

Feasibility for biological control of water celery (*Helosciadium nodiflorum*)

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Feasibility for biological control of water celery (*Helosciadium nodiflorum*)

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Summary

Project and client

• The prospects of developing a biocontrol programme against water celery (*Helosciadium nodiflorum*) in New Zealand were assessed by Manaaki Whenua – Landcare Research for Nelson City Council.

Objectives

- To review the literature to identify potential biocontrol agents for *H. nodiflorum* and assess the feasibility of their release in NZ.
- To assess the prospects of achieving successful biological control of *H. nodiflorum*.
- To estimate the cost of the programme for the biological control of *H. nodiflorum*.

Results

- *H. nodiflorum* is a sprawling, emergent, perennial aquatic herb in the carrot family, Apiaceae. The plant is native to Europe, northern Africa and western Asia. It is a weed in Chile and New Zealand, and is naturalised in parts of North and South America. *H. nodiflorum* is common in many river systems in its native range.
- The distribution of *H. nodiflorum* in New Zealand is scattered from Northland to Wellington. It is abundant in many areas, especially near the coast. It has also established in the north and west of the South Island.
- *H. nodiflorum* contributes to the degradation of water quality of streams in New Zealand. The plant also risks displacing endangered indigenous species in wetlands.
- In its native range *H. nodiflorum* is traditionally used as a medicinal herb and a culinary vegetable. It is also used to monitor and remediate pollution by heavy metals and inorganic dissolved nutrients. Any such uses in New Zealand are yet to be established.
- The New Zealand indigenous species closest to *H. nodiflorum* sit in the same tribe, Oenantheae, in the genus *Lilaeopsis*. *Lilaeopsis* is phylogenetically distant from *Helosciadium*. Other indigenous and/or economically important species in the family Apiaceae are further distant from *Helosciadium*. The genus *Apium*, in which celery and celeriac sit and in which *H. nodiflorum* was previously classified, sits in a different tribe, Apieae.
- Opposition to the biocontrol of *H. nodiflorum* may come from growers of economically important crops in the carrot family. The potential for opposition from medicinal herbalists and/or on cultural grounds for wild food harvest needs to be explored.
- *H. nodiflorum* is difficult to control chemically and mechanically. It regrows readily from detached shoots.
- Biological control has never been attempted against *H. nodiflorum.*
- Only nine pathogens were reported from *H. nodiflorum* in the literature, and only one, the smut fungus *Entyloma helosciadii*, appears to be sufficiently host specific. Screening this pathogen to assess the levels of damage is worth pursuing.

• Only 11 species of arthropods were reported from *H. nodiflorum* in the literature, and only one, the stem mining moth, *Depressaria ultimella*, appears to be sufficiently host specific and damaging. This moth is listed on the Ministry for Primary Industries' unwanted organisms register and would have to be removed from this list. In addition, *D. ultimella* may not provide sufficient control of infestations in fast-flowing water.

Conclusions

The range of *H. nodiflorum* may be expanding in New Zealand, and conventional management methods do not provide satisfactory long-term control. Aquatic weeds have been successful targets of biocontrol. *H. nodiflorum* is likely to be an intermediate target for biocontrol.

Despite this species being well studied in its native range, not many natural enemies have been reported in the literature and the plant is subject to management in parts of its native range. No other country has attempted biocontrol of *H. nodiflorum*, which will make this plant a high-cost target.

Two organisms have been identified from the literature as potential candidate biocontrol agents. It is possible that further potential agents may be discovered in native range surveys. Opposition to biocontrol of *H. nodiflorum* in New Zealand is likely to be minimal.

Recommendations

- Apply to remove the moth *Depressaria ultimella* from the list of unwanted organisms. Estimated cost: \$15,000-\$20,000.
- Undertake DNA studies to identify the geographical source of *H. nodiflorum* in New Zealand and whether the New Zealand material consists of pure *H. nodiflorum* or hybrids. Estimated cost: \$20,000-\$100,000.
- Undertake a survey of the natural enemies of *H. nodiflorum* in New Zealand and look for any potential biocontrol agents, and any other species living on the plant (such as predators) that might interfere with potential biocontrol agents. Note that this baseline information is vital for any subsequent application to release new biocontrol agents. Estimated cost: \$50,000-\$70,000.
- Analyse the costs of and benefits expected from biological control in comparison to conventional control of *H. nodiflorum*. This information will strengthen any future application to release new biocontrol agents. Estimated cost: \$15,000-\$25,000.
- Survey *H. nodiflorum* in its native range to identify more candidate biocontrol agents. Estimated cost: \$50,000-\$300,000.
- Identify and study the life cycle of prospective agents found during surveys. If required, undertake host-range testing of potential agents. This stage should include preliminary screening to test if the smut fungus *Entyloma helosciadii* isolates from *H. nodiflorum* are damaging to *H. nodiflorum* and whether this pathogen can infect indigenous *Lilaeopsis* species. Estimated cost: \$60,000-\$200,000 per agent.
- If testing shows agents are suitable, apply to release them in New Zealand. Estimated cost: \$55,000-\$75,000 for one or more species.

- Import into containment and gain permission to remove. Estimated cost: \$25,000-\$60,000 per species.
- Mass-rear and release agents. Estimated cost: \$100,000-\$250,000 per species.

Note: Estimated costs are exclusive of GST and are based on 2019/20 figures. New estimates will need to be provided if work is to be undertaken beyond those dates, and/or if complicating factors arise (e.g. disease infecting the imported agents).

1 Background

The prospects of developing a biocontrol programme against water celery, *Helosciadium nodiflorum* (L.) W.D.J. Koch (synonym *Apium nodiflorum* (L.) Lag.), in New Zealand were assessed by Manaaki Whenua – Landcare Research for Nelson City Council.

1.1 Global distribution and biology of *Helosciadium nodiflorum*

H. nodiflorum is native to Europe, northern Africa and western Asia (Ronse et al. 2010) (Figure 1). It is a weed in Chile (Global Register of Introduced and Invasive Species as Apium, via GBIF Secretariat 2019) and New Zealand. It is naturalised in parts of North, Central (Mexico) and South America (Ronse et al. 2010; Les 2017) and in southwestern Australia (GBIF Secretariat 2019). The plant is common in many river systems in its native range (e.g. Vlyssides & Bouranis 1998; Preece & Hick 2001; Comte et al. 2005; Ronse et al. 2010). It is considered a weed in Portugal and Spain, where it is native (Les 2017).



Figure 1. Global distribution of (top) *Helosciadium nodiflorum* (from GBIF: https://www.gbif.org/species/3631293) and (bottom) as *Apium nodiflorum* (from GBIF: https://www.gbif.org/species/7809784).

H. nodiflorum is a perennial herb in the carrot family, Apiaceae. It is a sprawling, emergent, perennial aquatic plant that can grow over 1 m in height, with glossy, bright-green leaves arranged in pairs up each stem, and white flowers close to leaf bases (Roy et al. 2004; New Zealand Plant Conservation Network 2020) (Figure 2). The stems are hollow, finely furrowed, may be up to 2 m long, and root at their lower nodes. The leaves are up to 70 cm long, with two to eight pairs of toothed, stalkless, oval to lance-shaped leaflets. The individual flowers are small (1 to 2 mm) with five white petals. Flowers occur in short-stalked clusters/umbels, 2 to 4 cm in diameter. The fruit are dark brown, small (2 mm long), ribbed, and ovoid/egg-shaped (Roy et al. 2004). Shoots die back in winter and regrow in spring (Newman 2004).

H. nodiflorum is insect-pollinated (Weber 1992). Seeds germinate from spring to autumn on damp substrates, or in shallow, slow-flowing water. Seedlings are usually outcompeted by mature plants in existing stands, but they can contribute to spread from the edges of existing stands. The plant regrows well from detached shoots, which readily form roots (Thommen & Westlake 1981; Newman 2004). *H. nodiflorum* grows in shallow-water ponds and lakes, drains, canals, ditches, the margins of slow-moving streams, the edges of rivers, in marshy areas, and around springs (Newman 2004). It can form submerged patches in slow-flowing deep water (Newman 2004). *H. nodiflorum* is a characteristic of nutrient-rich areas where the growth of other, taller species is restricted by erosion or disturbance of the margin (from flooding management). It is also abundant in fast-flowing chalk streams (Thommen & Westlake 1981; Newman 2004).

H. nodiflorum is a characteristic dominant species of specific wetland plant associations, and is an important member in other wetland plant associations in its native range of Italy (Landucci et al. 2013). In addition, *H. nodiflorum* provides a substrate for diverse communities of epiphytic algae and bacteria, which form important food sources for many aquatic invertebrates (Baker & Orr 1986; Comte et al. 2005). *H. nodiflorum* has been identified as an important host of the free-swimming larval stages (cercariae) of the liver fluke *Fasciola hepatica* L., and as such forms a part of the disease cycle (Hammami et al. 2007; Rondelaud et al. 2020).

H. nodiflorum is easily confused with watercress, *Nasturtium officinale* W.T. Aiton, hence its common name, fool's watercress. The two species grow in similar habitats and often grow together (Thommen & Westlake 1981). *H. nodiflorum* is well known for its extreme phenotypic variability (Ronse et al. 2010). It also hybridises easily with other *Helosciadium* species (e.g. Rita et al. 2018), and hybrids are often confused for *H. nodiflorum* (Riddelsdell 1914; Crackles 1976; Walters 1980). *H. nodiflorum* was also shown to hybridise with *Berula erecta* (Huds.) Coville, outside the genus *Helosciadium* (Desjardins et al. 2015). However, *H. nodiflorum* (as *Apium nodiflorum*) was noted as very difficult to hybridise with other *Apium* species/subspecies (in the context of breeding pest resistance into celery, Diawara et al. 1992). Recent experience in New Zealand has demonstrated that finding natural enemies for hybridised weeds can pose significant challenges.



Figure 2. Top: *Helosciadium nodiflorum* in flower (photo by Drepanostoma). Middle (L): leaf morphology (photo by Stephen Thorpe). (R): *H. nodiflorum* growing along a channel in the Whanganui region (photo by Phil Bendle). Bottom: a large infestation of *H. nodiflorum* in Nelson region (photo by Chris Ecroyd). All photos under CC-BY-NC licence.

