Manaaki Whenua
Landcare Research

Invertebrates and fungi associated with yellow flag iris, *Iris pseudacorus* L., in New Zealand

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Invertebrates and fungi associated with yellow flag iris, *Iris pseudacorus* L., in New Zealand

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Project and Client

- A survey of the invertebrate fauna and fungal pathogens associated with yellow flag iris, *Iris pseudacorus* L., in New Zealand was carried out during the autumn of 2021 and the summer of 2022 by Manaaki Whenua – Landcare Research for the National Biocontrol Collective and the Ministry of Primary Industries contract number S3F-20095.

Objectives

- To survey the invertebrate fauna and plant disease symptoms associated with yellow flag iris, *Iris pseudacorus*, in New Zealand and to identify the herbivores (and their associated predators and parasitoids) and plant pathogens present.

Methods

- The invertebrate fauna and plant disease symptoms associated with yellow flag iris were surveyed at 11 New Zealand sites, from Auckland to Southland, during autumn 2021 and summer 2022.
- Invertebrates were collected, reared and identified.
- Disease symptoms were photographed and recorded.
- Fungi were isolated from surface-disinfected symptomatic material.
- DNA sequences of pure fungal cultures were analysed.
- Literature was reviewed for information on life histories of the identified fungal cultures isolated from yellow flag iris tissues.

Results

- A total of 81 herbivorous invertebrate species, or groups of related taxonomic units, were recorded from yellow flag iris during the surveys.
- None of the herbivores recorded were yellow flag iris specialists.
- Very few plant disease symptoms were observed on yellow flag iris. These were mainly leaf spots and the occasional tuber lesions.
- A total of 113 pure fungal cultures was recovered from symptomatic tissues comprising 31 species from 19 genera. Three additional species were identified to the families Hypoxylaceae and Phaeosphaeriaceae, and the sub-class Sordariomycetidae.
- The predominant genera isolated were *Colletotrichum* and *Alternaria* from leaf spots. *Colletotrichum* and *Alternaria* species are common plant pathogens causing stem, leaf and fruit lesions.
- Only species belonging to the genera *Colletotrichum*, *Alternaria*, *Ilyonectria*, *Stemphylium, Nigrospora*, *Cadophora*, *Bipolaris*, *Ramularia* and *Fusarium* were considered as potential pathogens.

- None of the primary[1] plant pathogens found to be associated with disease symptoms of yellow flag iris are host-specific, and none had any major impact. The symptoms observed in the field were mainly "superficial" without any chronic disease symptoms observed killing whole plants.
- One fungal isolate, *Rachicladosporium iridis*, has only been isolated from leaf spots on *Iris* spp. overseas.
- The remaining species identified were considered either saprophytes or endophytes, or secondary[2] pathogens.

Conclusions

- No specialised yellow flag iris-feeding invertebrates were collected from yellow flag iris during these surveys.
- None of the primary pathogens found on yellow flag iris in the surveys are host specific and none had any major impact on this plant.

Recommendations

- Three invertebrate species were identified in a preliminary survey in Europe as potential biocontrol agents against yellow flag iris in South Africa.
- Given that no specialised yellow flag iris-feeding invertebrates and no damaging specialised pathogenic fungi have been recorded on yellow flag iris in New Zealand, we recommend that the invertebrate species identified in Europe as potential candidates for biological control should be investigated for their use in a classical biocontrol programme.

[1] A primary pathogen can cause disease on a healthy host.

[2] A secondary pathogen needs a wounded or weakened host to cause disease.

1 Introduction

A survey of the invertebrate fauna and fungi associated with yellow flag iris, *Iris pseudacorus* L., in New Zealand was carried out during the autumn of 2021 and the summer of 2022. This work was carried out by Manaaki Whenua – Landcare Research for the National Biocontrol Collective and the Ministry of Primary Industries contract number S3F-20095.

2 Background

Yellow flag iris, *Iris pseudacorus*, is a monocotyledonous species from the iris family Iridaceae. It is an erect, perennial, aquatic herbaceous plant that grows between 400 and 1,500 mm tall (Fig. 1), with bright yellow flowers. Yellow flag iris produces pink rhizomes of 10–40 mm diameter with 100–200 mm long roots (Fig. 2). These rhizomes can form dense clumps. An average of 10 upright, linear and smooth dark-green leaves are formed per ramet from the rhizomes, creating a fan shape from the base (Sutherland & Walton 1990; ISSG 2021). The 120-mm-wide yellow flowers have the typical iris shape, which consists of three sepals, three petals, and a leafy style (Fig. 2). In New Zealand, this plant flowers from September to December. The flowers need to be pollinated with pollen from another individual plant to form seeds (Stone 2009) while rhizome fragments can regenerate vegetatively. Yellow flag iris produces 40–80-mm long, 3-angled, green capsules with up to 120 brown, 7-mm long, disc-shaped seeds per capsule (Fig. 2). The buoyant seeds disperse through water, with flooding thought to be the prevailing means of both seed and rhizome dispersal (Gaskin et al. 2016). Seeds germinate in moist substrates with optimal temperatures between 20 and 30^{0}C; however, waterlogging inhibits seed germination (Coops & Van der Velde 1995; Lenssen et al. 1998).

Figure 1. Yellow flag iris along waterway at survey site in Mangawhai, Auckland (May 2021).

Figure 2. Yellow flag iris, *Iris pseudacorus*. (source: Flora Batava, Volume 5 (1828), www.BioLib.de)

Yellow flag iris is native to Europe, northern Africa, and western Asia (ISSG 2021). It has been introduced to Japan, Korea, India, the United States of America, Canada, South Africa, Chile, Argentina, Uruguay, Australia, and New Zealand (Fig. 3; USDA-APHIS 2013; Kew 2017). Its bright yellow flower contributed to its appeal for gardens and landscaped areas. *Iris pseudacorus* is considered a weed of significant concern in most of its introduced range due to its environmental and socioeconomic impacts (Gaskin et al. 2016; Hayasaka et al. 2018). However, this weed can still be purchased from online gardening stores and websites such as Amazon and eBay, regardless of its weed status (McGrannachan & Barton 2019).

