., *Paspalum distichum* L., *Elytrigia repens* (L.) Nevski, *Rumex pulcher* L. and *Nicandra physalodes* (L.) Gaertn. Of these low-risk species, eight are perennial species with principally vegetative reproduction (Table 2).

Cases of herbicide resistance for the listed species, for each mode of action (HRAC 2021) are shown in Table 3. Two herbicide mode of action groups are at high risk of selecting for weeds with resistance: acetohydroxyacid synthase inhibitors (group 2; e.g., nicosulfuron); and photosystem-II inhibitors (group 5; e.g., atrazine). A third group, synthetic auxins (group 4; e.g., dicamba) was classified as moderate risk. All other herbicide groups registered for maize in New Zealand are classified as low risk, including the commonly used herbicides acetochlor (group 15), pendimethalin (group 3), saflufenacil (group 14), topramezone (group 27) and mesotrione (group 27).

The weed species that generated the highest scores for potential herbicide resistance had globally documented cases of resistance to photosystem-II inhibitors (HRAC group 5; 114 cases) and/or acetohydroxyacid synthase inhibitors (group 2; 59 cases). Ten cases or less were reported for either microtubule assembly inhibitors (group 3) or synthetic auxins (group 4) and less than five cases for protoporphyrinogen oxidase inhibitors (group 14; PPO) or very long-chain fatty-acid synthesis inhibitors (group 15; VLCFA). None of the listed weeds were reported to have evolved resistance to hydroxyphenyl pyruvate dioxygenase inhibitors (group 27; HPPD).

The five weeds with the highest total risk scores are *Echinochloa crus-galli*, *Eleusine indica*, *Chenopodium album*, *Digitaria sanguinalis*, *Amaranthus powellii* (Figure 1). Two species with previous cases of resistance in New Zealand (*Chenopodium album* and *Digitaria sanguinalis*) are in the top five ranked weeds. *Solanum nigrum* and *Xanthium strumarium* were not placed high in the total risk ranking because many of the globally documented cases of resistance were to herbicides not used in maize. *Eleusine indica* and *Echinochloa crus-galli* had the highest risk of resistance against herbicides used in maize, but both also have many documented cases of evolved resistance to herbicides not used in maize.

DISCUSSION

The risk scores calculated here appear to have some predictive value, given that some high-risk weeds (*Chenopodium album*, *Solanum nigrum* and *Digitaria sanguinalis*) have already been found to be resistant in New Zealand in maize or other crops. A similar outcome

Table 3 Herbicide × weed species risk matrix for 24 of the 39 species listed in Table 2 where cases of resistance have been documented.

No.[1]	Species	Species Risk	Total number of cases of resistance of a weed species towards HRAC herbicide groups known to have resistance															Maize Cases	Global Cases	Total Risk Score
			HRAC herbicide group[2,3,4,5]: current numerical code/legacy letter code																	
			0/Z	1/A	2/B	3/K_1	4/O	5/C_1,C_2	9/G	10/H	13/F_4	14/E	15/K_3	22/D	27/F_2	29/L	34/F_3			
	Herbicide risk		1	3	**3**	**1**	**2**	**3**	3	1	1	**1**	**1**	2	**1**	1	1			
1	*Abutilon theophrasti*	2	0	0	**0**	**0**	**0**	**4***	0	0	0	**0**	**0**	0	**0**	0	0	4	4	6
2	*Amaranthus blitum* ssp. *oleraceus*	2	0	0	**1**	**0**	**0**	**2***	0	0	0	**0**	**0**	1	**0**	0	0	1	4	12
3	*Amaranthus powellii*	3	0	0	**7***	**0**	**0**	**9***	0	0	0	**0**	**0**	0	**0**	0	0	6	16	18
7	*Chenopodium album*	3	0	0	**6**	**0**	**1*⸙**	**45*⸙**	0	0	0	**0**	**0**	0	**0**	0	0	36	52	24
8	*Cirsium arvense*	2	0	0	**0**	**0**	**2**	**0**	0	0	0	**0**	**0**	0	**0**	0	0	0	2	4
12	*Datura stramonium*	2	0	0	**0**	**0**	**0**	**1***	0	0	0	**0**	**0**	0	**0**	0	0	1	1	6
13	*Digitaria sanguinalis*	3	0	7	**3*⸙**	**0**	**0**	**3***	0	0	0	**0**	**0**	0	**0**	0	0	3	13	18
14	*Echinochloa crus-galli*	3	2	13	**16***	**1**	**7**	**17***	1*	0	1	**0**	**3**	0	**0**	2	0	11	63	30
15	*Eleusine indica*	3	0	6*	**1**	**7**	**0**	**1**	15*	2	0	**2**	**0**	10*	**0**	0	0	8	44	24
17	*Fallopia convolvulus*	2	0	0	**2**	**0**	**0**	**2***	0	0	0	**0**	**0**	0	**0**	0	0	2	4	12
18	*Galinsoga parviflora*	2	0	0	**1**	**0**	**0**	**0**	0	0	0	**0**	**0**	0	**0**	0	0	0	1	6
23	*Panicum capillare*	2	0	0	**0**	**0**	**0**	**1***	0	0	0	**0**	**0**	0	**0**	0	0	1	1	6
24	*Panicum dichotomiflorum*	2	0	0	**0**	**0**	**0**	**1***	0	0	0	**0**	**0**	0	**0**	0	0	1	1	6
27	*Persicaria hydropiper*	2	0	0	**0**	**0**	**0**	**1***	0	0	0	**0**	**0**	0	**0**	0	0	1	1	6
28	*Persicaria lapathifolia*	2	0	0	**2**	**0**	**0**	**4***	0	0	0	**0**	**0**	0	**0**	0	0	3	6	12
29	*Persicaria maculosa*	2	0	0	**1**	**0**	**0**	**4*⸙**	0	0	0	**0**	**0**	0	**0**	0	0	2	5	12
30	*Polygonum aviculare*	2	0	0	**0**	**0**	**0**	**1***	0	0	0	**0**	**0**	0	**0**	0	1	1	2	6
31	*Portulaca oleracea*	2	0	0	**0**	**0**	**0**	**1**	0	0	0	**0**	**0**	0	**0**	0	0	0	1	6
33	*Rumex obtusifolius*	2	0	0	**1**	**0**	**0**	**0**	0	0	0	**0**	**0**	0	**0**	0	0	0	1	6
34	*Setaria pumila*	2	0	0	**1**	**0**	**0**	**4***	0	0	0	**0**	**0**	0	**0**	0	0	4	5	12
35	*Setaria verticillata*	2	0	0	**0**	**0**	**0**	**1***	0	0	0	**0**	**0**	0	**0**	0	0	1	1	6
36	*Solanum americanum*	2	0	0	**0**	**0**	**0**	**0**	0	0	0	**0**	**0**	2⸙	**0**	0	0	0	2	0
37	*Solanum nigrum*	3	0	0	**0**	**0**	**0**	**11*⸙**	0	0	0	**0**	**0**	3⸙	**0**	0	0	8	14	9
39	*Xanthium strumarium*	3	7	0	**13**	**0**	**0**	**0**	0	0	0	**0**	**0**	0	**0**	0	0	1	20	9
	Maize cases		0	1	**10**	**0**	**1**	**76**	6	0	0	**0**	**0**	2	**0**	0	0	97	97	
	Global cases		9	26	59	8	10	114	16	2	1	2	3	17	0	2	1	95	270	
	Total species		2	3	14	2	3	20	2	1	1	1	1	4	0	1	1	19	24	

[1] Code from Table 2; [2] Mode-of-action groups that do not have herbicide resistance cases in these species are not included; [3] An asterisk symbol (*) denotes cases within maize; [4] A fern symbol (⸙) denotes cases that occurred in New Zealand; [5] Entries in bold refer to herbicide groups used in New Zealand maize.

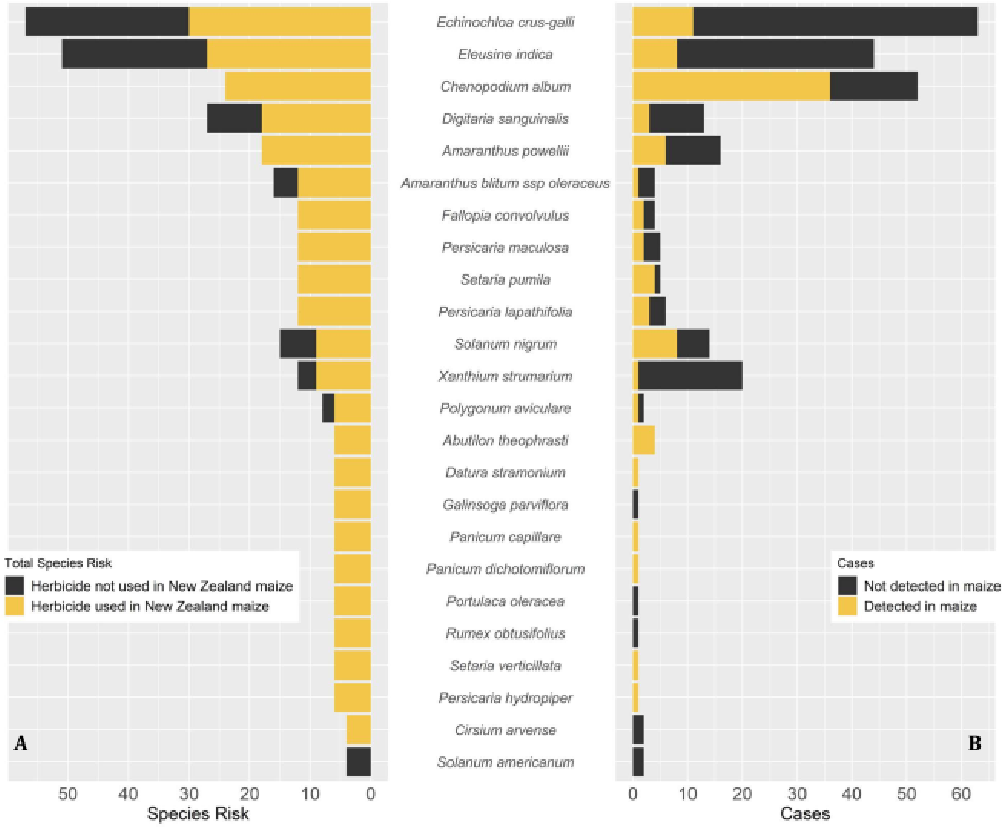

Figure 1 Detailed risk and case data for the 24 maize weeds classified as high or moderate risk of developing herbicide resistance in New Zealand. **A:** overall risk score of a weed species evolving herbicide resistance. High risk score: >10; moderate risk score: <10 but >0. **B:** number of published cases of herbicide resistance. *Note: cases of resistance in non-maize crops can still be toward herbicide groups used in maize.*

was seen in the risk assessment carried out for wheat and barley (Ngow et al. 2020). Weed species already resistant to at least one herbicide mode of action are at risk of becoming multiply resistant. *Chenopodium album* already has multiple resistance to herbicides from the triazine (i.e. atrazine) and synthetic auxin (i.e., dicamba, picloram) groups (James et al. 2005; Ghanizadeh & Harrington 2017), and has a high risk of developing resistance to acetohydroxyacid synthase inhibitors, as highlighted by recent cases of resistance in China (Huang et al. 2020) and Canada (Mo 2021). Despite widespread resistance globally for the weed species *Echinochloa crus-galli*, *Amaranthus powellii* and *Eleusine indica* there have not been any reports of resistance in New Zealand to date.

Only two high-risk herbicide mode-of-action groups (AHAS, PS-II inhibitors) and one moderate-risk group (synthetic auxins) are widely used in maize in New Zealand (Table 3). Most weed species ranked highly on this list have cases of resistance from either of these two high-risk groups. *Eleusine indica* is an exception – it is ranked second for total risk score (Figure 1), yet most resistance cases are in herbicides not widely used in maize. This species and *Echinochloa crus-galli* are the only species on this list to have evolved resistance to the low-risk herbicide groups used in maize (Table 3).

The weeds classified as high risk of becoming resistant are all annual forbs or grasses. Two perennial weed species *Rumex obtusifolius* L. and *Cirsium arvense* (L.) Scop. have a moderate risk, but the remaining eight perennial species listed in Table 2 are low risk. However, some of these weeds have been reported to possess a natural tolerance to herbicides, such as *Oxalis latifolia* and *Calystegia sepium* ssp. *roseata* (Rahman et al. 2002). Some herbicides are not expected to control certain taxonomic groups of weeds, e.g., ACCase inhibitors control grasses and Geraniaceae but not most other eudicots (Délye 2005). Overseas, atrazine can be used at high rates that permit control of *Digitaria sanguinalis*, although resistance cases are known (e.g., YanHui et al. 2017). In New Zealand, the label rates of atrazine are not sufficient to control *Digitaria sanguinalis* and other grass weeds. *Cyperus rotundus* is a common weed (Rahman et al. 1998) that is ranked as low risk here as it has never been reported as herbicide-resistant anywhere. Despite its low risk, it is difficult to control and only one herbicide is registered for its control in New Zealand. The propensity of a species to become herbicide resistant does not necessarily indicate that it is more problematic than other lower-risk species, nor does a low risk of developing resistance indicate the species is less problematic. *Eleusine indica* is a high-risk weed that is not often cited as a major problem in maize in New Zealand, yet it might become one if it evolves herbicide resistance.

Abutilon theophrasti and *Xanthium strumarium* are two uncommon, high-priority regulated weeds with moderate- and high-risk rankings respectively. *Abutilon theophrasti*, a quarantine weed, had two recent incursions in New Zealand, one in Waikato before 2011 and then across the country in 2015. It was introduced as a contaminant in imported Italian fodder beet seed (James & Cooper 2012, James & Pene 2018). Only four cases of resistance of this weed have been reported, all in maize crops and all toward atrazine in the United States (Anderson & Gronwald 1991). It is notable that some New Zealand *Abutilon theophrasti* populations were not controlled by high rates of atrazine (James & Cooper 2012). Although maize seed for sowing is rarely contaminated, other crops such as brassica may provide a pathway for the introduction of more common maize weeds i.e., *Chenopodium*, *Echinochloa*, *Persicaria*, *Amaranthus* in other crops, which may be herbicide resistant (Rubenstein et al. 2021). Seed for sowing could also bring in highly resistant species such as *Amaranthus palmerii* S.Watson and *Amaranthus tuberculatus* (Moq.) Sauer, neither of which are on the New Zealand quarantine weed list (MPI 2018).

This study does not account for the actual abundance of weeds, or herbicide use at the field level. High weed abundance in a field increases the likelihood of a population evolving herbicide resistance (Jasieniuk et al. 1996), and prevalence across regions in a weed species was identified as a predictor of herbicide resistance (Hulme & Liu 2021). Clearly herbicide use patterns affect the probability of resistance developing; unlike the wheat and barley risk assessment (Ngow et al. 2020), we do not have current data of herbicide field applications in maize. More insightful analysis of herbicide resistance risk would be possible if maize growers were to use an online platform for recording herbicide use. Our analysis may underestimate the risk for recently adopted mode-of-action groups with fewer reported cases of resistance documented. For example, HPPD inhibitors (mesotrione and topramezone) and PPO inhibitors (saflufenacil) were introduced to the market in the 2000s (Almsick 2009; Grossmann et al. 2010). Our risk assessment method relies on data that may contain biases that reflect regional differences in herbicide use, herbicide availability, resistance reporting and scientific attention.

Repeated use of any given method of weed control should be avoided where possible. Exposure over many years to a herbicide with a particular mode of action increases the likelihood of selecting plants with a resistance conferring mutation (Jasieniuk et al. 1996). Busi et al. (2020) found that pre-emergent herbicides, complex rotation between modes-of-action, and mixtures of these herbicides delay the onset of herbicide resistance. These practices can be followed for maize as there are several pre-emergent and post-emergent herbicide options (Table 1). Cultural practices of delayed planting, crop rotation, harvest weed-seed control, targeted tillage, increasing crop competitiveness and cover cropping can mitigate the evolution of herbicide resistance (Mhlanga et al. 2016; Trolove et al. 2017; Somerville et al. 2018; Moss et al. 2019; Walsh et al. 2020). Such integrated weed management techniques have long been promoted as a solution to herbicide resistance in weeds (Norsworthy et al. 2012; Harker 2013; Beckie et al. 2019; Moss et al. 2019).

CONCLUSIONS

Maize weeds in New Zealand are at a high risk of developing resistance to acetohydroxyacid synthase inhibitors (group 2) and photosystem-II inhibitors (group 5). Repeated selection for herbicide resistance is well documented internationally in the seven high-risk species, *Echinochloa crus-galli*, *Chenopodium album*, *Eleusine indica*, *Xanthium strumarium*, *Amaranthus powellii*, *Solanum nigrum* and *Digitaria sanguinalis*. Herbicide resistance surveys should focus on these high-risk weed species and herbicides. Extension efforts could help agrichemical suppliers, farmers and agronomists to preserve the efficacy of currently used herbicides by practising integrated weed management.

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Chapter 3: Weeds in maize in Bay of Plenty and Waikato

3.1 Introduction

Weeds are a major threat to production in maize and are considered to have potential to cause higher yield losses (40%) than pests and diseases (animal pests 16%, pathogens 9%, viruses 3%) (Oerke 2006). In New Zealand maize weeds have been estimated to cause yield losses from 30-70% (James et al. 2000). Weeds compete for resources reducing water and nutrient availability and light quantity and quality, prevent germination and restrict maize growth (Rajcan and Swanton 2001). Certain weed species are also able to produce allelochemicals (Qasem and Foy 2001). Weed competition is most damaging during seedling establishment, the critical period of weed control in maize is during the first few weeks after sowing (James et al. 2000, Rajcan and Swanton 2001).

Conventional weed control in New Zealand involves soil cultivation followed by pre-emergent herbicides, then post-emergent herbicides. Alternative control measures may incorporate soil conservation practices (no-tillage, minimum-tillage, strip-tillage), inter-row tillage, variation in planting densities and winter cover crops (Abdin et al. 2000, Harker 2013, Rahman 1985, Stone et al. 2000, Trolove et al. 2017). Despite employing these methods, certain weeds are able to persist in maize systems, due to traits such as natural tolerance to herbicides, evolved herbicide resistance, seed dormancy and perennial vegetative structures (Ghanizadeh and Harrington 2019b, Rahman 1985).

There is a long history of research on maize weeds in New Zealand, particularly in Waikato and Gisborne regions. *Chenopodium album* L., *Amaranthus powelli* (recorded as *Amaranthus retroflexus), Solanum nigrum, Echinochloa crus-galli* and *Nicandra physalodes* were major weeds in 1950s Gisborne maize (McKee 1955). In 1960s Waikato maize, triazine herbicides were the main control tools; *A. powelli* (recorded as *Amaranthus hybridus), C. album* and *Persicaria maculosa* were regarded as common, and annual grasses *Digitaria sanguinalis, Panicum capillare* and *Panicum dichotomiflorum* were emerging problems (Patterson 1961, Thompson 1962). Grass weeds continued to be problematic in the 1970s particularly as atrazine was not effective on *D. sanguinalis, Panicum* spp. and *Setaria pumila* although it did control broadleaf weeds and *E. crus-galli* (Patterson 1971, Woon 1971). In the 1980s Waikato researchers showed that *P. maculosa* and *C. album* populations had evolved herbicide resistance toward atrazine (Rahman et al. 1983a, Rahman and Patterson 1987).

A list of the most common maize weeds was published in 1985 (Rahman 1985). Seedbank studies in 1997 at 15 sites, and again in 1999-2002 at 30 sites recorded the following weeds as common: *C. album, P. maculosa*, *S. nigrum*, *D. sanguinalis*, *P. dichotomiflorum* (Rahman et al. 1997, 2006). *Chenopodium album* populations in eastern Waikato evolved resistance to dicamba in the 2000s (James et al. 2005). The weeds *Oxalis latifolia* (Waikato), *Abutilon theophrasti* (Waikato), *Panicum milleaceum* (Gisborne), *Xanthium strumarium* (Bay of Plenty, Waikato) and *Cyperus rotundus* (Bay of Plenty) were identified as problem weeds more recently (James et al. 2010b, 2016, James and Cooper 2012, Rahman et al. 1998b, 2002). Most recently, *D. sanguinalis* populations in Bay of Plenty and Waikato were shown to have evolved nicosulfuron resistance (Buddenhagen et al. 2021a, Harrington and James, T.K. 2022).

Some smaller studies have advanced knowledge of maize weeds in New Zealand, but no large study comparable to the study of the weed flora of wheat and barley fields in Canterbury (84 fields) has been published for maize (Bourdôt et al. 1998). The largest studies of a regional 'maize flora' were those seedbank studies in the late 1990s-early 2000s, which sampled 30 sites (Rahman et al. 1997, 2006). These studies were the main source of information for a list of important maize weeds assessed for their risk of evolving herbicide resistance (Chapter 2; Ngow et al. 2021). The weed seedbank can represent the potential weed composition of a field (Rahman et al. 2001a), and field surveying of weeds prior to crop harvest can identify current and emerging weed problems (Hanzlik and Gerowitt 2016). This study here combines assessments of weed seedbanks and weed cover on randomly selected farms at harvest time at 52 sites to update knowledge of the prevalence of maize weeds across the Bay of Plenty and Waikato regions.

3.2 Materials and Methods

3.2.1 Site selection

Landowners or farm managers were contacted to obtain access to sites that had cultivated maize for at-least two concurrent years. Farmer contact information was sourced from Agribase (Sanson 2000), which is based on voluntary surveys of farmers across the country and the Foundation for Arable Research member database, which holds information of members who pay a levy on maize seed. Database records were randomized, and farmers called in random order (in January) until permission for 20 sites in Waikato and 10 sites in Bay of Plenty, per database, was obtained. Waikato farms were visited in late February and Bay of Plenty farms in early March 2021 prior to harvest (Fig. 3-1). Sixty farms were selected but only 52 were available for sampling due to some farmers requiring an early harvest.

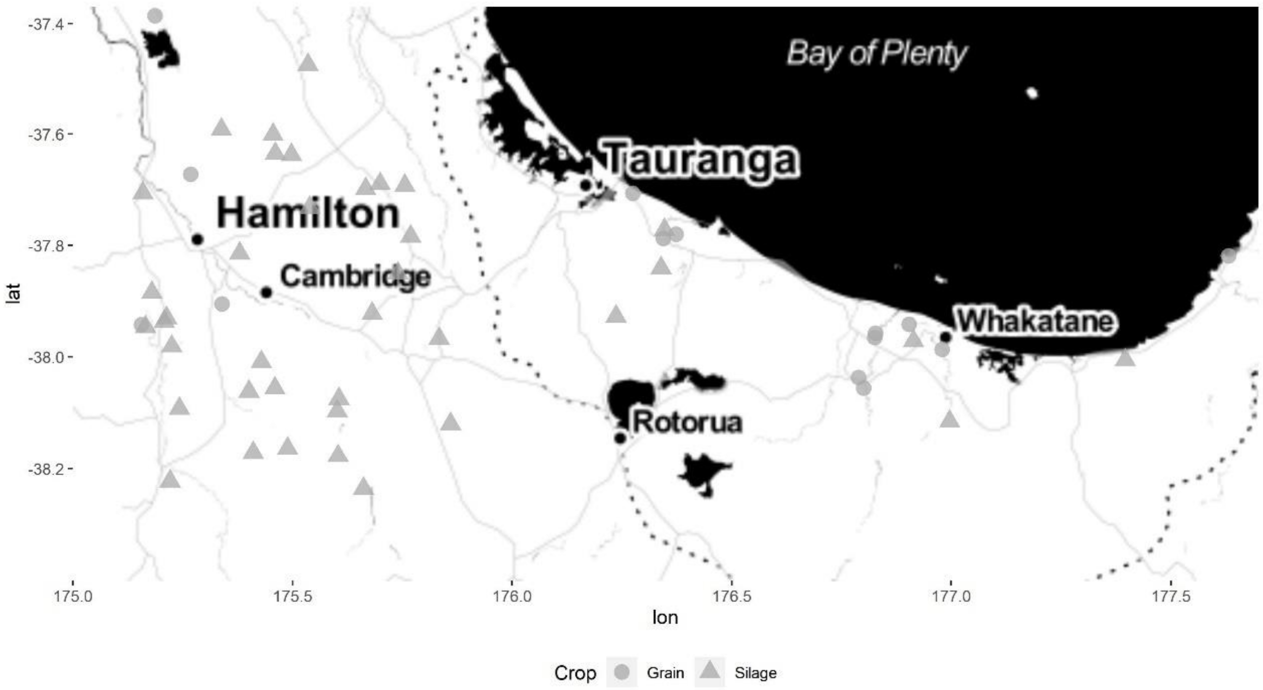

Figure 3-1. Map of grain and silage maize-growing farms sampled in the Waikato (36) and Bay of Plenty (16).

3.2.2 *Field sampling*

Percentage ground cover was estimated for any weed species present and soil cores were taken along transects in the headland (maize rows bordering the field edge) and the centre (or core) of maize fields. Gateways, breaks and corners were avoided, headland rows were differentiated from rows in the centre of the field, as the weed abundance and composition are known to differ between those areas. Between farms, measures were taken to maintain farm-farm biosecurity, such as cleaning equipment, boots and clothing of any weed seeds or soil.

For headlands the 6th and 10th row from the outside margin were selected for transects. Headlands were usually 16-rows wide, occasionally fields had 18 or 32 rows. Row selection for centre rows depended on field size. For large fields, a random number generator was used to select rows 30-130m from the corner where the headland borders the centre; for smaller fields the approximate centre of the field was estimated, and a row was selected. The fields were entered from the easiest access point, typically a gateway or corner.

Transect start positions were at a random distance from the edge. For the centre transect this was 0-50m from the internal border with the headland. For the headland transect this was 30-130m from a gateway or corner, to avoid areas where planting equipment make turns and cause extra disturbance (potentially facilitating uncharacteristically high weed cover). For both centre and headlands two transects were laid out and assessed for a total transect length of 132m (66m ×

2), which assuming a row width of 75cm covers a total area of 100m^2. Transects were 66m long and usually 0.75m wide (standard maize row spacing); if row spacing was 0.66m wide, transect length was adjusted to 75m to maintain the same sampling area. There were rarely farms with different row widths, these were sampled at a transect length to attain an area sampled of 100m^2.

Six soil cores were sampled, one at 0m, at 33m and at 66m in the first pass, then again in reverse in the second pass, which was four rows adjacent. The soil corer was set to 7.5cm depth and had a 7.5cm diameter; the total area sampled for each area was 0.0265m^2 and had a 1988cm^3 volume. One seed detected would therefore be equivalent to 37.7 seeds per m^2. Along transects, any weeds were identified and scored (Fig 3-3). Some weeds were particularly difficult to identify to species level, so were identified to genus level (*Amaranthus* spp., *Persicaria* spp., *Calystegia* spp., *Oxalis* spp.). Each weed was given a percentage cover estimate within each transect. For the herbicide resistance aspect of this survey (Chapter 4), mature weed seeds found on or near the transects were collected.