1.2 Distribution and pest status in New Zealand

H. nodiflorum was first recorded as naturalised in New Zealand in 1947 (Webb et al. 1988). In the North Island infestations are found scattered from Northland to Wellington, and the plant is locally abundant in many regions, especially near coastal areas (Roy et al. 2004) (Figure 3). *H. nodiflorum* has also established in the north and west of the South Island (Champion 2018; New Zealand Plant Conservation Network 2020). Its distribution in the South Island is confined to a small number of streams, while it is absent from nearby similar streams, suggesting that dispersal is effectively human assisted, with no evidence of natural dispersal (Champion 2018). *H. nodiflorum* was possibly introduced as a seed contaminant or a contaminant of ornamental pond plants (New Zealand Plant Conservation Network 2020). Because it can be mistaken for watercress, it can be collected and distributed as such (New Zealand Plant Conservation Network 2020).

H. nodiflorum contributes to degradation of water quality of streams in New Zealand, especially in combination with farm drainage coming into these streams. *H. nodiflorum* traps nutrients and depletes dissolved oxygen when it decomposes (Wilcock et al. 1995). The plant also risks displacing endangered indigenous species in wetlands (Champion et al. 2011; Champion 2018). In the North Island the plant is managed frequently to clear drainage networks (Champion 2018). If uncleared from slow-flowing streams, build-up of the plant reduces water flow, leading to floods (R. Frizzell, Environmental Programmes Adviser, pers. comm.).

H. nodiflorum is not currently regulated by any regional council (Champion et al. 2019), yet the plant is of concern and is controlled by regional councils and by the Department of Conservation in parts of the North Island (Champion et al. 2008; Champion et al. 2011; Champion et al. 2019). In a recent weed risk assessment of aquatic species, *H. nodiflorum* scored 47 points. Those species scoring above 50 are considered at high risk of becoming invasive; species scoring between 40 and 50 are considered a moderate risk (Champion et al. 2019). The capability of the model used for this risk assessment (Aquatic Weed Risk Assessment Model) to differentiate with high accuracy between invaders and non-invaders has been validated (Gordon et al. 2012).

It has been suggested that eradication from the South Island may be feasible via chemical control (Champion 2018).



Figure 3. New Zealand distribution of *Helosciadium nodiflorum* (from GBIF: https://www.gbif.org/species/3631293).

1.3 Beneficial uses

1.3.1 Pharmacological beneficial uses

H. nodiflorum is widely used in Moroccan folk medicine against abscesses, stomatitis (inflammation of the mouth and lips), gumboil (swelling on the gum) and mouth ulcer (Larhsini et al. 1996). In Italy the plant has been used in traditional medicine as a diuretic and decongestant of kidneys and the urinary tract, and it is believed to be used as a drug for hypersensitivity and as a remedy for arthritis (summarised in Afshar et al. 2017). It has also been used traditionally to treat digestive disorders, dysfunctions of the gastrointestinal tract and respiratory tract, cough, inflammation, and as a purifying/detoxifying herb (summarised in Maxia et al. 2012).

H. nodiflorum has been used as a stimulant and to treat diseases of the spleen ((Dafni 1985), cited in (Lev & Amar 2008)). In addition, extract of the plant has been shown to ameliorate osteoporotic symptoms in rats (Tsakova et al. 2015). It is used as a laxative in veterinary folk medicine in Italy (Viegi et al. 2003). *H. nodiflorum* is valued for its high phenolic and flavonoid content (Popova et al. 2014), which is probably responsible for its high antioxidant activity (Morales et al. 2012).

Essential oils from *H. nodiflorum* have shown antifungal activity against dermatophytes (Maxia et al. 2012), and inhibitory effects on *Helicobacter pylori* (previously known as *Campylobacter*) (Menghini et al. 2010). Extracts from *H. nodiflorum* have shown variable degrees of anti-fungal activity on a selection of species and strains of *Candida, Aspergillus* and *Scopurialopsis* (Larhsini et al. 1996), leading to exploration of the use of essential oils from this plant in the treatment of dermatophytosis and candidosis (Maxia et al. 2012). Essential oils from *H. nodiflorum* have also shown early potential for treating the

protozoan *Trypanosoma brucei*, the cause of human sleeping sickness (Kamte et al. 2018). The plant has shown a degree of antibiotic activity against *Staphylococcus aureus* (Mesmar & Abussaud 1991).

1.3.2 Non-pharmacological beneficial uses

Food

H. nodiflorum has traditionally been harvested as a fresh vegetable in the Mediterranean (e.g. de Cortes Sánchez-Mata & Tardío 2016), a use that has almost disappeared due to social changes, changes in taste, and due to pollution and the destruction of habitat (Benelli et al. 2017). Domestication and development as a modern vegetable is now being considered in Spain (Guijarro-Real et al. 2019a; Guijarro-Real et al. 2019b). It is considered a vegetable high in antioxidants (Guijarro-Real et al. 2019a).

Pest control

Extracts from *H. nodiflorum* were less effective than extracts from lettuce and watercress at attracting the snail *Biomphalaria glabrata* for snail control. The snail is the host of the blood fluke *Schistosoma mansoni* (Bousfield et al. 1980).

Components from the essential oils of *H. nodiflorum* have shown good potential as insecticides when tested on caterpillars of the cabbage looper moth (*Trichoplusia ni*) (Afshar et al. 2017) and against larvae of the disease-vectoring mosquito *Culex quinquefasciatus* (Benelli et al. 2017).

Environmental monitoring and remediation

H. nodiflorum can accumulate heavy metals, especially in its roots (Zurayk et al. 2001). It has been assessed as a bioindicator for monitoring heavy metal pollution (Zurayk et al. 2001; Pratas et al. 2010; Rodrigues et al. 2010; Bonanno et al. 2017; Baldantoni et al. 2018) and for removing heavy metals from polluted water bodies (Vlyssides & Bouranis 1998; Pratas et al. 2010; Rodrigues et al. 2010; Kara 2014) as well as for treating wastewater (Vlyssides et al. 2005). While other plants in the same community performed better as bioindicators and accumulators of heavy metals (Rodrigues et al. 2010; Kara 2014; Bonanno et al. 2017), *H. nodiflorum* did accumulate pollutants in quantities large enough to warrant caution harvesting this wild vegetable for food from areas that have been exposed to heavy metals (Zurayk et al. 2001).

H. nodiflorum was also assessed as a bioindicator for dissolved inorganic nutrients; for example, from effluent (Peipoch et al. 2014; Baldantoni et al. 2018), from agricultural leaching, and from the discharge of urban waste (Romero & Onaindia 1995). It was found to take up nitrate, which could contribute to the restoration of waterways contaminated by effluent (Peipoch et al. 2014).

1.4 Phylogeny and taxonomy

The Apiaceae is a large family with a global distribution, approximately 430 genera and over 3,780 species. *Helosciadium nodiflorum* (synonym *Apium nodiflorum*) is placed in the subfamily Apioideae tribe Oenantheae (Table1; Figures 4, 5, 6). The genus *Helosciadium* is restricted to Europe and comprises five species (Hardway et al. 2004; Ronse et al. 2010). Twelve genera in the Apiaceae have representatives in New Zealand's indigenous flora (Table 1), with *Lilaeopsis* being in the same subfamily and tribe as *H. nodiflorum* (Figures 5, 6). The other 11 genera are in other subfamilies or tribes (Table1; Figures 4, 5, 6), and none of these genera are closely related to *H. nodiflorum*: the subfamilies and tribes are significantly differentiated, and the genera/species in the different subfamilies and tribes are not closely related. *Helosciadium* is most closely related to the genus *Sium*, and these two genera are part of a larger group of genera whose relationships are not fully resolved (Hardway et al. 2004; Spalik et al. 2009; Ronse et al. 2010) (Figure 6).

Of the New Zealand indigenous Apiaceae genera, *Lilaeopsis* is in the tribe Oenantheae and is therefore the most closely related to *H. nodiflorum* (Figure 6). *Lilaeopsis* includes 14 species and occurs in the New World and Australasia; in New Zealand it comprises two indigenous (non-endemic) species, *L. ruthiana and L. novae-zelandiae* (Affolter 1985). The genus also includes two exotic species in New Zealand, as documented in Ngā Tipu o Aotearoa – New Zealand Plants¹: *L. brasiliensis* (Glaz.) Affolter (biostatus: sometimes present, casual) and *L. mauritiana* G. Petersen & Affolter (biostatus: present in captivity/cultivation/culture).

Lilaeopsis and *Helosciadium* are not especially closely related within the tribe Oenantheae (Hardway et al. 2004; Spalik et al. 2009) (Figure 6), and the phylogenetic distance between them increases the prospect that potential biocontrol agents for *H. nodiflorum* will be unlikely to pose a threat to the indigenous *Lilaeopsis* species. Note that in the phylogeny of Hardway et al. (2004), *Lilaeopsis* is represented by the North American species *L. occidentalis* and placed in a North American (NA) clade with other genera (Figure 6). The species *L. occidentalis* and the New Zealand species *L. novae-zelandiae* and *L. ruthiana* are not shown on the tree in Figure 6, but are also all members of *Lilaeopsis* (Bone et al. 2011).

1.4.1 Nomenclature

The taxonomy and nomenclature of *H. nodiflorum* has been unsettled for a long time and there have been significant taxonomic issues associated with this species. The recent phylogenetic and taxonomic studies of the Apiaceae have established that *Apium nodiflorum* should be placed in Apiaceae subfamily Apioideae tribe Oenantheae and assigned to the genus *Helosciadium* W.D.J.Koch (Hardway et al. 2004; Spalik et al. 2009; Ronse et al. 2010). Therefore, *Helosciadium nodiflorum* (L.) W.D.J.Koch is the accepted name and *Apium nodiflorum* (L.) Lag. should now be treated as a synonym. The New

¹ (https://nzflora.landcareresearch.co.nz/)

Zealand species of *Apium* and the cultivated culinary celery (*Apium graveolens*) are retained in *Apium* (tribe Apieae).

1.4.2 Relationship of the genus *Helosciadium* with the genus *Apium*

The New Zealand indigenous taxa of *Apium*, the genus in which *Helosciadium nodiflorum* was previously placed, belong to the tribe Apieae (Table 1; Figure 5) and are *Apium prostratum* subsp. *denticulatum* P.S.Short (biostatus: endemic) and *Apium prostratum* subsp. *prostratum* var. *filiforme* (A.Rich.) Kirk (biostatus: indigenous, non-endemic). *Apium prostratum* Labill. ex Vent. subsp. *prostratum* var. *prostratum* has been recorded as indigenous (Short 1979) but is now regarded as naturalised (Heenan et al. 2002). *Apium graveolens*, culinary celery, is widely cultivated in New Zealand.