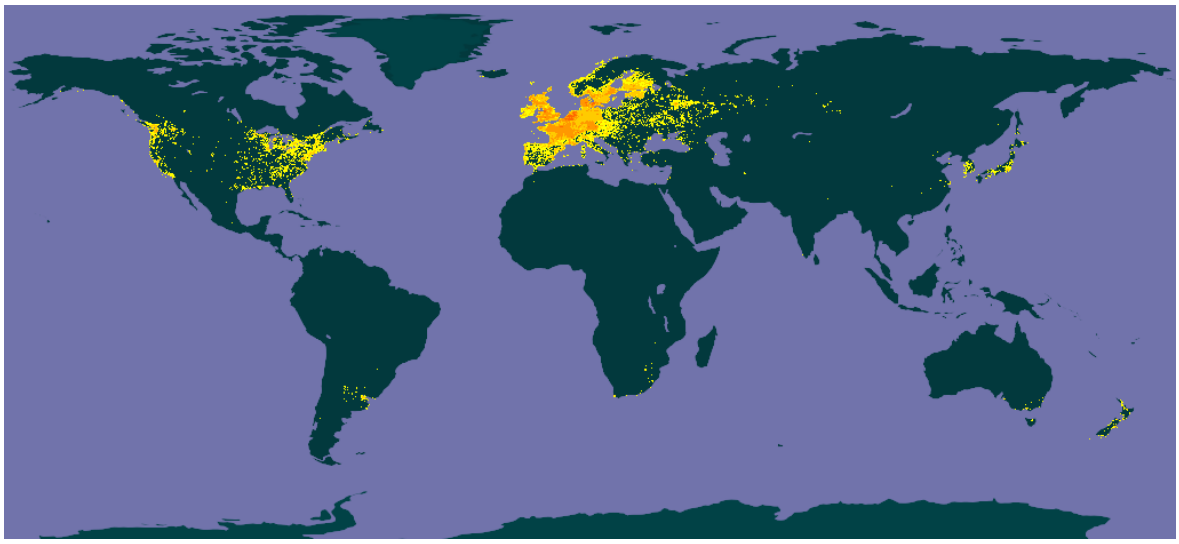

Figure 3. Global distribution of yellow flag iris (https://www.gbif.org/species/5298231).

Yellow flag iris grows in very wet conditions and is found on the shores of still water bodies such as lakes, ponds, and slow-flowing watercourses, wetlands, and soil marshes. It can tolerate low pH, submersion, drought and long-term anoxia (Schlüter & Crawford 2001; Minuti et al. 2021). It can grow on a range of water-deposited substrates, including silt, sand, gravel and cobbles (Sutherland & Walton 1990; Stone 2009). It is often found in meadows, wet pastures, ditches, and irrigation systems (King County Noxious Weed Control Program 2020).

In New Zealand, yellow flag iris is an unwanted organism under the Biosecurity Act (1993) and a National Plant Pest Accord Species, which prevents its propagation, sale, and distribution throughout the country. It is included in the Regional Pest Management Plans in seven regions with the following status:

- 'eradication' in Northland, with all yellow flag iris sites being controlled and monitored until the plant is eliminated (Bryn Gillard, biosecurity officer, Northland Regional Council, pers. comm.)
- 'sustained control' in Auckland and in Tasman-Nelson, with the aim to reduce its spread by on-going control (Nelson City Council & Tasman District Council 2019; Auckland Council 2021)
- 'progressive containment' in Waikato, Bay of Plenty and the West Coast, with the aim to contain and reduce the amount of yellow flag iris (West Coast Regional Council 2018, Waikato Regional Council 2019, Bay of Plenty Regional Council 2021)
- 'site-led control' in Gisborne (Gisborne District Council).

First discovered growing in the wild in Lower Hutt in 1938 (Te Waihora Co-Governance 2021), yellow flag iris is present across New Zealand from Stewart Island to Northland (Fig. 4), with localised, dense stands in Christchurch along the Avon River and in Waikato (NZPCN 2021). The largest stands of yellow flag iris are found along the Waikato River. An aerial survey of the distribution of yellow flag iris along the Waikato River between Ngaruawahia and Tuakau determined that the weed was present over an area of 258 ha

and could potentially occupy an additional 576 ha along the river (Wildland Consultants 2011). Additionally, isolated patches of yellow flag iris were spread across 50 km of riverbank and island shoreline.

Figure 4. Yellow flag iris distribution in New Zealand https://www.gbif.org/species/5298231).

Yellow flag iris forms dense stands and rhizome mats that displace native species and can alter habitats by compacting soil and increasing elevation by trapping sediments (King County Noxious Weed Control Program 2020). It can obstruct small streams and irrigation systems and can reduce stream width, increasing flooding risk (King County Noxious Weed Control Program 2020). Fishing and recreational activities may also be restricted in waterbody margins where yellow flag iris forms dense stands (Wildland Consultants 2011). It may degrade whitebait spawning areas (Te Waihora Co-Governance 2021).

Yellow flag iris contains large quantities of glycosides that are toxic to humans and animals. Its sap can cause skin irritation to humans (Te Waihora Co-Governance 2021) and if ingested it can cause moderate to severe abdominal pain, gastroenteritis, nausea spasms, vomiting, diarrhoea, staggering, and paralysis (Tu 2003).

