Survey of invertebrate and pathogenic fauna of tutsan (*Hypericum androsaemum*) and DNA analysis of New Zealand plant populations

Landcare Research
Manaaki Whenua

Survey of invertebrate and pathogenic fauna of tutsan (*Hypericum androsaemum*) and DNA analysis of New Zealand plant populations

Elizabeth Rendell

University of Birmingham, Intern with Landcare Research

Hugh Gourlay

Landcare Research

Prepared for:

Tutsan Action Group

Hikurangi House
Miriama Street
PO Box 221
Taumarunui 3946

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University of Birmingham Edgbaston, Birmingham B15 2TT, UK

Landcare Research, Gerald Street, PO Box 40, Lincoln 7640, New Zealand, Ph +64 3 321 9999, Fax +64 3 321 9998, www.landcareresearch.co.nz

Reviewed by:	*Approved for release by:*
Simon Fowler & Dagmar Goeke Researchers Landcare Research	Caroline Saunders Portfolio Leader Supporting Trade
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Contents

Summary

Project and Client

- A survey of the distribution and density of tutsan infestations, the invertebrate fauna and fungal pathogens associated with tutsan and genetic analysis of tutsan in New Zealand was carried out between November 2011 and March 2012 by Landcare Research for the Tutsan Action Group. This was a recommendation of the feasibility study investigating the prospects for the biological control of tutsan (*Hypericum androsaemum* L.) in New Zealand.

Objectives

- Identify the locations and severity of tutsan infestations in New Zealand.
- Survey the invertebrate fauna and fungi associated with tutsan, *Hypericum androsaemum*, in New Zealand and identify the herbivores (and their associated predators and parasitoids) and pathogens present.
- Complete genetic analysis of tutsan populations in New Zealand.
- Based on these findings, recommend further action to be taken in the Biological Control of Tutsan Programme.

Methods

- We surveyed 37 sites of tutsan (*Hypericum androsaemum* L.) from throughout New Zealand to record the distribution and density of tutsan in each region.
- The invertebrate fauna associated with tutsan was sampled at 13 of these sites; the samples were preserved and later identified.
- Signs of fungal pathogens associated with tutsan were searched for at all 37 sites.
- Leaf material was collected from all 37 sites, and later underwent genetic analysis.

Results

- The most severe tutsan infestations are in Taumarunui in the Ruapehu district and Eastern Bay of Plenty. Most sites of tutsan in the South Island consist of just a few plants.
- From the 13 sites sampled, we recovered fauna from 64 invertebrate groups. Few predatory groups were found associated with tutsan, the most abundant of which was spiders. Overall damage attributed to herbivory was found to be less than 4% at most sites.
- Two fungal species were recorded from 16 tutsan sites in New Zealand, *Melampsora hypericorum* and *Diploceras hypericinium*.

- There are two main strains of tutsan in New Zealand. One strain originated from the UK and the origin of the second strain will be determined from European surveys that are currently underway.

Conclusions

- Tutsan infestations are considerably more severe in the North Island.
- A range of exotic and native invertebrates attack tutsan in New Zealand, but none are specific to tutsan and overall damage is minimal.
- Although herbivory damage is minimal, most of that damage can be attributed to the bronze beetle and leaf roller caterpillars.
- There is scope for the introduction of host-specific biocontrol agents against tutsan in New Zealand. None of the herbivory niches on tutsan are well utilised and therefore a host-specific introduced biocontrol agent against tutsan is unlikely to face competition from existing herbivores in New Zealand.
- There is little genetic variation between tutsan populations in New Zealand.
- Tutsan plants in the South Island, Wellington and southern Hawke’s Bay originated from the UK. Analysis of tutsan plants in Europe will identify the origin of plants in the Ruapehu district and the rest of the North Island.
- Tutsan rust should be investigated further for biocontrol potential against tutsan in New Zealand.

Recommendations

- In light of our conclusion that there are no host-specific herbivorous invertebrates feeding on tutsan in New Zealand, we recommend the following actions (in order):
  1. Survey invertebrates associated with tutsan in parts of its native range, Europe. (These surveys have already begun and are being continued throughout 2013.)
  2. Analyse the DNA of tutsan plants in its native range (Europe) to identify the origin of tutsan plants in the North Island. (This work will be started in September 2012.)
  3. Survey tutsan rust present in Europe, to identify any strains of rust not currently present in New Zealand that may be more virulent against tutsan.
  4. For the most promising prospective invertebrate and fungal biocontrol agents, undertake host-range testing on non-target plant species of most importance to New Zealand.
  5. Introduce host-specific invertebrates and/or fungi to New Zealand as biocontrol agents against tutsan.

1 Introduction

In response to a recommendation of the feasibility study investigating the prospects for the biological control of tutsan (*Hypericum androsaemum* L.) in New Zealand, field surveys were carried out by Landcare Research during November and December 2011 throughout the North Island, and during February and March 2012 throughout the South Island, to collect information for the Tutsan Action Group.

Stage two of any biological control programme against a pest plant requires that invertebrates and fungal pathogens are surveyed in the pest's host range. These surveys look to identify whether (a) there are any invertebrates or fungi that are already acting as a biocontrol agent and (b) there are no invertebrates or fungi that would inhibit the success of a potential biocontrol agent in the future through competition, parasitism or predation.

As the first nationwide surveys to be conducted on tutsan in New Zealand, they were also conducted to identify how much of a problem tutsan is by providing an overview of the location and severity of infestations.

Since the tutsan rust fungus *Melampsora hypericorum* is a known biocontrol agent against tutsan in Victoria, Australia, tutsan rust samples were collected from New Zealand tutsan in an attempt to identify why the rust is not currently acting as a biocontrol agent in this country. The analysis of the rust collected is yet to be completed.

2 Background

Tutsan is a perennial, semi-evergreen shrub growing up to 1.5 m tall (Webb et al. 1988; Popay et al. 2010). It is frequently found on poor pasture, at forest margins and in wasteland in cool, temperate areas with high rainfall. It is unpalatable to stock, but has not been proven to be noxious to animals.

The name tutsan comes from the French words *tout* (all) and *sain* (healthy) in reference to various old medicinal uses for *Hypericum androsaemum.* Plants of the genus *Hypericum* (some species have been used since ancient times in the treatment of wounds and inflammations) were apparently gathered and burned to ward off evil spirits on the eve of St John's Day, thus giving rise to the genus' common name of St John's wort (Missouri Botanical Garden Plantfinder: http://www.missouribotanicalgarden.org).

Hypericum androsaemum is one of 10 species of *Hypericum* that have naturalised in New Zealand (Webb et al. 1988). There are also four native species – *H. involutum, H. pusillum, H. rubicundulum* and *H. minutiflorum. H. rubicundulum* and *H. minutiflorum* are both endemic to New Zealand, with *H. minutiflorum* listed as critically threatened (Heenan 2008; Groenteman et al. 2011).