3.2.3 *Processing soil seedbank samples*

When farms were sampled in Waikato, at the end of each day soil samples were spread out in trays for germination (Fig 3-2). Bay of Plenty samples were stored in insulated containers until we returned to the Plant Protection facilities at Ruakura. Plastic propagation trays (Egmont RXPROPT; 34cm × 20cm × 6cm) were filled with potting mix (Daltons Grass and Clover; ⅓ coco-coir, ⅓ bark, ⅓ pumice) to the depth of 3cm. Woven polyethylene weedmat was placed on the potting mix and sample soil added to near the top of the tray for another depth of 3cm. Trays were planted late-February/mid-March and irrigated and maintained in a temperature controlled glasshouse maintained at 18-22 °C. Seedlings were counted and identified as they emerged, and any unidentifiable seedlings were transplanted and grown until they flowered or were otherwise identifiable. For vegetatively reproducing species such as *Cyperus rotundus, Cynodon dactylon, Paspalum distichum* and *Oxalis latifolia,* emerging vegetative structures were counted. After identification and enumeration, plants were removed, soils were mixed, and trays were irrigated. This occurred at-least three times per tray, and the final assessment was on the 3rd of September (early spring, approximately 6 months after seedbanks were placed in the glasshouse).

Figure 3-2. Trays of weeds germinating from the soil seedbanks of 52 maize sites in the Ruakura Plant Protection glasshouse.

3.2.3 Data analysis

Soil order was sourced from S-map Manaaki Whenua Landcare Research (Ford 2019); climate data was sourced from NIWA (NIWA, 2012): median annual total rainfall, median annual average temperature and median annual total sunshine hours. The type of soil cultivation was recorded (no-till, strip-till or conventional), decimal-degrees latitude and longitude, years cultivated and maize type (grain or silage) during the survey. All data analysis was done in *R* (R Core Team 2021). Principal component analysis was done using the *vegan* package (Dixon 2003). For principal component analysis percentage cover estimates and seedbank counts were Hellinger transformed to standardize data and mitigate horseshoe effects (Durbecq et al. 2020, Legendre and Gallagher 2001, Mol et al. 2015). Environmental variables were fitted (multiple regression) onto ordinations using the '*envfit*' function (Dixon 2003).

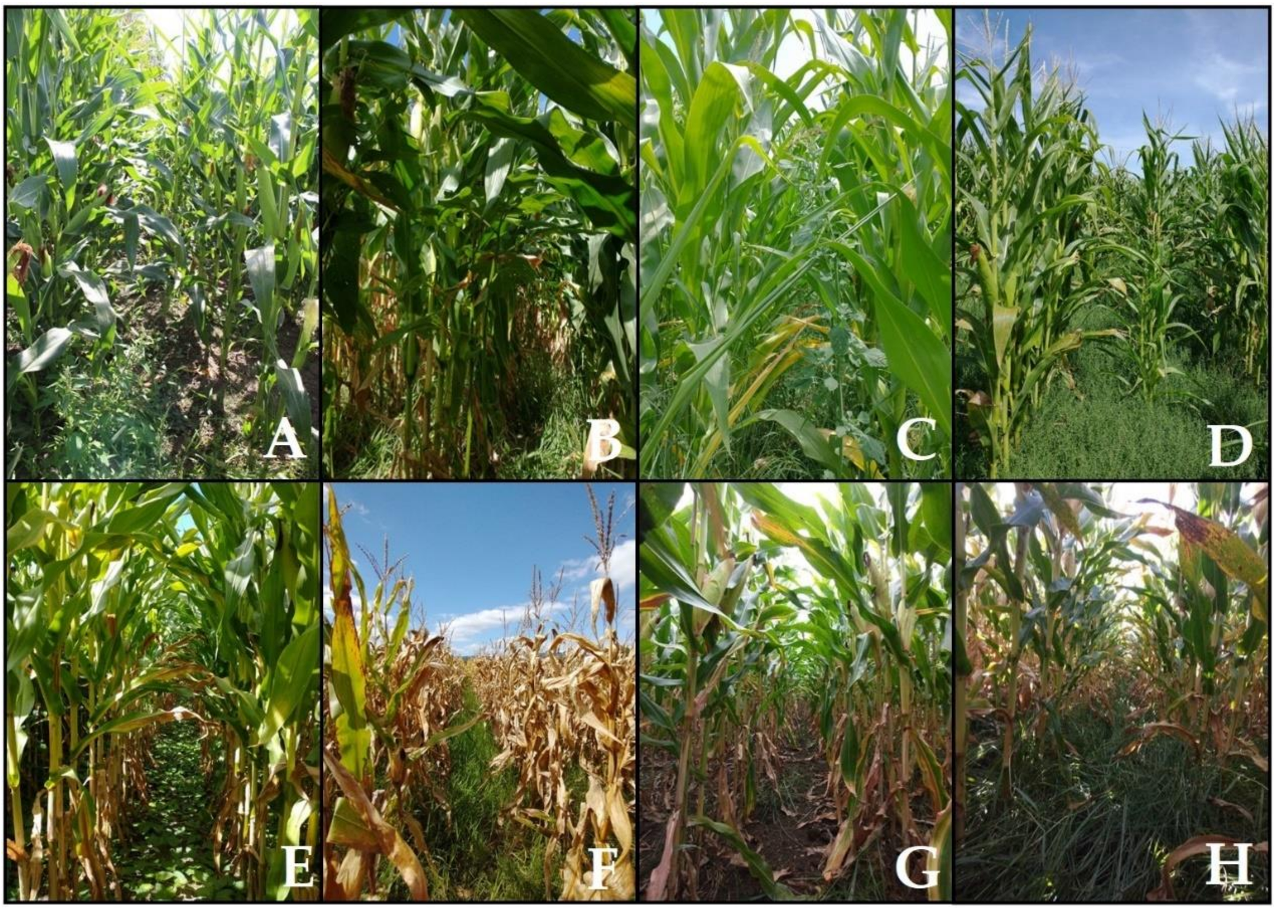

Figure 3-3. Example rows of eight maize farms with weeds pictured: *Chenopodium album* (**A**), *Xanthium strumarium* & *Digitaria sanguinalis* (**B**), *Echinochloa crus-galli* & *Chenopodium album* & *Digitaria sanguinalis* (**C**), *Polygonum aviculare* (**D**), *Calystegia* spp. (**E**), *Cynodon dactylon* & *Paspalum distichum* (**F**), *Cyperus rotundus* (low abundance; **G**) and *Cyperus rotundus* (high abundance; **H**).

3.3 Results

There were 134 plant species recorded as present in the maize paddocks: 90 in field, 96 in seedbank (Appendix 5). *Digitaria sanguinalis*, *Chenopodium album* and *Persicaria* spp. were the most widespread summer weeds in maize, present in >70% of farms (Table 3-1). Other common summer weeds that were present in over half of the maize farms are *Setaria pumila*, *Solanum nigrum* and *Rumex obtusifolius*. *Sonchus oleraceus* and *Erigeron* spp. were found in more than half of the farms but were not abundant in the field. Winter weeds *Juncus bufonius* and *Poa annua* were present in >70% of farms. There were some observations of 'out-of-season' weeds growing at harvest time, but they were never at a high density, except for one site with *P. annua*. Similarly, the spring weed *Stellaria media* was found in 15 farms at harvest time but identified from 34 farms in the soil seedbank. The most common plant family was Poaceae (57% of total cover, 44% of total seed count; Appendix 8). Amaranthaceae and Polygonaceae weeds were widespread, and common within field and seedbank assessments (9.3% and 8.2% of total cover, 6.8% and 8.9% of total seed count; Appendix 8).

There were 52 sites sampled (site information is in Appendix 6). Forty-three sites were observed to be conventionally cultivated, six no-till, one strip-till and two were not recorded. Waikato soils were mostly allophanic (18) and gley (8) with the remainder brown (2), granular (2), organic (3), pumice (1) and ultic (2); Bay of Plenty soils were mostly gley (5) and pumice (5) with the rest allophanic (1), brown (1), podzol (1) and recent (3). Most farms were planted at 75 cm row spacing but two Waikato sites had maize planted 50 cm apart. For all sites except one the years in cultivation was at-least two, but more information was only available for 16 sites, of these, three sites were cultivated for ≤5y, five ≤10y, four ≤15y four ≤20y, one site was cultivated for 58 years and one site was cultivated for the first year. Seventy-three percent of sites were silage maize; Bay of Plenty had more grain (10/16) and Waikato more silage maize sampled (32/36). Silage paddocks were often within dairy farms, but some were in maize cropping farms.

Table 3-1. Count of farms by weed for the twenty-five most common species observed in maize in a randomized survey of 16 Bay of Plenty and 36 Waikato farms, from soil seedbanks (>0 seeds) and field visual assessments (>0% cover). Summer weeds in bold font.

	Bay of Plenty (n=16)			**Waikato (n=36)**			**Total (n=52)**	
Species	**Field**	**Seedbank**	**Farms**	**Field**	**Seedbank**	**Farms**	**Farms**	**Percentage**
Digitaria sanguinalis	15	12	15	34	27	36	51	98%
Chenopodium album	12	8	13	28	24	31	44	85%
Juncus bufonius	0	13	13	0	28	28	41	79%
Poa annua	1	10	10	0	28	28	38	73%
***Persicaria* spp.**	6	4	7	27	22	30	37	71%
Stellaria media	4	10	11	10	24	26	37	71%
Setaria pumila	6	0	6	21	9	25	31	60%
Sonchus oleraceus	6	4	10	15	12	20	30	58%
Solanum nigrum	3	5	8	16	10	22	30	58%
***Erigeron* spp.**	1	8	9	2	16	18	27	52%
Rumex obtusifolius	8	4	8	16	12	18	26	50%
Oxalis latifolia	6	4	8	11	9	17	25	48%
Panicum dichotomiflorum	10	7	11	7	8	13	24	46%
Cirsium arvense	6	0	6	17	6	17	23	44%
Lolium spp.	0	1	1	3	15	22	23	44%
Amaranthus blitum* ssp. *oleraceus	3	6	8	10	11	15	23	44%
Echinochloa crus-galli	3	0	3	18	7	19	22	42%
Lepidium didymum	2	10	10	2	11	12	22	42%
Nicandra physalodes	5	1	6	13	5	14	20	38%
Plantago major	0	4	4	1	14	15	19	37%
Cerastium glomeratum	0	5	5	0	13	13	18	35%
Trifolium repens	2	2	4	7	10	13	17	33%
Modiola caroliana	8	0	8	8	0	8	16	31%
Phytolacca octandra	7	0	7	9	0	9	16	31%
***Calystegia* spp.**	8	0	8	7	0	7	15	29%

Few weeds had consistently high cover scores (Table 3-2). *Digitaria sanguinalis* is by far the most abundant weed, found with ≥1% cover in 50% of farms. *Chenopodium album* was seen in 27%

of farms with ≥1% cover. Willow weeds *Persicaria maculosa* and *Persicaria lapathifolia* were found with ≥1% cover in 19% of all farms, with *P. maculosa* being the more common species of the two. *Echinochloa crus-galli* was recorded with ≥1% cover in 17% of Waikato farms but only one Bay of Plenty farm; *Fallopia convolvulus* and *Setaria pumila* were also abundant in Waikato but not in Bay of Plenty farms (Table 3-2). Conversely, *Cyperus* spp., *Calystegia* spp., *Panicum dichotomiflorum, Cynodon dactylon,* and *Paspalum distichum* were abundant (≥1%) in the Bay of Plenty. *Digitaria sanguinalis* was the only weed present in >20% of farms with a ≥10% cover estimate.

The perennial spreading sedge *Cyperus rotundus* was found in seven of the sixteen Bay of Plenty farms with an average 22.6% cover and standard deviation of 25.9% (Table 3-2); it was found in one Waikato farm but was not abundant (0.6% cover). Other perennial spreading grasses *Cynodon dactylon* and *Paspalum distichum* were recorded with high mean cover estimates (>15%) in the Bay of Plenty (mean cover for farms with it present); *Elytrigia repens* was recorded in five Waikato farms where it had an 18% mean cover. The annual sprawling grass *Digitaria sanguinalis* had >3% mean cover but a similar grass *Panicum dichotomiflorum* only had <1% mean cover. The annual grass *Echinochloa crus-galli* was highly abundant in Waikato (5±10%), but another annual grass *Setaria pumila* was less abundant (1% in Waikato).

Digitaria sanguinalis had a mean >10 seeds (377 seeds m^{-2}) detected in farms from both regions, and was found on 13 farms with ≥10 seeds (Table 3-2). One farm had a *D. sanguinalis* seed density of 3056 seeds m^{-2} (81 seeds). *Chenopodium album* and *Persicaria* spp. had a mean >7 seeds. *Persicaria* spp. were found on nine farms with ≥10 seeds. The average count for *Cyperus rotundus* tubers was 3.8 in the Bay of Plenty, and the average bulb count for *Oxalis latifolia* was >2 for both regions. *Echinochloa crus-galli* and *Setaria pumila* were not common in the seedbank. Winter weeds *Poa annua* and *Juncus bufonius* had high mean seed densities (seeds m^{-2}) in Waikato (694±509, 287±185) and Bay of Plenty (1170±1872, 566±951). *Poa annua* was found in 22 farms with ≥10 seeds and 32 farms with ≥5 seeds. *Stellaria media* was abundant (≥10 seeds) on 8 farms and had a mean 8.3 seeds in Waikato farms. Neither *Juncus bufonius*, *Poa annua* or *Stellaria media* were abundant in summer (Table 3-2).

The mean (± standard deviation) total cover was 23.0±28.2%. There was no significant difference (unpaired t test: p-value = 0.7129) between Bay of Plenty (38.4±16.1) and Waikato farms (35.9±25.3). Four farms had an average total weed cover higher than 90%; three between 50-90%, sixteen between 10-50%, twenty-two between 1-10% and seven below 1% average total weed cover (Appendix 9). The average total seed density was 2810±2309 seeds m^{-2} (76±61 seeds counted). There was no significant difference (unpaired t test: p-value = 0.1526) between Bay of Plenty (2165±1609 seeds m^{-2}) and Waikato (3096±2528 seeds m^{-2}) seedbank densities. Eight farms had seed densities higher than 5000 total seeds m^{-2}, twelve between 2500-5000, twenty-three between 1000-2500 and nine below 1000 seeds m^{-2} (1000 seeds m^{-2} is equivalent to 26.5 seeds counted; Appendix 9).

The first and second principal components represented 16.0% and 12.1% of variation for the cover plot PCA (Fig 3-4A) and 19.0% and 11.5% for the seedbank PCA (Fig 3-4B). Some species appear to have have a disproportionate influence on the detected compositional differences between farms, and influence plot placement in ordination space. *Digitaria sanguinalis* (DIGSAN), *Cyperus rotundus* (CYPROT), *Persicaria* spp. (PERSP) and *Chenopodium album* (CHEALB) are distinct from the central cluster of species. *Echinochloa crus-galli* (ECHCRU), *Fallopia convolvulus* (FALCON), *Paspalum distichum* (PASDIS), *Cynodon dactylon* (CYNDAC), *Calystegia* spp. (CALSP) and *Elytrigia repens* (ELYREP) appear to be distinct. For the seedbank plot, *D. sanguinalis, Poa annua* (POANN), *Stellaria media* (STEMED) and *Juncus bufonius* (JUNBUF) are distinct. These are all species that are widespread and abundant (high cover and/or high seed count).

A multiple regression of environmental variables on PCA revealed some relationships for weed cover and seedbanks (Table 3-3). There was strong evidence that longitude (p-value = 0.001), crop type (grain vs silage; p-value = 0.001), region (p-value = 0.001) and annual sunlight hours (p-value = 0.007), are correlated with weed cover. These trends are likely driven by the high weed cover and presence of *Cynodon dactylon*, *Cyperus rotundus* and *Paspalum distichum* in the eastern Bay of Plenty (Table 3-2). There was evidence for a relationship between crop type and seedbanks (p-value = 0.003), and weak evidence for sunlight (p-value = 0.033). All of the environmental variables had poor goodness of fit for both percentage cover and seedbank ordinations (for most $R^2 < 0.1$), though sunlight ($R^2 = 0.2102$) and longitude ($R^2 = 0.3392$) had a better goodness of fit for weed cover.

Table 3-2. Number of farms where the weeds where present above certain cover thresholds (≥1%, ≥5% average cover of headland and centre) and seed counts (≥5, ≥10 average cover of headland and centre) and mean cover and seedbank density (seeds m^{-2}) by region for common weed species recorded in maize in Bay of Plenty and Waikato. Means are only for farms where the weed is present. Weeds selected are the fifteen most frequent weeds with cover ≥1% and five most common weeds in the seedbank (seed count ≥1) not already included.

	Count of Farms by Average Cover						**Count of Farms by Average Seedling Count**					
	Bay of Plenty			**Waikato**			**Bay of Plenty**			**Waikato**		
Species	**n≥1%**	**n≥5%**	**Mean ±SD**	**n≥1%**	**n≥5%**	**Mean ±SD**	**n≥5**	**n≥10**	**Mean ±SD**	**n≥5**	**n≥10**	**Mean ±SD**
Digitaria sanguinalis	9	4	3.9±5.5%	17	7	5.3±10.3%	6	4	423±551	12	9	528±777
Chenopodium album	6	1	4.8±12%	8	2	1.8±5.6%	4	1	275±392	8	4	309±642
Persicaria spp.	3	1	3.4±6.3%	7	2	1.6±4%	2	1	1257±2336	10	8	302±298
*Cyperus rotundus**	7	5	22.6±25.9%	0	0	0.7±0%	2	1	143±177	0	0	na
Echinochloa crus-galli	1	0	0.5±0.5%	6	4	5.0±10.1%	0	0	na	2	0	102±102
Fallopia convolvulus	0	0	0.5±0%	6	0	1.4±1.2%	0	0	na	0	0	na
Calystegia spp.*	3	0	1.0±1.4%	2	1	1.4±2.9%	0	0	na	0	0	na
Cirsium arvense	1	0	0.4±0.8%	4	2	1.1±2.4%	0	0	na	0	0	42±23
*Cynodon dactylon**	5	2	26.1±40%	0	0	0.3±0%	1	0	189±215	0	0	113±0
*Elytrigia repens**	1	0	2.3±3.2%	4	2	18.0±27.5%	0	0	na	0	0	151±0
*Paspalum distichum**	5	3	15.1±22.7%	0	0	0.1±0.1%	1	1	170±245	0	0	38±0
Setaria pumila	1	0	0.5±1%	4	1	1±2.7%	0	0	na	0	0	57±49
Solanum nigrum	2	1	3.2±3.9%	2	0	0.2±0.3%	1	0	94±128	2	1	136±117
*Oxalis latifolia**	1	1	0.9±2%	2	1	1.3±3%	0	0	75±30	2	0	128±106
Panicum dichotomiflorum	2	0	0.3±0.5%	1	0	0.6±1.1%	0	0	60±34	4	1	196±189
Juncus bufonius	0	0	na	0	0	na	8	5	287±185	15	9	566±951
Poa annua	0	0	na	0	0	0.1±0%	9	8	694±509	23	14	1170±1872
Stellaria media	0	0	0.1±0.2%	0	0	0.1±0%	4	1	170±140	12	7	313±332

Erigeron spp.	0	0	0.1±0%	0	0	0±0%	1	0	87±87	0	0	42±11
Lepidium didymum	0	0	na	0	0	na	2	2	242±355	5	2	211±226

*for these vegetative perennial species no seedlings were found but vegetative structures were counted; in the case of *Cirsium arvense* only seedlings were counted

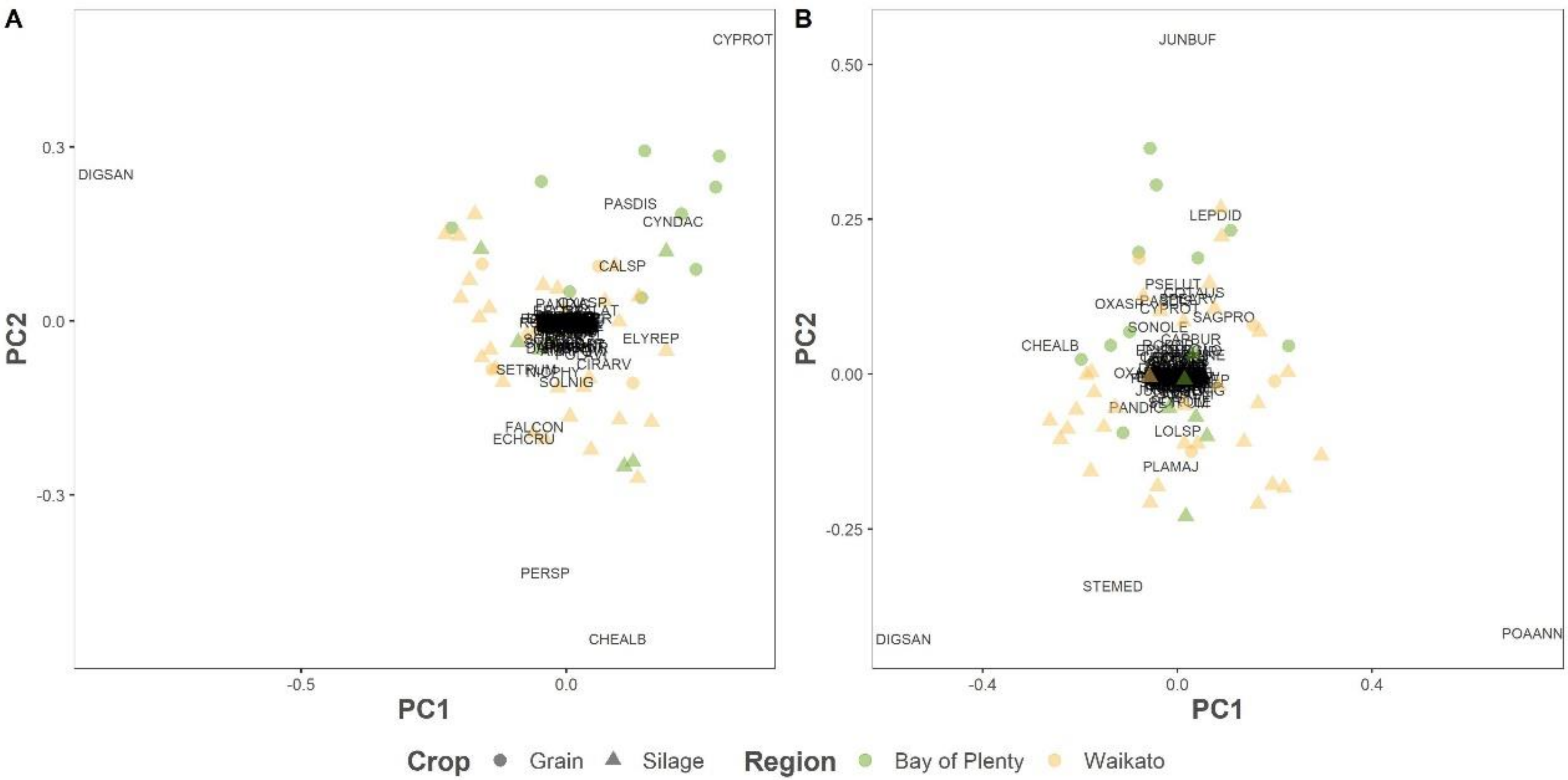

Figure 3-4. Principal component analysis (PCA) biplots for square-root transformed estimated percentage cover **(A)** and square-root transformed count seedbank (**B)** for weed species (text) across 52 maize fields (symbols) in the Bay of Plenty and Waikato. Species binomials are abbreviated to a six letter code.

Table 3-3. Centroids, R^2 and p-value for the multiple regression of environmental variables on ordination axes (999 permutations) for PCA ordination of percentage cover and seedbank counts.

	Estimated percentage cover (PCA)				Seed count (PCA)			
	PC1	**PC2**	**R^2**	**Pr(>r)**	**PC1**	**PC2**	**R^2**	**Pr(>r)**
Latitude	-0.99604	-0.08889	0.0323	0.451	0.29780	0.95463	0.0518	0.282
Longitude	0.77653	0.63008	0.3392	0.001	-0.07939	0.99684	0.0584	0.240
Rainfall	0.93256	-0.36102	0.0215	0.620	-0.49630	0.86815	0.0114	0.772
Temperature	0.20034	0.97973	0.0743	0.173	-0.04187	0.99912	0.1235	0.033
Sunlight	0.68534	0.72822	0.2102	0.007	-0.21184	0.97730	0.0960	0.078
Wind Speed	0.90218	0.43135	0.0343	0.428	0.58894	0.80818	0.0202	0.613
Grain	0.1602	0.2664	0.1581	0.001	-0.0053	0.2513	0.1104	0.003
Silage	-0.0590	-0.0981	0.1581	0.001	0.0020	-0.0926	0.1104	0.003
Bay of Plenty	0.1755	0.1821	0.1263	0.001	-0.0281	0.1381	0.0419	0.111
Waikato	-0.0780	-0.0810	0.1263	0.001	0.0125	-0.0614	0.0419	0.111
Soil type	-	-	0.1387	0.620	-	-	0.1810	0.293

3.4 Discussion

Digitaria sanguinalis, Chenopodium album and *Persicaria* spp. are the most important maize weeds of Waikato and Bay of Plenty. *Echinochloa crus-galli, Setaria pumila* and *Fallopia convolvulus* are notable in Waikato and *Panicum dichotomiflorum* and *Solanum nigrum* in the Bay of Plenty. Major perennial weeds in the Bay of Plenty are *Cyperus rotundus, Cynodon dactylon* and *Paspalum distichum* and *Elytrigia repens* in Waikato. *Sonchus* spp., *Erigeron* spp. and *Rumex obtusifolius* are widespread but not abundant.

The weeds observed here are not dissimilar from those previously documented. A list of 39 weeds compiled from literature sources was assessed for risk of herbicide resistance (Chapter 2: Ngow et al. 2021); thirty-five of those were observed in this field survey. Many of those listed weeds were obviously major weeds: annual grasses *D. sanguinalis, E. crus-galli, S. pumila, P. dichotomiflorum;* annual broadleaves *C. album, F. convolvulus, Persicaria* spp., *S. nigrum;* perennial grasses *C. dactylon, E. repens, P. distichum;* perennial sedge *C. rotundus;* perennial broadleaves *Calystegia* spp. *C. arvense* and *O. latifolia.*

Galinsoga parviflora, Bidens frondosa, Malva parviflora, Rumex pulcher and *Solanum americanum* were not common in Waikato and Bay of Plenty maize farms and could justifiably be removed from the list in Chapter 2: Ngow et al. 2021. Of those uncommon weeds, only *Solanum americanum* had evolved herbicide resistance before, and was ranked at a medium to low risk of developing resistance. *Persicaria hydropiper* was not commonly found in this study, but it is known to be more prominent in wet paddocks (Rahman 1985). The relatively common *Amaranthus blitum* ssp. *oleraceus* was included in Chapter 2: Ngow et al. 2021, but *Amaranthus deflexus* was not. *Amaranthus deflexus* was only observed in one farm, but it is more common south of Waikato and in the Hawkes Bay

(Webb et al. 1988). *Conium maculatum* was not commonly found, but is a known problem in Manawatu maize (James et al. 2007), while field bindweed (*Convolvulus arvensis*), broom corn millet (*Panicum milleaceum*) and witchgrass (*Panicum capillare*) were absent, but are known problems of the Hawkes Bay and Gisborne. Farms in the eastern Waikato had weeds observed that are regulated under Regional Management Plans. *Xanthium strumarium* was found in one farm (not in the sampled area), and another farm with *Abutilon theophrasti*, was harvested before it could be sampled. *Xanthium spinosum* was found in both regions in three farms.