Control methods include mechanical removal, which is deemed unsuitable for yellow flag iris in New Zealand (Williams & Champion 2008), and chemical control using glyphosate

or metsulfuron (Wildland Consultants 2011). Repeated applications are required to control seedlings from seed banks and to prevent re-infestation from nearby populations. Cost of chemical control has been estimated to vary between NZD 100 to NZD 340 per hectare for isolated patches to over NZD 1,350 per hectare for density cover over 40% (Wildland Consultants 2011).

Biological control could offer many advantages over current control methods for yellow flag iris, especially around waterways and given its rapid spread. It could offer continuous action and self-dispersal that current control methods do not provide. South Africa initiated a biocontrol programme targeting yellow flag iris in 2016 that resulted in surveys of natural enemies in Belgium and in northern Italy (Minuti et al. 2021). From these surveys, three insects were prioritised as candidate biocontrol agents: a sawfly (*Rhadinoceraea micans* Klug); a seed weevil (*Mononychus punctumalbum* Herbst); and a flea beetle (*Aphthona nonstriata* Goeze). The flea beetle has been imported to South Africa where it is currently undergoing host specificity testing, while the remaining two insects are also expected to undergo host specificity testing in South Africa (Minuti et al. 2021). *Rhadinoceraea micans* was found to cause severe defoliation in its native range; *M. punctumalbum* adults damage flowers and fruits and larvae feed on seeds and *A. nonstriata* adults eat strips of epidermis parallel to the leaf veins while larvae mine the stems down to the rhizome where they bore into the rhizome (Minuti et al. 2021).

This report describes the results of a survey of the invertebrate fauna and fungal pathogens associated with yellow flag iris in New Zealand.

3 Objectives

To survey the invertebrate fauna and plant disease symptoms associated with yellow flag iris (*Iris pseudacorus*) in New Zealand and identify the herbivores (and their associated predators and parasitoids) and plant pathogens present.

The main aims of the survey were to:

1 determine whether any specialist yellow flag iris invertebrates or pathogens are already present in New Zealand.

2 determine whether any generalist invertebrate herbivores or plant pathogens are exerting a significant adverse impact on yellow flag iris in New Zealand.

3 record the invertebrate parasitoids and predators associated with the herbivorous invertebrates on yellow flag iris in New Zealand.

Such information is useful at the early stages of a biological control programme to avoid wasting resources on importing something that is already present in New Zealand. Also, knowing whether a candidate agent has similar 'analogue' native arthropod herbivores utilising the exotic weed helps predict whether natural enemies of the 'analogue' could potentially interfere with the efficacy of any future agent released here (Paynter et al. 2010).

4 Methods

4.1 Invertebrates

The invertebrate fauna of yellow flag iris was surveyed at 11 sites across New Zealand, between May 2021 and January 2022 (Fig. 5 and Appendix 1). All sites (except Tuatapere scenic reserve) were inspected once. At each site, 10 collection locations were randomly selected by sampling 5–10-m distant plants in different directions. A collecting tray, 80 × 80 cm, was placed under suitable parts of selected plants, and the foliage above the tray was hit five times with a stick. Most invertebrates that fell onto the tray were collected with an aspirator and preserved in 95% alcohol. Caterpillars (Lepidoptera) were collected live, and placed, along with yellow flag iris leaves, into ventilated containers to rear through to adult for identification. Any parasitoids emerging from the caterpillars were identified.

A rapid visual inspection of foliage (generally less than a minute for each of the ten collection locations per site) was made for signs of invertebrates such as gall-formers, leaf miners, scale insects and stem/shoot borers. Invertebrates found during the visual inspections were collected live, along with the plant material they were on, for identification. At each site, a visual estimate was made of the amount of herbivore-related damage, and the likely cause of the damage was noted (e.g. slugs). The ten yellow flag iris plants per site were dug up and inspected for rhizome damage.

The invertebrates collected were identified to species or genus level where feasible. However, some invertebrates were placed into higher-level taxonomic units (e.g. 'spiders'). They were then ranked on a scale of abundance according to the total number of individuals collected, and the number of collecting events at which they were present. Invertebrates were classed as rare, occasional, common or abundant according to the definitions below:

rare: fewer than 5 individuals collected in total

occasional: 5–15 individuals collected in total, **or** present at fewer than 5 sites

common: 16–99 individuals collected **and** present at 5 or more sites

abundant: 100+ individuals collected **and** present at 7 or more sites

4.2 Plant pathogens

Plant pathogens associated with yellow flag iris were surveyed at the same 11 New Zealand sites and times as for invertebrates. At each site, plants were inspected for signs of pathogen damage and/or presence on either diseased leaves, rhizomes or fruits. Samples of symptomatic tissues were placed in paper bags, placed into labelled zip-lock bags, and kept cool in transit, and held at 4–10°C until processing. Collected material was usually processed within 5 days of collection.

In the laboratory, disease symptoms were recorded and photographed. A dissecting microscope was used to search necrotic areas for fungal reproductive structures especially, conidia and spores. Symptomatic tissues were surface disinfected to remove any fungi that were present on the surface of plant material but had not penetrated plant tissues, removing a number of saprophytes. This process was achieved by immersion in 70% ethanol for 30 s, 2% sodium hypochlorite for 1 min, and 70% ethanol again for 30 s, followed by two rinses in sterile reverse osmosis water. The tissue fragments were air-dried. placed on potato dextrose agar (PDA; Difco Labs, Detroit, MI, USA) amended with 0.02% streptomycin (Sigma, St Louis, MI, USA), contained in 9-cm Petri dishes. Plates were sealed with parafilm, and incubated under white light (12-hour photoperiod) at temperatures of 18 ± 2°C. This method allows fungi present in symptomatic tissue fragments to grow onto agar.