Hypericum perforatum (St John's wort) is an exotic species of *Hypericum* that became a serious pasture weed in New Zealand from the 1930s. In 1943 and 1965 the lesser (*Chrysolina hyperici*) and greater (*C. quadrigemina*) St John's wort beetles were successfully introduced to control the weed (Groenteman et al. 2011).

Tutsan is native to parts of Europe (UK, Ireland, Belgium, Switzerland, The Netherlands, Spain, France, Bulgaria, Italy, former Yugoslavia and Portugal), Asia (Turkey, Turkmenistan, northern Iran, western Syria) (Clapham et al. 1987; Blamey & Grey-Wilson 1989; Parsons & Cuthbertson 2001), north-west Africa (Tunisia and north Algeria) and Caucasia (Georgia, Armenia, Dagestan and Azerbaijan) (Groenteman 2009).

Tutsan is capable of spreading with ease from forest margins, roadsides and waste areas into the surrounding pasture land; it will rarely establish at undisturbed sites. In the majority of locations tutsan does not threaten diversity of indigenous species in native forests, although this has been known to occur in an area near Mansfield, Victoria (Australia) and in Taumarunui, New Zealand.

Within New Zealand, tutsan is abundant in the Taumarunui district where it has become a serious pasture weed. It is also present along most roadsides within the district. Tutsan is spread by machinery on the farm, the roadside mower along roads and by birds.

St John's wort beetles were released against tutsan in the 1940s, but they failed to establish (Groenteman et al. 2011). No-choice laboratory tests proved that tutsan was a less than adequate host for the beetles and for this reason the beetles did not establish when released.

The feasibility study for the potential biological control of *H. androsaemum* was conducted by Ronny Groenteman in 2009. She concluded that tutsan is suitable for biological control since it has no economically important close relatives. However, there are two endemic species of *Hypericum* in New Zealand, and therefore a high level of host specificity would be required of an introduced agent (Groenteman 2009).

3 Objectives

- Identify the locations and severity of tutsan infestations in New Zealand.
- Survey the invertebrate fauna and fungi associated with tutsan, *Hypericum androsaemum*, in New Zealand and identify the herbivores (and their associated predators and parasitoids) and pathogens present.
- Complete genetic analysis of tutsan populations in New Zealand.
- Based on these findings, recommend further action to be taken in the Biological Control of Tutsan Programme.

4 Methods

Regional councils, Department of Conservation (DOC) representatives and regional park rangers were consulted as to the location of tutsan (*Hypericum androsaemum*) in each region. Tutsan was sampled at a total of 37 sites across New Zealand's North and South islands between November 2011 and March 2012 to identify the location and severity of each infestation(Figure 1). Invertebrate surveys of 13 of these sites were conducted to identify whether there were any damaging invertebrates present. Signs of fungal pathogen attack were searched for at all 37 sites and where found samples were collected. Leaf material was collected at all 37 sites to later undergo genetic analysis.

4.1 Tutsan density and distribution

For each site visited, the GPS location was determined, the land use noted, along with aspect where appropriate.

A suitable place to lay a 5 × 5 m quadrat was chosen and marked out at each of 10 sites using a 30-m tape measure and four marking poles. The density of tutsan was estimated visually within this quadrat.

The density of tutsan in the area surrounding the quadrat was also estimated visually and with photographs. A chart categorising the severity of infestations was later constructed based on these data and sites were categorised according to the following definitions (Table 1). Photographic examples of each category are shown in Appendix 1.

The abundance of new tutsan shoots and seedlings at the site were also noted. Average plant height of five tutsan plants at each site was recorded.

Meetings with landowners, regional pest plant officers or DOC representatives were also helpful in providing information on the plant population history of the site.

Table 1 Categories of infestation severity

Category	Criteria
1	Up to 10 individual plants present
2	Tutsan present in sites of up to 1000 m^2, below 5% plant density or small roadside infestation
3	Between 5 and 20% plant density, less than 50 ha or large roadside infestation
4	50–100+ha covered, less than 60% density
5	Over 100ha covered with tutsan at 60% density or above