Eleusine indica was not common (observed in 6 farms). This species is one of the worst weeds of maize globally, and has a high propensity to evolve herbicide resistance (Chapter 2: Ngow et al. 2021). It is likely that it is still well controlled by herbicides here. The congeneric species *Eleusine tristachya* was seen in the gateway of one farm in the eastern Bay of Plenty. This species is widespread in the Hawkes Bay but is not known to be a cropping weed and was only recently recorded in Waikato (iNaturalist: Taupo 6362165) and the Bay of Plenty (iNaturalist: Kawerau 70801178, Whakatane 70992108, Rotorua 103928431). The weed is a minor maize weed in Argentina (Brunori and Puricelli 2020). A morphologically unusual *Persicaria* was seen on three maize farms in the Bay of Plenty. It had large, branching inflorescences, strigose ochrea, large lamina covered in short hairs with the red blotch and grew to a large size. This species may be one of the unidentified *Persicaria* aff. *decipiens* recorded in a recent checklist, 'Tall willow weed' or 'Waikato willow weed' (Lange et al. 2018, Nicol 1997, Taylor, R. L. 1980, Webb et al. 1988). The bird-dispersed subshrub *Phytolacca octandra* was found in 12 farms, albeit in low numbers. This is also a weed of maize in Taranaki (Densley et al. 2005), but it is more commonly a weed in pasture, neglected areas and bush gaps (Webb et al. 1988). The related species *Phytolacca americana* is a weed of maize in the United States (Patches et al. 2017).

A similar study to this, where seedbank and field observations were compared was completed in 2002 on 20 Waikato, 4 Bay of Plenty and 6 Poverty Bay sites sampled in spring (Rahman et al., 2006). In that study, they found 72 species in Waikato, we found 134 overall. Comparing results from this thesis to theirs (omitting Poverty Bay), we found *D. sanguinalis* have similar prevalence (97% compared to their 92%). *E. crus-galli* was observed more often (42% compared to 13%), *C. album* (85% compared to 67%) and *Persicaria* spp. (71% compared to 58%). *Panicum dichotomiflorum* was observed less frequently in this study (46% of sites compared to 83%). In the previous study *Panicum dichotomiflorum* was noted to be in high numbers in Waikato, and *E. crus-galli* in low numbers, but in this study the opposite was true. *Setaria pumila* was not recorded, although that weed experienced a large increase in abundance in the late 2000s (James et al. 2009).

Amaranthus powelli was only found in 25% (13/52) of sites sampled in this study (Table 3-2) compared to 45% (11/24) of Waikato and Bay of Plenty sites in Rahman et al., (2006). Rahman et al., (2006) did not record *Amaranthus blitum* ssp. *oleraceus*, *Fallopia convolvulus, Modiola cariolianensis* or *Stellaria media* to be common. Spring weeds were observed (i.e, *Veronica persica*, *Lepidium didymum*, *Cerastrium glomeratum* and *Lotus corniculatus*) less frequently. Perennial weeds were not recorded either in those seedbank studies, likely omitted as they do not rely on seed to regenerate; this study shows *Calystegia* spp., *Cirsium arvense, Cyperus rotundus, Cynodon dactylon, Oxalis latifolia, Paspalum distichum* are common perennial weeds. Another prior study of Waikato and Bay of Plenty maize had much higher average seedbank densities 32,930±64,321m^{-2} than ours 2810±2309 seeds m^{-2} (Rahman et al. 1997). It is possible that the reduced seedbank densities are due to differences in the studies' sampling period, as weeds germinate over the growing season diminishing their seedbank (Rahman and James 1993b), studies sampled in spring (Rahman et al. 1997, 2006), would likely have higher seedbank densities than those sampled in summer (this one). It is also possible that changes in weed composition over time occurred, reducing seedbank numbers.

It is likely that most weeds present at a site can be detected by germinating seedbank samples. A similar number of weed species were detected in the seedbank and in the field. Many of the common maize weeds in a previous study showed significant correlation between the soil seedbank and site abundance (Rahman et al. 2006). It is possible the timing of sampling in this study may have influenced results. Summer weeds germinate from October-February, reducing their soil seedbanks by February (when we sampled), however large amounts of seed can remain present (Rahman and James 1993b). The maturity of weeds varied between fields, in some fields it is possible that early shattered seed would have entered the seedbank. Weeds that shatter earlier or at higher rates, (*Chenopodium album* up to 50% seeds shattering, *Digitaria sanguinalis* 77% seeds shattering) compared to others (*Fallopia convolvulus* 18% seeds shattering; Maity et al. 2021), may be overrepresented in the seedbank. Seeds of summer weeds like *Chenopodium album* are highest in autumn (after shattering) and low in spring (after germination), and winter weeds (*Poa annua, Juncus bufonius)* remain stable at around 8% of the total seedbank (Rahman et al. 2001b).

Our study was sampled after weed control was applied. All weeds detected therefore had either survived or escaped herbicide control. A variety of factors could affect the number and composition of weeds observed growing in maize fields or in the seed banks; determining which factors may be influencing a species is not the subject of this study, but bears considering. In some cases, this could be due to poor herbicide control. Too much, or too little rainfall can reduce the efficacy of pre-emergent herbicides, as they leach from the soil, or are not activated, respectively

(Rahman 1985, Rahman et al. 2011). High seedling cover can reduce post-emergent herbicide efficacy, as weeds shield other weeds from spray droplets (James et al. 2000, 2006b). So too can weed size (James et al. 2000, 2006b), as weeds in advanced growth stages gain tolerance (Faccini and Puricelli 2007). Weeds can escape herbicide control by delaying germination, and this trait that is frequently under selection in arable fields (Darmency et al. 2017). Weed seed dormancy varies between species and between and within populations (James et al. 2006b, 2010a, Kon et al. 2007). The seedbank density of some weeds with seed dormancy may be underrepresented in this study. However, trays were disturbed each at least three times over six months, and most weeds emerge in the first two incubations (Rahman et al. 1998a). Another possibility is that residual herbicides sprayed earlier in the season could be present in the soil samples, as herbicide residues can remain even after months with little rainfall (Rahman et al. 2011). However, no obvious signs of residual herbicides were observed.

Like this study, earlier soil seedbank studies in Waikato and Bay of Plenty maize fields identified the winter weeds *Poa annua* and *Stellaria media* to be common in maize fields (Rahman et al. 2004, 2006). Continuously cropped maize is usually planted into a winter forage grass (Chakwizira et al. 2019), and herbicides may be used to control weeds in these pastures (Ghanizadeh and Harrington 2019c). A population of the winter weed *Stellaria media* in Waikato is believed to be resistant to flumetsulam (Buddenhagen et al. 2021a); this weed has become resistant to herbicides with the same mode-of-action in Southland and Canterbury (Buddenhagen et al. 2021a, Seefeldt et al. 2001). *Poa annua* is another common winter weed that has become resistant to herbicides in cereals in New Zealand (Buddenhagen et al. 2021a). High seedbank densities of these weeds may reflect poor control in winter, which could be due to herbicide resistance. *Sagina procumbens* and *Juncus bufonius* were also common in seedbanks but are diminutive in stature and unlikely to pose any problems to maize or winter crops.

Sunny and warm sites (eastern Bay of Plenty) are better suited to maize grain production, so differences in weed cover and seedbank composition may be explained by environmental conditions or different management of grain maize and silage maize. The management history of each site is likely to be highly important in explaining weed composition, as sites were variable for weed cover and seedbank cover. Arable environments are intensively managed, and likely selected for or against particular weeds. Mechanical and chemical weed control, crop competitivity and nutrient availability select for plants with particular traits. Weed compositions shift from pastoral weeds to weeds that can tolerate the maize growing environment (Rahman 1985). With the use of broadleaf active herbicides like atrazine and 2,4-D, broadleaf weeds become less common and grass weeds become more common (Rahman 1985). Perennial weeds able to tolerate herbicides such as *Calystegia* spp., *O. latifolia*, *C. rotundus*, *C. dactylon* and *P. distichum*, weeds with seed dormancy able to germinate after herbicides are applied such as *D. sanguinalis*, *E crus-galli*, *Persicaria* spp. and weeds with evolved herbicide resistance unaffected by herbicide application such as *C. album*, *D. sanguinalis* and *P. maculosa* appear to now be the common maize weeds in the Bay of Plenty and Waikato.

Similar studies identifying weed flora of maize have been done elsewhere. A German study found *E. crus-galli*, *Chenopodium* spp. and *S. nigrum* were common, standing out from other weeds with disproportionate influence on a PCA of weed densities (Mol et al. 2015). Those weeds were present in 49.0%, 78.9% and 32.7% of 1460 sampled fields, respectively. Many of the same weeds recorded were found in this study, but there was no *C. rotundus* or *P. distichum* present, and *C. dactylon*, *D. sanguinalis*, *Setaria* spp. and *P. dichotomiflorum* were far less frequent in German maize (0.1%, 5.5%, 8.2%, 0.5%). There was not as much *E. repens* (36.5%), *F. convolvulus* (53.6%) or *Matricaria* spp. (51.8%) in this study. *Persicaria* spp. were more common here than in German maize (71% compared to 17-24%). Maize weeds of China are similar to New Zealand, and include cosmopolitan species *D. sanguinalis*, *E. crus-galli*, *E. indica*, *P. aviculare*, *C. dactylon*, *C. rotundus*, *C. album* and *S. nigrum* but also some other species not found here (Zhang 2003). A survey of common weeds of crops including maize in the southern United States had many native US species that are not found in New Zealand (Webster and Nichols 2012). *Cyperus* spp., *Digitaria* spp. *C. dactylon*, *Persicaria* spp., *E. indica*, *Calystegia* spp. are common in the US and were also found in this study. An Ethiopian maize weed flora study featured many species that are absent in New Zealand (Tamado and Milberg 2000), though *C. rotundus*, *C. dactylon*, *D. stramonium*, *S. nigrum* were shared. Many weeds in New Zealand were introduced by European colonists (Brandt et al. 2021), and are likely continuously introduced through the seed trade (Rubenstein et al. 2021). The weed flora of Bay of Plenty and Waikato maize is similar to European and Asian maize (Mol et al. 2015, Zhang 2003), but less similar to American or African maize systems (Tamado and Milberg 2000, Webster and Nichols 2012). It is important to prevent introduction of new weeds, especially from places that weeds in New Zealand have not historically come from.

Some maize weeds are known to have herbicide resistant biotypes (*C. album*, *P. maculosa*, *D. sanguinalis*) (Harrington and James, T.K. 2022, James et al. 2005, Rahman and Patterson 1987), it is possible these weeds could be overrepresented due to their herbicide resistance. It is also possible that the evolution of resistance in those weeds was made more likely because of their abundance. The five weed species considered to have the highest risk of developing herbicide resistance were

E. crus-galli, E. indica, C. album, D. sanguinalis and *A. powelli* (Chapter 2: Ngow et al. 2021). All were found in this survey, but *E. indica* and *A. powelli* were uncommon, and should be less likely to evolve herbicide resistant populations locally than the other species. Other common weeds *F. convolvulus, P. dichotomiflorum, S. pumila, S. nigrum* were considered at moderate-high risk of evolving resistance, but perennial weeds *Calystegia* spp., *C. dactylon, C. rotundus, E. repens, Oxalis latifolia* and *P. distichum* are considered low risk.

Weeds that have frequently evolved herbicide resistance in maize that are not widespread in New Zealand are: *Abutilon theophrasti*; *Amaranthus* spp. (A. *hybridus*, *A. palmeri*, *A. tuberculatus*, *A. retroflexus*); *Ambrosia artemisifolia, Kochia scoparia*; *Setaria faberi*; *Setaria viridis*; *Sorghum* spp. (*Sorghum halapense, Sorghum bicolor*) (Heap 2022). As they have become herbicide resistant repeatedly overseas, it is likely that inadvertent importation of contaminant seeds from those countries could carry herbicide resistance traits. *Amaranthus* species, particularly *Amaranthus palmeri* are major weeds in the United States that are now known to have been accidentally exported, with resistant biotypes included, to Europe, Africa, Asia and South America (Iamonico and El Mokni 2017, Küpper et al. 2017, Shimono et al. 2020, Torra et al. 2020). *Amaranthus* spp. are common contaminants in seed lots entering New Zealand (Rubenstein et al. 2021), but no known incursion has occurred yet for this species in New Zealand. Unlike Europe and North America, *Ambrosia artemisifolia* is present but is not a major agricultural weed here; it has been known to be a seed contaminant but was not found in recent seed inspection data (Rubenstein et al. 2021, Webb et al. 1988). *Sorghum halapense* and *Abutilon theophrasti* are on the quarantine species list and are occasionally detected (Ministry for Primary Industries, 2018, Rubenstein et al. 2021). Two major incursions have spread *Abutilon theophrasti*, across Waikato in 2012 and across New Zealand in fodder beet in 2015 (James and Cooper 2012, James and Pene 2018b). There is a program to manage velvetleaf involving the Ministry for Primary Industries. *Sorghum halapense,* first found in Gisborne in 1972, then in 39 Waikato farms in 1980, was targeted for removal, then believed to be eradicated (Hulme 2020, Rahman 1985). After no detections over the past ten years, *S. halapense* was recently found in an Auckland garden (Ministry for Primary Industries 2022).

3.5 Conclusions

Random surveys of two major maize growing regions revealed *Digitaria sanguinalis, Chenopodium album* and *Persicaria* spp. to be the most abundant summer weeds in maize crops. The regions were similar in their suite of weeds, but some were common in Waikato and not Bay of Plenty. *Echinochloa crus-galli* and *Setaria pumila* are prominent in Waikato farms, while perennial weeds *Cyperus rotundus*, *Cynodon dactylon* and *Paspalum distichum* are problematic in Bay of Plenty farms.

Chenopodium album, *E. crus-galli* and *Persicaria* spp. may be increasing in abundance compared to the largest previous study in these regions only a little more than a decade earlier. *Amaranthus powelli* appears to have decreased in abundance. The most common species (*D. sanguinalis*, *C. album*, *P. maculosa*, *Setaria pumila*, *Solanum nigrum*) in maize fields also happen to be at a high risk of developing herbicide resistance (Chapter 2; Ngow et al. 2021), and four of those five have already evolved resistance in New Zealand. Farms varied in weediness, but with an average weed cover of 20.7%, this indicates weeds are not always well controlled. Maize fields had seedbanks with a mean density of 2810 seeds m^{-2}, with such large seed banks, rare resistance conferring mutations are more likely to occur and become selected for. Weed problems of the future can be prevented by targeted weed control, considering farm-farm biosecurity, implementing herbicide resistance mitigation practices and with effective border biosecurity to prevent invasions of highly resistant and invasive weeds.

Chapter 4: Prevalence of herbicide resistant weeds in maize in Bay of Plenty and Waikato

4.1 Introduction

Herbicide resistant weeds are a major challenge to the current chemical-based system of growing many arable crops. Herbicides were introduced to agriculture in the 1940s after the discovery of the synthetic auxin herbicides MCPA and 2,4-D (Kudsk and Streibig 2003), allowing selective control of particular weeds and reducing reliance on tillage and manual labour. In New Zealand 2,4-D was widely used in maize, removing broadleaf weeds but not annual grass weeds (Rahman 1985, Woon 1970). In the 1950s triazine herbicides were discovered (Kudsk and Streibig 2003). These became more popular as they were able to control broadleaf weeds and a problematic grass weed *Echinochloa crus-galli (*L.) Beauv if used at high rates (Patterson 1961, Rahman 1985). Other grass weeds *Digitaria sanguinalis* (L.) Scop., *Panicum* spp. and *Setaria* spp. were not controlled by atrazine or 2,4-D. New herbicides to control grass weeds were explored: a substituted urea herbicide linuron, thiocarbamates EPTC and butylate, and chloroacetamides alachlor, metolachlor, propachlor and dimethenamid-P (Capper 1975, Cumberland et al. 1970, Kitchener 1971, Rahman 1985, Rahman and James 1992, Rowe et al. 1976, Sumich 1963, 1966). The thiocarbamate herbicide EPTC began to show reduced control in soils where it had been applied repeatedly, due to microbial metabolism (Rahman and James 1983). A dinitroaline herbicide pendimethalin was introduced in the 1980s for grass weed control (Rahman 1985).

The non-selective herbicide glyphosate was made available in 1974, and was used for removing perennial grasses and other weeds before planting maize (Bishop and Field 1987, Duke 2017, Rahman 1985). Atrazine was the most widely used herbicide in the 1980s; wide-spectrum weed control relied on atrazine, with additional grass herbicides (thiocarbamate herbicides, chloroacetamide herbicides, pendimethalin and linuron) or broadleaf herbicides (triazines, synthetic auxins: 2,4-D, dicamba) used depending on the weed composition (Rahman 1985). In 1982, the first case of herbicide resistance was detected in New Zealand; the broadleaf weed *Chenopodium album* L. began surviving rates between 35 to 60 kg a.i. ha^{-1} of atrazine, up to 60× more resistant than susceptible populations (current recommended rates of atrazine in maize are 750-1500 g.a.i.ha^{-1}) in Waikato maize crops (Rahman et al. 1983c). Atrazine-resistant *C. album* are resistant to other triazines, but not bromoxynil, which has been placed in a different sub-group of photosystem-II inhibitors (Ghanizadeh and Harrington 2017b). It is not known what the mechanism for atrazine resistance in these populations is, but it is believed that a mutation of the target site gene (*psb*A) is responsible (Ghanizadeh and Harrington 2019b). Not long after, a *Persicaria maculosa* L. population in northern Waikato developed resistance toward atrazine (up-to 10× more resistant than a susceptible population), with cross-resistance to other triazine herbicides (Rahman and Patterson 1987). For both resistant weeds, alternative herbicides, including dicamba were used. By the 1990s, herbicide resistant *C. album* was thought to be a problem in almost all maize fields, but resistant *P. maculosa* was not thought to have spread widely (Harrington and James 2005, Rahman 1990 p. 199).

The sulfonylurea nicosulfuron started to be used in 1990s to control some annual and perennial grass weeds as well as broadleaf weeds (James et al. 2006b, James and Rahman 1997, Rahman and James 1993a). In 2005, dicamba-resistant *C. album* was reported from eastern Waikato (James et al. 2005). The original site had had dicamba applied for twenty years to treat atrazine resistant *C. album*; at the time it was estimated 100 hectares were affected (James et al. 2005). Dicamba-resistant *C. album* were 19× more resistant than susceptible populations (Ghanizadeh et al. 2015b). Those dicamba-resistant *C. album* populations were morphologically distinct, with lighter-coloured and smoother-margined leaves. They were also resistant to atrazine, and later studies showed that there was cross-resistance to pyridine carboxylic acid synthetic auxins (clopyralid, aminopyralid) but not phenoxy acid synthetic auxins (2,4-D, mecoprop; Ghanizadeh and Harrington 2017b, Rahman et al. 2014b). A significant fitness cost was imposed on the plants because of their evolved multiple resistance (Ghanizadeh and Harrington 2019a). Dicamba resistance in *C. album* populations is believed to be due to target-site resistance after investigation of some non-target site mechanisms (Ghanizadeh et al. 2018).

New herbicides entered the local market in the 2000s, a triketone herbicide mesotrione, a pyrazol herbicide topramezone and a pyrimidinedione herbicide saflufenacil (James et al. 2006a, Rahman et al. 2014a, Trolove et al. 2011). A trial showed evidence that atrazine-resistant *C. album* was present in the Bay of Plenty and Manawatu (James et al. 2007). Soils with a histories of repeated use of atrazine were shown to have increased degradation of atrazine (James et al. 2010c). Most recently an initial case of *Digitaria sanguinalis* resistant to the AHAS inhibitor nicosulfuron was reported in 2017 from a Bay of Plenty farm (Buddenhagen et al. 2021a, Harrington and James, T.K. 2022, Hutching 2017). The resistant population first identified was 2× more resistant than a susceptible population, surviving the label rate (60 g.ai.ha^{-1}; Harrington and James, T.K. 2020). New resistant populations (I1, I2 in this study) collected in 2019 were shown to be resistant to at least 60 g.ai.ha^{-1} (Buddenhagen et al. 2021a). A list of herbicides used in maize is presented in Chapter 2: Ngow et al. 2021 and the most common weeds in Chapter 3.

Randomized surveys aim to provide an estimate of the prevalence of weed resistance to herbicides in a geographical area (Buddenhagen et al. 2019). A recent survey in Canterbury cereals found widespread ACCase (40%) and AHAS (23) inhibitor resistant *Lolium* spp. in 88 randomly selected farms (Buddenhagen et al. 2021a). That survey also found unexpected new resistance, *Sonchus* spp. did not seem to be problematic in cereal fields, yet 14% of farms had *Sonchus* spp. resistant to AHAS inhibitors. Past studies of herbicide resistant weeds in maize did not describe the geographic extent, or scale, of resistance (Ghanizadeh and Harrington 2019b). Atrazine resistant *Chenopodium album* was believed to be present in almost all maize fields, while dicamba resistant *C. album* was only noted from farms near Matamata, Waikato and atrazine resistant *P. maculosa* from farms near Te Kowhai, Waikato (Rahman 1990, Rahman et al. 2014b). Nicosulfuron resistant *D. sanguinalis* is believed to be in Bay of Plenty and Waikato, having been found in a western Bay of Plenty farm and two eastern Waikato (Morrinsville, Matamata) farms (Buddenhagen et al. 2021a).

The study objectives are to reveal the presence (or lack of) of any new resistance and to determine the distribution of resistant weeds in maize in two of New Zealand's major growing regions (Waikato, Bay of Plenty). Results presented here will be the first randomized survey of herbicide resistant weeds in maize in New Zealand.

4.2 Materials and Methods

To select farms, farm managers or owners were contacted in randomized order from two databases to provide twenty Waikato and ten Bay of Plenty farms from each list (a total of 60). Paddocks needed to have had cultivated maize grown for at-least two years. Farmer contact information was sourced from Agribase (Sanson 2000), which is based on voluntary surveys of farmers across the country and the Foundation for Arable Research (FAR) member database, which holds information of members who pay a levy on maize seed. Farms were sampled before harvest, in late February (Waikato) and early March (Bay of Plenty) 2021. A total fifty-two sites were sampled in the survey. Additionally, in January, four sites in the Bay of Plenty surrounding a farm with suspected nicosulfuron-resistant *D. sanguinalis* were sampled specifically for *D. sanguinalis*, and sites previously detected as resistant in Buddenhagen et al. (2021) were included. Seeds were collected from mature weeds present within fields (in and near transects surveyed in Chapter 3) and stored at 5°C.

Seeds from 773 plants were collected for herbicide resistance testing (Appendix 7). A decision was made to test *Chenopodium album, Persicaria* spp. and *Digitaria sanguinalis* for herbicide resistance. These three weeds were the most abundant weed species (Chapter 3), they were assessed as high or moderate risk for developing herbicide resistance (Chapter 2), they had a history of herbicide resistance in New Zealand (Chapter 2), and were the most frequently collected (Appendix 7). Seeds were collected from 151 *D. sanguinalis*, 106 *C. album* and 131 *Persicaria* spp. plants. Multiple samples were collected from each farm, as separate individuals, and each were tested at herbicide label rates (Buddenhagen et al. 2019). Farms without a weed collected did not have the weed present in abundance, or the weed did not have mature seed. As field sampling was in summer prior to harvest, it is likely any weed not with mature seed would have germinated after spraying had occurred earlier in the season.

Susceptible (negative) and resistant (positive) controls were used to ensure herbicide treatments were effective. For *C. album*, atrazine-resistant *C. album* previously collected were used for resistant controls, and atrazine (and other herbicide) susceptible *C. album* were used for susceptible controls. For *Persicaria* spp., no resistant samples were available, but putative 'susceptible' controls were collected from a railway at Ruakura. For *Digitaria sanguinalis,* samples from the original nicosulfuron-resistant population and others recently confirmed as resistant (Buddenhagen et al. 2021) were used as resistant controls, and samples collected from gardens at Ruakura were used as susceptible controls.

Chenopodium album, Persicaria spp. and *D. sanguinalis* were tested for herbicide resistance in the Ruakura glasshouse facility using a 'tray method' (Buddenhagen et al. 2021a). Plastic propagation trays (Egmont RXPROPT; 34cm × 20cm × 6cm) were filled with potting mix (Dalton's grass and clover mix; 33.3% coco-coir, 33.3% pumice, 33.3% bark). Trays had six rows of four samples and a negative and positive 'control' if possible; samples were planted on the outer two rows and controls in the centre two rows.

Chenopodium album was soaked in a 0.1% potassium nitrate solution for 24 hours, planted on the surface of the potting mix, covered over with seed raising mix (Yates® Black Magic®) and immediately watered. The same process occurred for *D. sanguinalis*. *Persicaria* spp. were not treated, and were planted into trays of potting mix at a depth of 1cm. When plants were within the recommended size for herbicide treatment (3 leaf stage for the grass, and 4-leaf broadleaf weeds), they were counted and treated.

Trays were sprayed with a moving belt glasshouse sprayer using a single fan nozzle (TeeJet TT11002) at 200 kPa, positioned 440 mm above the top of the pots/trays to apply 200 L/ha, as in previous studies (Buddenhagen et al. 2021a). All herbicide treatments used the highest recommended label rate for the herbicide tested. *Chenopodium album* and *Persicaria* spp. were treated with the same herbicides: atrazine (Atraflow ®) at 1500 g.ai.ha^{-1}, nicosulfuron (Latro WG ®) at 60 g.ai.ha^{-1}, mesotrione (Mesoflex ®) at 96 g.ai.ha^{-1} and dicamba (Banvel 480 ®) at 600 g.ai.ha^{-1}; *Digitaria sanguinalis* samples were treated with nicosulfuron at 60 g.ai.ha^{-1} and 0.5% oil-based adjuvant (Bonza®), when plants had not yet tillered. Mortality was assessed after 3-5 weeks, but as that varied, final assessments were not made until it was clear susceptible controls had died.

Digitaria sanguinalis populations shown to be resistant in initial tests were further assessed for their level of resistance in a pot-based dose-response experiment. Seed dormancy was broken by being placed in 0.2% potassium nitrate solution for 24 hours, then up to three seeds each were planted onto the surface of small (5cm × 5cm × 8cm) pots filled with grass and clover mix and covered over with seed raising mix. Plants were grown out and sprayed at the pre-tillering growth stage. Germination varied, and some plants had >1 tiller, these were marked with tags and counted separately (results for large plants not presented here). Two pots, each with 1-3 seedlings were assigned to treatments. Pots were sprayed with 0, 15, 30, 60, 120 and 240 g.ai.ha^{-1} of nicosulfuron and 0.5% oil-based adjuvant. Pots were assessed for mortality 16 days later.

Resistant farms were mapped using the *ggmap* R package (Kahle and Wickham 2013). Soil seedbank seed count and estimated percentage soil cover of weeds (Chapter 3) in farms with and without resistance were compared using a Wilcoxon test (as they had a non-normal distributions) and plotted using *ggplot2* (Wickham 2016). 95% confidence intervals for estimates of resistance were calculated using the binomial distribution.

4.3 Results

4.3.1 Prevalence of resistance in farms

Twenty-three farms (44%; 95% confidence interval between 32-58%) had either atrazine-resistant *C. album* (22), nicosulfuron-resistant *D. sanguinalis* (7) or both (5). Eighteen farms had no resistance detected, and eight had none of those three weeds collected and tested (Table 1).

Table 4-1. Surviving plant count (left: survivor count, right: treated count) of weeds treated with herbicides by each farm (alive/tested) from Bay of Plenty (BP) and Waikato (WK). Abbreviations refer to the weed species: CA = *Chenopodium album,* PS = *Persicaria* spp, DS = *Digitaria sanguinalis*.