The New Zealand indigenous *Apium* taxa and culinary celery are unrelated to *Helosciadium* and are most closely related to the monospecific genus *Naufraga*, an endemic of the Balearic Islands (Downie et al. 2000; Spalik et al. 2010). Based on the phylogenetic distance between *Helosciadium* and *Apium* (see Figure 5), it is likely that potential candidate agents for *H. nodiflorum* can be found that will not pose a threat to the indigenous New Zealand *Apium* taxa or to celery (*A. graveolens*).

Subfamily	Tribe	Subtribe	NZ genera and Helosciadium
Apioideae Seemann	Oenantheae Dumort.	-	<i>Helosciadium</i> W.D.J.Koch, <i>Lilaeopsis</i> Greene
	Aciphylleae M.F.Watson & S.R.Downie	-	<i>Aciphylla</i> J.R.Forst. & G.Forst., <i>Anisotome</i> Hook.f., <i>Gingidia</i> J.W.Dawson, <i>Lignocarpa</i> J.W. Dawson, <i>Scandia</i> J.W.Dawson
	Apieae Takht. ex V.M.Vinogr.	-	Apium L.
	Scandiceae Spreng.	Daucinae Dumort	Daucus L.
		Scandicinae Tausch	<i>Chaerophyllum</i> L.
Azorelloideae Plunkett & Lowry			<i>Azorella</i> Lam.
Mackinlavoideae Plunkett			Actinotus Labill., Centella L.

Table 1. The placement of *Helosciadium* and New Zealand indigenous representatives of the family Apiaceae. This placement follows the Angiosperm Phylogeny website, where the family is divided into subfamilies (<u>http://www.mobot.org/MOBOT/Research/APweb/</u>), and the tribes follow Downie et al. (2010).

Eryngium L.

Saniculeae Burnett

& Lowry

Saniculoideae Burnett



Figure 4. Phylogenetic relationships of the Apiaceae subfamilies Mackinlayoideae, Azorelloideae, Saniculoideae, and Apioideae. *Helosciadium* is in the subfamily Apioideae. Other subfamilies that include New Zealand indigenous species and commercially important species are highlighted. Reproduced from Angiosperm Phylogeny website: http://www.mobot.org/MOBOT/Research/APweb/ (accessed February 2020).



Figure 5. Phylogenetic relationships of Apiaceae subfamilies Saniculoideae (tribe Saniculeae) and Apioideae (tribes Aciphylleae, Apieae, Oenantheae, Scandiceae). *Helosciadium* sits in the tribe Oenantheae, as does the genus *Lilaeopsis* with two New Zealand indigenous species. Other tribes with New Zealand indigenous species and species of economic importance are highlighted. (Reproduced from Downie et al. 2010)).



Figure 6. Phylogenetic relationships of the tribe Oenantheae showing the relationships between *Helosciadium nodiflorum* and the genus *Lilaeopsis*, where the closest New Zealand indigenous species sit. (Reproduced from Hardway et al. 2004).

1.5 Potential opposition to biocontrol

Opposition to biocontrol of *H. nodiflorum* could arise from growers of closely related crops in the carrot family Apiaceae: celery, celeriac, parsnip, carrot, and herbs like parsley and coriander. Such growers would have to be assured that any natural enemies would be host specific to *H. nodiflorum*. However, the revised taxonomic position of *H. nodiflorum* in the genus *Helosciadium*, and not in the genus *Apium* (in which celery and celeriac sit), increases the chances that sufficiently host-specific agents can be found.

H. nodiflorum is used in traditional herbal medicine in its native range, and so the herbal medicine community in New Zealand should be approached to explore whether *H. nodiflorum* is also valued here for medicinal use.

Preliminary information (Champion 2018) suggests that *H. nodiflorum* is not widely harvested as wild food in New Zealand, but further work is needed to establish whether the plant is valued here as a food source.

1.6 Control options

Mechanical control can be relatively easy in large streams by cutting and removing the cut material onto the bank. In the UK it is important to leave the cut material on the bank for a short period, to allow invertebrates to return to the water if they have been using the plant as habitat. In small streams mechanical control tends to be more difficult because the plant produces high biomass. In either case, cutting provides relief for less than one season because of rapid regrowth from cuttings (Newman 2004), so mechanical control can worsen the original problem (Dawson 1978; Champion 2018).

Chemical control with most herbicides approved for use in aquatic situations can give temporary relief for up to two to three seasons (Newman 2004). In spring, application of dichlobenil can give up to 3 years of control. If treating later in the season, glyphosate can control for up to two seasons. This species is also susceptible to 2,4-D, which should be applied before flowering (Newman 2004), but 2,4-D is not permitted for use in waterways in New Zealand.

Herbivorous fish such as grass carp are not effective in cooler temperatures (Dawson & Hallows 1983), and may therefore not be suitable in some parts of New Zealand. Livestock will eat this plant readily, however, and sustained grazing pressure over 2 to 3 years can eliminate *H. nodiflorum* locally (Newman 2004).

Dense shading will also lead the plant to disappear, and management that stabilises stream banks and limits erosion will reduce its competitiveness (Newman 2004). Plantings for shading take time to grow to an effective size, and it has been proposed as an intermediate measure to use artificial shading by covering sections of the waterway for several weeks (Dawson & Hallows 1983). Such artificial sequential covering of sections of the waterway was shown to be less disruptive to the ecosystem in comparison to mechanical and chemical control (Dawson & Hallows 1983). However, this method is noted to be 'aesthetically displeasing', not specific to any one species of rooted macrophyte, and does not lead to eradication (Dawson & Hallows 1983).

In New Zealand, mechanical and chemical control are used (Champion et al. 2008; Champion et al. 2011; Champion 2018; Champion et al. 2019). These methods provide temporary relief, with regrowth from remaining plant material or recolonisation from seeds occurring within less than a year (Champion et al. 2011; Champion et al. 2019). Mechanical removal increases the risk of spread to new locations from contaminated machinery and other human-assisted means (Champion 2018). The selection of products for chemical control is restricted and subject to regulatory approval (Champion 2018).

1.7 Potential advantages and disadvantages of biological control

Biological control could offer many advantages over current control methods for the management of *H. nodiflorum* given the difficulty of controlling this weed by conventional means, the growing public disapproval of using chemicals to control weeds in waterways, and the increasing risk of human-assisted spread. Classical biological control, if successful, is more cost-effective than other forms of control because it offers continuous action and self-dispersal to areas that are not likely to be targeted by other control programmes. Biological control is an attractive option for a target weed such as *H. nodiflorum* that is increasing in range and has yet to reach its full weedy potential. While biocontrol is often used as the last-resort management option once all else has failed, it may be more effective when the pest is targeted at the earlier stages of invasion (Henderson 1999; Delfosse 2005).

Despite its advantages, biological control may not be a 'silver bullet', although multiple analyses have indicated the success rate of weed biocontrol programmes has been greater than was previously supposed (Hoffmann 1995; McFadyen 1998; Briese 2000; Fowler et al. 2000; Schwarzländer et al. 2018). Complete successes, where biological control is so dramatic that no other control methods are required, only account for approximately onethird of all completed programmes (McFadyen 1998). Furthermore, although biological control is often perceived as an environmentally benign alternative to chemicals, some cases of predictable damage to non-target plants have been reported (e.g. Louda et al. 2003).

Nevertheless, the risk of failure and the impacts of non-target attack are likely to be minor compared with the potential benefits (Hinz et al. 2019; Paynter et al. 2020). For example, Paynter and Flanagan (2004) showed that, of the weed biocontrol programmes that did not deliver complete control, most resulted in substantial or partial control (i.e. biocontrol contributes to management, but other control methods are still required to achieve adequate control). An example of partial control in New Zealand (listed by Fowler et al. 2000) is that of alligator weed (*Alternanthera philoxeroides*), for which biocontrol of floating weed mats is often successful on static water bodies but agents do not attack terrestrial infestations. Failure (i.e. an inability to find or establish control organisms, or an absence of agent impact) is rarely an outcome of weed biocontrol programmes (Paynter & Flanagan 2004), and is often the result of funding running out before all options have been exhausted.

Studies on host use by arthropod biocontrol agents indicate that virtually all risk of nontarget attack is borne by plant species closely related to the target weeds, and that the risk to native and beneficial flora can be judged reliably before introduction (Pemberton 2000; Suckling & Sforza 2014). Similarly, a survey in New Zealand indicated that host-range testing in past weed biocontrol programmes was highly reliable overall. Only four instances of non-target attack were recorded on native plant species; all four examples were of plants very closely related to the target weeds that were predictable from host-range testing, and resulted in minor impacts (Paynter et al. 2004; Paynter et al. 2008; Paynter et al. 2018).

An economic analysis from Australia indicated that weed biocontrol programmes provide a strongly positive return on investment overall, with benefits from the programmes far outweighing the total costs: for every dollar invested in weed biocontrol in Australia, a benefit of \$23.10 is generated (Page & Lacey 2006). No such detailed analysis is available for New Zealand, but Suckling (2013) suggests that the net benefits to New Zealand from weed biocontrol range between \$11 million and \$217 million per annum.

1.8 Predicting establishment of biocontrol agents

Reliably predicting the likelihood of establishment and impacts of introduced arthropods and pathogens on plant populations has long been a goal of weed biocontrol programmes. Research has been conducted to determine the importance of both release size (e.g. Memmott et al. 1998; Memmott et al. 2005) and climate matching (e.g. van Klinken et al. 2003). In addition, parasitism, predation and disease in the new range can affect agent establishment (Paynter et al. 2010; Paynter et al. 2018).

The best current predictor of establishment success of new organisms is the size of releases (Paynter et al. 2016). In New Zealand, weed biocontrol agent establishment rates are very high, largely due to the extensive technology transfer programme that Manaaki Whenua – Landcare Research operates with regional councils, community and farmer groups, the Department of Conservation, and forestry companies (Fowler et al. 2000; Hayes 2000; Fowler et al. 2010). This approach allows numerous releases of biocontrol agents to be made quickly at many sites.