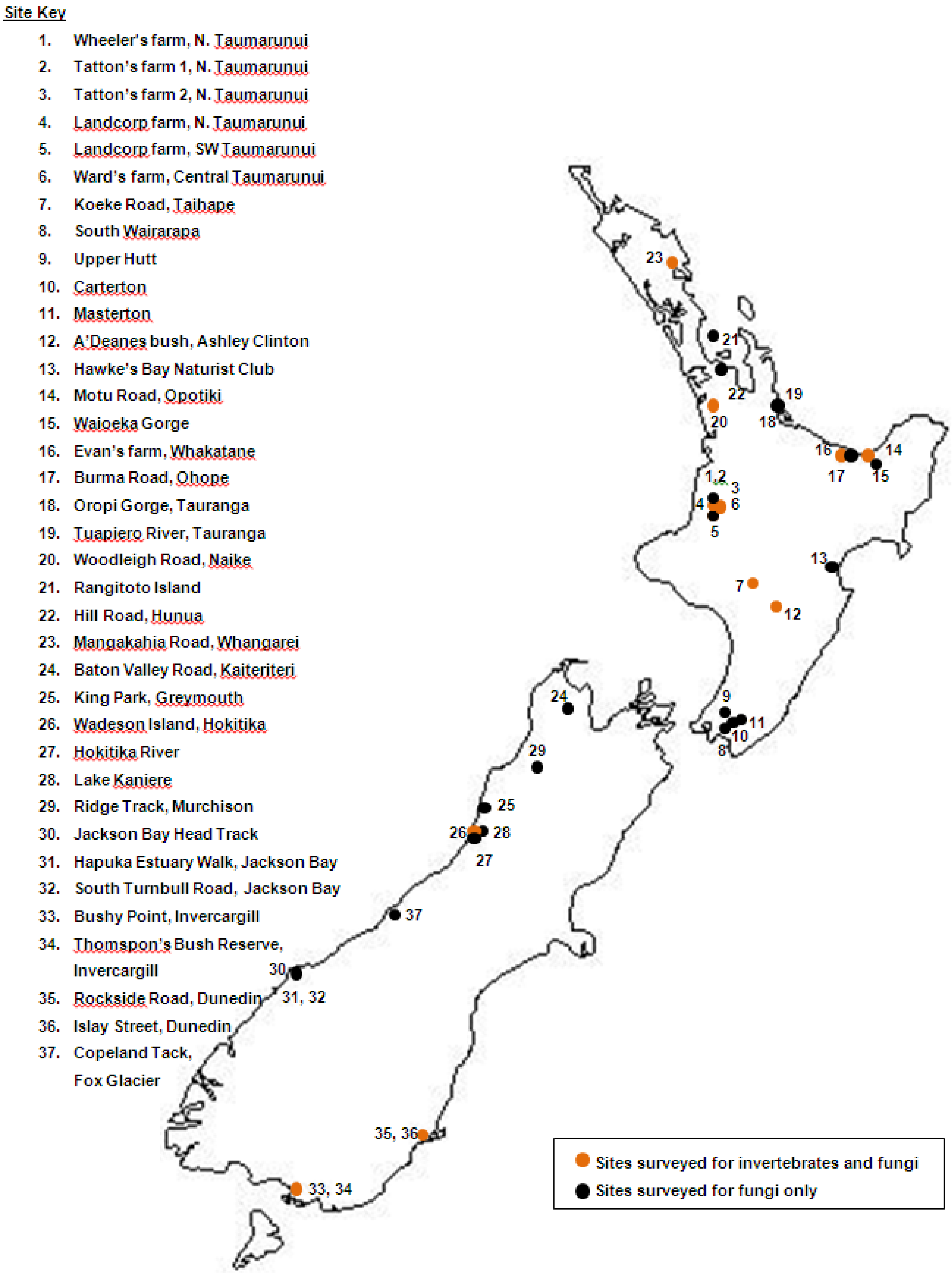

Figure 1 Sites where tutsan is present in New Zealand, visited in Summer 2011/12.

4.2 Invertebrates

A total of five plants at each site were randomly selected and sampled for invertebrates. A beating tray (80 × 80 cm) was placed under the plant and the plant was beaten five times with a solid stick. The invertebrates that dropped onto the tray were collected with the use of an aspirator, preserved in 95% ethanol, and later identified to genus or species level where possible.

Each of the five plants at each site were briefly inspected for signs of other invertebrates such as leaf miner, scale insects, gall-formers and stem-borers. If found these were preserved in ethanol on the plant material they were found on and later identified to genus or species level where possible.

At each site, five stems were selected randomly, and the youngest 10 leaves were inspected for herbivore damage. Where found, the damage was estimated as a percentage of each leaf and these estimates were later combined to give an average of the herbivore damage per site.

All invertebrates were then ranked using a scale of abundance according to the number of individuals collected and number of sites at which they were present. They were classed as rare, occasional, common or abundant according to the definitions below (Table 2).

Table 2 Categories for abundance analysis

Abundance	Criteria
Rare	<4 individuals collected
Occasional	4–15 individuals collected OR >15 individuals collected, but present at less than 4 sites
Common	16–119 individuals collected and/or present at more than 4 sites
Abundant	120+ individuals collected and present at more than 6 sites

4.3 Fungi

The tutsan plants at each site were inspected for signs of rust fungus. If found, up to five infected leaf samples were removed and placed in ziplock bags containing silica gel for immediate drying. The samples were sent to Landcare Research, Lincoln, for storage until the rust DNA could be analysed at a later date.

The extent of the fungal infection was noted for each site. Whether the infection was present on every plant in the quadrat or on every leaf of those that were infected was recorded. Plants that appeared to be controlled by the fungus were photographed and sampled. The data were later used to create an index of rust infection to categorise sites according to the definitions below (Table 3). Photographs of different categories of rust infection are shown in Appendix 2.

Table 3 Categories for extent of rust infection

Category	Criteria
1	Less than 10 rust pustules per leaf
2	Between 10 and 100 rust pustules per leaf
3	Over 100 rust pustules per leaf
4	A severe infection with over 100 pustules per leaf and leaves have started to deform or distort as a result of infection
5	Leaves are necrotic and abscise as a result of rust infection

4.4 Leaf material

Out of the 37 sites visited leaf material was collected from only 24, 16 in the North Island and 8 in the South, and used in the DNA analysis studies. This sample size is representative of all of the plant populations around the country. At each site, five plants were randomly selected and leaf samples taken for later DNA analysis. The leaves selected were those free of herbivore, fungal or any other damage symptoms. Once removed the leaves were placed in individual ziplock bags containing silica gel for immediate drying. The samples were sent to Landcare Research, Lincoln, for storage.

DNA was extracted in the molecular laboratory at Landcare Research, Lincoln, from the dried leaf samples collected from each site. These were sent to Landcare Research, Auckland, for sequencing.

5 Results

5.1 Tutsan density and distribution

A large number of the sites visited were roadside infestations, which made laying a quadrat difficult. The majority of sites in the South Island were not sampled using a quadrat since sites often consisted of just a few plants. Quadrat density at the sampled sites is shown in Figure 2.

From Figure 2 it can be seen that Hokitika appeared to host the highest density of tutsan. However, the dense scrub present at this site limited the size of the quadrat to 3 × 3 m as it had to be placed between trees. Had the full 5 × 5 m quadrat been surveyed at this site the density would have been lower due to the scrub utilising some of the space inside the quadrat.

Taumarunui hosted the highest densities of tutsan (Figure 2), with quadrats surveyed being up to 60% covered in tutsan. It was noted that the area surrounding the quadrats often appeared to have an even higher density of tutsan. This was due to the topography of the area as these sites were steep hillside pastures, which made laying a quadrat at the highest density area difficult, particulary at Sites 1 and 4 at Taumarunui. Opotiki and Whakatane also hosted tutsan in relatively high densities, with the quadrats having 45% and 50% tutsan cover respectively.

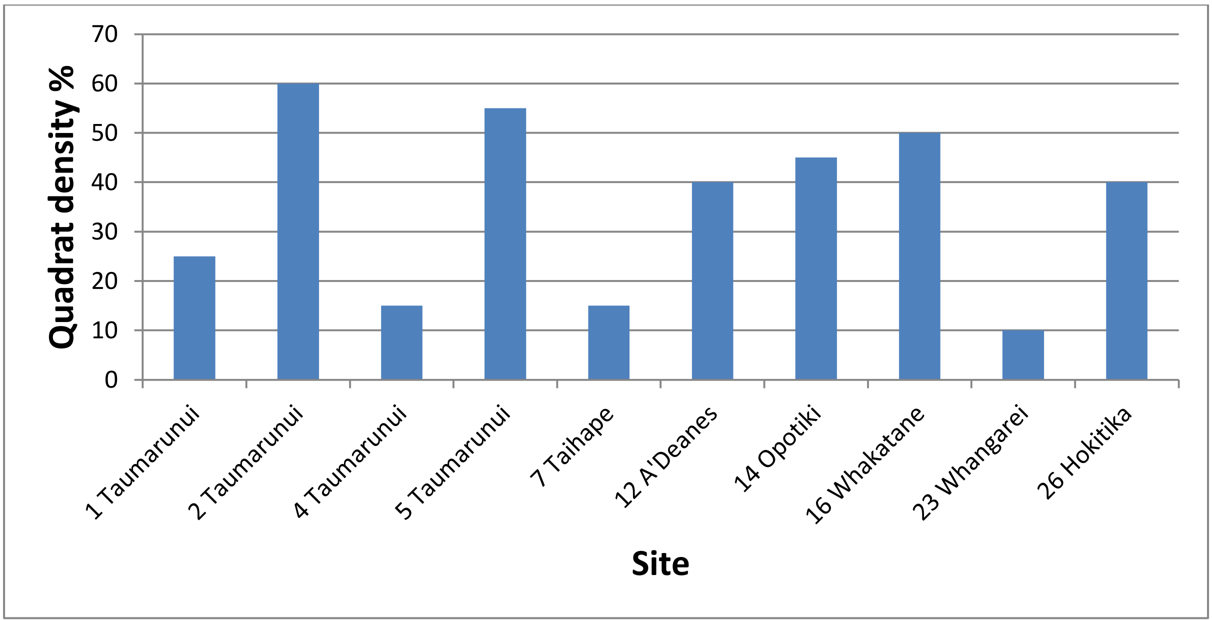

Figure 2 Quadrat density, a measure of tutsan peak density at the 10 sampled areas.