	CA				**PS**		**DS**		
ID	**atrazine**		**dicamba**		**atrazine**		**nicosulfuron**		**Resistance Summary**
BP1	3	35	0	27	0	28	0	29	CA atrazine (developing)
BP2					0	2	0	3	none
BP3	6	6	0	3					CA atrazine
BP4	5	5	0	3					CA atrazine
BP5	0	50	0	50	0	60			none

BP6									no weeds tested
BP7	51	76	0	87	0	14	0	18	CA atrazine
BP8	0	40	0	47	0	5	0	9	none
BP9	0	7	0	11			0	15	none
BP10									no weeds tested
BP11									no weeds tested
BP12							0	3	none
BP13							0	16	none
BP14	0	20	0	19			0	3	none
BP15									no weeds tested
BP16	0	5	0	3			0	4	none
WK1					0	2			none
WK2									no weeds tested
WK3	13	13	0	7	0	12			CA atrazine
WK4									no weeds tested
WK5							1	4	DS nicosulfuron (developing)
WK6	43	43	0	28	0	3			CA atrazine
WK7	12	26	0	30			0	28	CA atrazine
WK8	0	5	0	9					none
WK9	0	50	0	51	0	66			none
WK10					0	2	8	19	DS nicosulfuron
WK11	92	92	0	77	0	36			CA atrazine
WK12							0	27	none
WK13	69	69	0	65	0	13	0	2	CA atrazine
WK14	0	77	0	80	0	17	0	28	none
WK15	59	59	0	68	0	42	18	53	CA atrazine, DS nicosulfuron
WK16	0	15	0	18	0	12	0	12	none
WK17	26	26	0	28	0	4			CA atrazine
WK18	0	11	0	12	0	37	4	30	DS nicosulfuron (developing)
WK19	28	28	0	26	0	5	5	25	CA atrazine, DS nicosulfuron
WK20							11	59	DS nicosulfuron
WK21	19	41	0	37	0	38	0	9	CA atrazine
WK22					0	30	0	27	none
WK23	8	8	0	5	0	1	12	23	CA atrazine, DS nicosulfuron
WK24	35	35	0	37	0	6	7	25	CA atrazine, DS nicosulfuron
WK25									none
WK26	7	7	0	4			0	18	CA atrazine
WK27									no weeds tested
WK28	35	35	0	38	0	24	22	22	CA atrazine, DS nicosulfuron
WK29					0	9	0	7	none
WK30	21	21	0	21	0	2			CA atrazine
WK31	84	84	0	90	0	24	0	21	CA atrazine
WK32	45	45	0	40	0	31	0	13	CA atrazine
WK33	11	11	0	14			0	9	CA atrazine
WK34	17	30	0	30	0	13	2	50	CA atrazine, DS nicosulfuron (developing)
WK35									no weeds tested
WK36					0	14			none

4.3.2 Chenopodium album

Atrazine resistant *Chenopodium album* is common; of the 106 samples collected, 99 samples were tested and 65 of those were resistant. Forty-three percent of farms (95% binomial confidence interval is 30-56%) had atrazine-resistant *C. album*. 78% of Waikato farms (18/23) and 44% of Bay of Plenty farms (4/9) with mature *C. album* seed tested had atrazine resistance (Fig 4-1). Most Waikato and western Bay of Plenty farms have atrazine resistant *C. album,* but the eastern Bay of Plenty is mostly free of atrazine-resistant *C. album*. Only one farm in that area had 1/3 samples testing as resistant. All resistant populations were in silage maize except for one Waikato farm. Two additional sites outside of the survey tested negative for atrazine and dicamba resistance (Appendix 10).

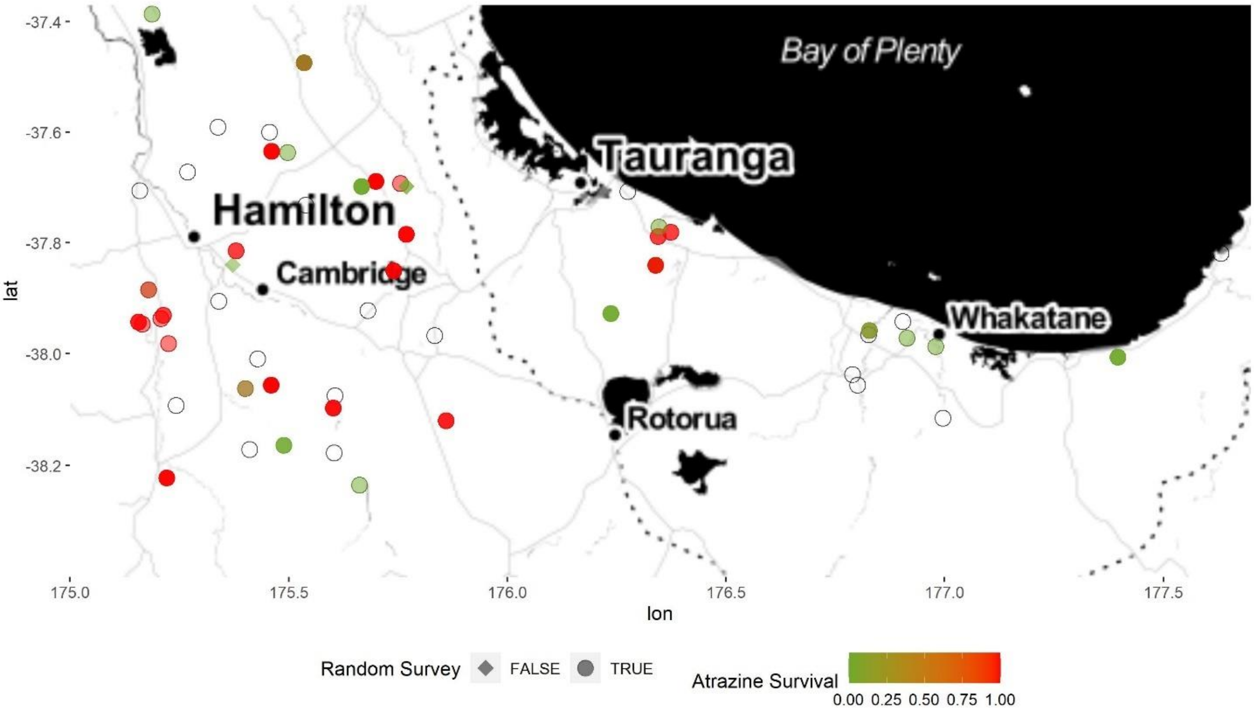

Figure 4-1. Atrazine resistance in 99 *Chenopodium album* samples collected from 32 farms in a survey of 52 randomly selected farms in Waikato and Bay of Plenty. Open circles are farms where *C. album* was not collected or otherwise able to be tested, closed circles are farms from the randomized survey and diamonds are farms from outside the survey.

Atrazine resistant *C. album* plants were not damaged by atrazine, but mesotrione, dicamba and nicosulfuron were effective on all populations (Fig 4-2). Typical dicamba symptoms were observed on all treated populations (extended stems, leaf cupping). Some *C. album* plants that were larger at time of treatment still produced flowers despite the damage from dicamba. Extensive damage was observed on all populations treated with dicamba in this survey, unlike prior studies by James et al. (2005) where no damage was observed. No dicamba resistance was detected.

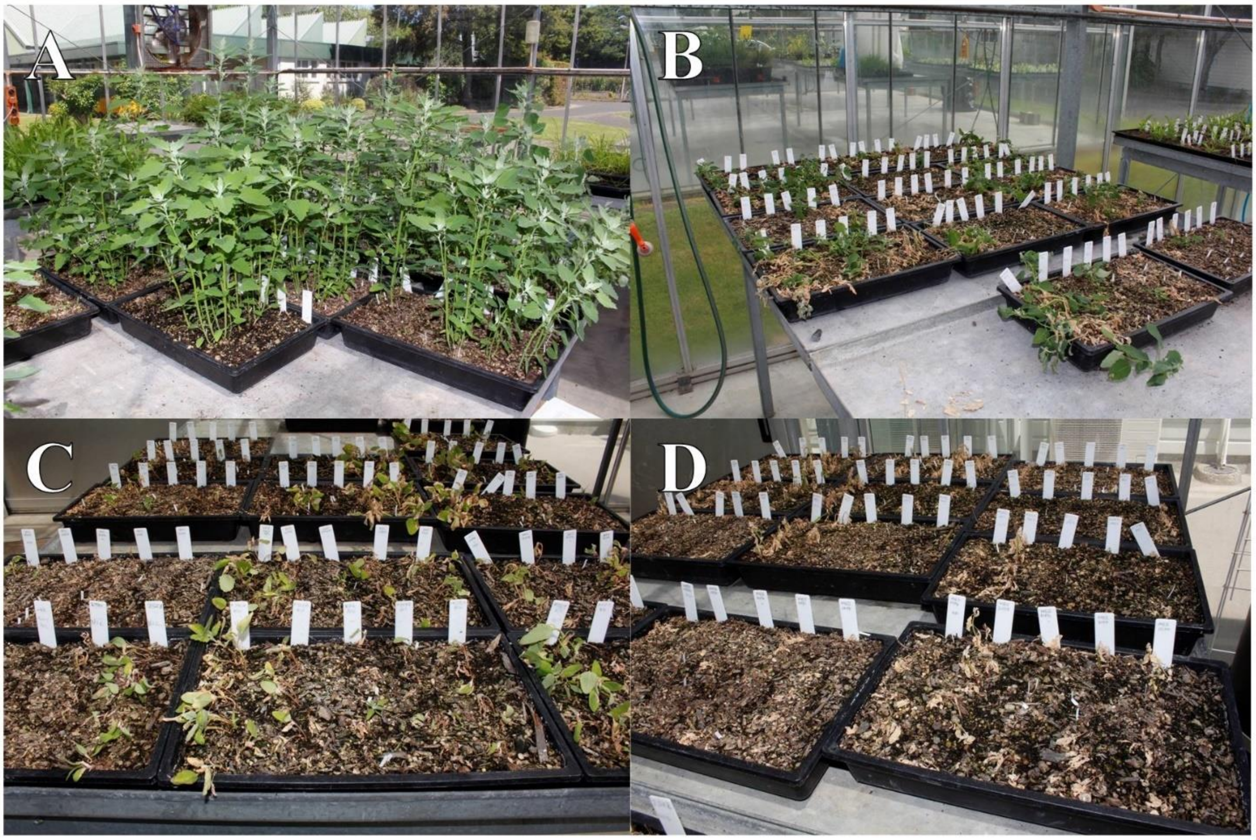

Figure 4-2. Response of *Chenopodium album* populations to (**A**) 1500 g.ai.ha^{-1} atrazine, (**B**) 600 g.ai.ha^{-1} dicamba, (**C**) 60 g.ai.ha^{-1} nicosulfuron and (**D**) 96 g.ai.ha^{-1} mesotrione. A known resistant control is in the middle row of each tray for the atrazine treatment (**A**).

For *C. album,* atrazine-resistance was correlated with increased abundance in the soil seedbank (Wilcoxon test: w-value 226.5, p-value 0.01756) but not percentage cover (Wilcoxon test: w-value 346.5, p-value 0.7157). There was no evidence of a difference between field headland (rows located near the edge) and centre areas for *C. album* percentage cover (Wilcoxon test: w-value 431, p-value = 0.9574); nor for seedbank density (Wilcoxon test: w-value 361.5, p-value = 0.2608). Resistant farms had a mean seed bank density close to four times that of susceptible farms. Most sites (resistant or susceptible) had percentage cover below 10% and seedbank density below 1000 seeds m^{-2} for *C. album*; the only two sites with higher values were those with atrazine resistant *C. album* (Table 4-1, Appendix 9).

4.3.3 Digitaria sanguinalis

Nicosulfuron-resistant *Digitaria sanguinalis* was found on ten farms (Table 1, Fig 4-3). No nicosulfuron resistant *D. sanguinalis* was found in Bay of Plenty farms (0/9) but ten Waikato farms (10/22 tested) had resistant *D. sanguinalis*, though plants from some of those farms had low rates of survival (WK5, WK18, WK34). Thirty-one farms were tested; of those farms not tested, all but three had low abundance (<5%; Appendix 9). With the more conservative estimate (not considering WK5, WK18, WK34 to have resistant *D. sanguinalis*), we estimate 0% (0/16) of Bay of Plenty farms and 19% (7/36) of Waikato farms have this a biotype (overall 13%, with a 95% binomial confidence interval of 7-25%). One farm located near Lichfield had much more resistant *D. sanguinalis* than others (WK28) with 100% survival. All sites with nicosulfuron-resistant *D. sanguinalis* were growing silage maize.

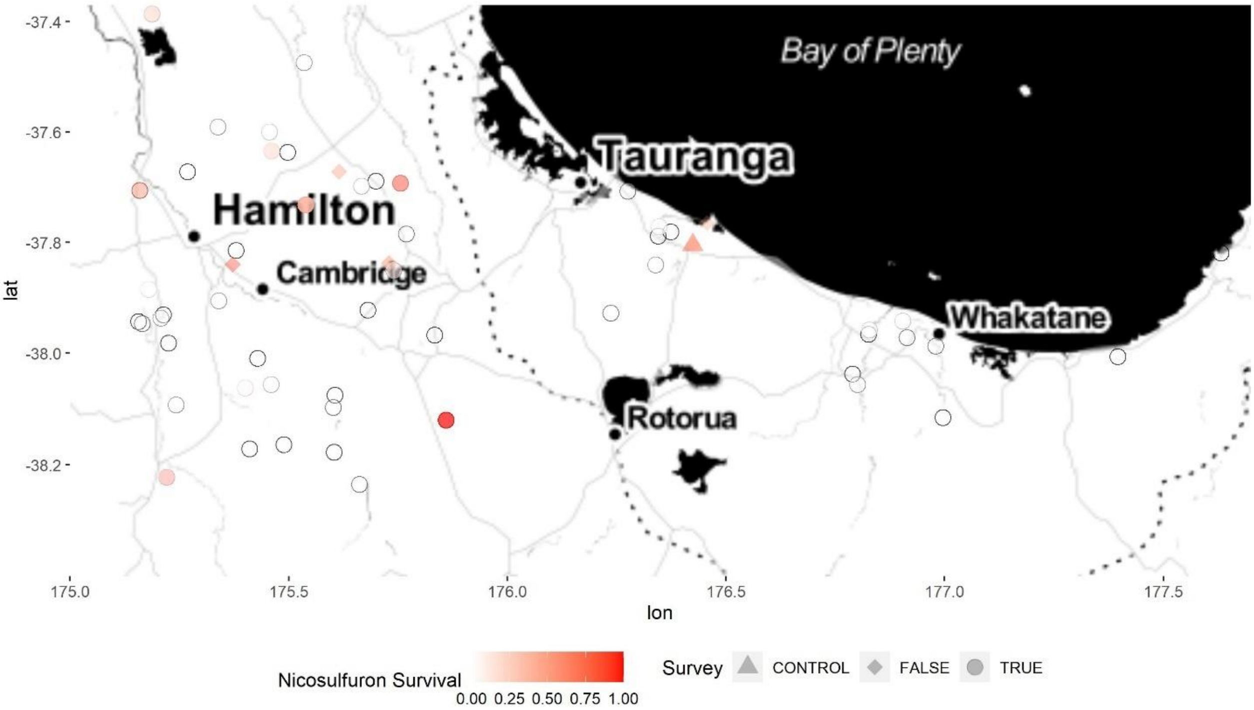

Figure 4-3. Nicosulfuron resistance in 139 *Digitaria sanguinalis* samples from 31 farms in a survey of 52 randomly selected farms in Waikato and Bay of Plenty. Open circles are farms where *D. sanguinalis* was not collected or otherwise able to be tested, closed circles are farms from the randomized survey, diamonds are farms from outside the survey and the triangle is the original site used as a resistant control.

Two farms outside of the randomized survey in the Bay of Plenty (NS1, NS3; Appendix 10) were did not have resistant *D. sanguinalis*, but an additional site managed by the contractor connected to the original nicosulfuron resistance case from 2017 had resistant plants (Harrington and James, T.K. 2022). Two additional samples from self-reporting growers in eastern Waikato tested positive (IN1, IN2), and one tested negative (IN3). Another non-survey sample from the Northern Crop Research Site may be resistant, but not enough samples were tested.

Figure 4-4. Response of *Digitaria sanguinalis* populations to 60 g.ai.ha^{-1} nicosulfuron. A known resistant control is in the middle row of each tray.

Seedbank densities of *D. sanguinalis* in farms with nicosulfuron resistance were significantly higher than farms with susceptible plants (Wilcoxon test: w-value = 1301, p-value = 0.001075; Fig 4-5) and similar differences were seen for percentage cover (Wilcoxon test: w-value = 1290.5, p-value = 0.001925; Fig 4-5). Percentage cover estimates for nicosulfuron resistant farms were more than twice those of farms with nicosulfuron susceptible *D. sanguinalis* (Fig 4-5). There was no evidence of differences in seedbank densities (Wilcoxon test: w-value 1186, p-value = 0.2687) or plant cover (Wilcoxon test: w-value 1146.5, p-value = 0.1817) between field headlands and centres.

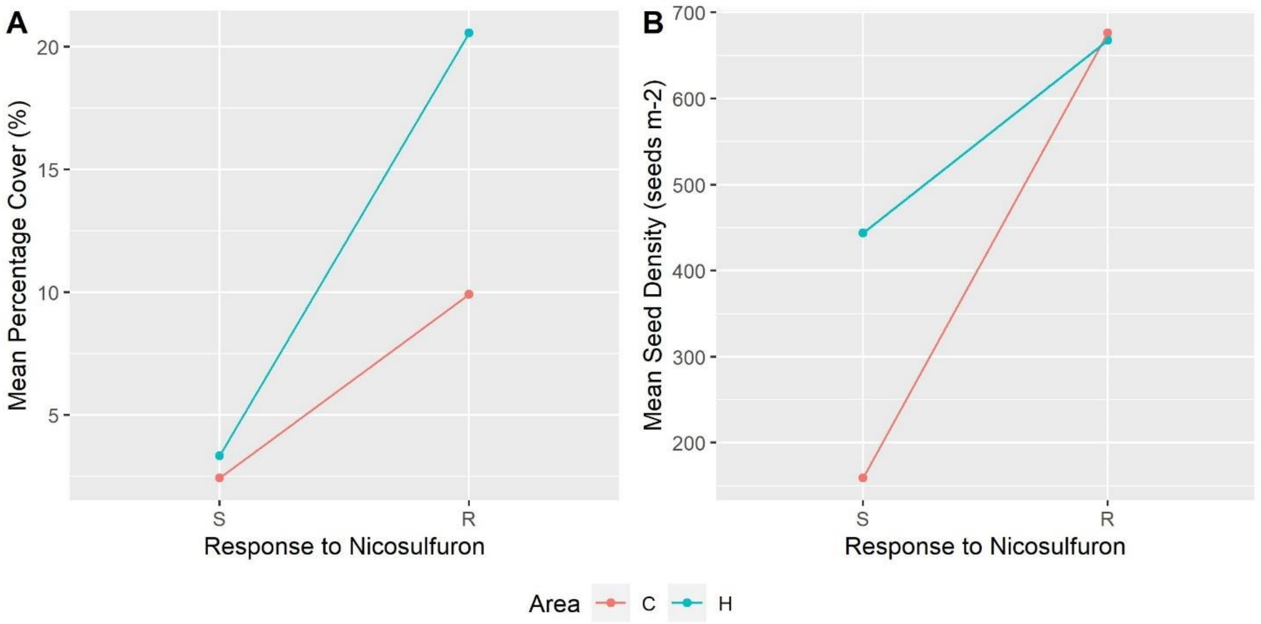

Figure 4-5. Interaction plots of (**A**) the mean percentage cover and (**B**) mean percentage seedbank density (seeds m^{-2}) of *D. sanguinalis* for nicosulfuron susceptible (S) and resistant (R) sampled sites in centre (C) and headland (H) transects.

A preliminary study to determine the amount of resistance *D. sanguinalis* populations has shown that there were differences between populations (Fig 4-6, Table 1). Resistant plants were usually stunted, with decreased growth compared to untreated (Fig 4-4, Fig 4-6). Samples from WK10 and WK28 (Fig 4-6), survived rates up to 240 g.ai.ha^{-1} (four times the recommended rate) and WK20 and WK23 to 60 g.ai.ha^{-1} (the recommended rate). The susceptible control collected from gardens did not survive the lowest rate (15 g.ai.ha^{-1}). The farmer-reported sample IN3 believed to be nicosulfuron-resistant was similar to the susceptible garden sample, failing to survive the lowest rate (Fig 4-6). Other field-collected populations that were not resistant had some survival to 30 g.ai.ha^{-1} nicosulfuron.

Figure 4-6. Response of (**top**) a susceptible sample and (**bottom**) the most resistant *Digitaria sanguinalis* sample treated with 4X, 2X, 1X (60g a.i. ha$^{-1)}$, 1/2X, 1/4X, 0X nicosulfuron and adjuvant (0.5% vol.) 16 days after treatment.

4.3.4 *Persicaria* spp.

Four willow weed (*Persicaria* spp.) species were collected (willow weed *Persicaria maculosa,* pale willow weed *Persicaria lapathifolia,* waterpepper *Persicaria hydropiper* and 'Giant willow weed' *Persicaria* sp.). There were no *Persicaria* spp. resistant to atrazine, mesotrione, nicosulfuron or dicamba in any of the 29 farms tested (Table 1, Fig 4-7).

Figure 4-7. Response of *Persicaria* spp. populations to (**A**) 1500 g.ai.ha^{-1} atrazine, (**B**) 600 g.ai.ha^{-1} dicamba, (**C**) 60 g.ai.ha^{-1} nicosulfuron and (**D**) 96 g.ai.ha^{-1} mesotrione.

4.4 Discussion

Herbicide resistant weeds are widespread in Waikato and Bay of Plenty farms. We detected 44% of all farms surveyed to have either atrazine-resistant *C. album* or nicosulfuron-resistant *D. sanguinalis.* The FAR member database listed 300 Waikato and 81 Bay of Plenty arable members and Agribase listed 570 Waikato and 202 Bay of Plenty farms that grow at-least one hectare of an arable crop. If the number of farms growing maize is correct, it can be estimated that between 122-448 Waikato and Bay of Plenty maize farms may have herbicide resistant weeds. It may be that 27-193 farms have nicosulfuron-resistant *Digitaria sanguinalis*.

It has long been known that atrazine resistant *C. album* are widespread in Waikato maize (James et al. 2005, Rahman 1990, Rahman et al. 2014b), this study confirms that. All resistant *C. album* appeared completely unaffected by the herbicide, which may suggest the resistant biotypes are the same as previously reported. Interestingly some Waikato farms still did not have atrazine resistant *C. album*. This study also shows that some western Bay of Plenty farms have atrazine-resistant *C. album*, but eastern Bay of Plenty farms do not. By chance, no farms along the Matatā straight were selected (between Te Puke and Whakatane). A previous study in a Matatā farm showed evidence of atrazine resistance in *C. album*, with some seedlings germinating after pre-emergent atrazine +

acetochlor (James et al. 2007). Past studies of seedbanks have shown high numbers of *C. album* in Bay of Plenty, Waikato and Hawkes Bay (Rahman et al. 2006), which may indicate atrazine resistance.

Dicamba resistant *C. album* were previously reported from farms near Matamata (Ghanizadeh et al. 2015b, James et al. 2005, Rahman et al. 2014b) In this survey, there were eight farms sampled within a 15km radius of Matamata. One had no *C. album* recorded, two had *C. album* present but with viable seed collected and tested seed, and five tested as susceptible. The smooth leaf margin morphotype that was previously associated with dicamba-resistant *C. album* was observed within some of these farms, though there was no systematic investigation of the morphology of *C. album* leaves across the whole survey. The lack of widespread dicamba resistant *C. album* could be due to their reduced fitness and the availability of other herbicide options (Ghanizadeh and Harrington 2019a, Rahman et al. 2014b). For all the *C. album* (atrazine resistant and susceptible) populations in this study, dicamba, mesotrione, nicosulfuron and likely topramezone and some non-triazine photosystem II inhibitors (bromoxynil) are viable post-emergent herbicide options (Rahman et al. 2008, 2014b). To avoid resistance, rotations between these effective modes-of-action is recommended.

Nicosulfuron resistant *D. sanguinalis* populations are sporadically distributed. Previous efforts revealed that resistance is low, at 2× the susceptible rate (Harrington and James, T.K. 2022). Earlier work found resistant *D. sanguinalis* in eastern Waikato and the western Bay of Plenty, with this survey, more locations were identified across the Waikato. Given that the first reports of nicosulfuron resistant *D. sanguinalis* are recent, it seems that the resistance is increasing. My investigation of farms near the original site revealed that no nearby farms had resistant *D. sanguinalis* but other sites that were being cropped by the same contractor had resistance. There is no natural ability for long-distance dispersal in *D. sanguinalis,* nor *C. album* (as there are in other herbicide-resistant species, for example wind-dispersed *Sonchus* spp.; Merriam et al. 2018; Buddenhagen et al. 2021). In addition, plants are largely self-pollinating, and unless resistance evolved separately many times, it is likely the seed has been spread between farms through machinery. Knowledge of farm-farm network connectivity (i.e. through shared contractors) could provide insights into farm biosecurity risks. Hygiene measures for equipment, vehicle and people moving between and within farms is important to avoid spreading resistant weeds further.

With emerging nicosulfuron resistance, there are some options still available to control resistant *D. sanguinalis*. Topramezone appears to be a viable option, though it is noted that higher rates are required for tillering plants (Rahman et al. 2013). Mesotrione alone is not an effective post-emergent or pre-emergent option for *D. sanguinalis* nor is the pre-emergent herbicide saflufenical effective (James et al. 2006a, Trolove et al. 2011). In both cases, mixtures with chloroacetamides gave good control. The chloroacetamide herbicides acetochlor, alachlor, dimethenamid-P and s-metolachlor are known to be effective on *D. sanguinalis* (James and Rahman 2009, Rowe et al. 1976). Pendimethalin remains a viable pre-emergence option, but it can be damaging to maize crops (Rahman 1985). It is important to not rely on one option, and to rotate between chemical groups to avoid nicosulfuron-resistant *D. sanguinalis* further evolving resistance to other groups. Even with these other options, post-emergent control is lacking, with later germinating seedlings potentially able to avoid pre-emergent herbicides. Whilst late germinating seedlings may be less impactful, as weed competition is most important in the early season when maize plants are small (Page et al. 2012), the ability of *D. sanguinalis* to emerge late will only help nicosulfuron resistant populations persist.

Increased seed dormancy is a trait that has evolved in response to intensive herbicide selection, just like evolved herbicide resistance. In some cases, herbicide resistant weed populations have concurrently evolved increased dormancy (Maity et al. 2022, Owen et al. 2011). *Digitaria sanguinalis* seeds exhibit variable levels of dormancy (James et al. 2007, Oreja et al. 2020), which selection is able to act on. Later germinating *D. sanguinalis* seedlings would avoid pre-emergent herbicides but might be too small to survive nicosulfuron (note that susceptible plants can survive nicosulfuron if they are large enough). With nicosulfuron-resistance, these late germinating seedlings can survive. Further, seedbank densities are higher with nicosulfuron resistance present (Fig 4-5), so there will be more seeds for selection to act on. Therefore, it is possible that selection for increased dormancy could have occurred in Bay of Plenty and Waikato maize and co-occurred with selection for nicosulfuron resistance.