In a broad sense, climate matching should rarely be a problem because co-evolved weed biocontrol agents and their host plants should, generally, be adapted to the same climatic conditions. However, van Klinken et al. (2003) noted that climate became an issue when agents were collected from a restricted part of the range of a plant species that occurs over a wide range of climatic and ecological conditions. Similar scenarios could also occur if the native distribution of a plant was restricted, compared to its introduced range. Problems can also arise if the target country has a climate that the plant can tolerate better than the agent. It is worth noting that an agent's sensitivity to climate may be confounded with photoperiodism, which can result in shifts (either up or down) to the number of generations per year in the new climate (Grevstad & Coop 2015). Given the Mediterranean and European distribution of *H. nodiflorum*, northern Spain and the UK may provide the best climatic matches between New Zealand and the native range.

1.9 Predicting the impact of biocontrol agents

Predicting the impact of an exotic organism in a new environment is more challenging than predicting the likelihood of its establishment (e.g. Cock et al. 2015). In addition to climate matching, an organism will face a host of other factors, such as predation, parasitism and competition, which might affect its ability to thrive in a new environment, as well as the ability of its host plant to compensate for attack. Information regarding the growth of *H. nodiflorum* in New Zealand compared with the native range is not available.

Denoth et al. (2002) found that the success of biocontrol against weeds increased with the number of agents released, although they argued that this result might be because of the increased likelihood of the most damaging species being released with the greater number of agent species released (lottery model), rather than the cumulative impact of multiple species of natural enemies. Spectacular biocontrol successes have been achieved with only one agent (Denoth et al. 2002), and a challenge for biocontrol practitioners is to identify the agent(s) most likely to have an impact on weed populations.

Crawley (1989) and Charudattan (2005) reviewed the use of insects and plant pathogenic fungi, respectively, in weed biocontrol. Crawley showed that certain insect groups have proved more successful at reducing host plant abundance than others; for example, around 50% of releases of Dactylopiidae (cochineal insects), Curculionidae (weevils) and Chrysomelidae (leaf beetles) that established resulted in marked or complete control of the target weed.

Weed biocontrol agents, especially rust fungi, can be so specialised that they show host specificity to genotypes within a given plant species. Thus, matching the target host's susceptibility to the candidate pathogen's virulence is of utmost importance for successful biocontrol with pathogen agents. Host–pathogen interactions at the species and subspecies levels are often governed by single-gene differences in rusts (e.g. varietal specificity, Charudattan 2005). For example, efficacy of the skeletonweed rust fungus, *Puccinia chondrillina*, varies from low to high depending on the form of the weed (Burdon et al. 1981). Eriophyid mites can show similar levels of specificity: at least one biotype of St John's wort, *Hypericum perforatum*, appears to be resistant to the eriophyid mite *Aculus hyperici* in Australia (Mayo & Roush 1997).

For certain candidate agents it is prudent to determine which subspecies and forms of a weed are present in the introduced range, and if possible survey for potential agents on similar material in the native range, to facilitate finding the correct agent biotype(s) and reduce the potential for incompatibility. Molecular techniques can be used to determine the diversity within and origin of a weed species. Some studies have used a cheaper method of directly testing the susceptibility of a weed to candidate agent species by planting out 'trap plants' collected in the weed's introduced range and monitoring damage to them in the native range. Logistics and biosecurity regulations can sometimes prohibit the use of trap gardens. In recent years the cost of conducting molecular tests has decreased markedly, and their use in determining the origin of weeds is becoming an integral component of biocontrol programmes.

Tapping into multiple experts' deep functional understanding of the system in question has been suggested as a way to assess the likelihood of success of biocontrol programmes (van Klinken et al. 2016).

Paynter et al. (2012) have developed a model with three traits that provides the ability to predict biocontrol impact against novel species in novel regions. According to their model, biocontrol impact varies according to:

- whether or not a weed was reported to be a major weed in its native range (a surrogate measure for relative abundance in the native and exotic ranges), and if so, whether a CAB Abstracts search listed five or more references that described the plant as a weed in the native range (i.e. excluding articles reporting native range surveys for biocontrol agents)
- mode of reproduction (sexual or asexual a surrogate measure of potential genetic diversity)
- ecosystem (aquatic/wetland versus terrestrial).

Using this system, success appears almost guaranteed against 'good target' weeds with the best combination of factors for success (aquatic, clonal species that are not major weeds in their native ranges), while most programmes against 'difficult targets' (i.e. weeds with the worst combination of factors for success) have failed to result in a measurable impact. Table 2 contains the predicted values for the proportion reduction achieved for each of the eight combinations of the three traits. The combination of traits for *H. nodiflorum* indicate it is a relatively good target (not a major weed in native range, sexual, aquatic/wetland, average percentage reduction due to biocontrol 77%).

Major weed in native range	Reproduction	Ecosystem	Percentage reduction from biocontrol
No	Asexual	Aquatic/wetland	93
No	Sexual	Aquatic/wetland	77
No	Asexual	Terrestrial	80
No	Sexual	Terrestrial	50
Yes	Asexual	Aquatic/wetland	69
Yes	Sexual	Aquatic/wetland	36
Yes	Asexual	Terrestrial	41
Yes	Sexual	Terrestrial	15

Table 2. Predictions of the proportion reduction achieved by biocontrol for each of the eight combinations of the predictor variables (Paynter et al. 2012)

Finally, Charudattan (2005) and Seastedt (2015) also concluded that the stakeholders' perceptions of the effectiveness of a biocontrol programme can be unpredictable, leading to conflicting views of 'success'. Therefore, the aims of a biocontrol programme against *H. nodiflorum* should be clearly defined from the outset, so that success or failure can be assessed objectively against a well-defined goal.

1.10 Biological control of *H. nodiflorum* elsewhere

Biological control of *H. nodiflorum* has not been attempted anywhere (Winston et al. 2014, and the updated 2019 online edition https://www.ibiocontrol.org/species/).

2 Objectives

- To review the literature to identify potential biocontrol agents for *H. nodiflorum* and assess the feasibility of their release in NZ
- To assess the prospects of achieving successful biological control of *H. nodiflorum*.
- To estimate the cost of the programme for biological control of *H. nodiflorum*.

3 Methods

3.1 Identifying fungal pathogens of *Helosciadium nodiflorum*

Information was compiled on the pathogens associated with *Helosciadium nodiflorum*. Data sources included several online databases:

- Ecological Flora of the British Isles (Fitter & Peat 1994): http://www.ecoflora.org.uk/
- Kew Royal Botanic Garden Plants and Fungi species browser: http://www.kew.org/science-conservation/plants-fungi/species-browser
- New Zealand Fungi and Bacteria Database (NZFUNGI2) (Landcare Research 2020): http://nzfungi2.landcareresearch.co.nz
- Plant Parasites of Europe: Leaf miners, Galls and Fungi (Ellis 2020): https://bladmineerders.nl/
- USDA Fungus-Host Database (FDSM) (Farr & Rossman 2020): https://nt.arsgrin.gov/fungaldatabases/fungushost/fungushost.cfm

Web of Science, Google, Google Scholar and CAB Abstracts were searched for associations and information using the terms '*Helosciadium nodiflorum*', '*Apium nodiflorum*' 'Water celery' and 'Fool's watercress' in combination with the terms 'Pathogen/s', 'Disease', 'Fungi/us', 'Bacteria/um' and 'Symptom/s'. The New Zealand Fungi and Bacteria Database was used to confirm the presence or absence in New Zealand of micro-organisms recorded on *H. nodiflorum*. Latin names, synonyms and authorities were all verified using the Species Fungorum website (http://www.speciesfungorum.org/Names/Names.asp).

3.2 Identifying arthropod biocontrol agents of *Helosciadium nodiflorum*

Unlike for fungal pathogens, comprehensive online databases for all arthropod herbivores do not exist. However, the following databases were searched:

- HOSTS a database of the world's lepidopteran host plants, the Natural History Museum's world listing (Natural History Museum London 2020): http://www.nhm.ac.uk/jdsml/research-curation/research/projects/hostplants/
- Database of Insects and Their Food Plants, Biological Records Centre (UK) (Biological Records Centre (BRC) 2020): http://www.brc.ac.uk/dbif/Interpreting_foodplant_records.aspx
- Plant Parasites of Europe: Leaf Miners, Galls and Fungi: http://bladmineerders.nl/
- Plant-SyNZ[™]: http://plant-synz.landcareresearch.co.nz/SearchForm.aspx

In addition, CAB Abstracts, Current Contents, Web of Science, Agricola, Science Direct, Google, and Google Scholar were searched using the terms '*Helosciadium nodiflorum* or *Apium nodiflorum* or water celery or fools watercress' and sub-searched using the terms 'invertebrate* or herbivor*'. Checklists of New Zealand fauna were referred to in order to determine whether any of the species recorded feeding on or infecting *H. nodiflorum* already occur in New Zealand.

4 Results

4.1 Pathogens attacking *Helosciadium nodiflorum*

Nine pathogenic micro-organisms associated with *H. nodiflorum* were found in the literature (Appendix 1). Note that species that are unsuitable for biocontrol (e.g. because they have a very broad host range or are saprophytic) are not discussed here. The species discussed in detail below appear in the following order: 1) species with the highest potential as biocontrol agents, then 2) species in the taxonomic order in which they appear in Appendix 1.

Entyloma helosciadii

This is the only pathogen that appears promising as a potential candidate agent. The genus *Entyloma* belongs to a group known as white smuts. *Entyloma* species can be highly damaging to their plant hosts (e.g. E. ageratinae in NZ, Barton et al. 2007). However, the symptoms caused by *E. helosciadii* on *H. nodiflorum* appear to be relatively minor. Images in Woods et al. (2018) show small, pale spots on both the upper and lower surfaces of otherwise green and vigorous leaves. Further, it is stated that leaf spots are easiest to find on the 'lower, older leaves', and that 'as the leaves senesce the areas colonised by the fungus become brown' (Woods et al. 2018). These descriptions suggest that this pathogen is not aggressive and not capable of killing entire plants. However, it is possible that field conditions were sub-optimal for the pathogen, or that the infected plants were more resistant than New Zealand plants would be, so it would be premature to reject the pathogen based on these symptom descriptions alone.