Once sites had been sampled, an index of infestation severity was drawn up to categorise the data collected for both distribution and density. This gave an overview of the severity of tutsan infestations in each area (Figure 3). Photographic examples of each category are shown in Appendix 1. Categories were assigned to each location according to the highest level of infestation found in that area.

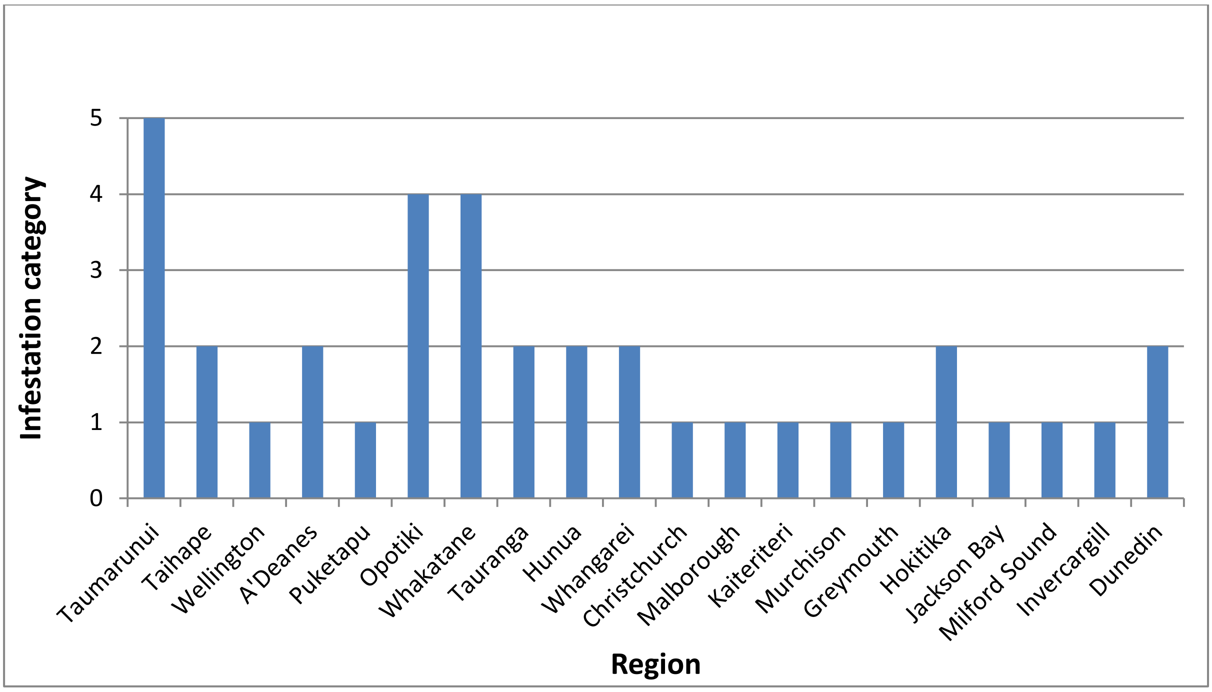

Figure 3 Infestations of tutsan in New Zealand.

Taumarunui hosts the worst infestations in New Zealand, assigned to Category 5. In 2008 it was estimated that 1500 ha in the district were infested with greater than 60% cover (see Figure 4) and new sites have been noted since. Tutsan has always been present throughout the area in lower densities than are experienced today. Population densities are reported to have increased markedly over the last 15 years.

Figure 4 Pasture in Taumarunui, infested with tutsan .

Eastern Bay of Plenty also suffers from a few large infestations of tutsan, with the weed invading both pasture and roadsides. Invasions on farmland in Opotiki and Whakatane are as high as Category 4 in some areas. Tutsan density is highest in these areas on properties of low fertility such as ex-forestry blocks. Its population growth in some areas such as the Waioeka Gorge has gone unnoticed, not being part of local pest plant management strategies since it was once not very abundant in the Bay of Plenty.

In Northland and Auckland, tutsan appears to be a common plant, found scattered on many roadsides and riverbanks. It is not considered problematic in these areas in comparison to other weeds. Regional council biosecurity staff monitor these sites annually for signs for population growth, as the populations in other areas have spread quite rapidly.

There are only a few sites where tutsan is known to grow in Hawke's Bay. Populations are small, and the most severe in A'Deanes bush has been assigned to Category 2. This population has been present for 15 years and is reported to be spreading. Control by spraying has occurred at this site.

Greater Wellington hosts the least tutsan of any region in the North Island, with no more than 10 individual plants known in the whole Greater Wellington area at the time of sampling. Plants are hand-pulled when found.

Only a few plants were located during surveys on Rangitoto Island; this population has been reported to have declined considerably since the 1990s.

The majority of sites in the South Island have been assigned to Category 1. These sites consist of a few individual plants. Tutsan was not found in Christchurch, Marlborough, or Milford Sound during these surveys since plants occur in such low densities in these areas, their locations are not well known. They have been included in the graph as they are known to grow in these areas despite not being found in this instance.

At the time of the surveys, sites of tutsan in the South Island that were larger than a few plants were present on the Hokitika River and on Rockside Road in Dunedin. These sites are relatively small compared with sites found in the North Island.

Tutsan was known to be a problematic pasture weed in Otago in the 1940s, and reports from landowners at the time suggest it disappeared very quickly due most likely to the impact of the tutsan rust. Since this marked decrease, tutsan has not been a problematic weed in South Island.

In theory the presence of many new seedlings indicated a possible growth in the population, whereas an absence implied a static or declining population. However, during the surveys seedlings were found to be present at all sites in varying numbers and did not seem to reflect a known growth or decline in the populations. The height of plants was also measured in an attempt to infer population growth or decline, but at most sites visited plants were of uniform height at 1.1 m tall (tutsan grows to about 1 m by 1 m; www.aussiegardening.com.au).

5.2 Invertebrates

A total of 13 sites were found to be suitable for invertebrate surveying. Only three suitable sites were found in the South Island, with the majority of locations consisting of 1–5 individual plants, which were mostly seedlings. Sites with very low densities of tutsan were also not sampled since this would not add significant information to the dataset.

