Molecular detection and pathology of the oomycete *Albugo candida* (white rust) in threatened coastal cresses

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ABSTRACT

This study describes the development and testing of a PCR-based assay for the detection of the internal transcribed spacer region 1 (ITS 1) of the ribosomal DNA (rDNA) of *Albugo candida*. Similar PCR-based approaches have become widely used to detect *Albugo* spp. and other fungi within plant tissue. Primers were designed based on differences in the ITS 1 sequences between *A. candida* and *Lepidium oleraceum*. The primers preferentially amplified the *A. candida* ITS 1 from tissue samples in which the plant ITS 1 template predominated. *Albugo candida* ITS 1 DNA was detected in symptomatic host tissue as well as in a number of asymptomatic plants collected from Auckland Regional Botanic Gardens, New Zealand. *Albugo candida* ITS 1 DNA was also detected in surface-sterilised seed of *L. oleraceum*. These findings suggest that in the native host *L. oleraceum*, *A. candida* can exist as a latent asymptomatic infection and can also be transmitted vertically through seed. With more development, this technique will have the ability to detect *Albugo* in wild and *ex situ* populations, and could assist in efforts to ensure that wild stocks are not placed at risk by contamination from restoration plantings. However, because the possibility of false negatives has not been eliminated, further research is required to ensure unambiguous detection. A study on the impact of *A. candida* infection on plant growth and mortality in *L. oleraceum* seedlings failed to conclusively demonstrate that the pathogen imposes a significant fitness cost. Further studies are required, including growing seedlings through to reproductive maturity and assessing performance in the field, before firm conclusions can be reached.

Keywords: *Albugo candida*, Brassicaceae, *Lepidium oleraceum*, New Zealand, conservation

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1. Introduction

The oomycete parasite *Albugo candida* (Pers.) Kuntze is a widespread pathogen of brassicaceous crops. It is an obligate parasite that attacks at least 29 genera of crucifers (Brassicaceae), including major agricultural crops, common weeds and native species (Farr et al. 1989), and is the causal agent of white rust. Saharan & Verma (1992) reported that as many as 241 species in 63 genera are known hosts of *A. candida*.

Albugo candida was first discovered in New Zealand in 1884 by William Colenso, possibly having been introduced accidentally on weeds or food crops. It has been confirmed that it infects wild and cultivated populations of all indigenous brassicaceous species, including the genera *Lepidium*, *Rorippa*, *Ischnocarpus* (endemic genus), *Cheesemania*, *Iti* (endemic genus), *Pachycladon* (endemic genus) and *Notothlaspi* (endemic genus). The majority of the species in these genera are listed as threatened, and *A. candida* has been identified as a serious threat to New Zealand's coastal cress (*Lepidium*) species (Norton & de Lange 1999: Objective 8).

Aside from the fact that *Albugo* can infect all these genera, little is known about the ecology and nature of the pathogen, although it is clear that the disease seriously afflicts some plants, reducing their reproductive vigour and ultimately leading to their death (Alexander & Burdon 1984). White pustules of sporangia erupting from under the epidermis are the characteristic symptoms of white rust. Pustules may be localised on any plant organ (leaves, stems, flowers or fruit), or they may occur as large contiguous pustules throughout the plant. In addition, so-called 'stagheads' of distorted, sterile inflorescences indicate systemic disease, often severely affecting plant reproduction. Stagheads can cause substantial yield loss in seed crops such as rapeseed (*Brassica napus* L.) (Harper & Pittman 1974; Petrie 1988). The progression of the disease from localised pustules to systemic stagheads is not well understood (Verma & Petrie 1980; Liu & Rimmer 1990).

Some research has led to the hypothesis that *A. candida* commonly forms systemic asymptomatic infections that are latent for long periods of time (Jacobson et al. 1998). If this is true, such indeterminate latent infections temporally separate pathogen infection and symptom expression. The environmental and genetic parameters controlling such distinct events may differ, adding a new level of complexity to the disease cycle in both *ex situ* and field situations.

Current management of *Albugo* in New Zealand involves spraying systemic fungicides such as Ridomil on cultivated and wild populations. Although these fungicides have apparently increased the vigour and fecundity of cress in some instances (e.g. *Lepidium banksii* in Golden Bay), it remains unclear how effective they really are. Furthermore, this approach is very expensive, unsustainable and environmentally damaging, and there are health risks to users. As a consequence, the use of these sprays is unpopular with staff of the Department of Conservation, the public and iwi.