Most of the hosts of *E. helosciadii* listed on the USDA database (Farr & Rossman 2020) are within the same tribe as *H. nodiflorum* (Oenantheae) (Hardway et al. 2004). The tribe Oenantheae includes plants native to New Zealand, in the genus *Lilaeopsis*. The smut also infects several species in the genus *Apium*, which is in a different tribe (Apieae). If this is the true host range of *E. helosciadii*, then this pathogen is probably not specific enough for use as a biocontrol agent.

Determining the true host range of *E. helosciadii* will require molecular analysis. *Entyloma* species tend to have narrow host ranges restricted to a single genus or species, and according to Savchenko et al. (2015), 'Additional sequencing is needed to resolve the taxonomy of *Entyloma helosciadii*, a complex of three species known to infect hosts from four different genera in the tribe Oenantheae'. Overall, there are about 20 *Entyloma* species that specialise on hosts in the Apiaceae family, and only two of these, not including *E. helosciadii*, have undergone molecular phylogenetic analyses (Savchenko et al. 2015).

Molecular phylogenetic analysis of collections of *E. helosciadii* from different hosts may well reveal sub-taxa within it that are specific to the tribe Oenantheae, or even the genus *Helosciadium*. Consequently, *Entyloma helosciadii* is the pathogen with the most promise as a classical biocontrol agent for *H. nodiflorum*. It would be worth at least doing preliminary testing to determine whether or not *E. helosciadii* isolates from *H. nodiflorum* are capable of causing significant damage to the target weed, and also whether or not it can infect *Lilaeopsis* species native to New Zealand.

Septoria apiicola

This fungus causes serious disease to the economically valued plants celery (*Apium graveolens*) and celeriac (*A. graveolens* var. *rapaceum*) (Sutton & Waterston 1964), and can also infect other valued plants such as parsley (*Petroselinum crispum*) and an *Apium* species indigenous to New Zealand (*A. prostratum*, Figures 7 & 8). Therefore, *S. apiicola* is not suitable as a classical biocontrol agent.

Septoria apiicola already occurs in New Zealand, so if it were to be utilised against *H. nodiflorum* here, it would need to be applied as a bioherbicide (i.e. mass produced in culture and applied to the weed as a formulated product in a manner similar to a chemical herbicide). There is no bioherbicide product based on *S. apiicola* currently available on the market or under development globally. Developing a bioherbicide is an expensive process that requires many years of research. It should only be considered as an option if either 1) the pathogen has already been developed/is being developed as a bioherbicide overseas and New Zealand can share resources with others, and/or 2) there are no good options for classical biocontrol. Neither of these scenarios applies here.



Figure 7. *Septoria apiicola* spores on host *Apium prostratum* (PDD 96509, Landcare Research 2020). Copyright: Jerry Cooper. Licence: CC BY 4.0.



Figure 8. *Septoria apiicola* symptoms on *Apium prostratum* (PDD 96509, Landcare Research 2020). Copyright: Jerry Cooper. Licence: CC BY 4.0.

Note also that a study on breeding resistance against pathogens on the crops celery and celeriac investigated incorporating genes from *H. nodiflorum* into the vegetables to increase resistance to *Septoria* species (Bruznican et al. 2020). This is because *H. nodiflorum* plants have shown far fewer symptoms of disease when infected by *S. apiicola* in laboratory trials than cultivated celery (Edwards et al. 1997). Given that *H. nodiflorum* plants have natural resistance to *S. apiicola*, simple glasshouse trials may be all that is required to indicate quickly if this pathogen is sufficiently damaging to be a useful bioherbicide.

Septoria umbelliferarum

This *Septoria* species does not occur in New Zealand and would need to be imported as a classical biocontrol agent. Since it is known to infect and damage seeds of a valued herb (caraway, *Carum carvi*) and this non-target host belongs to a different tribe to *H. nodiflorum* (Careae), this pathogen lacks sufficient host specificity. Note also comments above about *H. nodiflorum* having greater natural resistance to *Septoria* species than other plants in the Apiaceae.

Ascochyta helosciadii

The association between this pathogen and *H. nodiflorum* was only discovered because a collection of *Ascochyta helosciadii* on this host, from Turkey, is stored in the NZ Fungal and Plant Disease Collection (PDD) (Landcare Research 2020). There is also a collection from the same country at the Kew Botanic gardens (IMI 363888), but the two collections from Turkey have the same collection date (02/06/1944) and are almost certainly from the same source. Anecdotally, this most likely occurred because in the past IMI mycologists would often keep (as a reference) part of a collection when they were sent diseased plant material and asked to identify the causative organism.²

Both the original species description of *Ascochyta helosciadii* (as *Marssonina helosciadii*) (Fautrey & Lambotte 1896) and the collection from Turkey have the host *H. nodiflorum*. No further information on the host range of *A. helosciadii* could be found. It may (or may not) be specific to *H. nodiflorum*. Unfortunately, the collections in PDD and IMI would have been dried, and are almost certainly dead, and so this fungus would need to be recollected in the field before it could be investigated as a potential biocontrol agent. No disease symptoms are given in the species description or provided with the collection from Turkey.

The genus *Ascochyta* is large, containing almost 400 species (Kirk et al. 2008). Some species are significant pathogens (e.g. *A. pisi* causes Ascochyta blight of pea (*Pisum sativum;* Bretag et al. 2006), but most are not. While it is possible that *Ascochyta helosciadii* could be sufficiently host specific, its capacity to cause significant damage to *H. nodiflorum* would need to be tested. No *Ascochyta* species has been used previously

² http://www.herbimi.info/herbimi/home.htm.

for classical biocontrol (Winston et al. 2014, and the updated 2019 online edition: https://www.ibiocontrol.org/species/).

Buerenia inundata

Buerenia inundata belongs to a group within the Ascomycetes called the Protomycetales. They are parasitic, but can be grown on artificial medium (Ahmad 1974), which makes them easier to work with. The hosts of *B. inundata* sit in four genera that are all within the same subfamily as *H. nodiflorum* (subfamily Apioideae). Two of these genera, *Sium* and *Berula*, are in the same tribe as *H. nodiflorum*, Oenantheae. The third genus, *Apium*, is in the tribe Apieae, and the fourth genus, *Daucus*, is in the Scandiceae. Since there are plants native to New Zealand that are in genera within the tribe Oenantheae (*Lilaeopsis* spp.), this pathogen is probably not specific enough for use as a biocontrol agent.

Symptoms caused by *B. inundata* include brown/orange open galls on the leaves with translucent spore-like structures. These symptoms look superficially similar to those of white smuts such as *Entyloma* species (Preece & Hick 2001), but these pathogens are completely different taxonomically: the genus *Buerenia* is in the phylum Ascomycota while the genus *Entyloma* is in the phylum Basidiomycota. Both genera are associated with *H. nodiflorum* in the UK, and it will be important for researchers conducting pathogen surveys in the native range to be able to distinguish infections caused by *Buerenia inundata* from infections caused by *Entyloma helosciadii* (discussed above).

Observations suggest that infection by *B. inundata* remains localised near the infection site and is not systemic (Preece & Hick 2001). It has been noted that '*A. nodiflorum* is an exceedingly common plant in lowland Britain' yet 'the Shropshire Check list ... has only two records of the fungus' (Preece & Hick 2001), suggesting that damage caused to *H. nodiflorum* by *B. inundata* is inconspicuous, and that the plant is not significantly affected at the population level. Therefore *B. inundata* is unlikely to be sufficiently damaging.

Uromyces lineolatus

This pathogen is a rust (order Pucciniales). Rust fungi often make useful biocontrol agents due to their high host specificity, high virulence and efficient dispersal (Barton (née Fröhlich) 2004). At least 20 of the approximately 30 pathogen species moved from one country to another for the purpose of weed biocontrol have been rusts (Barton 2012; Winston et al. 2014 and the updated 2019 online edition: <u>https://www.ibiocontrol.org/species/</u>; Schwarzländer et al. 2018).

Uromyces lineolatus, however, apparently has a broader host range than most rusts. In addition, *U. lineolatus* is heteroecious (Jerling & Berglund 1994; Koike et al. 2006), which means it requires two unrelated host plant species to complete their life cycle. The secondary host of *U. lineolatus* is the bulrush, *Bolboschoenus maritimus* (family Cyperaceae) (Jerling & Berglund 1994). The primary and secondary plant hosts become infected by different types of spores (aeciospores and basidiospores, respectively). This complex life cycle would make any host-range tests prohibitively expensive. It would be necessary to apply aeciospores to all the close relatives of the target weed, and also apply

basidiospores to close relatives of its alternate host. Both the primary host (*H. nodiflorum*) and the secondary host (*B. maritimus*) have New Zealand indigenous congenerics.³.

Finally, *U. lineolatus* causes significant damage to carrots and parsnips, which are widely grown in New Zealand. Carrots, especially, are an important export seed crop. Introduction of this pathogen is therefore likely to attract opposition from growers of these crops. Consequently, despite the strong history of rust fungi as classical biocontrol agents, *U. lineolatus* is unlikely to be a good candidate agent.

4.1.1 Discussion

Only nine pathogens have been recorded from *H. nodiflorum*, which is a relatively small number compared to pathogens recorded from other weeds (J. Barton, pers. obs.). Often not much is known about the mycobiota of weedy plants in their home range because they are not valued or problematic there and so have been little studied. However, this seems unlikely for *H. nodiflorum*, which has been valued in Europe and the Mediterranean for centuries both as food and as a medicinal herb (see section 1.3 Beneficial uses). Also, the plant is common in the UK, which is home to many active mycologists: The British Mycological Society was established in 1896 and is still a vibrant organisation with around 400 members living in the UK (Dr Norman Porrett, BMS administrator, pers. comm.). It is therefore unlikely (albeit possible) that a pathogen that causes significant damage to *H. nodiflorum* throughout its home range has been overlooked.

It is plausible that the same compounds that make *H. nodiflorum* useful as a medicinal herb are also responsible for it having a poor mycobiota. Volatile chemicals produced by this plant have been shown to have insecticidal (Benelli et al. 2017), antibacterial (Rosato et al. 2018) and antifungal (Maxia et al. 2012; Salehi et al. 2019) properties.