Fungal colonies that grew out of the tissue fragments were transferred to fresh PDA plates. Each identified isolate was given a unique number. Isolates that produced spores were identified to species level where possible. Pure mycelial isolates had their nuclear ribosomal internal transcribed spacer (ITS) region sequenced, using a standard polymerase chain reaction (PCR) protocol with the fungal-specific primers ITS1F and ITS4 (White et al. 1990; Gardes & Bruns 1993). The ITS region is the most widely sequenced DNA region in the molecular ecology of fungi and is used as a universal fungal barcode sequence. All PCR products were sequenced in both directions using standard protocols established in the EcoGene® laboratory; and DNA sequences were assembled using Geneious v 10.2.6 (http://www.geneious.com; Kearse et al. 2012). Sequences were subjected to a GenBank BLASTn search (https://blast.ncbi.nlm.nih.gov/Blast.cgi; Altschul et al. 1990) to determine the closest sequence-based match to organisms whose sequences have already been deposited in this databank. Species identifications were confirmed using spore or cultural morphology combined with the sequence data where possible.

Taxonomic literature and fungal systematists were consulted to determine which of the identified fungi were likely to be causing the damage, and which of the fungi were associative saprotrophs, and/or endophytic organisms.

Figure 5. Map of the survey sites showing the extent of geographic spread.

5 Results

5.1 Invertebrates

A total of 81 invertebrate species or taxonomic units were recorded during the *Iris pseudacorus* surveys, roughly classified as herbivores, predators, parasitoids or saprophytes/fungivores (Appendix 2).

5.1.1 Herbivores

A total of 38 herbivorous (including omnivorous) invertebrate species and taxonomic units (where identification to species level was not feasible) were recorded from yellow flag iris during this survey. One herbivorous species, the meadow spittlebug (*Philaenus spumarius*), was classed as 'Common'. Five species or taxonomic units were 'Occasional' and 33 were 'Rare' (Appendix 2). No species or taxonomic unit was categorised as 'Abundant'.

Damage caused by herbivorous species was considered minimal and the overall amount of foliage consumed by herbivores was estimated to be less than 1%. There were no signs of gall-formers, leaf miners or scale insects. Only minor notching, sucking, or chewing damage was recorded on leaves or external surfaces of fruits (Fig. 6).

Figure 6. Herbivore feeding damage on yellow flag iris leaf. A and B. Chewing damage on leaves; C. Chewing damage on fruits.

The meadow spittlebug (*Philaenus spumarius*) was the most common species recorded, with 42 individuals collected across five sites. This is an introduced species to New Zealand and is native throughout the Northern hemisphere (Fig. 7). It is a polyphagous species that feeds on a wide variety of plant species and is therefore not host specific to yellow flag iris. Apart from *P. spumarius*, 16 other sap-feeding species, or groups of taxonomically related

sap-feeding species, were collected from yellow flag iris during the survey. No other sap-feeders were classed as 'Common', four were 'Occasional' and 13 were 'Rare' (Appendix 2). Ten of these sap-feeders are polyphagous while six (unidentified Acanthosomatidae, unidentified Aphididae, unidentified Berytidae, *Chaetedus* sp., unidentified Miridae and unidentified Hemipteran) are unknown.

Figure 7. *Philaenus spumarius*. Photo: Charles J. Sharp from Sharp Photography. https://en.wikipedia.org/wiki/Philaenus_spumarius

Nine herbivorous lepidopteran species or taxonomic units were collected from yellow flag iris during the survey, but damage attributed to lepidopterans was minimal. These species are either host-specific to native plants (e.g. *Pasiphila* sp.) or polyphagous (e.g. *Ctenopseustis* sp.) and unlikely to damage yellow flag iris. All nine Lepidopteran species collected during the survey were classed as 'Rare'.

Six species or taxonomic units of herbivorous adult beetles were collected during this survey but no foliage damage that could be attributed to beetles was observed. However, some beetle species may have been feeding on yellow flag iris. Fuller's rose weevil (*Naupactus cervinus*) and the plantain weevil (*Mecinus pascuorum*) are relatively large weevils with wide host ranges. Similarly, the native grass grub beetle, *Costelytra zealandica* and the exotic bronze leaf beetle *Diachus auratus* have a wide host range that could include yellow flag iris. Some of the other beetle species probably would not have fed on yellow flag iris but were transient on the plant or used the plant as shelter. These include the weed biocontrol agent *Cassida rubiginosa* (thistle tortoise beetle) and *Mecinus pascuorum*, the latter being monophagous on *Plantago lanceolata* (Nieminen and Vikberg 2015). The two potential biocontrol agents for yellow flag iris – the iris flea beetle *Aphthona nonstriata* and the iris seed weevil *Mononychus punctumalbum* – were not found during the survey.

Three orthopteran species were found during the survey, but only one specimen of each species was found, and feeding damage to yellow flag iris is likely to be very minor. Two of these species are polyphagous, while the unidentified orthopteran's host preference is unknown.

At each survey site, 10 plants were dug up and their roots cut open lengthwise in search of root feeders, but none were found.

Snails were classed as 'Rare' and found at only one site during the survey and no evidence of mollusc 'slime trails' was recorded. Earwigs (order Dermaptera) are omnivorous and can cause damage to living plant tissue. The European earwig, *Forficula auricularia*, was found but classed as 'Rare'. Slaters (order Isopoda) were classed as 'Occasional' and may have been causing some damage to living tissue but are likely to have been feeding mostly on dead and decaying material.

5.1.2 Parasitosis

Parasitoids and predators were recorded to help identify factors that may inhibit introduced biological control agents. The two candidate biocontrol agents currently being considered for introduction into New Zealand are both Coleopterans so Coleopteran parasitoids are of particular interest. However, no parasitoids of Coleoptera were found during the survey. A total of six parasitic wasps were collected and identified: three are parasitoids of Dipterans; two of Lepidopterans; and one of Hemipterans (Appendix 2). Two of these – *Microgastrinae* sp. and an unidentified Encrytidae – hatched from larval stages. All the other parasitoids recorded during the survey were collected directly from the plants by beating.