Roadside sites are obscure ecological niches and the invertebrates found there are not usually representative of invertebrates associated with the plant in other locations such as pastures. There is also danger involved in sampling at roadside sites without a footpath. For these reasons roadside sites were not sampled for invertebrates.

A total of 64 groups of invertebrates were collected from across New Zealand (Table 4). None of these were found to be specialists on tutsan. There were individuals for which identification was not possible due to excessive damage or juvenile stages that were too immature to identify.

Table 4 Invertebrates associated with *Hypericum androsaemum* in New Zealand (Indet. = indeterminate)

Taxon	Common name	Feeding site	Origin	Site	Abundance	Individuals
Phylum Arthropoda						
Class INSECTA	Insects					
COLEOPTERA	Beetles					
Indet. larvae		Species dependent	-	4,14,16,23	Occasional	6
Chrysomilidae	Leaf beetles					
Eucolaspis brunnea	Bronze beetles	Phytophagous	Native	2,5,7,14,20,23	Common	31
Curculionidae	Weevils					
Rhopalomerus sp.		Pollen feeder	Native	35	Rare	1
Irenimus sp.		Phytophagous	Endemic	5	Rare	1
Heplocneme sp.		Phytophagous	-	5	Rare	1
Indet. sp. 1		Phytophagous	-	35	Rare	1
Indet. sp. 2		Phytophagous	-	7	Rare	1
Scarabidae	Scarab beetles					
Pyronota sp.		Phytophagous	Native	3	Rare	1
Cryptophagidae	Cryptic beetles					
Indet. sp. 1		Mycetophagous	-	4,16,26	Rare	4
Indet. sp. 2		Mycetophagous	-	35	Rare	1
Melandridae	Leaping beetles					
Indet. sp.		Species dependent	Native	14	Rare	1
Nitulidae	Sap beetle					

Taxon	Common name	Feeding site	Origin	Site	Abundance	Individuals
Hisparonia hystrix		Sap/fungal feeder	Endemic	14	Rare	1
Melyridae	Flower beetle					
Halyles nigrescens		Phytophagous	Native	5	Rare	1
Scirtidae	Marsh beetles					
Indet. sp.		Predatory	-	35	Rare	1
Anthicidae	Ant beetle					
Sapintus pellucidipes		Omnivorous	Endemic	5	Rare	1
Latridiidae	Mildew beetles					
Corticaria sp.		Mycetophagous	-	35	Rare	2
Indet. sp. 1		Mycetophagous	-	4	Rare	1
Indet. sp. 2		Mycetophagous	-	16,2	Rare	2
Indet. sp. 3		Mycetophagous	-	16	Rare	1
Staphylinidae	Rove beetle					
Indet. sp.		Predacous	-	35	Rare	1
Corylophidae	Hooded beetle					
Indet. sp.		Mycetophagous	-	35	Rare	1
Anthribidae	Fungus weevils					
Sharpus sp.		Mycetophagous		35	Rare	1
Coccinellidae	Ladybirds					
Indet. sp.		Mostly predacous	-	35	Rare	1

Taxon	Common name	Feeding site	Origin	Site	Abundance	Individuals
HEMIPTERA						
Aphrophoridae	Spittle bugs					
Philaenus spumarius	Meadow spittlebug	Phytophagous	Adventive	26,35	Occasional	6
Carystoterpa sp.		Phytophagous	Endemic	5	Rare	1
Indet. juveniles		Phytophagous	-	2,4,5,7,14,16	Common	27
Cixiidae	Plant hoppers					
Aka dunedinensis		Herbivorous	Endemic	35	Rare	1
Parasemo sp.		Herbivorous	Endemic	26	Rare	1
Acanthosomatidae	Shield bugs					
Oncacontias vittatus	Forest shield bugs	Phytophagous	Endemic	26,35	Rare	3
Miridae	Mirid bugs					
Diomocoris sp.		Sap feeder	Endemic	6	Rare	1
Indet. sp.		-	-	5	Rare	1
Lygaeidae	Seed bugs					
Indet. sp.		Phytophagous	-	5	Rare	1
Aphidea	Aphids					
Indet. sp.		Sap feeders	-	2,4,5,16,23,26,35	Common	58
Psyllidae	Psyllid					
Indet. sp.		Phytophagous	-	12	Occasional	14
Cicadidae	Cicadas					
Indet. sp.		Sap feeder	Endemic	1	Rare	1

Nabidae	Damsel bugs					
Indet. sp.		Predatory	-	16	Rare	1
ORTHOPTERA	Crickets, grasshoppers, weta					
Indet. Orthoptera		-	-	2,5	Rare	2
Tettigoniidae	Long-horned grasshoppers					
Conocephalus sp.	Field grasshopper	Phytophagous	Native	16	Rare	1
Anostostomatidae	Giant weta					
Hemideina sp.	Native tree weta	Omnivorous	Endemic	1	Rare	2
Raphidophoridae	Cave weta					
Indet. sp. (juvenile)		Omnivorous	-	1,6,23	Occasional	4
Phasmatodea	Stick insect					
Indet. juvenile		Phytophagous	Native	5	Rare	2
ARCHAEOGNATHA	Bristle tails					
Indet. juvenile		Scavenger	-	35	Occasional	26
Subclass PTERYGOTA						
DIPTERA	True fly					
Indet. sp.		Various	-	2,4,23,26,33,35	Occasional	11

Taxon	Common name	Feeding site	Origin	Site	Abundance	Individuals
LEPIDOPTERA	Butterflies, moths					
Geometridae	Looper moths					
Pseudocoremia sp. 1		Phytophagous	Endemic	16,23,33,35	Occasional	4
Declana leptomera		Phytophagous	Endemic	14	Rare	1
Declana floccosa	Forest semilooper	Phytophagous	Endemic	4	Rare	1
Tortricidae	Leaf rollers					
Epalxiphora axenana	Sharp tipped bell moth	Phytophagous	Endemic	2	Rare	1
Planotortrix excessana		Phytophagous	Endemic	26	Rare	1
Ctenopseustis herana	Brown headed leaf roller	Phytophagous	Endemic	35, 37	Rare	3
Pyrgotis plagiatana	Painted wedge	Phytophagous	Endemic	35	Rare	1
Noctuidae	Armyworms, cut worms					
Graphania ustistriga		Phytophagous	Endemic	4,26	Rare	2
Stathmopodidae						
Stathmopoda sp.		Phytophagous	E/a	35	Rare	1
Gracillariidae?	Leaf mining moths					
Indet. sp.		Phytophagous	-	37	Rare	1
NEUROPTERA						
Micromus tasmaniae	Brown lacewing	Predatory	Exotic	2, 4, 12, 14,35	Occasional	8