The lack of atrazine resistant *Persicaria maculosa* is not surprising. It is believed that the initial resistant *P. maculosa* infestation had not spread far (Rahman 1990). It is unknown if atrazine-resistant *P. maculosa* had reduced fitness, or increased susceptibility to other herbicides as atrazine-resistant *C. album* has (Ghanizadeh 2015). The use and availability of other herbicide options for *Persicaria* spp. may have prevented the resistant biotypes' spread. Both *P. maculosa* and *P. lapathifolia* were recorded from Waikato and Bay of Plenty maize (Chapter 3), though the former appeared more common than the latter. *Persicaria maculosa* and *P. lapathifolia* were classed as moderate risk weeds, and placed in the middle of the ranking in the herbicide resistance risk assessment (Chapter 2: Ngow et al. 2021). Elsewhere in the world, both species have evolved atrazine resistance multiple times in maize, and AHAS-inhibitor resistance in cereals (Heap 2022). As *Persicaria* spp. are extremely common in Waikato and Bay of Plenty maize, they may yet develop resistance to other herbicides, or atrazine again, but results from this thesis suggests common post-emergent herbicides used now are effective.

Resistant *D. sanguinalis* populations were found with high abundance (>10-20% cover and >650 seeds m^{-2}, Fig 4-5). This is not surprising, as resistant weeds are more likely to set seed, leading to higher seed loads and higher cover. Additionally, high proportions of *D. sanguinalis* seed are known to survive for three years in the seedbank (Dowsett and James 2012) and the weed is known for the ability to germinate late, after herbicide application (James et al. 2007). Unlike *D. sanguinalis*, resistant *C. album* populations did not show higher percentage cover than susceptible populations, but they did show higher seedbank densities. Otherwise, the success of other herbicides (in other mode-of-action groups) in controlling *C. album* may explain similar weed cover in farms with atrazine-resistant and susceptible populations. Farms still using atrazine on atrazine-resistant *C. album* would have high field populations of the weed. A resistant farm with low field presence of the weed but high seed bank may have historically had an atrazine problem but have since used effective weed control methods, suggesting a long lived seedbank. Farms with resistance and low seedbank and field abundance of the weed likely have a successful control regime for that weed; farms with no resistance and low abundances also have successful control.

It seems herbicide resistance in New Zealand is mostly due to local selective pressures, and not the invasion of foreign herbicide-resistant weeds (Ghanizadeh and Harrington 2019b). Two counterexamples exist. An invasive population of the cereal weed *Alopecurus myosuroides* Huds. in Canterbury cereals and ryegrass seed crops is known to have resistance to an AHAS-inhibitor (iodosulfuron), and another population is believed to have ACCase inhibitor resistance (Buddenhagen pers. comm). Both of those arrived via contaminated seed lines. Invasive populations of the maize weed *Abutilon theophrasti* Medik. expressed tolerance to the label rate of atrazine (James and Cooper 2012), though this may not be resistance as the rate survived (1500 $g.ai.ha^{-1}$) is similar to the LD_{50} (1100 $g.ai.ha^{-1}$) of a susceptible population in another study in the United States (Gray et al. 1995). It is unknown how exactly those populations came into New Zealand, but a later incursion came through fodder beet seed (James and Pene 2018a). All other cases of resistance are assumed to have evolved in New Zealand (Buddenhagen et al. 2019, 2021a, Ghanizadeh and Harrington 2019b). Our border biosecurity is effective, with lower risk than many other countries for importing resistant weeds (Buddenhagen et al. 2021b), but the aforementioned *A. myosuroides* incursions show that there are still opportunities to accidentally import herbicide resistant weeds (FAR 2020b, Foundation for Arable Research, 2022).

Another example of weeds that could cause major problems in maize in New Zealand are the *Amaranthus* spp. from the United States. In particular, *Amaranthus palmeri* is highly competitive, and has evolved resistance to nine herbicide mode-of-action groups (Heap 2022, Ward et al. 2013). There are *A. palmeri* populations resistant to five mode-of-action groups (Kumar et al. 2019). This weed is now being spread globally, in Japan with glyphosate resistance (Shimono et al. 2020), Spain with nicosulfuron resistance (Torra et al. 2020), Brazil with glyphosate and AHAS inhibitor resistance (Küpper et al. 2017) and Botswana and South Africa with glyphosate and AHAS inhibitor resistance (Reinhardt et al. 2022). Thankfully, contaminated seedlots are rare, with less than 2% seed samples having detectable weeds; maize seed lots only had 0.2% contamination rates (Rubenstein et al. 2021). Pasture species *Medicago* (27.3%), *Trifolium* (19.8%), *Lolium* (15.9%) and feed crops *Beta* (8%) have higher contamination rates than maize (Rubenstein et al. 2021). Border biosecurity and crop monitoring can prevent a weed like *A. palmeri* from establishing in New Zealand.

It is possible that there are undetected resistant weed populations, for instance previously reported atrazine-resistant *P. maculosa* or dicamba resistant *C. album*. The seed collection process in this study collected from multiple plants throughout the field to increase the chance of sampling from multiple genotypes. Sampling was randomized across the Bay of Plenty and Waikato, therefore there is a chance farms or fields with resistance may have been missed. There were between 381-772 farms in Waikato and Bay of Plenty according to the FAR and Agribase databases, meaning 6.7-13.6% of farms were sampled in the two regions. Even assuming our detectability rates for a site were good (>90%), we may have under-estimated the true prevalence of resistant weeds (Buddenhagen et al. 2019).

Other species in maize may have herbicide resistance. Weeds predicted to have a high likelihood for resistance are recorded in Chapter 2: Ngow et al. 2021. Seeds were collected in this study (Appendix 7), for all of the high-risk species not already tested: *Amaranthus* spp. (24), *E. crus-galli* (54), *Eleusine indica* (L.) Gaertn. (1), *Fallopia convolvulus* (L.) Á.Löve (36) and *Setaria pumila* (Poir.) Roem. et Schult. (44). Some other species with previously recorded resistance *Datura stramonium* L. (13), *Panicum dichotomiflorum* Michx. (43), *Polygonum aviculare* L. (14), *Setaria verticillata* (L.) P. Beauv. (11) and *Solanum nigrum* L. (22) were also frequently collected. Higher-risk and more abundant and widespread species should be prioritized for future testing, particularly *E. crus-galli*. Perennial weed species in maize are less likely to evolve resistance (Chapter 2: Ngow et al. 2021). Most perennial weeds in the survey did not have seeds collected, but there were some *Calystegia* spp. (7), *Cirsium arvense* L. (8), *Cynodon dactylon* (2), *Cyperus rotundus* (<23), *Elytrigia repens*

(1), *Paspalum distichum* (13) and *Rumex* spp. (22) seeds collected. For some, it is unlikely they will germinate; *Cyperus rotundus* seeds were planted but none germinated (Chapter 3). It is not impossible that they would evolve herbicide resistance, but of those species, only *Rumex obtusifolius* and *Cirsium arvense* are known to have elsewhere (Chapter 2: Ngow et al. 2021). *Stellaria media* and *Poa annua* are two winter weeds found in maize seedbanks (Chapter 3) that have a history of herbicide resistance in New Zealand seedbank samples were grown to collected seed from for future herbicide resistance testing. Weeds collected in a similar randomized survey were found to be resistant (cereals in Canterbury) despite not reported for resistance in New Zealand before (Buddenhagen et al. 2021a), some of which were assessed to be moderate-high risk (Ngow et al. 2020).

The first identified nicosulfuron resistant *D. sanguinalis* population was 2× resistant, and preliminary studies of the populations found here show that some may be more resistant. More extensive dose-response tests for nicosulfuron-resistant *D. sanguinalis* populations could better confirm the level of resistance. Tests for cross-resistance within AHAS-inhibitors may not be important, as other AHAS-inhibitors are not used to control *D. sanguinalis* in maize. It is already known that atrazine resistant *C. album* are cross-resistant to other triazines, but it is unknown if other non-triazine PS-II inhibitors (for instance bromoxynil) are effective on atrazine-resistant *C. album* populations identified here. Pre-emergence herbicides were not tested. They are considered less likely to have resistance evolve (Busi et al. 2020), and are ranked as low risk in Chapter 2: Ngow et al. 2021. These herbicides are an important component of weed control regimes in maize. Testing *D. sanguinalis* for chloroacetamide resistance would be particularly worthwhile, as herbicides of this group are currently some of the most effective on *D. sanguinalis* and other grass weeds. Examining the seed dormancy of *D. sanguinalis* populations with and without nicosulfuron resistance could unveil a theorized relationship between the two. Further studies could determine the presence of any fitness cost, the inheritance of resistance and the molecular mechanisms causing resistance for *D. sanguinalis*.

Other important maize growing regions within New Zealand are Gisborne, Hawkes Bay, Taranaki and Manawatu-Whanganui (Millner and Roskruge 2013). The extent of weed resistance to herbicides in maize is poorly understood in those regions. It is known that some *Solanum nigrum* L. populations are resistant to atrazine in Manawatu pea crops (Harrington et al. 2001) and it is believed those populations have not become widespread (Harrington and James 2005). Atrazine-resistant *C. album* are believed to be present everywhere maize is grown in New Zealand (James et al. 2005), and there is evidence of resistant *C. album* in a Manawatu trial (James et al. 2007). This study shows there are areas where atrazine-resistant *C. album* is absent (eastern Bay of Plenty). The weed flora of those regions are different to Waikato-Bay of Plenty maize, with weeds such as *Setaria verticillata* (L.) P.Beauv. and *Panicum capillare* L. present (Rahman et al. 2006). Surveys for weeds from Napier southward to Martinborough were carried out in the summer of 2022.

Strategies to prevent or delay the evolution of herbicide resistance in maize weeds are well known (Harrington and James, T.K. 2022, Norsworthy et al. 2012). The usage of pre-emergent herbicides in maize is widespread, and these herbicides are believed to be at lower risk of evolving resistance (Chapter 2: Ngow et al. 2021). Following pre-emergent herbicides with post-emergent herbicides limits the population size of weeds, which further reduces the risk of them evolving herbicide resistance. These sequences need to be constantly changed, with different pre-emergent and post-emergent herbicides rotated between (Beckie and Reboud 2009, Busi et al. 2020). Non-chemical weed management (IWM) practices can be adopted (Harker 2013), which will decrease likelihood of weeds evolving resistance. Reducing transmission of resistant weeds between farms is possible by upholding strict farm biosecurity. Equipment can bring in weeds (Thill and Mallory-Smith 1997), it is important to ensure those equipment are clean of weed seeds. Monitoring for new weed problems can prevent a small infestation from becoming endemic.

4.5 Conclusion

Twenty-three (44%) farms in a randomized survey of 36 Waikato and 16 Bay of Plenty farms have herbicide resistant weeds. Atrazine-resistant *Chenopodium album* are widespread and also found in 18 Waikato farms and 4 western Bay of Plenty farms. Nicosulfuron-resistant *Digitaria sanguinalis* populations are sporadically distributed, in ≥7 farms in Waikato. There were no atrazine-resistant *Persicaria maculosa* populations detected, nor were there dicamba-resistant *C. album* in the randomized list of farms sampled. Five farms had atrazine-resistant *C. album* and nicosulfuron-resistant *D. sanguinalis*. No new resistance was detected. Atrazine-resistant *C. album* are well studied, but nicosulfuron-resistant *D. sanguinalis* are not. Dose-response studies, characterization of mechanism of resistance, studies of fitness costs and inheritance of resistance may be important follow-up studies. Future work should involve examining the other species collected for herbicide resistance, particular the high risk *Echinochloa crus-galli* and *Solanum nigrum,* and testing for resistance to pre-emergent herbicides, particularly for *Digitaria sanguinalis.* A comparable study of Hawkes Bay maize weed populations is being done. Herbicide resistance mitigation strategies such as rotating herbicide groups, alternative weed control and strict biosecurity practices will help prevent the evolution and limit the spread of herbicide resistant weeds.

Chapter 5: Discussion

5.1 Introduction

Despite an abundance of research into maize weeds, there have been few systematic studies of weeds across multiple farms in New Zealand. The first was in 1997, the second from 1999-2002 (Rahman et al. 1997, 2006). The weed survey presented in this thesis twenty years from the previous survey presents updated knowledge of weed prevalence and is the first systematic survey for herbicide resistance in maize. Three weeds were known to have evolved herbicide resistance in maize in New Zealand. Many other weeds present in maize have repeatedly evolved resistance to herbicides globally, and assessments of weeds for their risk of evolving herbicide resistance have been done previously (Moss et al. 2019, Ngow et al. 2020), but not specifically for maize. Previous studies focused on resistance detected, studies were done at individual sites or several sites near newly discovered resistant weed populations (Ghanizadeh and Harrington 2019a, James et al. 2005, Rahman et al. 1983c, Rahman and Patterson 1987). It was believed that atrazine resistant *C. album* is present in most maize farms; the recent occurrence of nicosulfuron resistant *Digitaria sanguinalis* in western Bay of Plenty and eastern Waikato farms (Buddenhagen et al. 2021a) and multiple resistant *C. album* in eastern Waikato warranted further investigation into the prevalence of this resistant weed. The study presented here is the first systematic survey for herbicide resistant weeds in maize in New Zealand.

The study objectives were to:

- Determine which weeds of maize in New Zealand are most likely to develop resistance.
- Carry out weed surveys and seed bank studies in randomly selected farms in two major maize producing regions (Waikato and Bay of Plenty) to determine which weeds are important for maize growers.
- Determine the regional prevalence of herbicide resistant weeds on maize farms in those regions by testing seed collected from weeds that have survived control measures.

5.2 The risk of maize weeds evolving herbicide resistance

5.2.1 Risks of maize weeds evolving herbicide resistance

The risk of maize weeds evolving herbicide resistance was assessed in Chapter 2: Ngow et al. 2021. A list of maize weeds was generated from literature, field observations and discussion with weed scientists. Weeds were assessed for their risk of developing herbicide resistance based on an adapted risk assessment protocol (EPPO 2015, Moss et al. 2019, Ngow et al. 2020), using a global database of herbicide resistance cases (Heap 2022). Weeds in this risk assessment evolved resistance to herbicides 270 times; 97 of those cases were in maize (Heap 2022). Of a list of 39 maize weeds, the 'species risk' was high for 7 weeds, moderate for 17 and low for 15 weeds. These high risk weeds were *Amaranthus powelli, Chenopodium album, Digitaria sanguinalis, Echinochloa crus-galli, Eleusine indica, Solanum nigrum* and *Xanthium strumarium*. Herbicide mode-of-action groups (HRAC 2022), had the same scores as a previous study (Ngow et al. 2020); high risk herbicide groups were AHAS inhibitors and photosystem-II inhibitors, synthetic auxins were moderate risk and PPO inhibitors, HPPD inhibitors, microtubule assembly inhibitors and VLCFA synthesis inhibitors were low risk. When weeds were ranked in order of 'cumulative risk' wherein the species risk scores were multiplied by the herbicide risk scores per species where a case of resistance had occurred in that herbicide group, the highest risk weed was *Echinochloa crus-galli* with a score of 30, followed by *Eleusine indica* (24), *Chenopodium album* (24), *Digitaria sanguinalis* (18) and *Amaranthus powelli* (18); the remaining weeds had scores of 12 and below, and 'low-risk' weeds had scores of zero.

The risk of weeds based on their history of resistance can be combined with their actual prevalence and abundance for a more accurate assessment. Highly abundant weeds are more likely to evolve resistance (Jasieniuk et al. 1996), and widespread weeds were found to also be more likely to evolve resistance (Hulme and Liu 2021). The most recorded weed species in Bay of Plenty and Waikato maize farms were *Digitaria sanguinalis*, *Chenopodium album* and *Persicaria* spp. (*P. lapathifolia* and *P. maculosa*; Chapter 3). Those first two species *C. album* and *D. sanguinalis* were ranked as high risk, and have both evolved resistance already in New Zealand maize (Buddenhagen et al. 2021a, James et al. 2005, Rahman et al. 1983a), and were found to be resistant in 42% and 13% of Bay of Plenty and Waikato farms sampled in the herbicide resistance survey, respectively (Chapter 4). *Persicaria* spp. were ranked as moderate risk, and were not found to have herbicide resistance despite evolving resistance previously (Rahman and Patterson 1987).

Digitaria sanguinalis has evolved herbicide resistance in maize three times (excluding New Zealand), and another ten times in other crops (Heap 2022, Chapter 2: Ngow et al. 2021). Three of the thirteen cases were to AHAS inhibitors, three to (triazine) photosystem-II inhibitors and seven to ACCase inhibitors (not used in maize; Heap 2021). Resistant *D. sanguinalis* populations have evolved to AHAS-inhibitors in China, France and Australia (Heap 2022, Hidayat and Preston 2001, Mei et al. 2017), and in New Zealand (Chapter 4). Photosystem-II inhibitor resistance for *D. sanguinalis* is practically irrelevant in a New Zealand context as the species is considered 'tolerant' to field rates of atrazine (Woon 1971). Likewise, ACCase inhibitors are not used in maize. The first case of glyphosate resistance (with a novel target site mutation) in *Digitaria sanguinalis* was recently reported in soybean-maize cropping system in Argentina (Yanniccari et al. 2022), but again, this herbicide is only used pre-planting in maize in New Zealand. Nonetheless, these cases of resistance in other herbicide groups provide evidence that *D. sanguinalis* is able to evolve herbicide resistance repeatedly and should be viewed as a high risk weed. No cases of *D. sanguinalis* resistant to any other herbicide groups (for instance chloroacetamides) used in maize in New Zealand have been reported.

Chenopodium album has evolved resistance to herbicides thirty-six times in maize, and fifty-two times overall, of which forty-five were to photosystem-II inhibitors, six to AHAS inhibitors and once to synthetic auxins (Heap 2022, Chapter 2: Ngow et al. 2021). Atrazine resistant *C. album* populations have repeatedly evolved in maize globally, but the only time *C. album* evolved synthetic auxin (dicamba) resistance was in eastern Waikato maize (James et al. 2005). AHAS inhibitor resistant *C. album* has evolved repeatedly in soybean and cereal crops, but not yet in maize (Heap 2022). Nicosulfuron is widely used in New Zealand maize and its repeated use may inadvertently select for resistant *C. album*. As atrazine and dicamba resistance has already evolved in *C. album* in maize in New Zealand (though the latter was not observed in the randomized survey), more cases of evolved resistance could occur.

Persicaria maculosa and *Persicaria lapathifolia* were ranked as moderate risk weeds, having evolved herbicide resistance five and six times, respectively (Heap 2022, Chapter 2: Ngow et al. 2021). Both species have evolved photosystem-II inhibitor resistance and AHAS inhibitor resistance globally (Heap 2022). *Persicaria maculosa* evolved atrazine resistance in Waikato maize in the 1980s (Rahman and Patterson 1987), but populations were not found to be resistant to atrazine (nor other herbicides) in the herbicide resistance survey (Chapter 4) despite the weed being extremely common (Chapter 3). *Persicaria lapathifolia* is not referred to in journal articles about maize in New Zealand, though it is known to be present there (Taylor, R. L. 1980). No *P. lapathifolia* populations were resistant to atrazine, nor other herbicides, despite also being common. In Southland, *P. maculosa* populations are believed to have evolved resistance to AHAS inhibitors (FAR 2018). Both species are at risk of evolving resistance to herbicides, particularly high risk herbicides atrazine and nicosulfuron.

Aside from the three most widespread and abundant weeds *D. sanguinalis*, *C. album* and *Persicaria* spp. (Chapter 3), there are several other weeds with moderate-high risks of evolving herbicide resistance (Chapter 2: Ngow et al. 2021). *Echinochloa crus-galli* was the highest risk weed, with sixty-three cases of herbicide resistance, and was common in the weed survey (Chapter 3), so should be highly prioritized in future research to proactively manage resistance. *Eleusine indica*, *Amaranthus powelli* and *Solanum nigrum* were also high risk weeds but were not as common and therefore are less likely to be resistant in Bay of Plenty and Waikato maize than predicted solely by their histories of resistance. Annual grasses *Panicum dichotomiflorum* and *Setaria pumila* and annual broadleaf weeds *Amaranthus blitum* ssp. *oleraceus, Datura stramonium, Fallopia convolvulus* and *Polygonum aviculare* were assessed to be moderate risk but were not found to be abundant in this study. Perennial weeds problematic in eastern Bay of Plenty farms *Cyperus rotundus, Cynodon dactylon* and *Paspalum distichum* were all low risk weeds; similarly *Oxalis latifolia* and *Calystegia* spp. were low risk. The only perennial weeds with some risk were *Rumex obtusifolius* and *Cirsium arvense*, both widespread across fields (Chapter 3). All weeds that were abundant in maize crops (Table 3-2), were on the weed species list.

5.2.2 Risks of weeds evolving resistance

In Chapter 2; Ngow et al. (2021), 'species risk' was estimated by the number of cases of resistance, rather than the biological characteristics, of a weed. Biological traits like annuality, fecundity and mating system appear to be important (Holt et al. 2013, Jasieniuk et al. 1996, Kreiner et al. 2018). Hulme and Liu (2021) ran analyses on 222 species resistant or susceptible to herbicides to determine which plant traits are predictive of resistance. They found that weeds that are outcrossing, monoecious/diecious, wind-pollinated, larger seeded and with high chromosome numbers are more likely to be resistant (Hulme and Liu 2021).

Many weeds are facultatively self-pollinating/outcrossing and vary between populations (Whitehead et al. 2018). For example, the most common weeds (Chapter 3), *Digitaria sanguinalis* can outcross and self-pollinate (Mas and Verdú 2018), *Chenopodium album* can do both but has limited outcrossing (0.16%) at 15m (Yerka et al. 2012) and *Persicaria* spp. are insect- or self-pollinated (Simmonds 1945a, 1945b). Plant mating systems influence how new mutations are inherited, outcrossing weeds can have higher effective population sizes and standing variation than self-pollinating weeds, but self-pollinating weeds are able to utilize recessive mutations (Ghanizadeh et al. 2019, Hawkins et al. 2019, Kreiner et al. 2018). Predominantly self-pollinating weeds may be more likely to evolve independent adaptations (Kreiner et al. 2018). Self-pollinating plants may have increased mutation rates, smaller genomes and reduced 'selfish genetic elements' (Wright et al. 2008), the former being more conducive to and the latter two being less conducive herbicide resistance evolution. Outcrossing weeds could be more easily spread resistance through pollen-mediated gene flow reaching distances of 300m for *Amaranthus palmeri* and 3000m for *Lolium rigidum* (Busi et al. 2008, Sosnoskie et al. 2012). Those species are obligate outcrossing species, *Amaranthus palmeri* a dioecious species (Sosnoskie et al. 2012), and *Lolium rigidum* with a genetic self-incompatibility system (McCraw and Spoor 1983). Resistant weeds were shown to be more likely to be outcrossing than susceptible weeds (Hulme and Liu 2021).

Plant ploidy is believed to affect herbicide resistance evolution but was a not a trait that explained the pattern of herbicide resistance in United States weeds (Hulme and Liu 2021). *Digitaria sanguinalis* is tetraploid or hexaploid, *C. album* diploid (Pellicer and Leitch 2020), although variation has been recorded (Rice et al. 2015). *Persicaria maculosa* is tetraploid, with *Persicaria lapathifolia* diploid (Kim et al. 2008). Self-pollinating polyploid species are more likely to have resistance than outcrossing polyploid species (Kreiner et al. 2018). In Oregon populations of the outcrossing weed *Lolium* spp., no polyploid, but many diploid populations were deemed resistant (Bobadilla et al. 2021). Similarly to *D. sanguinalis*, *Avena* spp. are often polyploid (Pellicer and Leitch 2020); hexaploid *Avena fatua*, a primarily self-pollinating species, experienced a 'dilution' effect, where individual resistance-conferring alleles provided weak levels of resistance (Yu et al. 2013). However, the hexaploid *A. fatua* could also have up-to three different resistance alleles (Yu et al. 2013); the same outcomes are possible for *D. sanguinalis*. For diploid *C. album* or *P. maculosa* a single mutation would likely have a stronger effect.

In Chapter 2; Ngow et al. (2021), the 'herbicide risk' was calculated from the proportion of species resistant to different herbicide mode-of-action groups. However, within mode-of-actions, there are chemical groups that have evolved resistance more often than others, like sulfonylureas in AHAS inhibitors, 'fops' in ACCase inhibitors and triazines in photosystem-II inhibitors (Heap 2022). There are also clusters of herbicide groups that appear to evolve resistance in similar suites of weeds (Hulme 2022a). AHAS inhibitors and photosystem-II inhibitors were clustered closely (Hulme 2022a); these two groups are used in maize in New Zealand, and were classed as high risk (Chapter 2; Ngow et al. 2021). The close link between the groups may indicate that other groups should be used to avoid multiple-resistant weeds. The problem with linking these herbicide groups in a clustering analysis, is that the mechanism for the link is not clear, they act on different enzymes, so the link may have more to do with herbicide use patterns in specific crops.

An article that aimed to identify agronomic factors in herbicide resistance evolution was published (Hulme 2022b). Research intensity (number of articles on herbicide resistant weeds) and years since first record of resistance are explanatory variables for herbicide resistant weeds in maize (Hulme 2022b). Area in cultivation and herbicide input were positively associated with resistance, but fertilizer input negatively, suggesting that fertilization increases crop-competitivity and decreases weed fecundity (Hulme 2022b).

The invasion of herbicide resistance weeds from other countries with a longer history of herbicide resistance selection adds an additional threat. The United States is the first country where herbicide resistant weeds in maize appeared (Heap 2022), and is the country with the most resistant weeds in maize (Hulme 2022b). Certain weeds seem to be emerging globally, for example *Amaranthus palmeri* which recently has invaded multiple countries outside of its original range in North America (Iamonico and El Mokni 2017, Shimono et al. 2020, Torra et al. 2020). The risk of importing weeds is low for maize seed, which had extremely low weed contaminant detection rates, but other forage and vegetable crop seeds like *Lolium* and *Trifolium* have higher rates of contamination (Rubenstein et al. 2021). In addition, the risk of importing resistance to New Zealand is relatively low compared to other countries due to the topography of its trade network (Buddenhagen et al. 2021b). Despite this, recent introductions of quarantine listed weeds (MPI, 2018) *Alopecurus myusoroides*, *Cuscuta pedicellata*, *Abutilon theophrasti*, show that our system is not impermeable (Buddenhagen et al. 2021b, James and Pene 2018a). *Alopecurus myosuroides* has been introduced into New Zealand with imports of ryegrass and linseed, with the first infestation (FAR 2020) believed to have AHAS inhibitor resistance and the other with ACCase inhibitor resistance (Buddenhagen unpublished). Local responses to these have all been fairly successful, but these weeds are difficult to remove; *Sorghum halapense* was considered eradicated (Hulme 2020, Rahman 1985), until recently (Ministry for Primary Industries 2022) and *Alopecurus myusoroides* had containment efforts showing success, until new infestations were recorded (FAR 2020b). Genetic tests on imported seed lots could help to detect the hard to identify problem plants e.g., *Amaranthus palmeri* (Brusa et al. 2021). The risk for introducing new weeds into maize through seed is low, thus the focus should be on internal biosecurity, preventative management and rapid control for newly resistant weeds.