The primary antifungal chemical *H. nodiflorum* produces is called dillapiole (Maxia et al. 2012). This chemical has been shown to inhibit some human pathogens (Maxia et al. 2012). 'However, information on the antifungal activity of dillapiole against plant pathogenic fungi is still quite limited' (Vizcaíno-Páez et al. 2016). Nonetheless, it is reasonable to hypothesise that dillapiole would be toxic to at least some plant pathogens, and that *H. nodiflorum* may not host as many microbes with biocontrol potential as a plant that did not produce this anti-microbial chemical.

³ https://nzflora.landcareresearch.co.nz/

4.2 Arthropods attacking *Helosciadium nodiflorum*

Eleven species of arthropods were found in the literature associated with *H. nodiflorum* and its synonyms (Appendix 2).

4.2.1 Moths

The most promising candidate is the stem mining moth *Depressaria ultimella* Stainton (Figure 9), which is fairly common on *H. nodiflorum* in the UK (Heckford 1983). Caterpillars begin mining the stems of side shoots, which wither and fill with orange-brown frass. Later the caterpillar mines into the main stem, where it makes an exit hole and plugs it with brown silk for adult emergence (Kimber 2020). Observations suggest only one larva is found in each infested plant (Heckford 1983). Mined plants collapse later in the season, and uninfested plants may grow to cover the area freed up by collapsing stems (Heckford 1983). This moth has one generation per year, with adults emerging during late summer (Heckford 1983). *D. ultimella* may prefer *H. nodiflorum* growing in slow-flowing water such as small roadside ditches over plants growing in streams (Heckford 1983).

This moth has been associated with other genera in the same tribe, Oenantheae, as *H. nodiflorum*. These are:

- Oenanthe (Oenanthe aquatica, which is present in New Zealand, exotic, naturalised, and well established in Marlborough⁴, (Webb et al. 1988)), and O. crocata, which is not present in New Zealand)
- *Sium* (*Sium latifolium*), which is not present in New Zealand).

These two genera are represented in New Zealand by other species, all either naturalised or weedy, except for skirret (*Sium sisarum* L), which is cultivated by home growers.⁵ The tribe Oenantheae is represented in New Zealand by two indigenous and two exotic species in the genus *Lilaeopsis*.

Depressaria ultimella may turn out to be sufficiently host specific to *H. nodiflorum*. Heckford (1983) questions earlier associations of *D. ultimella* with *Oenanthe crocata*, and notes that in a habitat with both hosts, *O. crocata* and *H. nodiflorum* (as *A. nodiflorum*), *H. nodiflorum* was associated with *D. ultimella* while *O. crocata* was associated with another closely related species, *D. daucella*. The adults of these two congeneric moth species look very similar, but their larvae look different.

D. ultimella is attacked by at least three species of larval parasitoids in the UK: *Barichneumon lepidus* (Gravenhorst), *Phaeogenes stripator* Wesmael, and *Dicaelotus morosus* Wesmael (Heckford 1983). These parasitoids are not present in New Zealand. If the moth can be released from its own natural enemies, and if the risk of the moth accumulating parasitoids from ecological analogues in New Zealand is low (Paynter et al.

⁴https://nzflora.landcareresearch.co.nz/default.aspx?selected=NameDetails&TabNum=0&NameId=DD4A5714-4BE7-4680-9A71-17919869394A,

⁵ For example, seed exchange, <u>http://www.southernseed.org.nz/apiaceae/sium-sisarum</u>

2010), then the effectiveness of *D. ultimella* as a biocontrol agent could be high, at least in infestations in slow-flowing water.

D. ultimella is listed as an unwanted organism by the Ministry for Primary Industries, so removal from the unwanted organisms list would be required before this moth could be assessed as a candidate biocontrol agent. The reasons behind *D. ultimella* being regarded as an unwanted organism are unclear. Most likely the old classification of *H. nodiflorum* as *Apium* has meant that its natural enemies were likely to be pests of celery. This reason no longer stands, and it should therefore be a formality to remove this moth from the list of unwanted organisms.



Figure 9. Adult of the stem miner *Depressaria ultimella*. (Photo by Jon Baker, with permission).

A closely related species, *Depressaria radiella* (Goeze), (Synonym *D. pestinacella* (Duponchel), the parsnip webworm, has also been recorded from *H. nodiflorum*. This pest species is present in New Zealand and is not suitable as a candidate biocontrol agent.

4.2.2 Beetles

Three species of beetles have been recorded from *H. nodiflorum*.

The stem weevil *Lixus iridis* Olivier can be highly damaging. This species was considered for biological control of the hogweed *Heracleum sosnowskyi* Manden in Russia (Krivosheina 2011), but its host range may not be sufficiently specific. It has been recorded from other genera in the family Apiaceae, including celery and parsnip (Hoffmann 1954; Scherf 1964). Other species in the genus *Lixus* have been used as biocontrol agents for other weeds. It may be worth exploring whether a host-specific ecotype (a distinct population within a species with specific adaptations) or cryptic species (separate species that cannot be distinguished morphologically) exist.

Adults of the brooklime leaf beetle *Prasocuris junci* (Brahm) feed on various hosts from various plant families. The main larval hosts are brooklime, *Veronica beccabunga* L., and *Veronica anagallis-aquatica* L. (family Plantaginaceae) (Cox 2007). One observation from Wales describes the beetle in high abundance on *H. nodiflorum* (Cox 2007). It may be possible to explore whether this is a host-specific ecotype or cryptic species.

The weevil *Hypera adspersa* (F.) can cause significant damage. It does, however, feed on other genera in the family Apiaceae, including carrot, and on one genus in the family Asteraceae (Skuhrovec 2005).

4.2.3 Flies

Two species of flies have been recorded from *H. nodiflorum*. Neither is suitable as a biocontrol agent. One is the pest *Psila rosae* (F.), the carrot fly. The other is the oligophagous gall fly *Euleia heraclei* (L.) (family Tephritidae) which has been recorded from many genera in the family Apiaceae, including several economically important species.

4.2.4 Aphids

Six species of gall-forming aphids have been associated with *H. nodiflorum* in the literature. Two of these species' records could not be confirmed through cross-referencing and are not listed in Appendix 2. All six gall-forming aphids are host-alternating aphids. Aphids with a life cycle that includes host alternation move between two, usually distantly related, host species. The primary host is often woody, and the secondary host is often herbaceous. Sexual reproduction occurs on the primary host. On the secondary host reproduction is asexual (Hałaj & Osiadacz 2013). The gall-forming aphids recorded from *H. nodiflorum* use this plant as a secondary host.

4.2.5 Discussion

H. nodiflorum is rich in terpenes and phenylpropanoids (Guijarro-Real et al. 2019b) which play a role in plant defence against herbivores and pathogens. This may limit the number of arthropods that are able to feed on *H. nodiflorum*.

5 Conclusions

5.1 Prospects of achieving biological control of *Helosciadium nodiflorum* in New Zealand

A small number of arthropods and pathogens have been recorded from *H. nodiflorum*, and only one arthropod and one pathogen appear to be sufficiently host specific. *H. nodiflorum* appears to be well studied, so it is surprising that so few natural enemies have been recorded.

H. nodiflorum is noted as common in many parts of its native range and even weedy in some, and has also been the subject of control and management operations in parts of its native range (Dawson 1978; Dawson & Hallows 1983; Newman 2004). This suggests that even in the native range, under the impact of its natural enemies, *H. nodiflorum* can build up dense populations. Species that can become weedy in their native range tend to be more difficult targets for biocontrol (Paynter et al. 2012).

Biocontrol has not been attempted against *H. nodiflorum* anywhere else. A programme in New Zealand would therefore be at the expensive end of the range, expected to cost around \$500,000 per agent; on average, two to three agents are needed to achieve control (Paynter et al. 2015).

There are numerous indigenous plants in the family Apiaceae, but none are closely related to the genus *Helosciadium*. The two indigenous species in the genus *Lilaeopsis* sit in the same tribe as *Helosciadium*, the tribe Oenantheae, yet the phylogenetic distance between these two genera increases the chances that sufficiently host-specific agents can be found. There are several commercially valuable crops in the family Apiaceae in New Zealand, including the significant carrot seed export industry. None of the commercially valuable species are closely related to the genus *Helosciadium*. Importantly, since water celery was moved from the genus *Apium* to the genus *Helosciadium*, celery, which remains in the genus *Apium*, is no longer in the same genus as water celery.

The significance of *H. nodiflorum* to herbal medicine and to wild food harvest has yet to be established. No other opposition to biocontrol of *H. nodiflorum* is anticipated.

It may be that *H. nodiflorum* is expanding its range further south. It is possible that with warming climate *H. nodiflorum* will invade more southern areas but modelling to project the potential range of this weed has not been performed. Chemical and mechanical methods fail to provide long-term control. Biocontrol can offer such long-term solution.

The issue of replacement weeds should be considered. There is the possibility that the niche vacated by *H. nodiflorum* after biocontrol may be taken up by worse weeds. Conversations with staff at Nelson City Council and Greater Wellington Regional Council suggest that *H. nodiflorum* is the one of the worst if not *the* worst of the suite of weeds they are managing in these freshwater systems, and so there is no concern that a replacement weed may be worse (R. Frizzell, Environmental Programmes Adviser, and A. Mattsen, Project Engineer – Flood Protection, pers. comm.).

H. nodiflorum hybridises easily with other *Helosciadium* species in the native range, so it will be important for a biocontrol programme to examine whether the populations in New Zealand are pure *H. nodiflorum* or hybrids. This will be especially important when searching for pathogens as biocontrol agents.

The European native range of *H. nodiflorum* has clear regulations and mechanisms supporting exporting biocontrol agents. Having these regulations and mechanisms in place simplifies undertaking native-range surveys, and collecting and exporting candidate biocontrol agents.

Two organisms, an arthropod and a pathogen, have been identified from the literature as potential candidate biocontrol agents, but it is possible that further potential agents would be discovered in native range surveys. It is also important to note that aquatic species tend to be good biocontrol targets (Paynter et al. 2012).