Taxon	Common name	Feeding site	Origin	Site	Abundance	Individuals
PLECOPTERA	Stonefly					
Indet. sp. 1		Scavenger	-	4, 23	Rare	2
Indet. sp. 2		Scavenger	-	7	Rare	1
EPHEMEROPTERA	Mayfly					
Indet. sp.		No mouth parts	-	1	Rare	1
HYMENOPTERA	Ants, wasps, sawflies					
Indet. Hymenoptera		-	-	35	Rare	1
Ichneumonidae	Ichneumon wasps					
Indet. sp. 1		Parasitic	-	35	Rare	1
Indet. sp. 2		Parasitic	-	26	Rare	1
Figitidae	Gall wasps					
Indet. sp.		Parasitic	-	35	Rare	2
Braconidae	Parasitic wasps					
Indet. sp.		Parasitic	-	35	Rare	1
Diapriidae?						
Indet. sp.		Parasitic	Endemic	35	Rare	1
Formicidae	Ants					
Indet. sp.		Omnivorous	-	1	Rare	1

Taxon	Common name	Feeding site	Origin	Site	Abundance	Individuals
AMPHIPODA						
Taltridae	Lawn shrimp					
Parorchestia ihurawao		Scavenger	Endemic	35	Occasional	26
Puhuruhuru aotearoa		Scavenger	Endemic	26	Rare	4
ISOPODA	Slaters					
Armadillidium vulgare	Pill bugs	Scavenger	Introduced	35	Occasional	52
Indet. sp.	Slaters	Scavenger	-	2,35	Occasional	5
Class GASTROPODA	Slugs and snails					
Indet. snails	Snails	Phytophagous	-	16,35	Rare	3
indet. slugs	Slugs	Phytophagous	-	35	Rare	1
Class ARANEIDA	Spiders					
Indet. sp.		Predatory	-	all sites	Common	185
Class DIPLODA	Milipedes					
Indet. Diplopoda		Saprophytic	-	35	Occasional	15

Herbivores

A total 28 groups of herbivorous invertebrates were collected from tutsan during this survey. One species, the bronze beetle (*Eucolaspis brunnea*), was classed as 'common', along with unidentified Aphrophoridae that were grouped together in a single taxonomic unit. Two species were listed as occasional – *Pseudocoremia* sp. and *Philaenus spumarius*, along with an unidentified species of juvenile psyllid found present at site 13 (in Hawke's Bay). A further 23 herbivorous groups found were listed as 'rare'.

The amount of herbivore damage recorded on tutsan plants attributable to herbivorous invertebrates was considered minimal. It was very unusual for herbivore damage to be over 4% of the total leaf area, with damage at most sites recorded as less than 1%. However, herbivore damage at Site 9 Upper Hutt was recorded at 20% and was attributed to the bronze beetle (Figure 5).

Figure 5 Damage to tutsan at Upper Hutt caused by herbivory by the bronze beetle (*Eucolaspis brunnea*).

Eucolaspis brunnea: This species was found to cause most herbivore damage to tutsan plants in the North Island. It is a species of leaf beetle commonly known as bronze beetle and is endemic to New Zealand (Rogers et al. 2006). It is an herbivorous insect that has been found to feed on a range of exotic and native, broadleaved and coniferous plants (Kay 1980). It was a significant pest in apple orchards around the 1920s and 1930s, requiring the use of organochlorine pesticides to control high population numbers (Clearwater & Richards 1984; Rogers et al. 2006). Its pest status and generalist feeding habits mean it is unsuitable for inundation as a biocontrol agent.

Leaf rollers: In the field surveys conducted in the South Island in February 2012, leaf roller caterpillars were found responsible for most herbivore damage to plants (Figure 6). A number of leaf roller species were identified; they are all generalist feeders on conifers and dicotelydonous angiosperms with wide host ranges (J. Dugdale, pers. comm.) and are therefore not appropriate for use as a biocontrol agent.

Figure 6: Evidence of leaf-roller caterpillars feeding on tutsan plants.

Sap-feeders: Aphids as a collective group were found to be common on tutsan plants. Further identification was not possible due to the fragile nature of aphid antennal and tarsal structures. It is these structures that are most needed for identification purposes. Furthermore, the damage caused by sap-feeders either through direct removal of nutrients or indirectly through entry of pathogens into stem punctures is very difficult to quantify.

Fruit feeders: Only one individual fruit feeder was found during this survey; it was identified as *Ctenopseustis herana*. The species is a generalist feeder with a wide host range in New Zealand and is therefore not appropriate for biological control. One species of Lygaeidae (unidentified) was recorded from this survey, a family whose members are known to feed on seeds; however, only one individual lygaeid was found across all field sites.

Leaf-miners, stem-borers, gall-formers and scale insects: No leaf-mining, stem-boring, gall-forming or scale invertebrates were recorded during this survey of tutsan plants. Juvenile Psyllidae are known to form galls in the leaves of plants but no evidence of this behaviour was recorded during the surveys despite their presence at Site 6 Central Taumarunui. There is scope for an introduced agent to utilise these niches without competition from the existing fauna associated with tutsan.

Non-herbivorous insects

Predators: Five predatory groups were recorded from this survey (Table 1); this list provides a reference when prioritising possible biocontrol agents in the future. Four of the five predatory groups were classed as 'rare'. '*Araneida*' were classed as 'common' and were found at all sampled sites. A relatively low number of predatory groups are found associated with tutsan, therefore it appears unlikely that the success of a potential biocontrol agent will be inhibited by predation.

Parasitoids: Most families of *Hymenoptera* that were collected are endoparasitic. The list in Table 1 provides a reference of parasitic species that may inhibit an introduced agent. However, with relatively low numbers, this appears unlikely.

Fungal-feeders: Nine groups of fungal-feeding invertebrates were recorded from tutsan plants in this survey. The low abundance of fungal feeders recorded suggests that it is unlikely that fungal feeders would have a detrimental effect of the success of a potential fungal agent. These insects also have the potential to aid dispersal of fungal spores.

5.3 Fungi

Signs of fungal infection were searched for at all 37 sites in New Zealand. A total of two fungal species were identified from 16 sites. The most common fungus, *Melampsora hypericorum*, was identified from symptomatic plant tissues and the remaining fungus, *Diploceras hypericinium*, was identified by being isolated out of plant tissues into pure culture by Peter Johnston, Landcare Research, Auckland.

Diploceras hypericinium

Diploceras hypericinium was found present on tutsan plants at four sites across New Zealand. It appears as brown spots on leaves (Figure 7) and can cause leaf blight (Groenteman 2009). *D. hypericinium* is known to attack other species of *Hypericum* and is not virulent to *H. androsaemum* and would have to be used as a bioherbicide – which is unlikely to be economically viable in the case of tutsan (Groenteman 2009). Furthermore the topography of the Taumarunui area, where the most severe infections lie, has previously inhibited successful application of herbicides.