After Chapter 2: Ngow et al. 2021 was submitted (April 2021), twenty-seven more cases of resistance were added to the International Survey for Herbicide Resistant Weeds database (Heap 2022). None of these were for any of the species on the list, but related weeds *Digitaria insularis* and *Amaranthus retroflexus* evolved resistance in soybean (a crop commonly rotated with maize overseas), and weeds recorded in the survey (Chapter 3), *Poa annua* and *Lolium multiflorum* were recorded with multiple resistance (Heap 2022). The database has requirements to ensure accurate identification of evolved resistance and relevance to actual herbicide use: resistance must have evolved (not natural tolerance), it must be inheritable, resistance must be confirmed (i.e. a dose-response experiment) and it must be resistant to actual field rates of herbicides (Heap 2005). These criteria may mean that some cases might be missed, or added later than first reported; for example in the Iberian peninsula there are 77 cases of herbicide resistant weeds, but only 46 of those are in the global database (Torra et al. 2022). Similarly, in New Zealand, the database reports twenty-two cases (twenty-four if mode-of-actions are counted individually as in Chapter 2; Heap 2021), but with recently reported cases (Buddenhagen et al. 2021a), the number of individual 'cases' rises to thirty-five. Many of these have not yet met the evidential criteria for inclusion in the International Database for Herbicide Resistant Weeds. For example, despite being reported earlier (Buddenhagen et al. 2021a, Harrington and James, T.K. 2022), nicosulfuron resistant *Digitaria sanguinalis* is not yet in the database (Heap 2022); with the results of the dose-response experiment in Chapter 4, the criteria required to add this case to the database should be fulfilled. Risk assessments like Moss et al. (2019), Ngow et al. (2020); Hulme and Liu (2021) and Chapter 2: Ngow et al. (2021) may underestimate risk because of incomplete information.

5.3 Weed distribution and abundance in maize

5.3.1 Weeds of maize

Weed distribution and abundance was determined for Bay of Plenty and Waikato maize, from fifty-two randomly selected farms (Chapter 3). Weeds were recorded in the field (as a percentage cover estimate) and from soil seedbank samples. The three most common weeds were *Digitaria sanguinalis, Chenopodium album* and *Persicaria* spp., found in 98%, 85% and 71% of 52 Bay of Plenty and Waikato farms, respectively. The next most common summer weeds *Setaria pumila*, *Solanum nigrum*, *Oxalis latifolia*, *Panicum dichotomiflorum*, *Cirsium arvense* and *Echinochloa crus-galli* are found in 40-60% of Bay of Plenty and Waikato farms (in descending order). The spring weeds *Stellaria media* (71%) and *Lepidium didymum* (42%) and winter weeds *Poa annua* (73%) and *Juncus bufonius* (79%) were widespread. Summer weeds *Digitaria sanguinalis*, *Chenopodium album*, *Persicaria spp.*, *Echinochloa crus-galli* and *Solanum nigrum* were abundant in fields with average percentage covers of >3% in any region, and summer weeds *D. sanguinalis*, *C. album*, *Persicaria* spp. and spring and winter weeds *Juncus bufonius*, *Poa annua* and *Stellaria media* abundant in soil seedbanks with densities of >300 seeds m^{-2}. Other weeds *Sonchus oleraceus*, *Erigeron* spp., *Rumex obtusifolius*, *Amaranthus blitum* ssp. *oleraceus*, *Nicandra physalodes* were widespread but not abundant within fields. Perennial monocotyledonous weeds *Cyperus rotundus*, *Paspalum distichum*, *Cynodon dactylon* were rarely found outside of eastern Bay of Plenty farms but were highly abundant within fields.

Digitaria sanguinalis was found in all farms except one, half of all farms had *D. sanguinalis* ≥1% percentage cover and 11 farms had *D. sanguinalis* ≥5% cover. Eighteen farms had seedbank densities of *D. sanguinalis* above 188 seeds m^{-2} (or 5 seeds detected). First recorded in 1855, *D. sanguinalis* was already spread across New Zealand in 1900s and experienced a major increase in abundance in the late 1980s (Field and Forde 1990). *Digitaria sanguinalis* is a common weed of Waikato and Bay of Plenty pastures (Tozer et al. 2011, 2015b) and is found in many environments (Edgar and Connor 2010). The overuse of atrazine in maize from the 1970s led to increased abundance *D. sanguinalis* and other Panicoid grasses with tolerance to atrazine (Rahman 1985, Thompson et al. 1971). To control *D. sanguinalis* pre-emergent herbicides and post-emergent herbicides are used. The herbicide resistance survey in Chapter 4 has shown that ten Waikato farms had *D. sanguinalis* plants surviving nicosulfuron.

Chenopodium album was found in 44 Waikato and Bay of Plenty farms, and 14 farms with cover ≥1% and 12 farms with seedbank density >188 seeds m^{-2} (5 seeds detected). Despite weed management using broadleaf active herbicides selecting for tolerant annual grasses and against susceptible broadleaf weeds (Rahman 1985), *C. album* is widespread and abundant. The evolution of resistance to atrazine in *C. album* populations is likely a contributing factor. No farms had dicamba resistant *C. album* detected, though it was previously found in eastern Waikato (James et al. 2005).

Persicaria spp. were found in 37 farms Waikato and Bay of Plenty farms, and 10 with cover ≥1% and 12 farms with seedbank density >188 seeds m^{-2} (5 seeds detected). The two *Persicaria* species are similar, with *P. maculosa* (willow weed) having more often pink-red perianth, ciliate ochrea and an absence of glands on peduncles as opposed to *P. lapathifolia* (pale willow weed) with more often white perianth, glabrous ochrea and yellow glands on peduncles (Webb et al. 1988). *Persicaria lapathifolia* is not recorded in journal articles about maize weeds, but is known to be present, often co-occuring with *P. maculosa* (Popay et al. 2004, Taylor, R. L. 1980, Webb et al. 1988). An earlier study found *Persicaria maculosa* was not present in south Waikato seedbanks (Rahman et al. 2004, 2006), but it was present in the randomized survey in this study. Despite being so common, no *Persicaria* spp. population had resistance to atrazine, dicamba, nicosulfuron or mesotrione.

Solanum nigrum is common in maize. This weed has a high risk of developing herbicide resistance and has already become resistant to atrazine in Manawatu peas (Harrington et al. 2001). Some fields had high densities of *Solanum nigrum*, but it was not as problematic as other weeds. *Amaranthus* spp. are rarely abundant in maize in Bay of Plenty and Waikato; in the past, they were major weeds, but triazine and hormonal herbicides appear to have reduced these weeds presence. Amaranthus powelli is much less common than previously recorded (Rahman et al. 2006). There are two main *Amaranthus* in Waikato and Bay of Plenty. *Amaranthus powelli* and *Amarnathus blitum* ssp. *oleraceus*. *Amaranthus deflexus* was found in one farm but is found more commonly in crops south of Waikato. *Amaranthus retroflexus* is also present in New Zealand and is difficult to distinguish from *A. powelli* but is less common. Worldwide, *Amaranthus* spp. have repeatedly evolved herbicide resistance (Heap 2022). As they are not as abundant in New Zealand, they may be less likely to evolve herbicide resistance.

Echinochloa crus-gall, Panicum dichotomiflorum and *Setaria pumila* are Panicoid grasses found in 42%, 46%, 60% of farms, respectively. *Echinochloa crus-galli* is rare in Bay of Plenty (3 farms) but was found in >50% of Waikato farms. It had an average cover of 5% but may have a small seedbank (germination was observed in the seedbank of 7 farms). As *E. crus-galli* is known to exhibit dormancy, it is possible that some seedbank samples had dormant seeds (Martinkova et al. 2006). Later germinating *E. crus-galli* seedlings do not cause yield losses as high as earlier emerging plants (Bosnic and Swanton 1997). This species frequently evolves herbicide resistance (Chapter 2: Ngow et al. 2021). *Panicum dichotomiflorum* was found in 69% of Bay of Plenty farms but was not abundant in maize fields prior to harvest (mean cover <1%), though it is common in Bay of Plenty pastures (Tozer et al. 2011). Similarly, *Setaria pumila* was found in 69% of Waikato farms, but was also not abundant (mean cover ≤1%), though it is common in Waikato pastures (Tozer et al. 2015b). *Erigeron* spp. have become major weeds of maize and other crops globally, with evolved herbicide resistance, but do not seem to be problematic in New Zealand maize. They are present in more than half of maize farms but were never seen with high field abundance or seedbank densities. Similarly other weeds assessed as moderate-high risk for developing herbicide resistance like *Amaranthus* spp. and *Eleusine indica* were not abundant, thus may pose less of a risk than predicted in Chapter 2: Ngow et al. 2021.

The most important perennial weed was *Cyperus rotundus*, found in seven Bay of Plenty farms and one Waikato farm. Where it was found in the Bay of Plenty, average cover was 22.6±25.9% (Table 3-2). Halosulfuron is able to be applied annually to reduce *C. rotundus* populations (Rahman et al. 1998b). However, as halosulfuron is the only herbicide available for *C. rotundus*, there is risk that resistance may evolve. Elsewhere, a related species *C. esculentus* has evolved resistance to halosulfuron (Tehranchian et al. 2015); which suggests that resistant biotypes could develop for *C. rotundus*. Admittedly this risk is low, as the species reproduces vegetatively in maize and rarely produces viable seed in New Zealand (FAR 2020a). While some seed was produced by *C. rotundus* in Bay of Plenty maize, none was viable (Chapter 3). *Cyperus rotundus* can be spread from farm to farm through rhizomes and tubers. Only one farm in Waikato had this weed, with a low estimated cover (0.7%) compared to Bay of Plenty farms (mean 22.6%). The Waikato *Cyperus rotundus* infested farm had no emergence of tubers from its soil seedbank, compared to an average 3.8 tubers per sample (or 143.4 m^{-2}). Frosts and lower average annual temperatures and sunlight hours may mean that this nutsedge will not become as problematic in Waikato. Regardless, it is important that this weed doesn't spread further, in Bay of Plenty and Waikato.

Perennial grasses *Cynodon dactylon, Paspalum distichum* and *Elytrigia repens* were not widespread but could be abundant where present. High cover of eastern Bay of Plenty farms by perennial grasses reflects poor herbicide control in those farms. Perennial grasses are often removed with glyphosate before planting maize (Rahman 1985). During the maize growing season, nicosulfuron has been proven to control *E. repens* and *P. distichum* (James and Rahman 1997). Mesotrione has poor efficacy on *E. repens*, but a mixture of mesotrione and nicosulfuron was shown to control *P. distichum* (James et al. 2006a). No herbicide resistance has evolved for any of those perennial grasses (Heap 2022), and those species reproduce primarily vegetatively, so resistance is unlikely to explain their poor control. Perennial broadleaf weeds *Oxalis latifolia*, *Cirsium arvense* and *Calystegia* spp. were widespread but were not abundant like the grasses. These perennial weeds can also be difficult to control with herbicides during the maize growing season (Rahman 1985). Post-emergent herbicides nicosulfuron and dicamba are effective on *Calystegia sepium* ssp. *roseata* and nicosulfuron on *Oxalis latifolia* (Rahman et al. 2002); outside of the maize growing season glyphosate was effective on *C. sepium* ssp. *roseata* when applied pre-planting but not when applied post-harvest (Rahman et al. 2002).

There are no estimates of abundance or prevalence for maize weeds in New Zealand prior to Rahman's seedbank studies (Rahman et al. 1997, 2006), but common weeds like *A. powelli, C. album, P. maculosa* regularly appeared in maize weed literature. Broadleaf-active synthetic auxin and photosystem-II herbicides shifted the weed composition of many maize fields toward grass weeds *D. sanguinalis* and *P. dichotomiflorum* and selected for atrazine resistant *C. album* and *P. maculosa* and dicamba resistant *C. album* (James et al. 2005, Rahman 1985, Rahman et al. 1983c, Rahman and Patterson 1987, Woon 1971). Weed seedbank studies in 1999-2002 identified *A. powelli*, *C. album*, *P. maculosa*, *S. nigrum*, *D. sanguinalis* and *P. dichotomiflorum* to be common weed species in Bay of Plenty and Waikato farms (Rahman et al. 2006). Since those studies (two decades prior), *C. album, E. crus-galli* and *Persicaria* spp. seem to have become more common, while *P. dichotomiflorum* and *A. powelli* seem to have become less common. Perennial weeds not regenerating from seed were not the subject of that study (Rahman et al. 2006), so the studies may be difficult to compare for perennial weeds. However, *Cyperus rotundus* does appear to have emerged as a major weed in eastern Bay of Plenty farms. The study presented in Chapter 3 is the first systematic, randomized survey of weeds in Bay of Plenty and Waikato farms, and records annual and perennial weeds present in field during late summer and in the seedbank.

Future studies of a regional maize weed 'flora' should use randomized sampling like the study here and use both field observation and seedbank sampling. Future studies of maize farms in Waikato and Bay of Plenty could show changes in the prevalence of weeds and any new weeds. Uncommon weeds now, like *A. powelli* and *E. indica*, could become more widespread if they were to evolve herbicide resistance. Potential new introductions of weeds like *Abutilon theophrasti*, *Sorghum halapense* and *Amaranthus palmeri* could become weed problems in maize especially if they are imported with herbicide resistance. The maize weed 'flora' of other regions has not been systematically studied, except Hawkes Bay which had six sites sampled by Rahman et al. (2006). Regions most important to sample are those that grow the most maize, Hawkes Bay, Gisborne and Manawatu-Whanganui. It is also unknown what the prevalence of herbicide resistance in maize weeds is within other regions.

5.4 Herbicide resistance in maize

5.4.1 Surveying for resistance

Results from the randomized survey for herbicide resistance are presented in Chapter 4. Motivating this study was the evolution of resistance in three maize weeds: *Chenopodium album*, *Persicaria maculosa* and *Digitaria sanguinalis* (Buddenhagen et al. 2021a, James et al. 2005, Rahman et al. 1983c, Rahman and Patterson 1987). These three weeds were the most common weeds in maize fields in the Bay of Plenty and Waikato, found in >70% of 52 fields sampled (Chapter 3). Of the 773 seed samples collected from weeds in 16 Bay of Plenty and 36 Waikato maize paddocks, 151 were *Digitaria sanguinalis*, 106 *Chenopodium album* and 131 were *Persicaria* spp. seeds, representing more than half of the seed collected. Resistance was confirmed for atrazine in *C. album* and nicosulfuron in *D. sanguinalis*, but not other herbicides for any of the weeds (including no atrazine resistant *P. maculosa* nor dicamba resistant *C. album*). No damage was observed in response to atrazine by atrazine-resistant *C. album* populations, but nicosulfuron-resistant *D. sanguinalis* populations were stunted. Resistant *D. sanguinalis* populations were able to survive twice the rate of nicosulfuron as susceptible populations (60 compared to 30 g.ai.ha^{-1}), though two populations appeared to have survivors at 240 g.ai.ha^{-1}. Twenty-two sites had atrazine-resistant *C. album* (42%), seven sites had nicosulfuron-resistant *D. sanguinalis* (13%), of those five sites had both, so a total twenty-four sites (46%) had herbicide resistant weeds.

The most recent weed to evolve resistance in maize in New Zealand is *D. sanguinalis*. The first resistant population was identified in 2017 (Harrington and James, T.K. 2022, Hutching 2017).

This first population was from a western Bay of Plenty farm, and was resistant to 60 g.ai.ha^{-1} nicosulfuron, twice that of a susceptible population (Harrington pers. comm.). Two years later, two farms from east Waikato were identified to have nicosulfuron resistant *D. sanguinalis* (Buddenhagen et al. 2021a). Both of those Waikato farms had some survival to higher rates than field rates (Buddenhagen et al. 2021a), but had stunted growth. *Digitaria sanguinalis* seeds were collected from Bay of Plenty and Waikato farms in the randomized survey for nicosulfuron testing. With this study, nicosulfuron resistant *D. sanguinalis* was identified in 7 farms out of 31 farms with seed collected (out of 52 surveyed). Three other farms had some survival, but at low rates. Farms from outside the randomized survey were also tested, including several locations around the original 2017 case, a self-reported sample (I3) suspected of resistance and one from the FAR Northern Crop Research Site (NCRS). The self-reported sample (I3) was not resistant, but the NCRS sample showed some survival. Two farms near the original case did not have resistance, but sites managed by the same contractor as the original case did have resistant *D. sanguinalis*. Rates of resistance were to recommended field rates for most populations, except two sites that survived to 240 g.ai.ha^{-1} nicosulfuron (4× the field rate).

It is unknown yet what mechanism causes nicosulfuron resistance in Bay of Plenty and Waikato *Digitaria sanguinalis* populations. Target site mutations often confer high rates of AHAS-inhibitor herbicide resistance (Murphy and Tranel 2019), but resistant *Digitaria sanguinalis* populations in this study appeared strongly affected by dosage, and most were resistant at only 2× the rate of susceptible populations. The low rate of resistance observed may imply a non-target site mechanism is responsible for resistance in *D. sanguinalis*. *In-vitro* enzymatic assays measuring AHAS activity and target site sequencing can confirm target-site resistance (Murphy and Tranel 2019), and have been done for *D. sanguinalis* in China (Mei et al. 2017). Non-target site mechanisms are diverse (Délye et al. 2013b, Ghanizadeh and Harrington 2017a), but some mechanisms can be identified by relatively simple tests. Malathion, a cytochrome P450 inhibitor, can be applied prior to herbicide spraying to test for cytochrome P450 mediated herbicide metabolism, and NBD-Cl, a glutathione-S-transferase (GST) inhibitor, for GST (Ghanizadeh et al. 2022). Nicosulfuron resistant *D. sanguinalis* populations in China were affected by malathion (Mei et al. 2017). Assays with radio-labelled herbicides can diagnose some herbicide translocation and absorption mechanisms (Ghanizadeh et al. 2018).

Persicaria spp. were found in 37 farms Waikato and Bay of Plenty farms in the randomized survey; four species were found, but only *Persicaria maculosa* and *Persicaria lapathifolia* were common. *Persicaria maculosa* attained atrazine resistance in Waikato maize in the 1980s (Rahman and Patterson 1987), but was not believed to have spread far (Harrington and James 2005, Rahman 1990). The results here suggest the problem, if even present, is restricted in range. Both weeds have a moderate risk of developing herbicide resistance (Chapter 2: Ngow et al. 2021), but neither *Persicaria maculosa* or *Persicaria lapathifolia* had any herbicide resistant populations in the survey (Chapter 4). It is not obvious why *Persicaria* was so widespread and abundant despite not having herbicide resistance. Later germinating seeds in maize fields is a possible explanation, *Persicaria maculosa* germinates at higher rates as temperature increases (Vleeshouwers 1998).

Estimates of resistance for *C. album* are 43% of all farms with atrazine resistant *C. album* and 13% of all farms with nicosulfuron resistant *D. sanguinalis*. These estimates are conservative, for several reasons. Farms usually had 5 or so plants collected from and tested, meaning there is a chance a resistant plant could be missed. Farms that were untested either had no plants present with viable seed, or poor germination from seed collected, in the former case resistance could be missed if other herbicides/control measures are successful and in the latter case weeds may be resistant even if they have poor germination. On top of that, the estimate for nicosulfuron resistant *D. sanguinalis* of 13% is from 7 resistant farms, but an additional three farms had a few plants surviving nicosulfuron, if this was used the estimate would become 19%. The problem of potentially under-estimating resistance rates seems more likely than over-estimating it (Buddenhagen et al. 2019).

Maize is often followed by a winter feed crop (Chakwizira et al. 2019), and is grown in close association to dairy pastures. There were weeds common in seedbanks that were not observed in fields in summer (Chapter 3), particularly *Juncus bufonius*, *Stellaria media* and *Poa annua*. These weeds are also pasture weeds (Bourdôt et al. 2007, Ghanizadeh and Harrington 2019c), and have evolved resistance to herbicides before in New Zealand. *Poa annua* can grow in many environments (Edgar and Connor 2010), and has evolved herbicide resistance in cereals to iodosulfuron and turf to haloxyfop in New Zealand (Buddenhagen et al. 2021a, Ghanizadeh et al. 2020). *Stellaria media* has evolved resistance to the AHAS inhibitors in Southland cereals (chlorsulfuron), Canterbury (flumetsulam) and Waikato (flumetsulam) autumn-sown ryegrass feed crops (Buddenhagen et al. 2021a, Seefeldt et al. 2001). In pastures and winter ryegrass, two herbicide groups are commonly used to control weeds: synthetic auxins and AHAS inhibitors (Ghanizadeh and Harrington 2019c). In permanent pastures, gaps left by winter weeds like *Poa annua* and *Stellaria media* can be colonized by summer annual grasses *Digitaria sanguinalis* and *Setaria pumila*, and vice versa. Herbicide resistant biotypes of these weeds may cause problems in pasture. Seeds were collected for both species from maize seedbanks to test for herbicide resistance

This study is now the second systematic herbicide resistant survey in New Zealand crops following a randomized survey of 82 Canterbury arable farms (Buddenhagen et al. 2021a). That study revealed that ≥48% of farms had at-least one of seven herbicide resistant weeds identified, with ACCase inhibitor resistant (17%) and AHAS inhibitor resistant (28%) *Lolium* spp. the most widespread resistant weeds. The study presented here showed a similar number of farms with resistance (44%), but only for two weeds. Future study of responses to herbicides in other weeds collected in the randomized survey are planned and may reveal additional farms with resistance. Clearly herbicide resistance is a widespread issue in New Zealand cropping farm systems.

5.5 Synthesis

Weeds can evolve resistance on site, or resistant weeds (or genetic elements) can invade a site. Strategies to prevent maize fields being saturated with resistant weeds should address both causes.

5.5.1 Managing herbicide resistance

Recently, with the rise of herbicide resistant weeds, there is increased focus on developing new herbicides. Regulation, reduced market availability and herbicide resistant weeds (reducing herbicide efficacy) all play a part in limiting herbicide availability, as is seen as a problem in Canterbury arable farms (Espig et al. 2022). Due to the efficacy of glyphosate, few new herbicides were being developed (Duke 2012). Since then, there has been renewed focus on herbicide discovery, using genomic technology (Duke et al. 2019, Yan et al. 2018). Farmers in Canterbury (Espig et al. 2022) do not hold techno-optimist views, instead favouring preserving current herbicide efficacy. This attitude, which may be shared in maize growers, aligns with best-practice guidelines; simply adding more mode-of-action groups without adjusting practices will still result in herbicide resistance (Gaines et al. 2021).

There are more and more herbicide resistant weeds being detected in New Zealand in various crops (Buddenhagen et al. 2019, 2021a, Ghanizadeh et al. 2020, Ghanizadeh and Harrington 2019b). Atrazine resistant *Solanum nigrum* may become a problem in maize, though there are many other effective herbicides available. Glyphosate-resistant *Lolium* spp. may cause problems before planting, but again, other herbicides should be effective on *Lolium*. Many of those are weeds of other crops, and in regions where maize is not the major arable crop, thus not likely to pose a threat to maize.

Risks of evolved herbicide resistance may appear to differ between weed species (Holt et al. 2013, Moss et al. 2019, Chapter 2: Ngow et al. 2021) but weed management is the only controllable aspect. A more cautious approach should be taken for higher risk weed species or herbicide groups. Repeated application of a single herbicide, which may select for resistance in a weed, should be avoided. Herbicide rotations and mixtures are commonly advocated for to reduce the risk of resistance evolving (Harrington and James 2005).

The first case of nicosulfuron resistant *D. sanguinalis* in New Zealand was identified from a field with six years of nicosulfuron usage (Hutching 2017). Other cases of resistance in maize were under selection for multiple years: dicamba resistant *C. album* for twenty years (James et al. 2005), atrazine resistant *P. maculosa* for ten years (Rahman and Patterson 1987), and atrazine resistant *C. album* for 'several' years (Rahman et al. 1983c). The speed in which weeds develop resistance to herbicides is down to many factors, most obviously herbicide programme and weed population size. Populations with larger seed seedbanks are more likely to evolve resistance (Diggle et al. 2003, Jasieniuk et al. 1996). Limiting the size of weed seedbank populations is a consistent strategy in herbicide resistance management (Beckie et al. 2019).

Growers who repeatedly apply the same herbicide are more likely to select for resistance (Diggle et al. 2003, Moss et al. 2019), as was done for all the cases of resistance in maize in New Zealand. There is a risk of mixtures and rotations selecting for 'generalist' resistance (i.e. some types of non-target site resistance; Comont et al. 2020, Neve 2007). Utilizing herbicide mixtures, rotations and sequences to reduce the likelihood of resistance evolving has long been promoted (Harrington and James 2005). However, in reality, decisions to use herbicides has been based on efficacy and cost, and farmers may not always decide (Espig et al. 2022). Atrazine worked on *P. maculosa*, until it didn't. Atrazine worked on *C. album*, until it didn't. Dicamba worked on *C. album*, until it didn't. Nicosulfuron worked on *D. sanguinalis*, until it didn't. In all of these cases, the herbicide that weeds evolved resistance to was the most effective option at the time. They were applied for decades with success, until luck ran out, and a rare mutation increases in weed populations to force growers to use other chemicals.

Lower effective herbicide rates are known to select for herbicide resistance rapidly (Gressel 2011, Neve and Powles 2005a, 2005b). Reduced effective herbicide rates can be from lower applied rates or by some factor reducing the efficacy of herbicides. Sprayer equipment inefficacy caused by incorrect nozzle choice may reduce effective herbicide rates. Some soil types are known to reduce herbicide efficacy, high-organic matter soils in Waikato known to reduce atrazine efficacy (Rahman et al. 1978, 1980). Soils with long histories of herbicide use are known to have reduced herbicide efficacy (James et al. 2010c, James and Rahman 2009, Rahman and James 1983), possibly due to selection for herbicide-degrading micro-organisms present. Weeds may cover other weeds from foliar-herbicides (James et al. 2000), reducing effective rates.

Herbicide resistance is an evolutionary phenomenon (Baucom 2016), as such eco-evolutionary knowledge is useful (Neve et al. 2009, 2014). Reduced fitness as a pleiotropic effect of herbicide resistance suggests that some resistant weeds would be outcompeted by weeds without resistance (Ghanizadeh and Harrington 2019a). Dicamba-resistant *C. album* have demonstrated reduced fitness (Ghanizadeh and Harrington 2019a); it may be that their lack of spread (no detection in the survey) could be in part due to their reduced fitness. No investigation yet of any possible fitness costs has been undertaken for *D. sangunialis*. Fitness costs are not always present, for example glyphosate-resistant *Amaranthus palmeri* (Vila-Aiub et al. 2014).

The results from Chapter 3 and Chapter 4 established a baseline spatial understanding of weeds, and resistant weeds in maize. Future studies will be able to use this study to trace the inevitable spread of weeds, and resistant weeds, as part of an 'environmental epidemiology' style approach suggested by Comont and Neve (2021). Unlike wind-dispersed thistles (Harrington and Hewage 1997), or long distance pollen-mediated gene flow in *Lolium* (Busi et al. 2008), resistant genes in the maize weeds in this study are not believed to be able to disperse far naturally. The three species with resistance have seeds that are gravity-dispersed. However, these seeds can travel long distances by being carried by people, vehicles or equipment. It is local evolution of resistance, and dispersal through networks of interconnected sites that determines the prevalence of herbicide resistant weeds in maize.