At present, the survival of many *Lepidium* populations in the wild cannot be assured. Therefore, one major component of current recovery plans involves the *ex situ* cultivation of stocks for future use in population enhancement or translocations. All cultivated stocks of *Lepidium* have developed *Albugo* infestations, and in some cases valuable plants have been lost to this disease. This places disease-free wild populations at great potential risk when nursery stocks are used for population enhancement.

2. Objectives

The objectives of this study were to:

- Test the hypothesis of systemic asymptomatic *Albugo candida* infection in *Lepidium oleraceum* by developing a PCR (polymerase chain reaction)-based assay for the presence of the pathogen's DNA. The development of a successful assay will allow researchers to determine whether the pathogen can exist in *L. oleraceum* as a latent asymptomatic infection under field and glasshouse conditions.
- Quantitatively assess the effect of *A. candida* infection on the growth and survival of *L. oleraceum* plants.
- Determine whether *Albugo* can be transferred vertically through seed.

3. Methods

3.1 DEVELOPMENT OF A PCR ASSAY FOR *A. candida* DNA IN *L. oleraceum*

3.1.1 DNA extraction, sequencing and primer design

Genomic DNA was extracted using standard CTAB methods (Doyle & Doyle 1990). The universal primers ITS 1 (5′-TCCGTAGGTGAACCTGCGG3′) and ITS 2 (5′-GCTGCGTTCTTCATCGATGC-3′) were initially used to amplify the internal transcribed spacer region 1 (ITS 1) between the 18S and 5.8S ribosomal genes (White et al. 1990). DNA extracted from inflorescences of *Lepidium oleraceum* that were heavily infected with *Albugo candida* typically yielded two PCR products, one derived from the host genome and the other putatively from the genome of the pathogen. These copies were of different sizes (c. 269 base pairs (bp) and c. 197 bp, respectively), allowing them to be separated via agarose gel electrophoresis and sequenced individually.

Following initial amplification, separated bands corresponding to the plant and pathogen copies of ITS 1 were manually excised and purified using Concert® Gel purification columns prior to re-amplification and direct sequencing (Big

Dye Terminator method) using an ABI 310 automated sequencer at Landcare Research, Mt Albert.

Recovered sequences were then aligned and compared using the program Sequencher (Version 3.2 Applied Biosystems). The identities of the plant and oomycete sequences were verified using previously generated *Lepidium* sequences (unpubl. data) as well as *Albugo* spp. sequences lodged on Genbank (BLAST/NCBI: www.ncbi.nlm.nih.gov).

This verification procedure confirmed that the pathogen was *A. candida*. Four separate *A. candida* sequences were generated, which were derived from different host plants growing at Auckland Regional Botanic Gardens, Manurewa (hereafter referred to as ARBG). Once aligned together with the sequences from *L. oleraceum,* regions that differed between *A. candida* and the plant sequence, but were identical within the four sequences of *A. candida*, were identified at the opposite ends of the molecule. Based on this observation, internal primers that were specific for *A. candida* were designed for the ITS 1 region using the unique nucleotide differences apparent between host and pathogen sequences present across the border of ITS 1 and the 5.8S ribosomal gene. The objective was to develop a PCR test that was specific only to the presence of *A. candida* DNA. To do this, a 20-mer oligonucleotide primer was designed and called 'ACAN-1' (5′CCACTGCTGAAAGTTTGTG-3′). This spanned the border of ITS 1 and 5.8S. Internal to the border of 18S ribosomal gene and ITS 1, another 20-mer primer (ACAN-2) was designed (5′CTATCCACGTGAACCACTTG-3′) (Fig. 1).

Figure 1. A schematic representation of the priming sites used in this study

3.1.2 Primer effectiveness and specificity trials

The efficacy and template specificity of these primers was assessed by conducting a wide PCR assay of both symptomatic and asymptomatic leaf material of *L. oleraceum*, as well as of DNA from other plant species for which *Albugo* infection is unknown. This assay of symptomatic, asymptomatic and fungicidally treated material also served as a test for the presence of *A. candida* as a latent asymptomatic systemic infection. Assays were also performed on juvenile plants from the fitness trials (see section 3.2) and surface-sterilised seeds obtained from ARBG (DNA from surface-sterilised seeds was extracted individually using the above methods). The material assayed is listed in Table 1. In each case, random subsets of 20 plants from each of the growth experiment treatments (see section 3.2.1) were assayed for the presence of the pathogen. The PCR assay procedure involved DNA extraction from the target tissue sample (usually leaves) followed by PCR amplification using the primers specific for *A. candida*. PCR products were run out via agarose gel electrophoresis using TAE buffer at a constant voltage of 4 V/cm. PCR products could then be visualised under UV light after staining with ethidium bromide. A 1-Kb (kilobase) DNA ladder (NEB) was run out alongside each PCR product to facilitate product size determination.