6 Recommendations

- Apply to remove the moth *Depressaria ultimella* from the list of unwanted organisms. Estimated cost: \$15,000-\$20,000.
- Undertake DNA studies to identify the geographical source of *H. nodiflorum* in New Zealand and whether the New Zealand material consists of pure *H. nodiflorum* or hybrids. Estimated cost: \$20,000-\$100,000.
- Undertake a survey of the natural enemies of *H. nodiflorum* in New Zealand and look for any potential biocontrol agents, and any other species living on the plant (such as predators) that might interfere with potential biocontrol agents. Note that this baseline information is vital for any subsequent application to release new biocontrol agents. Estimated cost: \$50,000-\$70,000.
- Analyse the costs of and benefits expected from biological control in comparison to conventional control of *H. nodiflorum*. This information will strengthen any future application to release new biocontrol agents. Estimated cost: \$15,000-\$25,000.
- Survey *H. nodiflorum* in its native range to identify more candidate biocontrol agents. Estimated cost: \$50,000-\$300,000.
- Identify and study the life cycle of prospective agents found during surveys. If required, undertake host-range testing of potential agents. This stage should include preliminary screening to test if the smut fungus *Entyloma helosciadii* isolates from *H. nodiflorum* are damaging to *H. nodiflorum* and whether this pathogen can infect indigenous *Lilaeopsis* species. Estimated cost: \$60,000–\$200,000 per agent.
- If testing shows agents are suitable, apply to release them in New Zealand. Estimated cost: \$55,000-\$75,000 for one or more species.
- Import into containment and gain permission to remove. Estimated cost: \$25,000-\$60,000 per species.
- Mass-rear and release agents. Estimated cost: \$100,000-\$250,000 per species.

Note: Estimated costs are exclusive of GST and are based on 2019/20 figures. New estimates will need to be provided if work is to be undertaken beyond those dates, and/or if complicating factors arise (e.g. disease infecting the imported agents).

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Phylum/ order/ family	Species ^a	Symptoms or lifestyle ^b	Geographic range on <i>H. nodiflorum</i>	Likely to be sufficiently host specific?	Present in NZ ^c ? Likely to be highly damaging?
ASCOMYCOTA					
Capnodiales					
Mycosphaerellaceae	Septoria apiicola Speg. Synonyms = Septoria apii Chester = Septoria apii- graveolentis Dorogin [as 'apii graveolentes]	Leaf spots/splotches (see Figure 8 above and other images at Landcare Research 2020)	Chile, UK (Farr & Rossman 2020).;NZ (Landcare Research 2020)	No. Hosts on USDA database are all in the Apiaceae family but are widely distributed therein. Hosts are: several <i>Apium</i> species (Apieae) including <i>A. graveolens</i> (celery), 1 or 2 <i>Bupleurum</i> spp. (Bupleureae), carrot (<i>Daucus carota,</i> Scandiceae), <i>Hydrocotyle</i> <i>salwinica</i> (pennywort, Araliaceae), 3 species of <i>Petroselinum</i> , including <i>P. crispum</i> (parsley, Apieae) and a species of <i>Pternopetalum</i> sp. (tribe undetermined except that it is not Oenantheae). In NZ this fungus has only been reported on <i>H. nodiflorum</i> and <i>Apium</i> species.	Present (exotic) in NZ. Of the 47 collections of <i>Septoria apiicola</i> found in PDD ⁴ or ICMP ⁵ , only two are from <i>H. nodiflorum</i> . This implies the fungus does not often cause conspicuous damage on this host in NZ. In contrast, a disease called 'late blight' caused by <i>S. apiicola</i> is apparently 'totally detrimental under poor crop management practices' to both celery and celeriac (<i>A. graveolens</i>) (Bruznican et al. 2020). There are also collections of <i>S. apiicola</i> in PDD & ICMP on NZ celery (<i>Apium</i> <i>prostratum</i> Labill. Ex Vent.) a species indigenous to NZ.

Appendix 1 – Records of pathogens associated with *Helosciadium nodiflorum*

^a Synonyms (old, invalid names for a taxon) are only given here where that (old) name is the one reported in the literature.

^b Information in this column is from: the author's general knowledge as a plant pathologist, Farr & Rossman 2020, or the reference provided.

^c Information in this column is from <u>https://nzfungi2.landcareresearch.co.nz</u> unless otherwise stated.

⁴ PDD = New Zealand Fungal and Plant Disease Collection – dried specimens

⁵ ICMP = International Collection of Microorganisms from Plants – living cultures of fungi and plant-associated bacteria

Phylum/ order/ family	Species ^a	Symptoms or lifestyle ^b	Geographic range on <i>H. nodiflorum</i>	Likely to be sufficiently host specific?	Present in NZ ^c ? Likely to be highly damaging?
Mycosphaerellaceae (cont.)	<i>Septoria umbelliferarum</i> Kalchbr.	Leaf spot	Libya (Farr & Rossman 2020)	No. While hosts are in the Apiaceae, they include caraway (<i>Carum carvi</i> , tribe Careae) (Mačkinaitė 2011) and <i>Coriandrum</i> sp. (genus containing coriander, tribe Coriandreae) (Farr & Rossman 2020).	Absent from NZ. Very little information could be found on this fungus. Its ability to damage <i>H. nodiflorum</i> is unknown.
Pleosporales					
Didymellaceae	Ascochyta helosciadii (Fautrey & Lambotte) Petr. synonym = Marssonia helosciadii Fautrey & Lambotte = Marssonina helosciadii (Fautrey & Lambotte) Magnus	The genus <i>Ascochyta</i> includes saprobes and pathogens. The latter generally cause leaf spots.	Turkey (Landcare Research 2020); Côte-d'Or, France (Fautrey & Lambotte 1896)	Possible. Only relevant literature discovered was the original species description (Fautrey & Lambotte 1896).	Absent from NZ (apart from the dried specimen from Turkey [PDD 60802]). Ability to damage <i>H. nodiflorum</i> unknown.
Taphrinales					
Protomycetaceae	Buerenia inundata (P.A. Dang.) M.S. Reddy & C.L. Kramer (original description gives genus name as 'Burenia'). Synonyms = Protomyces inundatus P.A.Dang. + Taphridium inundatus (Dangeard) von Buren	Members of the genus <i>Buerenia</i> are 'parasitic on Umbelliferae causing blisters or swellings on stems and leaves' (Reddy & Kramer 1975).	UK (Farr & Rossman 2020). The Encyclopedia of Life (https://eol.org/pages/191 715) shows 34 collections on <i>H. nodiflorum</i> in England and one in Ireland. Original species description also includes France, West Germany and Switzerland in range of pathogen, but this may be on other hosts (Reddy & Kramer 1975).	No. <i>B. inundata</i> is also parasitic on species of <i>Apium, Daucus</i> (although not <i>D. carot</i> a = carrots), <i>Berula</i> and <i>Sium</i> (water parsnips) (Reddy & Kramer 1975; Preece & Hick 2001).	Absent from NZ. Symptoms of <i>B. inundata</i> on <i>H. nodiflorum</i> described as 'spots' (Fitter & Peat 1994). Not likely to be highly damaging: symptom description and photographs of infected leaves in original description describe/show mild damage.

Phylum/ order/ family	Species ^a	Symptoms or lifestyle ^b	Geographic range on <i>H. nodiflorum</i>	Likely to be sufficiently host specific?	Present in NZ ^c ? Likely to be highly damaging?
BASIDIOMYCOTA					
Cystofilobasidiales					
Cystofilobasidiaceae	<i>Itersonilia perplexans Derx</i>	Saprobe and pathogen. Causes black streak disease of edible burdock (<i>Arctium</i> <i>lappa</i>) (Horita & Yasuoka 2002)	NZ (Landcare Research 2020)	No. 25 hosts listed on USDA website (Farr & Rossman 2020) and 9 additional host species are listed on the NZFungi2 database (Landcare Research 2020). Hosts belong to a range of families additional to the Apiaceae; e.g. <i>Arctium lappa</i> and <i>Chrysanthemum</i> spp. are in the Asteraceae while <i>Brassica oleraceae</i> (cabbage) is in the Brassicaceae.	Present, exotic in NZ. There are several collections of this fungus, on a variety of hosts, stored in PDD ⁴ and ICMP ⁵ . Only one of these, ICMP 15787, is on <i>H. nodiflorum</i> . Symptoms on <i>Arctium lappa</i> are leaf spots, which develop into necrotic streaks that cause 'significant damage' (Horita & Yasuoka 2002). However. <i>I.</i> <i>perplexans</i> is considered a 'saprobe or weak pathogen' on other hosts (Horita & Yasuoka 2002). Ability to damage <i>H. nodiflorum</i> unknown.
Entylomatales					
Entylomataceae	<i>Entyloma helosciadii</i> Magnus	White smut	Algeria, Austria, England, France, Germany, Ireland, Italy, Kenya, Morocco, North Africa & Portugal (Farr & Rossman 2020); Wales (Woods et al. 2018)	Maybe. Additional hosts listed on USDA website are: <i>Apium graveolens, Apium</i> <i>repens, Berula angustifolia, Helosciadium</i> <i>repens</i> (sometimes considered a synonym of <i>A. repens</i>), <i>Oenanthe crocata, Oenanthe</i> <i>silaifolia</i> and <i>Sium latifolium</i> (Farr & Rossman 2020).	Absent from NZ. Symptoms on <i>H. nodiflorum</i> described as small whitish or brownish spots on the leaves (Woods et al. 2018); and also 'Sori as small needle-shaped pustules on both surfaces of leaves' (Watson 1971).
Uredinales					
Pucciniaceae	<i>Uromyces lineolatus</i> (Desm.) J. Schröt.	Gall-forming rust	Europe (country not specified) (Ellis 2020)	No. Most, but not all of the hosts of this rust are in the Apiaceae (Farr & Rossman 2020). For example, the host <i>Lysimachia</i> <i>maritima</i> (formerly <i>Glaux maritima</i>) is in the Primulaceae. Note also that this rust completes part of its life cycle on a distantly	Absent from NZ. Rusts tend to cause significant damage to their hosts. However, no information could be found specifically on the

Phylum/ order/ family	Species ^a	Symptoms or lifestyle ^b	Geographic range on <i>H. nodiflorum</i>	Likely to be sufficiently host specific?	Present in NZ ^c ? Likely to be highly damaging?
				related host: <i>Bolboschoenus maritimus</i> (L.) Palla (= <i>Scirpus maritimus</i> L.) a bulrush in the Cyperaceae (Jerling & Berglund 1994). It also has a wide range within the Apiaceae, including commercially important taxa such as carrots (<i>Daucus carota</i>) and parsnips (<i>Pastinaca sativa</i>) (Ellis 2020; Farr & Rossman 2020).	impact of <i>Uromyces lineolatus</i> on <i>H. nodiflorum</i> .
BLASTOCLADIO-MY	СОТА				
Blastocladiales					
Physodermataceae	<i>Physoderma vagans</i> J. Schröt.	Gall	Europe (country not specified) (Ellis 2020)	No. Polyphagous (Ellis 2020). Hosts outside the Apiaceae include <i>Argentina anserina</i> (Rosaceae), <i>Ranunculus</i> spp. (Ranunculaceae) and <i>Trifolium fragiferum</i> strawberry clover, Fabaceae)	Absent from NZ. Symptoms on <i>H. nodiflorum</i> not given. Symptoms on other plants are small blisters and plant disfigurement, so unlikely to cause host mortality (Ellis 2020).
CHYTRIDIOMYCOTA					
Synchytriales					
Synchytriaceae	<i>Synchytrium aureum</i> J. Schröt.	Obligate parasite which causes galls (Karling 1964)	Europe (country not specified) (Ellis 2020)	No. Polyphagous on herbs (Ellis 2020). There are 567 records of <i>P. vagans</i> from plants in numerous families in the USDA database (Farr & Rossman 2020), and a monograph of <i>Synchytrium</i> spp. stated that <i>S. aureum</i> had been reported from 186 flowering plant species across 110 genera in 33 families, although many of these identifications may be inaccurate (Karling 1964).	Present, exotic in NZ. Not reported here associated with <i>H. nodiflorum</i> . Symptoms of <i>S. aureum</i> on <i>H. nodiflorum</i> not reported but while all <i>Synchytrium</i> species are parasitic, very few cause economically important levels of disease (Karling 1964).