Farmers in Canterbury are aware of herbicide resistance, but many are not knowledgeable about herbicide chemical groups (Espig et al. 2022). It is possible the same is true for North Island maize growers. Weeds, such as atrazine-resistant *C. album* and ACCase-inhibitor resistant *Lolium* spp., are often cross-resistant to other herbicides in the same chemical group (Beckie and Tardif 2012, Gunnarsson et al. 2017, Rahman et al. 1983c). Different herbicides, that are in the same mode-of-action group to the now ineffective herbicide (with evolved weed resistance), will often fail to work. Consequentially, unaffected weeds will produce seed, and increase in abundance. In the early stages of herbicide resistance, where resistant weeds are most vulnerable, it is critical to use herbicides from different groups if a resistant weed problem is suspected.

Non-chemical weed management, or integrated weed management, consists of many strategies to reduce weed populations (Harker 2013, Liebman et al. 1997, Moss 2019). These strategies have long been promoted, but uptake is slow (Liebman et al. 1997, Moss 2019, Norsworthy et al. 2012). Crop rotation is a strategy suggested to reduce the likelihood of weeds evolving resistance (Harrington and James 2005). Maize growers often crop the same paddock annually, though maize grown in dairy farms is often planted in fresh paddocks (Booker 2009, Densley et al. 2005).

Increased crop competitivity, through planting more competitive cultivars, narrower row spacing and increasing planting populations, can reduce weed growth (Mhlanga et al. 2016). However, higher crop densities approach an upper limit of yield as the maximum sunlight is intercepted and moisture stress becomes apparent and where weeds are controlled by herbicides, row spacing has no effect on maize yield (Stone et al. 2000). Crop competition is most viable in sites where there is high fertility and low moisture stress, or with maize hybrids that can tolerate inter- and intra-species competition (Mhlanga et al. 2016, Stone et al. 2000). In the case of *Cyperus esculentus*, a close relative of the Bay of Plenty maize weed *Cyperus rotundus,* increased planting rates were able to significantly reduce *C. esculentus* numbers (Ghafar and Watson 1983). The same approach may be necessary, as only one herbicide is available for *C. rotundus* control in New Zealand (Rahman et al. 1998b). Data were not collected of maize plant populations or maize varieties, and only two farms were recorded with a more narrow row spacing (Appendix 6).

Harvest weed seed control (HWSC) systems collect chaff (containing weed seeds) and burn it in narrow rows (Walsh et al. 2018); these may be a possible alternative weed control method in grain maize .(Shergill et al. 2020). Similarly, crop stubble burning is an effective tool to remove crop residues and weed seeds (Starch and Kurtz 1929), but it can cause adverse environmental impacts (Abdurrahman et al. 2020). The practice is rarely used in North Island maize, but is more common in Canterbury cereal crops (FAR 2013). Flame- and steam-weeding techniques are commonly used pre-emergence in organic farming (Ascard 1995, Kerpauskas et al. 2006); recent trials in New Zealand of a steam-weeder that applies a fine mist appears effective on some weeds (Merfield et al. 2017). However, other weeds including *Chenopodium album* become tolerant at the four-leaf stage (Merfield et al. 2017). Electric weeding is receiving more attention (Eberius 2017). Inter-row cultivation appears to be another method with potential to reduce weed populations (Abdin et al. 2000). Recent studies show that residues from winter cover crops can suppress weed germination (FAR, 2020, FAR 2021, Trolove et al. 2017).

Biological control of weeds in maize is not common, but classical biological control using natural enemies has been successful for some weed species in New Zealand (Hayes et al. 2013). Heavy defoliation was observed on *Cirsium arvense* inside a maize crop in the western Bay of Plenty, that appeared to have been caused by the green turtle beetle *Cassida rubiginosa*, a species released as a biological control agent (Cripps et al. 2019). Fungal biological agents have been researched for major pasture weeds *C. arvense* and *Ranunculus acris* (Hayes et al. 2013). Specific problematic weeds may benefit from biocontrol, *Cyperus rotundus* may be aided by mycoherbicides as *Cyperus esculentus* has (Phatak et al. 1987), though there is possibly risk of off-target effects with the large number of native *Cyperus* in New Zealand. For thistles this is not the case as there are no native Cardueae (Cripps et al. 2013).

These alternative weed control practices will become necessary if more weeds become resistant to herbicides in maize, however, no approach is a 'silver bullet'. Not only is there resistance to chemicals but there is also evolved 'resistance' to other control mechanisms. In early times, weeds removed by manual labour began to evolve mimicry to crops (Baker 1965). Weeds can evolve increased seed dormancy to escape herbicide control (Maity et al. 2022, Owen et al. 2015). Weeds have evolved resistance to harvest-seed-weed-control tactics by simply shattering early (Ashworth et al. 2016). Diversity in weed control is necessary to disrupt weed evolution.

Maize growers have many competing interests to balance, and it may not always be possible or preferred to pursue the most effective weed control practices. Despite this, weeds have potential to significantly reduce yields (James et al. 2000), more so than other pests (Oerke 2006), and are an important consideration. Reducing the likelihood of herbicide resistance evolving and limiting weed migration and gene flow from other farms can go a long way.

5.5.2 Weed spread

The abundance and prevalence of weeds in Bay of Plenty and Waikato maize farms is now known (Chapter 3). Weeds become common in maize crops by selection, for instance atrazine applied annually will select for annual grasses and resistant weeds, and against broadleaf weeds. Herbicide resistant weeds were common in Bay of Plenty and Waikato farms (Chapter 4), and may become more common if they are to spread between farms.

Weed seeds and other propagules can be spread between and within farms by natural and anthropic dispersal events (Benvenuti 2007, Thill and Mallory-Smith 1997). Weeds like *Setaria pumila* can spread by natural dispersal and by machinery along roadsides (James et al. 2019). Weeds like *Erigeron* spp. and *Sonchus* spp. can disperse seeds using wind (anemochory) across long distances (Benvenuti 2007). Weeds can spread through water (hydrochory) within sediments or by floating on top of water (Benvenuti 2007). Weeds can spread through animals externally (epizoochory), like *Setaria verticillata* and *Xanthium strumarium,* or internally (endozoochory) (Hogan et al. 2011). Weed seeds eaten by cattle can survive passage, especially hard-seeded weeds (Hogan et al. 2011), and cattle transport vehicles can spread weeds through effluent discharge, though roadside effluent disposal facilities prevent this (Environment Waikato 2010). Birds can spread weeds, like those with fleshy fruit *Phytolacca octandra* and *S. nigrum* eaten by frugivores

(Wotton and McAlpine 2015), but even non-frugivores like gulls can pass viable weed seeds (Martín-Vélez et al. 2021, Twigg et al. 2009).

Unlike other crops, weed seed contamination through imported maize seed lines is unlikely (Rubenstein et al. 2021). It is common practice to feed out hay on grain maize stubble (Underwood 1985); hay can have large amounts of weeds (Conn et al. 2010) and weeds that grow in both maize and pasture like *Setaria pumila* and *Digitaria sanguinalis* could be spread in hay. Maize silage is often grown on-farm, but as much as 37% is traded from silage cropping farms (Booker 2009). Weed seeds can be spread along roadsides and pathways through open-top silage trucks as cut maize is transported to silage pits (James et al. 2015). Weeds especially common in chopped maize were *C. album*, *D. sanguinalis*, *E. crus-galli*, *P. maculosa*, *S. pumila* and *S. nigrum* (James et al. 2015). Ensiling is able to reduce seed viability for some species (*C. album*, *S. pumila*) but not others with physical dormancy (*Abutilon theophrasti*; Trolove and Dowsett 2015; Hahn et al. 2021). Fresh maize and poorly ensiled maize, however, may have viable weed seeds present.

Vehicles can carry weed seeds long distances (Lippe and Kowarik 2007). In a study in Victoria, Australia, 41% of vehicles and 25% of machinery had noxious weeds (Moerkerk 2006). During as busy a time as silage harvest, machinery may go between sites without being cleaned, spreading weed seeds between sites. Grain maize is harvested later, from March-June (compared to silage from February), where many weed seeds may have already shattered and entered the seedbank (Rahman et al. 2001b). Grain maize debris remains on the site as only the grain is carried away, though weed seeds can still be carried with machinery and vehicles. These weeds when present in the seedbank can be spread through soil clumps on machinery, for instance an imported excavator contained high amounts of *Eleusine tristachya* and *Juncus bufonius* seed (Aalders et al. 2012). Harvesting and cultivation can spread weeds further within fields; in Dutch maize silage harvesting and cultivation was able to spread weed seeds 100m (Heijting et al. 2009). Weeds can not only move horizontally in fields but also vertically; it was proven that cultivation by plough could bury seeds in larger numbers compared to no-tillage or cultivation by power-harrow or rotary-hoe (Rahman et al. 2000). Some weeds can disperse seeds through time, such as *C. album* with heteromorphic seeds with different degrees of dormancy (Yao et al. 2010), and *Cirsium arvense* able to germinate after twenty-eight years in sandy soil (James et al. 2010a).

It is not enough to only consider weeds themselves being spread, but particular genetic components like herbicide resistance conferring alleles can spread through pollen or seed (Thill and Mallory-Smith 1997). Pollen-mediated gene flow is a possible way for resistant alleles to spread between locations (Jasieniuk et al. 1996, Jhala et al. 2020). *Chenopodium album* and *Digitaria* *sanguinalis* are both facultative outcrossers, but *C. album* has outcrossing rates of 3%, and *D. sanguinalis* to 42.5-100% (Mas and Verdú 2018, Yerka et al. 2012). It seems there may be a higher risk of pollen-mediated gene flow for *D. sanguinalis*.

Some resistance mutations are maternally inherited, like target site photosystem-II inhibitor resistance in *C. album*, and some are able to be paternally inherited, like synthetic auxin resistance (Ghanizadeh et al. 2019, Ghanizadeh and Harrington 2019b). Dominant or partially dominant alleles can be functional with one copy, and are easily spread in outcrossing species, whereas recessive mutations require two copies to be functional, and are more likely to fix in self-pollinating species than outcrossing species (Ghanizadeh et al. 2019, Kreiner et al. 2018). Inheritance of polygenic traits (like some non-target site resistance) becomes more complicated. Some weeds have mating systems that allow for more frequent outcrossing, such as self-incompatible *Lolium perenne* (Manzanares 2013) or diecious *Amaranthus palmeri* (Ward et al. 2013); conversely *C. album* and *Avena fatua* have perfect flowers and low outcrossing rates (Raju et al. 1985, Yerka et al. 2012). Outcrossing rates can vary between populations of the same species (Whitehead et al. 2018).

Many maize weeds (*Digitaria sanguinalis*, *Chenopodium album*, *Persicaria maculosa*) are already found in most farms. These weeds are not necessarily worrisome, but herbicide-resistant populations of these weeds exist. Resistant weeds are inconspicuous, except dicamba-resistant *C. album* with different morphology. Atrazine resistant *C. album* is already widespread, but nicosulfuron resistant *D. sanguinalis* populations appear to be emerging across Waikato (Chapter 4). *Digitaria sanguinalis* was found in 51 farms but was resistant in >7 farms. Particularly difficult to control weeds are also worth preventing the spread of. *Cyperus rotundus* is found in eastern Bay of Plenty farms and one Waikato farm. Weeds under regional pest management like *Abutilon theophrasti*, *Sorghum halapense* (presumed eradicated until recently) and *Xanthium strumarium* are not widespread in maize (Chapter 3), and current efforts appear to be working. To avoid spread any farm- farm contact should ensure there are measures taken to reduce the likelihood of weed spread.

5.5.3 Further work

The Bay of Plenty and Waikato grow a large amount of maize (up-to 30% of grain and 61% of silage tonnage), but other regions Hawkes Bay, Gisborne and Manawatu-Whanganui also grow a significant amount (up-to 52% & 15% of grain and 7% & 25% of silage tonnage, respectively; AIMI 2021). Early maize weed research was concentrated in Gisborne and Waikato, but recent research has mostly been in Waikato. There is a lack of current knowledge of the weed flora of those other regions, and no weed survey of other maize growing regions has been done. There have also been no herbicide resistance surveys for other maize-growing regions. Gisborne maize weeds are well studied yet there has been no study as extensive as this survey (Chapter 3) has been done in Gisborne. A previous effort sampled six sites finding *Amaranthus powelli, Chenopodium album, Solanum nigrum, Echinochloa crus-galli*, and notably *Setaria verticillata* in Gisborne, but not *Digitaria sanguinalis, Panicum dichotomiflorum* or *Persicaria maculosa* (Rahman et al. 2006). *Setaria verticillata* was rare in Waikato and Bay of Plenty (2 farms; Chapter 3, Appendix 5), but is known to be a major weed in Gisborne and the Hawkes Bay (Rahman et al. 2003). Other weeds found in Gisborne and Hawkes Bay but not Waikato and Bay of Plenty are *Panicum capillare* and *Panicum milleaceum* (James et al. 2010b, Woon 1971). Silage maize grown in Canterbury will probably have a similar weed flora to other arable crops in Canterbury, which includes grass weeds *Lolium* spp., *Avena* spp. and *Bromus* spp. and *C. album* (Bourdôt et al. 1998, Buddenhagen et al. 2021a, Ngow et al. 2020).

As *D. sanguinalis* is in 98% of Waikato-Bay of Plenty farms and has evolved nicosulfuron resistance therein, it will be of interest to determine the prevalence of the weed in Gisborne and Hawkes Bay. It is also unknown how prevalent the atrazine-resistant biotype of *C. album* is outside of Waikato and Bay of Plenty. It was claimed to be present in almost all maize farms in New Zealand (James et al. 2005), but no strong evidence from other regions, except for sites in the Bay of Plenty and Manawatu (James et al. 2007), were published. There have not yet been any reports of nicosulfuron-resistant *D. sanguinalis* outside of the Bay of Plenty and Waikato. It is possible, as similar herbicides would be used, that convergent evolution of resistance in these weeds would occur. It is not known how much gene flow there is between weed populations of different fields, sites and regions, but it is also possible that resistant weeds could spread into other regions (for example atrazine resistant *C. album* or nicosulfuron resistant *D. sanguinalis*. A herbicide resistance survey in other regions, particularly Gisborne and Hawkes Bay where a large amount of grain maize is grown, would show if these resistant weeds are present elsewhere in New Zealand.

It is known that nicosulfuron resistant *D. sanguinalis* have 2× the resistance of susceptible populations from a dose-mortality experiment, but this experiment only tested a few plants and only recorded mortality, not plant biomass as other experiments have. Observation showed that plants were stunted (Fig 4-6), but this was not quantified. Further work would involve a more involved dose-response experiment to assess the level of resistance more accurately in *D. sanguinalis* populations. It is unknown what mechanism causes nicosulfuron resistance in the populations found here (Chapter 4), or populations previously identified in Bay of Plenty and Waikato maize (Buddenhagen et al. 2021a). Enzyme activity bioassays and target-site sequencing may prove target-site resistance, as done previously for Chinese nicosulfuron resistant *D.*

sanguinalis (Mei et al. 2017). As resistance is only at field rates (2× susceptible) for most resistant populations, it may be that the resistance is non-target site. Investigations, including testing for cytochrome P450 inhibitor and GST inhibitor activity, may reveal non-target site mechanisms of resistance. As multiple *D. sanguinalis* populations have nicosulfuron resistance, it may be that they convergently evolved resistance, and could have different mutations or mechanisms of resistance. Recombination between different biotypes is possible, as *D. sanguinalis* can outcross (Mas and Verdú 2018), thus if populations have different mechanisms, increased resistance could occur with gene flow. Polyploidy may mean that resistance is 'diluted'; it could be worth testing the ploidy of resistant populations. Herbicide resistance genetic elements can come with pleiotropic effects (Vila-Aiub et al. 2009). It is unknown if nicosulfuron resistant *D. sanguinalis* have reduced fitness, as dicamba-atrazine and atrazine resistant *C. album* do (Ghanizadeh and Harrington 2019a).

For *C. album*, much is already known about atrazine and dicamba resistance. Target site mutations have not yet been identified for atrazine or dicamba resistant *C. album*. It is likely, as suggested (Ghanizadeh and Harrington 2019a), that atrazine resistance might be conferred by a common psbA mutation. It may also be possible that resistance could also have non-target site components. None of the non-target site mechanisms investigated for dicamba resistant *C. album* appear to be present (Ghanizadeh et al. 2018). Identification of target site resistance is recent for synthetic auxins, with the first in *Kochia scoparia* in 2018 (LeClere et al. 2018, Murphy and Tranel 2019). It may be that a similar target site mutation could have occurred for dicamba resistant *C. album*. For atrazine resistant *P. maculosa*, no resistant populations were found, so any further research would not be possible, and would also be practically irrelevant.

Herbicide resistance testing of other weed species commonly collected in the survey especially high risk species *A. powelli*, *E. crus galli* and *S. nigrum* (Chapter 2: Ngow et al. 2021) but also winter weeds *S. media* and *P. annua* would be worthwhile. Other grass species *S. pumila* and *P. dichotomiflorum* are not high risk species, but are worth testing. Pre-emergent herbicides were not assessed, but are a major contributor to weed control in maize. Future work should involve testing pre-emergent herbicides.

5.6 Conclusions

The studies that form this thesis on maize weeds have addressed key questions of weed abundance and prevalence of herbicide resistance. A risk assessment of common maize weeds could help scientists and farmers to identify new threats beyond the three resistant weeds already reported, and to avoid new resistance cases. Recording the extent of the resistant weed problem is the first phase in managing the issue, and results here show nearly half of maize farms have resistant weeds.

Growers need to rotate and/or mix pre-emergent and post-emergent herbicide chemical groups, use integrated weed management practices where feasible, maintain robust farm biosecurity and monitor for problematic weeds to avoid having a resistance problem.

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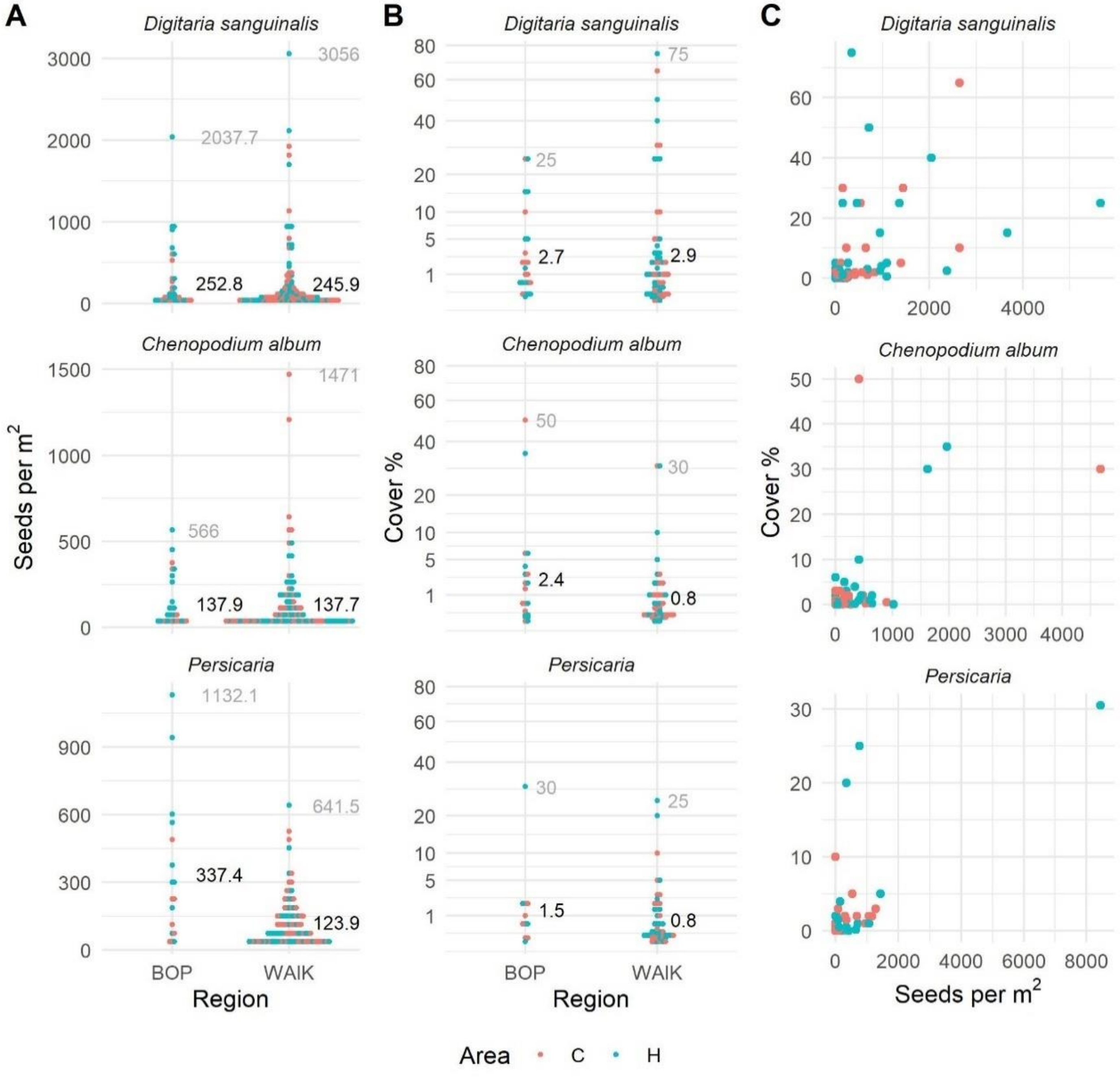
Appendices

Appendix 1. Summer grass *Digitaria sanguinalis* (L.) Scop. in the headland of a maize crop. Yellow bristle grass *Setaria pumila* (Poir.) Roem. & Schult. can be seen in the foreground.

Appendix 2. Fathen or huainanga *Chenopodium album* L. growing above crop height in a Whakatane maize crop.

Appendix 3. *Persicaria* panicles: willow weed *Persicaria maculosa* Gray (**above**) and pale willow weed *Persicaria lapathifolia* (L.) Delarbre (**below**).

Appendix 4. Beeswarm plots of seed density (m-2) of soil samples [A] and cover estimates (%) of transects [B] and the two plotted against each other [C] within the headland (H) and centre (C) of maize fields of the three most important weed species (*Digitaria sanguinalis*, *Chenopodium album*, *Persicaria* spp.) in the Bay of Plenty (BOP) and Waikato (WAIK). Mean and max values are added. Note: y-axis is square-root transformed for the cover plots [B].



Appendix 5. Complete list of all weed species observed in 52 randomly selected maize (kānga) paddocks in Bay of Plenty and Waikato; weeds were observed within the paddocks or identified from exhumed soil samples. Common names in English are sourced from Healy (1969) and Te Reo Māori names are sourced from Ngā Tipu Whakaoranga (2022) and (Beever 1991). Species difficult to identify (*Calystegia* spp., *Persicaria* spp) are given upper bounds.

Species	Common Name	Family	Growth Form	Sites
Achillea millefolium L.	yarrow	Apiaceae	perennial forb	3
Agrostis capillaris L.	browntop	Poaceae	perennial grass	1
Amaranthus blitum subsp. *oleraceus* (L.) Costea	purple amaranth	Amaranthaceae	annual forb	23
Amaranthus deflexus L.	prostrate amaranth	Amaranthaceae	annual/perennial forb	1
Amaranthus powellii S. Watson	redroot	Amaranthaceae	annual forb	13
Aphanes australis Rydb.	parsley piert	Rosaceae	annual forb	4
Apium graveolens L.	wild celery	Apiaceae	biennual forb	1
Bellis perennis L.	daisy	Asteraceae	perennial forb	1
Bidens frondosa L.	beggars’ ticks	Asteraceae	annual forb	1
Bromus catharticus Vahl	prairie grass	Poaceae	perennial grass	5
Calystegia sepium subsp. *roseata* Brummitt	pink bindweed, pōhue	Convolvulaceae	perennial vine	≤15
Calystegia silvatica subsp. *disjuncta* Brummit	Great bindweed	Convolvulaceae	perennial vine	≤15
Capsella bursa-pastoris (L.) Medik.	shepherd’s purse	Brassicaceae	annual forb	8
Cardamine flexuosa With.	wavy bitter cress	Brassicaceae	annual/perennial forb	2
Cardamine hirsuta L.	bitter cress	Brassicaceae	annual forb	9
Cerastium glomeratum Thuill.	annual mouse-ear chickweed	Caryophyllaceae	annual forb	18
Chenopodium album L.	fathen, huainanga	Amaranthaceae	annual forb	44
Cichorium intybus L.	chicory	Asteraceae	perennial forb	3
Cirsium arvense (L.) Scop.	Californian thistle	Asteraceae	perennial forb	23
Conium maculatum L.	hemlock	Apiaceae	annual/biennial forb	1
Cotula australis (Sieber ex Spreng.) Hook.f.	soldier’s button	Asteraceae	annual forb	5
Crepis capillaris (L.) Wallr.	hawksbeard	Asteraceae	annual/biennial forb	4
Cynodon dactylon (L.) Pers.	Indian doab	Poaceae	perennial grass	7
Cyperus eragrostis Lam.	umbrella sedge, puketangata	Cyperaceae	perennial sedge	1

Cyperus rotundus L.	purple nut grass	Cyperaceae	perennial sedge	8
Datura stramonium L.	thorn apple	Solanaceae	annual forb	13
Daucus carota L.	wild carrot	Apiaceae	annual/biennial forb	5
Digitaria sanguinalis (L.) Scop.	summer grass	Poaceae	annual grass	51
Dysphania pumilio (R. Br.) Mosyakin & Clemants	clammy goosefoot	Amaranthaceae	annual forb	6
Echinochloa crus-galli (L.) P. Beauv.	barnyard grass	Poaceae	annual grass	22
Eleusine indica (L.) Gaertn.	crowfoot grass	Poaceae	annual grass	6
Elymus repens (L.) Gould	couch, herewhenua	Poaceae	perennial grass	7
Epilobium ciliatum Raf.	tall willow herb, pukatea	Onagraceae	perennial forb	≤11
Epilobium billardiereanum subsp. *cinereum* (A.Rich.) P.H.Raven et Engelhorn	willow herb	Onagraceae	perennial forb	≤11
Erigeron bonariensis L.	wavy-leaved fleabane, hāka	Asteraceae	annual/biennial forb	≤27
Erigeron sumatrensis Retz.	broad-leaved fleabane, hāka	Asteraceae	annual/biennial forb	≤27
Erodium moschatum (L.) L'HÃƒÂ©r.	musky storksbill	Geraniaceae	annual forb	2
Euphorbia lathyris L.	caper spurge	Euphorbiaceae	annual/biennial forb	1
Euphorbia peplus L.	milkweed, kaikaiatua	Euphorbiaceae	annual forb	1
Fallopia convolvulus (L.) Ã©. LÃ¶ve	cornbind	Polygonaceae	annual forb	11
Fumaria capreolata L.	rampant fumitory	Papaveraceae	annual forb	2
Fumaria muralis Sond. ex W.D.J.Koch	scrambling fumitory	Papaveraceae	annual forb	1
Galinsoga quadriradiata Cav.	galinsoga	Asteraceae	annual forb	2
Gamochaeta coarctata (Willd.) Kerg.	purple cudweed	Asteraceae	annual/biennial forb	4
Geranium molle L.	dove's foot, namunamu	Geraniaceae	annual forb	1
Glyceria sp.	floating sweet grass	Poaceae	perennial grass	1
Helianthus annuus L.	sunflower	Asteraceae	annual forb	1
Hypericum perforatum L.	St John's wort	Hypericaceae	perennial forb	1
Hypochaeris radiata Falk	catsear	Asteraceae	perennial forb	3
Isolepis reticularis Colenso	*none known*	Cyperaceae	annual sedge	1
Juglans regia L.	walnut	Juglandaceae	perennial tree	1
Juncus acutus L.	sharp rush, wīwī	Juncaceae	perennial rush	1
Juncus articulatus L.	jointed rush, wīwī	Juncaceae	perennial rush	6