TABLE 1. MATERIAL OF *Lepidium oleraceum* (AND OTHER SPECIES WHERE SPECIFIED) ASSAYED FOR THE PRESENCE OF *Albugo candida* USING PRIMERS SPECIFIC FOR *A. candida*.

Each replicate consists of a single leaf from a different plant or an individual seed (where specified).

DNA SOURCE	NO. OF REPLICATES
Leaf material from **symptomatic** adult plants growing at ARBG	20
Leaf material from **asymptomatic** adult plants growing at ARBG	20
Leaf material from **asymptomatic** glasshouse-grown juvenile plants treated with systemic fungicides (i.e. fitness trial control plants)	20
Leaf material from **asymptomatic** glasshouse-grown juvenile plants inoculated with *A. candida* and not treated with systemic fungicides (i.e. fitness trial inoculated plants)	20
Surface-sterilised seed from plants growing at ARBG	10
Leaf material from a range of taxa where *Albugo* infection is unknown:	
Kunzea sp.	5
Cordyline sp.	5
Ranunculus sp.	5

3.2 ASSESSING THE FITNESS CONSEQUENCES OF *A. candida* INFECTION IN *L. oleraceum*

3.2.1 Trial design

A glasshouse growth trial was conducted to quantify the effect of *A. candida* infection on the fitness (measured as plant growth and mortality) of *L. oleraceum* plants. Seedlings used in this study were sourced from three regions (provenances) encompassing much of the morphological and molecular variation present in the species (pers. obs.): Stephens Island (Takapourewa), the Otago Peninsula and the Chatham Islands. Seeds and seedlings from these provenances were provided by ARBG. The trial was conducted using 120 seedlings (40 seedlings from each provenance) that were of a similar age and stage of development. These seedlings were randomly selected from germination trays and planted individually into 15-cm pots filled with a growing medium consisting of peat, sand and vermiculite in a 2:2:1 ratio.

Sixty seedlings (20 from each provenance) were then assigned to a random position within two separate treatments, which were located in separate glasshouse units at Mt. Albert. The treatments were placed in separate glasshouse units to prevent cross-infection by white rust and cross-contamination with fungicide. The plants in both treatments were watered two or three times per week throughout the 3-month experimental period. After a period of 3 weeks, an equal amount of general-purpose liquid fertiliser was applied to each pot. All 60 plants from one treatment were then inoculated (see section 3.2.2) with *A. candida.* The plants in the other treatment served as controls against which the effect of *Albugo* infection could be measured. After 3 months, plant growth and survival were recorded. No plant had initiated flowering during the course of the trial.

3.2.2 Inoculum preparation

An inoculum was prepared as per Singh et al. (1999). Heavily *Albugo*-infected sporulating inflorescences were collected from mature *L. oleraceum* plants growing at ARBG. Infected material was stored in sealed plastic bags at 4°C before use.

This sporulating material was macerated in sterile distilled water containing 0.1% Tween-80 wetting agent (Irish & Morelock 2002) using a household blender to form an aqueous suspension of active zoosporangia (Singh et al. 1999). Immediately before inoculation, a sterilised pin was used to make up to ten small incisions in each of the leaves of the seedlings, breaking the epidermis to facilitate pathogen entry into plant tissues (Irish & Morelock 2002). The inoculum was sprayed on both the upper and lower surfaces of the leaves of each plant. Inoculated seedlings were kept at 18°C and 100% humidity for a period of 24 h immediately post inoculation (Singh et al. 1999).

The negative control treatment, which consisted of 60 uninoculated seedlings, was isolated from the inoculated treatment in a separate glasshouse compartment. Because effective isolation could not be guaranteed (and also because *A. candida* is thought to be vertically transmitted through seed), negative control seedlings were sprayed three times during the trial with the systemic fungicides Mancozeb and Ridomil, in accordance with the manufacturers' specifications.

3.2.3 Measures of plant growth

At the end of the experimental period, the number of plants surviving was recorded and all surviving plants were harvested and air-dried at room temperature in individual paper bags. Where necessary, soil was removed from roots under a running tap before drying. Total plant dry weight was recorded after a 20-day drying period.