5.1.3 Predators

Ten species of predatory invertebrates or taxonomic units were recorded on yellow flag iris during this survey. Four taxonomic groupings – spiders, snout mites (Bdellidae), the damsel bug *Nabis* sp., and the Tasmanian lacewing (*Micromus tasmaniae*) – were classed as 'Occasional'. The remaining six predatory species or taxonomic units were classed as 'rare' and included three rove beetle species (Staphylinidae), one coccinellid, one pseudoscorpion and one mite (Appendix 2).

5.1.4 Saprophytic and fungivorous invertebrates

Several saprophytic and fungivorous invertebrates were recorded during this survey (Appendix 2), and they would most probably have been associated with decaying material and not damaging living plant material.

5.2 Plant pathogens

Very few symptoms caused by pathogens on yellow flag iris were observed in the field. These symptoms consisted of leaf spots, leaf lesions and leaf yellowing on mature leaves

and occasionally rhizome rot (Fig. 8). No symptomatic tissue was obtained from Porritt Park in Canterbury.

From a total of 113 fungal pure cultures recovered from symptomatic tissues, 31 species were identified from 19 genera (Appendix 3). Two isolates were identified to the families Hypoxylaceae and Phaeosphaeriaceae, and one isolate was identified to the sub-class Sordariomycetidae as no close matches were found for these isolates using BLAST. The predominant genera isolated were *Colletotrichum* and *Alternaria*. Species belonging to the genera *Colletotrichum*, *Alternaria*, *Ilyonectria*, *Nigrospora, Stemphylium*, *Stagonospora*, *Cadophora*, *Ramularia*, *Bipolaris*, and *Fusarium* were considered as potential pathogens; the remaining species identified were saprophytes, endophytes or secondary pathogens.

The only fungal culture isolated from yellow flag iris that seemed to be specific to *Iris* species was *Rachicladosporium iridis*. This fungus causes leaf spots on plants from the genus *Iris* (Crous et al. 2020) and was found on yellow spots on yellow flag iris in one location in this survey.

Figure 8. Yellow flag iris pathogen symptoms. A. leaf yellowing, B. lesions on seed capsules, C. leaf lesions, D. yellow leaf spots, E. rhizome rot.

Alternaria species usually target leaves (Abbasi et al. 2018; Chen et al. 2018; Garibaldi et al. 2018; Moccellin et al. 2018; Garibaldi et al. 2020; Matić et al. 2020) as spores are dispersed by wind or water splashes but they can also lead to stem lesions, fruit lesions, damping off and collar rot (Laemmlen 2001). They are more inclined to target tissues that are stressed, senescent or wounded (Laemmlen 2001). However, *Alternaria* species are also considered to be part of fungal endophytic communities associated with different plant species (Zhang et al. 2014; Varanda et al. 2016; Hamzah et al. 2018). The three different *Alternaria* species recovered from yellow flag iris were isolated from leaf material and seed capsules at six different sites. They are likely responsible for these symptoms.

Colletotrichum spp. are responsible for anthracnose foliar blight and fruit and stem rots on over 3000 plant species (Liang et al. 2018). The different species isolated from yellow flag iris are likely to be responsible for spots and lesions on leaves and were isolated from five sites. Three *Colletotrichum* species isolated from leaves were not identified to species level as only the ITS region was sequenced, and more gene loci are needed to be able to determine species names of these isolates.

Stemphylium vesicarium was recovered from leaf spots in this survey. This species causes leaf spots and lesions, seed pod lesions and stem necrosis on herbaceous plants and trees (Plantwise Knowledge Bank 2022).

Species from the genus *Nigrospora* can be pathogens, endophytes and saprophytes of various hosts. *Nigrospora oryzae* and *N. sphaerica* have been reported to cause leaf spots, stem blight, and foliar blight on a wide range of plants (Hao et al. 2020). These two *Nigrospora* species were recovered from lesions and dead patches on leaves from two sites.

Several species from the genus *Stagonospora* are plant pathogens and can cause leaf lesions and root rots (Cunfer 2000; Pusz & Plaskowska 2010; Davis et al. 2017). One isolate was recovered from leaf yellowing.

Cadophora luteo-olivacea has been associated with wood lesions and discoloration on grapevines (Maldonado-González et al. 2020) and more recently as a post-harvest pathogen of pear and apples (Amaral Carneiro et al. 2022). It was found once on leaf spots.

Species from the genus *Ramularia* can cause leaf spots, leaf yellowing and leaf dieback (Bayer 2020; Koike & Bari 2020; Hills 2021). The *Ramularia* isolate ITS sequence showed 98.5% sequence identity to that of *Ramularia pusilla* Unger, which is responsible for leaf spots and premature leaf loss on grass species in New Zealand (Latch 1964). It was isolated once from leaf spots on yellow flag iris.

Species from the genus *Bipolaris* include pathogens associated with leaf spots, leaf blights, root rots and seedling blights on various host plants (Manamgoda et al. 2014; Sun et al. 2020). *Bipolaris cynodontis* has been associated with leaf spot diseases on grasses (Vann 2022). It was isolated from dead patches on yellow flag iris leaves at one site.

Among the fungal species isolated from rhizome rot on yellow flag iris, *Ilyonectria* spp. have been associated with root rot and decay of woody and herbaceous plants (Cabral et

al. 2012). *Ilyonectria* spp. were only recovered twice from rhizome rot on yellow flag iris during this survey.

Fusarium species are known to have a wide host range and include pathogens, saprophytes and endophytes. *Fusarium lateritium* has been reported to cause wilt, tip and branch dieback, and cankers on a wide range of hosts, including trees and shrubs (Vitale et al. 2011). This fungus was considered as a potential bioherbicide against malvaceous weeds (Walker 1981). In New Zealand, it is has been isolated from a wide host range (Anonymous 2022). In this survey, it was isolated at one site on fruits.