Figure 7 Tutsan leaf infected with *Diploceras hypericinium.*

Melampsora hypericorum

The highest level of damage to tutsan caused by fungi was from *Melampsora hypericorum*, 'tutsan rust'. It was found at 13 sites across both islands. The symptoms of the tutsan rust fungus first appear as raised orange pustules on the underside of *H. androsaemum* leaves. As the infection progresses, the number of pustules becomes more prevalent and leaf shape can begin to distort as a result (Appendix 2). The final stages are necrosis of the leaves and some leaf abscission.

The extent of infection by *M. hypericorum* is exhibited in Figures A2.1–3 (Appendix 2). Infection by tutsan rust was found to be severe at three of the sites visited: Hawke's Bay Naturist Club, Woodleigh Road (Naike) and South Turnbull Road (Jackson Bay). A greater number of sites in the South Island were infected by the rust fungus than in the North Island (Figure 8). However, this could be attributed to the timing of sample collection. Symptoms of rust infection first appear on tutsan from December. North Island sites were sampled in November and December so it is possible that samples were taken before rust symptoms had a chance to show, but would have been present if sampling had been done later in the year. Contact with pest plant officers at regional councils was helpful in providing information and samples of the fungus in March 2012, although some still reported no disease symptoms present on infestations in their area at this time.

Genetic analysis of the tutsan rust collected during these surveys is due to be carried out in 2012.

Melampsora hypericorum has been reported to control invasive populations of *H. androsaemum* in Victoria, Australia. As a rust fungus it is likely to be highly host specific, as many species of rust fungus attack a single species or single biotype within a species (Groenteman 2009). Tests into infection of other *Hypericum* species by *M. hypericorum* suggest that its host range is restricted to tutsan.

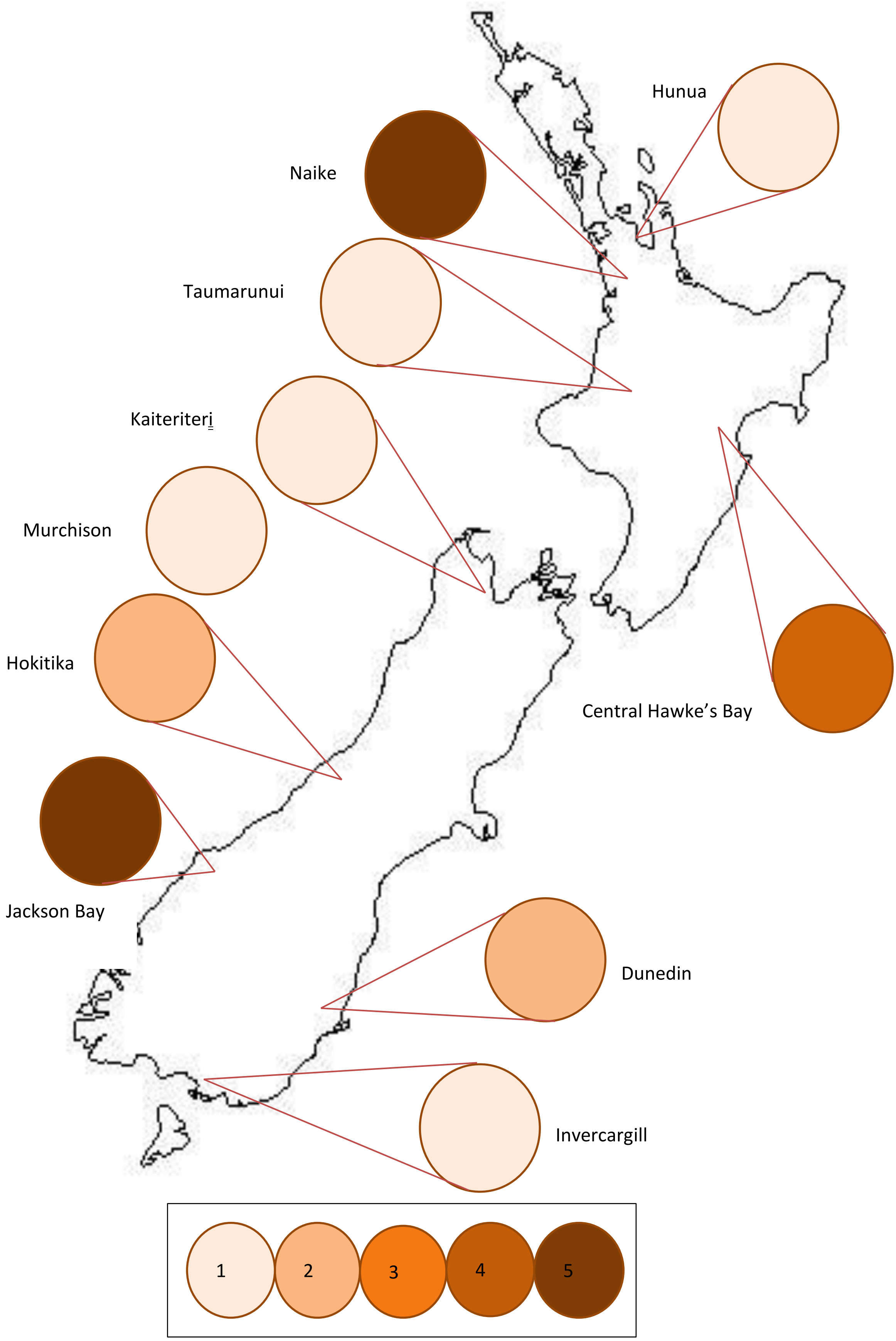

Figure 8 Abundance of tutsam rust found on tutsan (*Hypericum androsaemum*) in New Zealand.

5.4 Populations genetics

The DNA of tutsan plants was isolated and sequenced for 24 populations of plants in New Zealand. The results showed that there are four biotypes of tutsan in New Zealand (Table 4).

The genetic analysis of tutsan samples shows that there is little variation between plant populations in New Zealand. Comparisons with known sequences for tutsan plants in the UK shows that tutsan plants in the South Island, Wellington, and southern Hawke's Bay have originated from the UK.

Type 1 and 4 plants differ from each other by one nucleotide in an ~800-base-pair chain of nucleotides. This minimal difference suggests that Type 4 plants have originated from a nearby location to that of Type 1 plants.

Table 5 Location of tutsan (*Hypericum androsaemum*) biotypes in New Zealand

Location	Plant strain
Taumarunui	Type 1
Taihape	Type 1
Bay of Plenty	Type 1
Auckland	Type 1
Northland	Type 1
Hawke's Bay Naturist Club	Type 2
Naike	Type 3
A'Deanes bush, South Hawke's Bay	Type 4
Greater Wellington	Type 4
Kaiteriteri	Type 4
Murchison	Type 4
West Coast	Type 4
Invercargill	Type 4
Dunedin	Type 4

6 Conclusions

6.1 Tutsan distribution and density

Tutsan is widely distributed around New Zealand. In most regions populations are small and manageable with current control methods.