Juncus bufonius L.	toad rush, wīwī	Juncaceae	annual rush	41
Juncus ensifolius Wikstr.	iris-leaved rush, wīwī	Juncaceae	perennial rush	1
Juncus usitatus L.A.S.Johnson	leafless rush, wīwī	Juncaceae	perennial rush	1
Lactuca sp.	wild lettuce	Asteraceae	annual/biennial forb	1
Lamium amplexicaule L.	henbit	Lamiaceae	annual forb	1
Leontodon taraxacoides (Vill.) MÃ©rat	hawkbit	Asteraceae	perennial forb	3
Lepidium didymum L.	twin cress	Brassicaceae	annual/biennial forb	22
Leucanthemum vulgare (Vaill.) Lam.	oxeye daisy	Asteraceae	perennial forb	1
Lolium multiflorum Lam.	annual ryegrass	Poaceae	annual/biennial grass	≤23
Lolium perenne L.	perennial ryegrass	Poaceae	perennial grass	≤23
Lotus pedunculatus Cav.	lotus	Fabaceae	perennial forb	4
Ludwigia sp.	water purslane/water primrose	Onagraceae	perennial forb	2
Lysimachia arvensis (L.) U. Manns & Anderb.	scarlet pimpernel	Primulaceae	annual forb	3
Lythrum hyssopifolia L.	loosestrife	Lythraceae	annual forb	2
Malva neglecta Wallr.	dwarf mallow	Malvaceae	annual/perennial forb	4
Matricaria discoidea DC.	rayless chamomile	Asteraceae	annual forb	3
Medicago sativa L.	lucerne	Fabaceae	perennial forb	1
Mentha pulegium L.	pennyroyal	Lamiaceae	perennial forb	8
Modiola caroliniana (L.) G.Don	creeping mallow	Malvaceae	annual forb	16
Nasturtium officinale W.T.Aiton	watercress, kōwhitiwhiti	Brassicaceae	perennial forb	1
Nicandra physalodes (L.) Scop.	apple of Peru	Solanaceae	annual forb	20
Oxalis latifolia Kunth	fishtail oxalis, pink shamrock	Oxalidaceae	perennial forb	25
Oxalis exilis A.Cunn.	creeping oxalis	Oxalidaceae	perennial forb	≤10
Panicum dichotomiflorum Michx.	smooth witchgrass	Poaceae	annual grass	24
Paraserianthes lophantha (Willd.) I.C. Nielsen	brush wattle	Fabaceae	perennial tree	2
Paspalum dilatatum Poir.	paspalum	Poaceae	perennial grass	3
Paspalum distichum L.	Mercer grass	Poaceae	perennial grass	12
Pastinaca sativa L.	wild parsnip	Apiaceae	biennial forb	2
Pelargonium inodorum Willd.	kōpata	Geraniaceae	annual/biennial forb	1

Persicaria 'Giant'	Waikato willow weed, giant swamp willow weed	Polygonaceae	annual/perennial forb	3
Persicaria hydropiper (L.) Delarbre	water pepper	Polygonaceae	annual forb	3
Persicaria lapathifolia (L.) Delarbre	pale willow weed	Polygonaceae	annual forb	≤37
Persicaria maculosa Gray	willow weed	Polygonaceae	annual forb	≤37
Phalaris aquatica L.	phalaris	Poaceae		2
Phytolacca octandra L.	inkweed	Phytolaccaceae	perennial subshrub	16
Plantago lanceolata L.	narrow-leaved plantain, kopakopa	Plantaginaceae	perennial forb	4
Plantago major L.	broad-leaved plantain, kopakopa	Plantaginaceae	perennial forb	19
Poa annua L.	annual poa	Poaceae	annual/perennial grass	38
Polygonum arenastrum Boreau	small-leaved wireweed	Polygonaceae	annual forb	1
Polygonum aviculare L.	wireweed, mākākaka	Polygonaceae	annual/biennial forb	12
Portulaca oleracea L.	purslane	Portulacaceae	annual forb	6
Pseudognaphalium luteoalbum (L.) Hilliard & B.L. Burtt	Jersey cudweed, pukatea	Asteraceae	annual forb	10
Ranunculus acris L.	giant buttercup, kawariki	Ranunculaceae	perennial forb	2
Ranunculus flammula L.	spearwort, kawariki	Ranunculaceae	perennial forb	1
Ranunculus repens L.	creeping buttercup, kawariki	Ranunculaceae	perennial forb	8
Raphanus raphanistrum L.	wild radish	Brassicaceae	annual/perennial forb	3
Robinia pseudoacacia L.	robinia	Fabaceae	perennial tree	1
Rorippa sylvestris (L.) Besser	panapana, creeping yellow cress	Brassicaceae	perennial forb	5
Rubus fruticosus L. agg.	blackberry, tārewarewa	Rosaceae	perennial vine	3
Rumex obtusifolius L.	broad-leaved dock, paewhenua	Polygonaceae	perennial forb	26
Rumex pulcher L.	fiddle dock	Polygonaceae	perennial forb	2
Sagina procumbens L.	pearlwort	Caryophyllaceae	perennial forb	15
Salix sp.	willow	Salicaceae	perennial tree	1
Senecio bipinnatisectus Belcher	Australian fireweed	Asteraceae	annual/perennial forb	1
Senecio vulgaris L.	groundsel	Asteraceae	annual forb	2
Setaria gracilis Kunth	knot-root bristle grass	Poaceae	perennial grass	1
Setaria pumila (Poir.) Roem. & Schult.	yellow bristle grass	Poaceae	annual grass	31

Setaria verticillata (L.) P. Beauv.	rough bristle grass	Poaceae	annual grass	2
Silene gallica L.	catchfly	Caryophyllaceae	annual forb	5
Sisymbrium officinale (L.) Scop.	hedge mustard	Brassicaceae	annual forb	7
Solanum americanum Mill.	small-flowered nightshade, raupeti	Solanaceae	annual/perennial forb	3
Solanum nigrum L.	black nightshade, raupeti	Solanaceae	annual/perennial forb	30
Solanum nitidibaccatum Bitter	hairy nightshade	Solanaceae	annual forb	1
Solanum tuberosum L.	potato, rīwai	Solanaceae	perennial forb	2
Sonchus asper (L.) Hill	prickly sow thistle, pūhā tiotio	Asteraceae	annual/biennial forb	10
Sonchus oleraceus L.	sow thistle, pūhā	Asteraceae	annual forb	30
Spergula arvensis L.	spurrey	Caryophyllaceae	annual forb	6
Spergularia rubra (L.) J. Presl & C. Presl	sand spurrey	Caryophyllaceae	annual/perennial forb	1
Stellaria media (L.) Vill.	chickweed, kohukohu	Caryophyllaceae	annual forb	37
Symphyotrichum lanceolatum (Willd.) G.L. Nesom	sea aster	Asteraceae	perennial forb	2
Taraxacum officinale F.H. Wigg.	dandelion, tawao	Asteraceae	perennial forb	8
Trifolium pratense L.	red clover	Fabaceae	perennial forb	2
Trifolium repens L.	white clover	Fabaceae	perennial forb	17
Trifolium subterraneum L.	subterranean clover	Fabaceae	annual forb	2
Verbena incompta P.W. Michael	Purpletop	Verbenaceae	perennial forb	1
Veronica anagallis-aquatica L.	water speedwell	Plantaginaceae	perennial forb	1
Veronica persica Poir.	scrambling speedwell	Plantaginaceae	annual forb	7
Veronica serpyllifolia L.	turf speedwell	Plantaginaceae	perennial forb	6
Vulpia myuros (L.) C.C. Gmel.	ratstail fescue	Poaceae		1
Xanthium spinosum L.	Bathurst bur	Asteraceae	annual forb	3
Xanthium strumarium L.	Noogora bur	Asteraceae	annual forb	1

Appendix 6. Attributes of farms sampled in Bay of Plenty and Waikato. Soil type is estimated from S-map (smap.landcareresearch.co.nz), annual rainfall, annual mean temperature and sunshine hours are sourced from CliFlo (cliflo.niwa.co.nz) (Ford 2019, Seers and Shears 2015). Row spacing and tillage method were assessed on site and years cultivated is listed where known. Latitude and longitude are rounded to one decimal places.

Farm	Crop	Latitude	Longitude	Date sampled	Soil type	Row spacing	Tillage	Years	Rainfall (mm)	Mean temperature (°C)	Sunshine hours (h)
Bay of Plenty											
BP1	Grain	-38.0	176.8	8/03/2021	Recent	75	Cultivated	10	1300	14	2250
BP2	Grain	-37.7	176.3	28/02/2021	Gley	75	Cultivated	-	1400	14	2250
BP3	Grain	-37.8	176.3	1/03/2021	Allophanic	75	Cultivated	-	1400	14	2250
BP4	Grain	-37.8	176.4	2/03/2021	Pumice	75	Cultivated	-	1400	14	2250
BP5	Silage	-38.0	177.4	9/03/2021	Gley	75	Cultivated	-	1400	14	2250
BP6	Silage	-38.1	177.0	8/03/2021	Recent	75	Cultivated	-	1500	13	2125
BP7	Silage	-37.8	176.3	1/03/2021	Pumice	75	Cultivated	-	1800	13	2250
BP8	Silage	-37.9	176.2	28/02/2021	Pumice	75	No till	4	2200	12	2125
BP9	Silage	-37.8	176.3	1/03/2021	Gley	75	Cultivated	-	1400	14	2250
BP10	Grain	-37.8	177.6	9/03/2021	Brown	75	Cultivated	-	1400	14	2175
BP11	Grain	-38.0	176.8	8/03/2021	Pumice	75	Cultivated	-	1500	14	2225
BP12	Grain	-38.1	176.8	8/03/2021	Pumice	75	Cultivated	-	1500	14	2200
BP13	Grain	-37.9	176.9	9/03/2021	Gley	75	Cultivated	-	1200	14	2250
BP14	Silage	-38.0	176.9	10/03/2021	Podzol	75	Cultivated	-	1300	14	2250
BP15	Grain	-38.0	176.8	8/03/2021	Recent	75	Cultivated	-	1300	14	2250
BP16	Grain	-38.0	177.0	9/03/2021	Gley	75	Cultivated	-	1400	14	2250
Waikato											
WK1	Grain	-37.7	175.3	25/02/2021	Organic	50	Cultivated	-	1400	14	2000
WK2	Silage	-38.2	175.6	22/02/2021	Allophanic	75	-	-	1400	12	1925
WK3	Silage	-38.0	175.2	23/02/2021	Brown	75	No till	-	1500	13	1950
WK4	Silage	-38.2	175.4	22/02/2021	Allophanic	75	Cultivated	10	1400	13	1875
WK5	Silage	-38.1	175.2	24/02/2021	Allophanic	75	Cultivated	20	1400	13	1925
WK6	Grain	-37.9	175.2	23/02/2021	Allophanic	75	Cultivated	-	1500	13	1925

WK7	Silage	-37.9	175.2	22/02/2021	Gley	75	Cultivated	-	1500	14	1925
WK8	Silage	-37.6	175.5	25/02/2021	Granular	75	Cultivated	-	1000	14	2000
WK9	Silage	-38.2	175.5	22/02/2021	Allophanic	75	Cultivated	10	1400	13	1900
WK10	Silage	-37.7	175.2	25/02/2021	Gley	50	Cultivated	15	1400	14	1975
WK11	Silage	-37.7	175.7	17/02/2021	Allophanic	75	Cultivated	-	1100	14	2025
WK12	Grain	-37.9	175.3	18/02/2021	Allophanic	75	No till	-	1200	13	1950
WK13	Silage	-37.8	175.8	18/02/2021	Allophanic	75	Cultivated	14	1200	14	2000
WK14	Silage	-37.7	175.7	17/02/2021	Gley	75	Cultivated	-	1100	14	2025
WK15	Silage	-38.2	175.2	24/02/2021	Gley	75	Cultivated	-	1500	13	1850
WK16	Silage	-38.2	175.7	22/02/2021	Pumice	75	Cultivated	10	1500	12	1925
WK17	Silage	-37.9	175.2	22/02/2021	Gley	75	Cultivated	5	1400	13	1950
WK18	Grain	-37.4	175.2	19/02/2021	Organic	75	Cultivated	-	1100	14	1950
WK19	Silage	-37.6	175.5	17/02/2021	Ultic	75	Cultivated	-	1000	14	2000
WK20	Silage	-37.7	175.5	17/02/2021	Allophanic	75	No till	-	1100	13	2000
WK21	Silage	-37.5	175.5	18/02/2021	Gley	75	Cultivated	-	1100	14	2000
WK22	Silage	-37.6	175.5	17/02/2021	Ultic	75	Cultivated	-	1000	14	2000
WK23	Silage	-37.7	175.8	17/02/2021	Gley	75	Strip till	20	1200	14	2025
WK24	Silage	-37.9	175.7	12/02/2021	Allophanic	75	-	-	1200	13	2025
WK25	Silage	-37.9	175.7	18/02/2021	Gley	75	Cultivated	15	1100	13	1975
WK26	Silage	-37.9	175.2	23/02/2021	Granular	75	Cultivated	5	1500	13	1925
WK27	Silage	-38.0	175.8	18/02/2021	Allophanic	75	Cultivated	14	1300	13	1975
WK28	Silage	-38.1	175.9	23/02/2021	Allophanic	75	No till	-	1400	13	1950
WK29	Silage	-37.6	175.3	18/02/2021	Organic	75	Cultivated	-	1000	14	2000
WK30	Silage	-37.8	175.4	25/02/2021	Allophanic	75	Cultivated	-	1200	13	1975
WK31	Silage	-38.1	175.5	23/02/2021	Allophanic	75	Cultivated	8	1100	13	1900
WK32	Silage	-38.1	175.6	23/02/2021	Allophanic	75	Cultivated	20	1300	13	1950
WK33	Silage	-37.9	175.2	22/02/2021	Allophanic	75	Cultivated	58	1500	13	1950
WK34	Silage	-38.1	175.4	24/02/2021	Allophanic	75	Cultivated	20	1100	13	1900
WK35	Silage	-38.0	175.4	24/02/2021	Allophanic	75	Cultivated	0	1100	13	1925
WK36	Silage	-38.1	175.6	23/02/2021	Brown	75	No till	-	1300	13	1950

Non-survey Bay of Plenty farms sampled for nicosulfuron resistant *Digitaria sanguinalis*								
NS1	Silage	-37.8	176.5	18/01/2021	Gley	75	Cultivated	Dairy farm with a silage maize paddock.
NS2A	Silage	-37.8	176.4	18/01/2021	Pumice	75	Cultivated	Original nicosulfuron-resistance site.
NS2B	Silage	-37.8	176.5	18/01/2021	Allophanic	75	Cultivated	Site leased to grow maize by the same contractor.
NS2C	Silage	-37.8	176.5	18/01/2021	Allophanic	75	Cultivated	Site leased to grow maize by the same contractor.
NS2D	Silage	-37.8	176.5	18/01/2021	Allophanic	75	Cultivated	Site leased to grow maize by the same contractor.
NS3A	Silage	-37.9	176.4	18/01/2021	Pumice	75	Cultivated	Additional site nearby, managed by a different contractor.
NS3B	Silage	-37.9	176.4	18/01/2021	Pumice	75	Cultivated	Additional site nearby, managed by a different contractor.
NS3C	Silage	-37.9	176.5	18/01/2021	Pumice	75	Cultivated	Additional site nearby, managed by a different contractor.

Appendix 7. Weed seed collected from mature plants pre-harvest in 36 Waikato (late February) and 16 Bay of Plenty (late March) maize farms.

Species	Farms	Total Samples
Achillea millefolium	1	1
Amaranthus powellii	10	16
Amaranthus blitum ssp. *oleraceus*	8	8
Arrhenatherum elatius	1	1
Bidens frondosa	1	1
Calystegia spp.	5	7
Capsella bursa-pastoris	1	1
Chenopodium album	34	106
Cirsium arvense	6	8
Cynodon dactylon	1	2
Cyperus conglomeratus	1	1
Cyperus rotundus	4	16
Datura stramonium	10	13
Digitaria sanguinalis	45	151
Echinochloa crus-galli	23	54
Eleusine indica	1	1
Elymus repens	1	1
Fallopia convolvulus	14	36
Lepidium didymium	1	1
Lolium multiflorum	3	4
Lolium perenne	2	3
Malva sp.	1	1
Nicandra physalodes	12	20
Panicum dichotomiflorum	19	43
Paspalum dilatatum	2	3
Paspalum distichum	7	13
Persicaria 'Giant'	3	7
Persicaria hydropiper	3	3
Persicaria lapathifolia	5	21
Persicaria maculosa	17	46
Persicaria sp.	17	64
Polygonum aviculare	10	14
Portulaca oleracea	1	1
Rumex obtusifolius	15	18
Rumex pulcher	1	1
Rumex sp.	1	3
Setaria pumila	15	44
Setaria verticilliata	3	11
Solanum americanum	1	1
Solanum nigrum	6	22
Sonchus oleraceus	2	2
Stellaria media	3	3

Appendix 8. All plant families observed in 52 maize sites, summed by percentage of total estimated cover and percentage of total seedbank count (rounded to two significant figures).

Family	% total estimated percentage cover	% total seedbank count
Poaceae	57%	44%
Cyperaceae	13%	0.6%
Amaranthaceae	9.3%	6.8%
Polygonaceae	8.2%	8.9%
Oxalidaceae	2.7%	1.8%
Ranunculaceae	2.7%	0.6%
Solanaceae	2.6%	1.4%
Asteraceae	2.1%	2.5%
Convolvulaceae	1.4%	0%
Apiaceae	0.2%	>0%
Papaveraceae	0.2%	0.1%
Malvaceae	0.1%	0%
Phytolaccaceae	0.1%	0%
Caryophyllaceae	0.1%	11%
Fabaceae	0.1%	0.3%
Plantaginaceae	>0%	2.9%
Rubiaceae	>0%	0%
Brassicaceae	>0%	4.9%
Geraniaceae	>0%	>0%
Euphorbiaceae	>0%	0%
Hypericaceae	>0%	0%
Portulaceae	>0%	0.2%
Lamiaceae	>0%	1.3%
Juglandaceae	>0%	0%
Salicaceae	>0%	0%
Verbenaceae	>0%	0%
Juncaceae	0%	13%
Onagraceae	0%	0.3%
Rosaceae	0%	0.1%

Appendix 9. Percentage cover (**left**) and seedbank density estimates (**right**; seeds m^{-2}) of the five most important weeds for each of the 52 Waikato and Bay of Plenty farms averaged over headland and centre plots. Percentage cover estimates are rounded to two significant figures, if the weed was not observed 'nil' is recorded.

ID	***Digitaria sanguinalis***		***Chenopodium album***		***Persicaria* spp.**		***Echinochloa crus-galli***		***Cyperus rotundus***		**All weed species**	
BP1	15%	264	3.1%	0	1.0%	19	nil	0	nil	0	46%	1849
BP2	1.0%	189	nil	0	nil	19	nil	0	nil	0	1.1%	1321
BP3	14%	75	0.5%	19	nil	0	nil	0	8.5%	19	24%	566
BP4	1.0%	57	2.5%	283	nil	0	nil	0	1.5%	0	13%	566
BP5	0.1%	38	2.8%	283	16%	4755	0.1%	0	nil	0	23%	6566
BP6	0.8%	887	nil	19	0.0%	189	nil	0	nil	0	95%	4377
BP7	1.3%	38	43%	1189	0.8%	0	0.5%	0	nil	0	46%	1868
BP8	13%	792	1.3%	0	1.3%	0	0.3%	0	nil	0	23%	2415
BP9	3.5%	755	0.1%	0	nil	0	nil	0	nil	0	4.9%	2962
BP10	nil	0	nil	0	nil	0	nil	0	60%	434	91%	1679
BP11	0.1%	0	0.0%	0	nil	0	nil	0	nil	0	0.2%	1642
BP12	0.5%	0	0.1%	170	nil	0	nil	0	1.0%	0	94%	1094
BP13	7.8%	1830	0.0%	19	nil	0	nil	0	60%	189	69%	3321
BP14	0.1%	113	0.0%	19	0.0%	0	nil	0	nil	0	0.5%	906
BP15	0.0%	0	nil	0	nil	0	nil	0	15%	38	20%	2981
BP16	0.3%	19	4.5%	0	nil	0	nil	0	10%	38	19%	528
WK1	0.1%	0	0.0%	0	0.0%	0	nil	0	nil	0	0.4%	57
WK2	0.0%	0	0.5%	57	nil	0	0.0%	0	nil	0	24%	2472
WK3	28%	3547	30%	3151	15%	170	10%	226	nil	0	98%	8849
WK4	6.5%	113	nil	0	nil	0	nil	0	nil	0	7.1%	3264
WK5	2.5%	132	nil	170	nil	38	1.5%	0	nil	0	4.0%	887
WK6	0.1%	0	0.4%	774	0.2%	57	0.1%	0	nil	0	8.6%	2925
WK7	0.4%	151	0.1%	38	0.1%	0	nil	0	nil	0	1.9%	1509
WK8	0.0%	0	nil	19	nil	0	nil	0	nil	0	0.0%	1660
WK9	nil	57	1.5%	94	15%	642	26%	264	nil	0	75%	3189
WK10	1.6%	38	0.0%	0	0.1%	75	nil	0	nil	0	3.2%	6151

WK11	0.1%	19	0.6%	75	0.1%	0	nil	0	nil	0	2.0%	264
WK12	1.8%	38	0.0%	0	0.0%	0	0.0%	0	nil	0	2.6%	906
WK13	14%	245	2.5%	264	1.5%	509	nil	0	nil	0	22%	2208
WK14	1.0%	245	0.1%	0	0.1%	0	nil	0	nil	0	2.9%	1000
WK15	35%	1094	2.6%	94	4.0%	755	13%	38	nil	0	59%	3264
WK16	0.2%	0	0.1%	94	0.0%	38	nil	0	nil	0	3.0%	1377
WK17	0.2%	57	0.2%	774	0.1%	38	0.1%	0	nil	0	2.3%	2075
WK18	2.8%	245	0.2%	0	0.1%	1075	0.6%	19	nil	0	4.1%	9528
WK19	25%	774	1.0%	94	nil	245	18%	0	nil	0	45%	6226
WK20	13%	698	nil	0	nil	0	nil	0	nil	0	13%	1094
WK21	0.1%	0	5.5%	245	0.8%	642	0.1%	0	nil	0	7.7%	4038
WK22	0.1%	19	nil	0	1.1%	396	0.3%	0	nil	0	1.8%	6226
WK23	1.5%	1660	0.0%	0	0.0%	151	nil	0	0.7%	0	2.3%	2226
WK24	1.5%	94	0.4%	19	0.1%	0	nil	0	nil	0	2.9%	340
WK25	0.0%	0	nil	0	nil	0	nil	0	nil	0	10%	9340
WK26	0.5%	132	0.1%	19	0.1%	0	nil	0	nil	0	4.1%	1094
WK27	0.1%	0	nil	38	nil	19	nil	0	nil	0	0.9%	2038
WK28	38%	1491	1.5%	358	2.0%	245	1.5%	0	nil	0	46%	4811
WK29	0.8%	132	nil	0	0.2%	57	nil	0	nil	0	5.5%	5453
WK30	0.1%	0	0.8%	19	0.0%	0	nil	0	nil	0	1.0%	2226
WK31	3.0%	774	0.8%	283	0.5%	528	0.1%	75	nil	0	7.0%	2057
WK32	1.8%	1396	1.5%	415	1.8%	623	0.5%	0	nil	0	9.1%	4585
WK33	0.2%	151	0.1%	170	0.1%	19	0.1%	0	nil	0	1.4%	1000
WK34	nil	698	0.0%	75	0.0%	38	0.3%	19	nil	0	0.7%	1132
WK35	0.5%	132	0.1%	0	nil	0	nil	38	nil	0	12%	3509
WK36	1.0%	94	0.1%	94	0.3%	57	0.3%	0	nil	0	20%	2472

Appendix 10. Surviving plant count (left: survivor count, right: treated count) of weeds treated with herbicides by each farm (alive/tested), for non-survey farms.

ID	Species	Herbicide	Survival		Summary
Bay of Plenty farms sampled for nicosulfuron resistant *Digitaria sanguinalis*					
NS1	*Digitaria sanguinalis*	nicosulfuron	0	11	susceptible
NS2A	*Digitaria sanguinalis*	nicosulfuron	10	43	resistant
NS2C	*Digitaria sanguinalis*	nicosulfuron	3	5	resistant
NS3A	*Digitaria sanguinalis*	nicosulfuron	0	11	susceptible
Self-reported eastern Waikato farms suspected of nicosulfuron-resistant *Digitaria sanguinalis*					
IN1	*Digitaria sanguinalis*	nicosulfuron	215	418	resistant
IN2	*Digitaria sanguinalis*	nicosulfuron	49	91	resistant
IN3	*Digitaria sanguinalis*	nicosulfuron	0	6	susceptible
Other non-survey farms					
OT1	*Chenopodium album*	atrazine	0	28	susceptible
OT1	*Chenopodium album*	dicamba	0	25	susceptible
NCRS	*Chenopodium album*	atrazine	0	3	susceptible
NCRS	*Chenopodium album*	dicamba	0	3	susceptible
NCRS	*Digitaria sanguinalis*	nicosulfuron	2	3	resistant (possibly)

Appendix 11. Nicosulfuron dosage-response (mortality) of *Digitaria sanguinalis* populations at 0, 15, 30, 60, 120 and 240 g.ai.ha^{-1} nicosulfuron with 5% oil-based adjuvant.

Farm	**Sample**	**Prior result (60)**	**0**	**15**	**30**	**60**	**120**	**240**
Survey samples								
BP9	3033	0/5	0/0	0/1	0/1	0/1	0/1	0/0
WK5	2841	0/1	1/1	2/2	0/1	0/3	0/3	0/3
WK10	2844	5/15	3/3	7/7	5/5	3/4	2/5	3/5
WK15	3098	0/11	0/0	2/2	2/3	0/4	0/5	0/1
WK18	2879	0/3	3/3	1/1	1/2	0/4	0/3	0/3
WK19	2713	0/2	2/2	1/1	1/2	0/5	0/2	0/2
WK20	2748	0/3	0/0	4/4	3/3	2/3	0/3	0/1
WK22	2651	0/11	2/2	4/5	0/1	0/3	0/3	0/2
WK23	2954	0/4	0/0	3/3	2/2	1/5	0/2	0/2
WK28	2606	5/5	4/4	4/5	1/2	6/6	4/6	2/5
WK31	2533	not tested	0/0	0/0	0/1	0/1	0/0	0/0
WK34	3091	0/8	1/1	1/1	2/3	0/3	0/5	0/3
Non-survey samples								
NS2C	W3	1/8	5/5	3/3	2/2	3/5	0/4	0/6
NCRS	1957	2/2	1/1	1/1	1/1	1/2	0/2	0/1
I3	3308	no germination	1/1	0/1	0/1	0/2	0/2	0/1
Susceptible and resistant control samples								
RK	‘Sand’	0/247	2/2	0/3	0/0	0/6	0/4	0/4
NS2A	2431	328/428	2/2	4/4	5/5	4/6	1/7	0/5