6 Conclusions

6.1 Invertebrates

A range of native and introduced invertebrates are associated with yellow flag iris in New Zealand but no specialised yellow flag iris-feeding invertebrates were recorded in the New Zealand survey. Most herbivorous invertebrates recorded in our survey occurred in small numbers and the damage they caused to yellow flag iris was considered minimal.

Damage to yellow flag iris that could be attributed to Coleopterans was minimal. The two candidate biocontrol agents currently being considered for introduction into New Zealand are both Coleopterans. The iris flea beetle (*Aphthona nonstriata*) adults feed on leaves, particularly leaf tips, while the larvae mine the plant stem and bore the external parts of the rhizomes to feed within (Minuti 2021). The iris seed weevil (*Mononychus punctumalbum*) adults mine petals and sepals and lay eggs in fruits where the larvae feed on seeds (Minuti 2021). These potential biocontrol agents are likely to cause complementary damage to yellow flag iris and are unlikely to meet any significant competition from other Coleopterans in New Zealand. Furthermore, we found no parasitoids of Coleopterans during the survey.

None of the herbivorous invertebrates identified associated with yellow flag iris can be recommended for further testing as biological controls for yellow flag iris as they are polyphagous and the damage they cause to yellow flag iris is minimal.

6.2 Pathogens

The main primary plant pathogens associated with yellow flag iris disease symptoms have a wide host range and none had any major impact on this plant. One species isolated from leaf spots on yellow flag iris has only been found on plants from the genus *Iris*. However, this species did not seem to have a significant impact on yellow flag iris and was only found at one location.

No symptoms were observed on young yellow flag iris leaves. Leaf spots, leaf yellowing, leaf lesions, and dead patches were restricted to mature leaves. Very few fungal damages were observed on fruits (with no effects on seed production) and on rhizomes. These

symptoms were mainly superficial without any chronic disease symptoms observed killing whole plants.

None of the main primary pathogens identified associated with yellow flag iris can be recommended for further testing as biological controls for yellow flag iris as they have a very broad host-range, including valued horticultural species and their combined impact on this plant in the field was minimal.

7 Recommendations

In light of our conclusions that no specialised yellow flag iris-feeding invertebrates and no specialised pathogenic fungi have been recorded on yellow flag iris in New Zealand, we recommend that a classical biological control programme for yellow flag iris should proceed.

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Appendix 1 – Yellow flag iris survey (New Zealand 2021–2022) site details

Site number	Site name	Collection date	GPS coordinates (NZTM)
1	Mangawhai	20/05/2021	1742137E 5995527N
2	Kairanga	26/05/2021	1811921E 5530432N
3	Karamu	27/05/2021	1933131E 5607205N
4	Mercer	02/06/2021	1779933E 5872643N
5	Avon river	14/06/2021	1575781E 5183498N
6a	Tuatapere (autumn)	31/05/2021	1189249E 4878315N
6b	Tuatapere (summer)	17/01/2022	1189249E 4878315N
7	Pokeno	16/12/2021	1777582E 5871888N
8	Ashley Clinton	13/01/2022	1879363E 5570246N
9	Waimauku	20/01/2022	1734355E 5932758N
10	Porritt Park	24/01/2022	1574564E 5181936N
11	Waingaro	28/01/2022	1773604E 5833505N

Note: one site, Tuatapere (6a and 6b), was sampled on two occasions – in autumn 2021 and in summer 2022.

Appendix 2 – Invertebrates associated with yellow flag iris, *Iris pseudacorus*, at 11 New Zealand sites (2021–2022)

Taxon	Common name	Feeding mode	Abundance category[1] (number of individuals)	Collection sites[2]
Phylum Mollusca	**Molluscs**			
Class Gastropoda	**Slugs and snails**			
Unidentified Gastropoda	snail	Herbivorous	Rare (1)	6a
Phylum Arthropoda				
Class Crustacea				
Isopoda	**Slaters**			
Unidentified Isopoda		Saprophytic	Occasional (8)	1,2,7
Class Arachnida				
Acarina	**Mites and ticks**			
Bdellidae				
Unidentified Bdellidae	Snout mites	Predatory	Occasional (6)	6b,11
Unidentified Acarina		Predatory	Rare (1)	2
Araneida	Spiders			
Unidentified Araneida		Predatory	Occasional (34)	2,3,4,8
Amphipoda	Landhoppers			
Unidentified Talitridae		Saprophytic	Rare (1)	2
Pseudoscorpiones	Pseudoscorpions			
Unidentified Pseudoscorpiones		Predatory	Rare (1)	2
Class Collembola				
Unidentified Collembola	Springtails	Saprophytic	Rare (3)	2
Class Insecta	**Insects**			
Coleoptera	**Beetles**			
Anthicidae	Ant beetles			
Pseudocyclodinus glaber (King)		Saprophytic	Rare (3)	1
Saptinus deitzi (Werner and Chandler)		Saprophytic	Rare (4)	4,7
Anthribidae	Fungus weevils			
Euciodes suturalis Pascoe		Fungivorous	Rare (1)	11
Sharpius brouni (Sharp)		Fungivorous	Rare (1)	7
Chrysomelidae				
Cassida rubiginosa (Müller)	Thistle tortoise beetle	Herbivorous (adults pollen)	Rare (1)	10
Diachus auratus Fabricius	Bronze leaf beetle	Herbivorous	Rare (2)	3
Coccinellidae	Ladybirds			
Halmus chalybeus (Boisduval)	Steel blue ladybird	Predatory	Rare (1)	9
Corylophidae	Hooded beetles			
Sericoderus apicalis Lea		Fungivorous	Rare (4)	1
Sericoderus sp.		Fungivorous	Rare (1)	2