Order and Family	Species	Type of organism	Geographic range on <i>H. nodiflorum</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
COLEOPTERA					
Chrysomelidae	<i>Prasocuris junci</i> (Brahm), brooklime leaf beetle	Leaf beetle	UK (Cox 2007).	No. Adults feed on various hosts from various plant families. The main larval hosts are brooklime, <i>Veronica beccabunga</i> L. and <i>Veronica anagallis-aquatica</i> L. (Cox 2007) (https://www.coleoptera.org.uk/species/prasocu ris-junci). One observation from Wales describes the beetle in high abundance on <i>H. nodiflorum</i> (Cox 2007). It may be possible to explore whether this is a host-specific ecotype or cryptic species.	Not present in NZ. Damage level unknown. Adults feed on leaves. Larvae remove the lower epidermis of leaves (Cox 2007).
Curculionidae	<i>Hypera adspersa</i> (F.)	Weevil	Czech Republic (Skuhrovec 2005); Central Europe (Hoffmann 1954; Scherf 1964); UK, as syn <i>H. pollux</i> (F.) (Lee & Scott 2007).	No. Feeds on other genera in the family Apiaceae, including carrot, and on one genus in the family Asteraceae (Skuhrovec 2005)	Not present in NZ. Damage can be significant (e.g. on <i>Oenanthe crocata</i> <u>http://ecoflora.org.uk/search phytophagy2.php?insect species=Hypera%20adspersa</u>). <i>Hypera adspersa</i> (as <i>H. pollux</i>) is the obligatory prey of the mason wasp <i>Odynerus simillimus</i> Morawitz (Lee & Scott 2007).
Curculionidae	<i>Lixus iridis</i> Olivier	Stem weevil	Central Europe (Hoffmann 1954; Scherf 1964)	Possibly not. Feeds on other genera in the family Apiaceae, including celery and parsnip (Hoffmann 1954; Scherf 1964).	Not present in NZ. Potentially highly damaging. Was considered for biological control of <i>Heracleum sosnowskyi</i> Manden in Russia (Krivosheina 2011). Other species in the genus <i>Lixus</i> have been used as biocontrol agents for other weeds. May be worth exploring if host- specific ecotype or cryptic species exist.

Appendix 2 – Records of invertebrates feeding on *Helosciadium nodiflorum*

Order and Family	Species	Type of organism	Geographic range on <i>H. nodiflorum</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
DIPTERA					
Psilidae	<i>Psila rosae</i> (F.), carrot root fly	Root feeder	UK (Hardman & Ellis 1982)	No. Attacks many species and genera in the family Apiaceae, including carrot, parsnip, celery, parsley, coriander, as well as a small number of species in other plant families (Biological Records Centre (BRC) 2020) (http://www.brc.ac.uk/dbif/invertebratesresults.a spx?insectid=7228)	Present in NZ. Likely to be damaging. A severe pest of carrot.
Tephritidae	<i>Euleia heraclei</i> (L)	Gall fly (leaf galls)	England (Shcherbakov 2002)	No. Broadly oligophagous, feeding on many genera in the carrot family, including parsley, coriander, parsnip (e.g e.g Shcherbakov 2002; Ellis 2020) (https://bladmineerders.nl/parasites/animalia/art hropoda/insecta/diptera/brachycera/tephritidae /euleia/euleia-heraclei/)	Not present in NZ. Damage levels unknown.
HOMOPTERA					
Aphididae	<i>Aphis fabae fabae</i> Scopoli	Gall-forming aphid (leaf galls)	Europe (Ellis 2020)	No. Although this aphid has a narrow hot range for its primary host, it is polyphagous on many secondary hosts, including cultivated crops such as broad bean, beetroots and <i>Chenopodium</i> species (Dransfield & Brightwell 2020). <i>H.</i> <i>nodiflorum</i> is one of many secondary hosts (Blackman & Eastop 2018; Ellis 2020)	Not present in NZ. This aphid is known to transmit plant viruses. It is listed by MPI as an unwanted organism.

Order and Family	Species	Type of organism	Geographic range on <i>H. nodiflorum</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
Aphididae (cont.)	<i>Cavariella aegopodii</i> (Scopoli), willow-carrot aphid	Gall-forming aphid (leaf galls)	Europe (Ellis 2020); Portugal (Rodrigues et al. 2006)	No. Although this aphid has a narrow host range for its primary host, it is broadly oligophagous on secondary hosts in the carrot family, including carrot, celery, fennel, parsnip and parsley. <i>H. nodiflorum</i> is one of those secondary hosts (Blackman & Eastop 2018; Ellis 2020).	Present in NZ (exotic). Can be highly damaging. This aphid is a major pest of cultivated crops in the carrot family (Blackman & Eastop 2000).
	<i>Hyadaphis foeniculi</i> Passerini, fly honeysuckle aphid, fennel aphid	Gall-forming aphid (leaf galls)	Europe (Ellis 2020). Portugal (Rodrigues et al. 2006)	No. The primary hosts of this aphid are in honeysuckles, primarily fly-honeysuckle, <i>Lonicera xylosteum</i> . The aphid is oligophagous on secondary hosts in the carrot family, including <i>H. nodiflorum</i> , as well as fennel, celery, parsnip and parsley (Blackman & Eastop 2018).	Not present in NZ. Damage level undetermined.
	<i>Pemphigus protospirae</i> Lichtenstein, poplar spiral- gall aphid	Gall-forming aphid (leaf sheath galls)	Europe (Blackman & Eastop 2018; Ellis 2020).	No. The primary host of this aphid is poplar (Hałaj & Osiadacz 2013). Secondary hosts are aquatic plants in the carrot family. <i>H. nodiflorum</i> is one of the 3 main secondary hosts for this aphid (Ellis 2020). Native range distribution probably limited by availability of habitat for the secondary hosts (Blackman & Eastop 2018).	Not present in NZ. Damage levels unknown, Galls form as spirals in the leaf sheaths (Hałaj & Osiadacz 2013; Ellis 2020). It is listed by MPI as an unwanted organism.

Order and Family	Species	Type of organism	Geographic range on <i>H. nodiflorum</i>	Likely to be sufficiently host specific?	Present in NZ? Likely to be highly damaging?
LEPIDOPTERA					
Elachistidae	<i>Depressaria pestinacella</i> (Duponchel) Synonym <i>D. radiella</i> (Goeze), parsnip webworm	Flower and seed feeder	UK (Emmet & Langmaid 2002)	No. Attacks only a small number of species in a small number of genera in the family Apiaceae, including the economically important parsnip.	Present in NZ. Potentially damaging. A pest of parsnip.
	<i>Depressaria ultimella</i> Stainton	Stem miner	UK (Heckford 1983; Emmet & Langmaid 2002; <u>Lewis 2018</u> ; Natural History Museum London 2020)	Oligophagous. Recorded on <i>H. nodiflorum</i> and other hosts in the tribe Oenantheae (<i>Oenanthe</i> <i>aquatica, O. crocata</i> and <i>Sium latifolium</i>) (Ellis 2020)[<u>Sp. Page</u>]. <i>H. nodiflorum</i> is the main host in the UK (Heckford 1983; Kimber 2020). Association with <i>O. crocata</i> in the UK appears to have been made in error. The moth on <i>O. crocata</i> is more likely the congener <i>D.</i> <i>daucella</i> (Heckford 1983).	Not in NZ. Causes wilting of side stems, and later main stems (Kimber 2020). Several parasitoids have been reared from <i>D. ultimella</i> (Heckford 1983). If no indigenous analogues exist in NZ, then release from parasitism can increase the potential impact of this moth. The moth is abundant in the UK despite parasitism (Heckford 1983). Damage in NZ by the congener <i>D. radiella</i> , the parsnip webworm, is sometimes described as 'dramatic'. This exotic pest has no parasitoids here. <i>Depressaria ultimella</i> is listed by MPI as an unwanted organism.

Appendix 3 – Steps in a biocontrol project

A classical biocontrol programme typically works through the following steps. This is usually done in a sequential manner, but some activities may occur concurrently.

- Explore the feasibility of project. If the project looks feasible, proceed.
- Survey the weed in places where biocontrol is desired. If any potential agents are found, explore ways to maximise them. If any likely impediments are found, look for ways to mitigate them.
- Undertake molecular studies of the weed to help narrow down the best place in the native range to find natural enemies.
- Unless natural enemies are already well known, survey the weed in its native range. Identify and study the life cycles of the natural enemies found.
- Determine the host range for potential agents. Abandon any species that do not appear to be safe or effective enough.
- Apply to authorities for permission to release the agents.
- If permission is granted, import, clear through containment, and develop rearing techniques for the new agents (if not already known).
- Mass-rear and release agents over several years.
- Monitor the establishment success and dispersal of agents over several years.
- Harvest and redistribute agents.
- Evaluate the success of the project. Decide if further agents are needed.