In the South Island, tutsan plants are widely distributed, but the density of these plants is very low. Most sites visited during the surveys consisted of a few individual plants and are not considered problematic. The largest known infestation of tutsan in the South Island is on the Hokitika River. Tutsan plants were not located in Canterbury, Marlborough or Fiordland during these surveys since their populations are very sparse, not problematic, and tend to be dealt with when found.

Tutsan is also widely distributed around the North Island and the density of plants is much more varied. Infestations in parts of the North Island are severe, the most severe infestations occurring in the Ruapehu district, around Taumarunui.

Infestations around Taumarunui are very severe and not manageable under current control methods due to the size of the infestations, topography of the region, and the less than adequate herbicides available to control tutsan effectively.

Tutsan populations appear to be increasing in density in eastern Hawke’s Bay around Opotiki and Whakatane. These populations should be monitored for signs of increase as this can be rapid.

6.2 Invertebrates

The invertebrate surveys carried out on tutsan populations in New Zealand revealed that there do not appear to be any specialised tutsan-feeding invertebrates among the existing associated fauna. *Eucolaspis brunnea*, a leaf beetle, and leaf-rolling caterpillars are responsible for the majority of herbivore damage to tutsan in New Zealand; however, none of these are host-specific to tutsan. Since there are two native and two endemic species of *Hypericum* present in New Zealand, a high degree of host specificity would be required of any potential agents. With herbivory recorded at less than 4% within the majority of populations in New Zealand, damage incurred by these existing herbivores is not considered significant.

A future biocontrol agent against tutsan is unlikely to face any threats from competition with existing invertebrates associated with the weed in New Zealand since the herbivory niches are not well utilised. Some niches, such as leaf-mining, gall-forming and stem-boring, are not currently utilised at all. Therefore there is scope for the successful introduction of a future agent to markedly reduce the vigour of tutsan in New Zealand through the use of these niches.

The combined effect of generalist predators found associated with tutsan is unlikely to inhibit an introduced biocontrol agent due to the relatively low numbers found at present. However, any potential agents discovered with future research would need to be assessed with regard to the predators found in this survey in order to make further conclusions.

From the low abundance of recorded parasitoids associated with tutsan, it can be assumed that the success of an introduced biocontrol agent is unlikely to be inhibited by parasitism. If a potential agent is discovered in future research, the list of parasitoids given here (Table 1) can be used to identify any potential host–parasitoid interactions between the introduced agent and existing parasitoids.

6.3 Fungi and future prospects

The tutsan rust fungus (*Melampsora hypericorum*) is used currently as a method for controlling infestations of tutsan in Victoria, Australia. The fungus has autonomously spread to and among many populations of tutsan in New Zealand. The rust is capable of administering a high level of control on tutsan populations, as seen in Australia, and has the necessary highly host specific characteristic required of a potential agent in this programme. *M. hypericorum* could potentially be an effective biocontrol agent against tutsan in New Zealand. Currently the rust fungus is not naturally administering the level of control necessary to reduce large populations of tutsan, such as those in the Ruapehu district, in New Zealand. Further research investigating the strains of tutsan rust present in New Zealand and in tutsan's native range would need to occur to establish the reason for this.

6.4 Population genetics

There is little variation between populations of tutsan present in New Zealand, and the analysis shows that one strain of plants was probably introduced from the UK (Type 4). This population is found predominantly in the South Island. Genetic analysis of tutsan populations in Europe will provide more information on the origin of the plant populations in the North Island (Type 1).

The lack of significant variation between populations is advantageous if a rust strain is to be introduced as a potential biocontrol agent since they tend to be highly specific and are known to attack a single plant strain within a species.

Genetic analysis of plant populations in the UK and Europe are planned to be carried out in 2012 to enable the origin of Type 1 (main North Island strain) plants to be established and the origin of type 4 plants confirmed.

7 Recommendations

In light of the conclusion that there is no significant invertebrate damage on tutsan in New Zealand, it is suggested that *Hypericum androsaemum* be sampled for its associated invertebrates in its native range. This would provide information as to whether there are any natural enemies that are controlling native populations and that have the potential to be introduced into New Zealand as biocontrol agents.

The status of *Melampsora hypericorum* in Victoria, Australia, as a biocontrol agent against tutsan provides a possible direction to further the study into the biological control of this species.

Suggestions for further study (in order) are:

1. Survey invertebrates associated with tutsan in parts of its native range, Europe, to look for any potential biocontrol agents. (These surveys have already begun and are being continued throughout 2012.)

2. Analyse the DNA of tutsan plants in its native range (Europe) to identify the origin of tutsan plants in the North Island. (This work will be started in September 2012.)

3. Survey tutsan rust present in Europe, to identify any strains of rust not currently present in New Zealand that may be more virulent against tutsan.

If a suitable biocontrol agent is discovered through future research, further steps in this biological control programme would proceed as follows:

4. For the most promising prospective invertebrate and fungal biocontrol agents, undertake host-range testing on non-target plant species of most importance to New Zealand.

5. Introduce host-specific invertebrates and/or fungi to New Zealand as biocontrol agents against tutsan.

8 Acknowledgements

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Appendix 1 – Infestation index for tutsan in New Zealand

Category	Criteria
1	Up to 10 individual plants present
2	Tutsan present in sites of up to 1000 m^2, below 5% plant density or small roadside infestation
3	Between 5 and 20% plant density, less than 50 ha or large roadside infestation
4	50–100+ ha covered, less than 60% density
5	Over 100 ha covered with tutsan at 60% density or above

Figure A1.1 Category 1 Infestation: Kaiteriteri roadside.

Figure A1.2 Category 2 Infestation: Opotiki pasture.

Figure A1.3 Category 2 Infestation: Tauranga roadside (similar density continues along the roadside for a few kilometres**).**

Figure A1.4 Category 3 Infestation: Taumarunui pasture.

Figure A1.5 Category 3 Infestation: Tauranga roadside (similar density continues along the roadside for a few kilometres).

Figure A1.6 Category 4 Infestation: Whakatane (note bronze patches on opposite hillsides are also tutsan plants).

Figure A1.7 Category 5 Infestation: Taumarunui farmland

Appendix 2 – *Melampsora hypericorum* Index

Table A2 *Melampsora hypericorum* Index based on New Zealand observations

Category	Extent of Infection
Category 0	No rust infection present
Category 1	Less than 10 rust pustules per leaf
Category 2	Between 10 and 100 rust pustules per leaf
Category 3	Over 100 rust pustules per leaf
Category 4	A severe infection with over 100 pustules per leaf and leaves have started to deform or distort as a result of infection
Category 5	Leaves are necrotic and absise (defoliate through abscission) as a result of rust infection

Figure A2.1 Category 3: Over 100 pustules.

Figure A2.2 Category 4: Leaf shape starting to distort as a result of the infection; over 100 pustules are visible on the underside of the leaf.

Figure A2.3 Category 5: Most of the plant defoliated as a result of the infection.