Taxon	Common name	Feeding mode	Abundance category[1] (number of individuals)	Collection sites[2]
Cryptophagidae	Cryptic beetles			
Atomaria lewisi Reitter	Silken fungus beetle	Fungivorous	Rare (1)	6b
Paratomaria crowsoni Leschen		Pollen/ fungus feeder	Rare (2)	7
Curculionidae	Weevils			
Chalepistes compressus (Broun)	Compressed weevil	Herbivorous	Rare (3)	7
Mecinus pascuorum (Gyllenhal)	Plantain weevil	Herbivorous	Occasional (7)	11
Naupactus cervinus Boheman	Fuller's rose weevil	Herbivorous	Rare (3)	1
Latridiidae	Mildew beetles			
Bicava sp.1		Fungivorous	Rare (1)	11
Bicava sp.2		Fungivorous	Rare (1)	11
Cartodere bifasciata (Reitter)		Fungivorous	Occasional (28)	1,2,3,6a
Cartodere sp.		Fungivorous	Rare (1)	2
Corticaria sp.		Fungivorous	Rare (3)	11
Cortinicara meridianus Johnson		Fungivorous	Occasional (8)	7,11
Cortinicara sp.		Fungivorous	Rare (2)	11
Unidentified Latridiidae		Fungivorous	Rare (1)	6b
Mycetophagidae	Fungus beetles			
Litargus vestitus (Sharp)		Fungivorous	Occasional (8)	1,4
Scarabaeidae	Scarab beetles			
Costelytra zealandica (White)		Herbivorous	Rare (4)	7,9
Scirtidae	Marsh beetles			
Contacyphon variegatus Sharp		Saprophytic	Rare (1)	1
Silvanidae	Silvan flat bark beetles			
Cryptamorpha desjardinsi Guerin		Fungivorous	Rare (3)	4
Staphylinidae	Rove beetles			
Astenus guttulus (Fauvel)		Predatory	Rare (1)	2
Carpelimus corticinus (Gravenhorst)		Predatory	Rare (1)	1
Carpelimus sp.		Predatory	Rare (1)	11
Dermaptera	Earwigs			
Forficula auricularia Linnaeus	European earwig	Omnivorous	Rare (1)	11
Diptera	**Flies**			
Culicidae	Mosquitos			
Culex quinquefasciatus Say		Ectoparasite/ Nectar	Rare (4)	1,2
Limoniidae	Crane flies			
Dicranomyia sp.		Saprophytic	Rare (1)	1
Psychodidae	Drain flies			
Unidentified Psychodidae			Rare (2)	2
Unidentified Diptera			Occasional (40)	1,6b,11

Taxon	Common name	Feeding mode	Abundance category[1] (number of individuals)	Collection sites[2]
Hemiptera	**Bugs**			
Acanthosomatidae	Shield bugs			
Oncacontias vittatus Fabricius	Forest shield bug	Sap feeder	Rare (4)	6b
Rhopalimorpha lineolaris Pendergrast	Linear sedge shield bug	Sap feeder	Occasional (5)	6b
Unidentified Acanthosomatidae		Sap feeder	Rare (1)	3
Aphididae	Aphids			
Aulacorthum solani (Kaltenbach)		Sap feeder	Rare (1)	6b
Idiopterus nephrelepidis Davis		Sap feeder	Rare (2)	3,9
Unidentified Aphididae		Sap feeder	Occasional (14)	1,3,6b,9
Aphrophoridae	Spittle bugs			
Philaenus spumarius (Linnaeus)	Meadow spittlebug	Sap feeder	Common (42)	1,6b,7,8, 11
Berytidae	Stilt bugs			
Unidentified Berytidae		Sap feeder	Rare (4)	3
Flatidae	Planthoppers			
Siphanta acuta (Walker)		Sap feeder	Rare (2)	1
Miridae	Mirid bugs			
Chaetedus sp.		Sap feeder	Occasional (8)	11
Stenotus binotatus (Fabricius)		Sap feeder	Occasional (19)	6b,7,8,11
Unidentified Miridae		Sap feeder	Rare (3)	6b,11
Nabidae	Damsel bugs			
Nabis sp.		Predatory	Occasional (6)	6b
Pentatomidae	Shield bugs			
Dictyotus caenosus (Westwood)	Brown shield bug	Sap feeder	Rare (1)	7
Nezara viridula (Linnaeus)	Green vegetable bug	Sap feeder	Rare (1)	10
Ricaniidae	Planthoppers			
Scolypopa australis	Passion vine hopper	Sap feeder	Rare (3)	2,9
Rhyparochromidae				
Remaudiereana sp.		Sap feeder	Rare (1)	2
Unidentified Hemipteran		Sap feeder	Rare (2)	2
Hymenoptera	**Bees, wasps, ants**			
Bethylidae	Aculeate wasps			
Goniozus jacintae Farrugia		Parasitoid (of lepidoptera)	Occasional (7)	10
Braconidae	Parasitic wasps			
Aspilota parecur Berry		Parasitoid (of diptera)	Rare (1)	6b
Chorebus sp.		Parasitoid (of diptera)	Rare (1)	2
Microgastrinae sp.		Parasitoid (of lepidoptera)	Occasional (17)	1

Taxon	Common name	Feeding mode	Abundance category[1] (number of individuals)	Collection sites[2]
Diapriidae				
Stylaclista sp.		Parasitoid (of diptera)	Rare (1)	2
Encrytidae				
Unidentified Encrytidae		Parasitoid (of hemiptera)	Occasional (100+)	9
Formicidae	Ants			
Tetramorium sp.		Omnivorous	Rare (1)	9
Unidentified Formicidae		Likely omnivorous	Rare (1)	1
Lepidoptera	**Moths and butterflies**			
Elachistidae	Grass-miner moths			
Elachista sp.		Herbivorous (on grasses)	Rare (2)	8,11
Geometridae	Looper moths			
Orthoclydon praefectata Walker	White moth	Herbivorous (on *Phormium tenax*)	Rare (1)	8
Pasiphila sp.		Herbivorous	Rare (1)	11
Oecophoridae	Concealer moths			
Tingena sp.		Saprophytic	Rare (2)	11
Psychidae	Bagworm moths			
Lepidoscia sp.		Herbivorous	Rare (1)	10
Tineidae	Fungi moths			
Opogona omoscopa (Meyrick)		Saprophytic	Rare (1)	11
Tortricidae	Leafroller moths			
Cnephasia jactatana Walker	Lyre moth	Herbivorous	Rare (1)	5
Ctenopseustis sp.		Herbivorous	Rare (1)	6b
Unidentified Tortricidae (larva collected)		Herbivorous	Rare (3)	6b,9
Xyloryctidae	Timber moths			
Gymnobartha sp.		Herbivorous	Rare (1)	6b
Unidentified lepidopteran caterpillar		Herbivorous	Rare (1)	2
Neuroptera	**Lacewings**			
Hemerobiidae	Brown lacewings			
Micromus tasmaniae (Walker)	Tasmanian lacewing	Predatory	Occasional (11)	3,6b,9
Orthoptera	**Grasshoppers, crickets, weta**			
Tettigoniidae	Long-horned grasshoppers			
Caedicia simplex (Walker)		Herbivorous	Rare (1)	7
Conocephalus bilineatus	Field grasshopper	Herbivorous	Rare (1)	7
Unidentified orthopteran		Herbivorous	Rare (1)	11

Taxon	Common name	Feeding mode	Abundance category[1] (number of individuals)	Collection sites[2]
Trichoptera	**Caddisflies**			
Hydroptilidae	Micro-caddisflies			
Unidentified Hydroptilidae		Unknown	Occasional (20)	11

Appendix 3 – Fungal species isolated from symptomatic yellow flag iris tissues at 11 New Zealand sites (2021–2022)

Species recovered	Symptoms associated	Plant material isolated from	Collection sites	Comments
Colletotrichum spaethianum (Allesch.) Damm, P.F. Cannon & Crous	spots and black lesions	leaves	1, 2, 3	pathogen
	rot	rhizome	7	
Colletotrichum boninense Moriwaki, Toy. Sato & Tsukib.	yellowing	leaves	7	pathogen
Colletotrichum Corda sp. 1	black lesions	leaves	1	pathogen
Colletotrichum Corda sp. 2	yellow spots	leaves	6a	pathogen
Colletotrichum Corda sp. 3	yellow spots	leaves	6a	pathogen
Ilyonectria P. Chaverri & C. Salgado sp.	rot	rhizome	2	pathogen
Ilyonectria europaea	rot	rhizome	7	pathogen
Thelonectria P. Chaverri & C. Salgado	rot	rhizome	2	saprophyte/ weak pathogen
Penicillium rubens Biourge	spots	leaves	2	saprophyte/ endophyte
Penicillium atrosanguineum B.X. Dong	rot	rhizome	7	saprophyte/ endophyte
Alternaria rosae E.G. Simmons & C.F. Hill	spots	leaves	3, 5	pathogen
Alternaria alternata (Fr.) Keissl.	spots	leaves	3, 4, 5 ,6	pathogen
	lesions	capsule	11	
Alternaria infectoria E.G. Simmons	spots	leaves	8, 11	pathogen
Cladosporium allicinum (Fr.) Bensch, U. Braun & Crous	spots	leaves	3, 5, 6b, 7, 8	saprophyte/ endophyte
Cladosporium cladosporioides (Fresen.) G.A. de Vries	spots, dead patches	leaves	11	saprophyte/ endophyte
Epicoccum layuense Q. Chen, Crous & L. Cai	spots	leaves	3, 5	endophyte
Epicoccum nigrum Link	spots	leaves	6a, 6b, 8	endophyte
Epicoccum italicum Q. Chen, Crous & L. Cai	spots	leaves	6a	endophyte
Stemphylium vesicarium (Wallr.) E.G. Simmons	spots	leaves	4	pathogen
Botrytis cinerea Pers.	sclerotia	leaves	6a	wound pathogen (grey mould)
Itersonilia Derx	orange lesions	leaves	6a	saprophyte/ weak pathogen
Nigrospora sphaerica (Sacc.) E.W. Mason	black lesions	leaves	6a	pathogen

Species recovered	Symptoms associated	Plant material isolated from	Collection sites	Comments
Nigrospora oryzae (Berk. & Broome) Petch	black lesions	leaves	6a	pathogen
Nigrospora Zimm.	dead patches	leaves	11	pathogen
Didymella Sacc.	black lesions	leaves	6a	wound pathogen
Stagonospora (Sacc.) Sacc.	yellow spots	leaves	6a	pathogen
Cadophora luteo-olivacea (J.F.H. Beyma) T.C. Harr. & McNew	spots	leaves	2	pathogen
Rachicladosporium iridis (Auersw.) Crous	yellow spots	leaves	7	leaf spots on *Iris* species
Sporormiella australis (Speg.) S.I. Ahmed & Cain (=*Preussia australis* (Speg.) Arx)	yellowing	leaves	7, 9	saprophyte
Bipolaris Shoemaker	dead patches	leaves	11	pathogen
Bipolaris cynodontis (Marignoni) Shoemaker (= *Dreschlera cynodontis* (Marignoni) Subram. & B.L. Jain)	dead patches	leaves	11	pathogen
Fusarium lateritium Nees	lesion	capsule	11	pathogen
Sordariomycetidae O.E. Erikss. & Winka	brown spots	leaves	9	saprophyte/ pathogen
Phaeosphaeriaceae M.E. Barr	pycnidia	leaves	6a, 7	saprophyte/ pathogen
Hypoxylaceae DC.	brown spots	leaves	6b	saprophyte/ endophyte
Ramularia Unger	black spots	leaves	11	pathogen