3.2.4 Statistical tests

Effects of both *Albugo* infection (*Albugo* inoculation) and provenance on plant survival and biomass accumulation were modelled using regression analysis. Where necessary, $\log_e$ transformations were applied to the dry weight data to approximate normal error distributions in the models. All statistical analyses were conducted using the statistical package JMP version 3.0.1 (Sall et al. 1994).

4. Results

4.1 DNA SEQUENCES

The ITS 1 DNA sequences that were recovered from the host species (*Lepidium oleraceum*) and pathogen (*Albugo candida*) are presented in Table 2.

TABLE 2. HOST AND PATHOGEN ITS 1 SEQUENCES.

TAXON	ITS 1 SEQUENCE
Lepidium oleraceum (269 bp)	3′-tcgatacctg tccaaaacag aacgaccccc gaaccaacta tcatcacttg cggtggaccg gtttcttagc tgatcccgtg cccgccgaat ccttggtttc gcgtgccgtt cggaacggga gatctctctc ggaccggtyg tccgcttagc tgaaggatat cacaaaacca cggcacgaaa agtgtcaagg aacatgcaac cgaacggccg gcgttcgcct tcccggagac ggtgcgagcg agaacgctnt gctgcgatct aaagtct–5′
Albugo candida (197 bp)	3′ccacaccaaa aactatccac gtgaaccact ttgtataggt tttttgtcgc catatgcaac gcctcttcga ggagtgtttg tgyatgtgtg ggctaaccga aggcttttga ccgtaaggtt ggaagctgat gttttttctc atccttnacc tcaatatgaa tttcgcaaga acgaaagttt ttgcgtttac ttccac-5′

4.2 SPECIFICITY AND EFFICACY OF THE *A. candida* ITS 1 PRIMERS

4.2.1 Field-grown adult plants

The ability of the primers specific for *A. candida* to detect the pathogen in both symptomatic and asymptomatic host tissue was assessed using PCR assay tests. The *A. candida*-specific primers yielded single length products only (single bands), which corresponded in size to the ITS 1 region in *A. candida* (i.e. approximately 200 bp). In no case did these primers also amplify plant DNA (i.e. approximately 270 bp). The results of these assays are presented in Table 3.

In 18 of the 20 assays, *A. candida* DNA was detected in heavily infected tissue collected at ARBG (Table 3). However, *A. candida* DNA was also detected in 14 of the 20 leaf-tissue DNA samples from plants exhibiting no symptoms of the disease (Table 3).

4.2.2 Juvenile plants from the glasshouse trial

Of the 20 juvenile plants inoculated with *A. candida*, 17 assayed positively for the presence of the pathogen's DNA, even though none of these plants displayed obvious symptoms of the disease (Table 3). Two of the 20 control juvenile plants (i.e. that had not been inoculated and had been treated with fungicide) also assayed positively for the presence of white rust.

TABLE 3. PCR DETECTION OF *Albugo candida* IN HOST TISSUE USING PRIMERS SPECIFIC TO *A. candida*.

DNA SOURCE	NUMBER OF	
	REPLICATES	POSITIVE PCR AMPLIFICATIONS
Leaf material from clearly **symptomatic** adult *L. oleraceum* growing at ARBG	20	18
Leaf material from **asymptomatic** adult *L. oleraceum* growing at ARBG	20	14
Leaf material from **asymptomatic** glasshouse-grown juvenile *L. oleraceum* plants inoculated with *A. candida* and not treated with systemic fungicides	20	17
Leaf material from **asymptomatic** glasshouse-grown juvenile *L. oleraceum* plants treated with systemic fungicides	20	2
Seed from *L. oleraceum* plants growing at ARBG	10	7
Leaf material from a range of taxa where *Albugo* infection is unknown:		
Kunzea sp.	5	0
Cordyline sp.	5	0
Ranunculus sp.	5	0

4.2.3 Vertical transmission through seed

Seven of ten surface-sterilised seeds assayed positively for the presence of *A. candida* DNA (Table 3).

4.2.4 Tissue from species not susceptible to white rust

To eliminate the possibility of false positives, DNA from other plant species not known to be susceptible to *A. candida* was assayed. DNA of *A. candida* was not detected in any of the 15 samples assayed (five each of *Kunzea*, *Ranunculus* and *Cordyline* species) (Table 3).

4.3 EFFECT OF *A. candida* INFECTION ON GROWTH AND SURVIVAL OF *L. oleraceum*

By the end of the growth trial (3 months), very few plants (5 of 60) from the inoculated treatment displayed any signs of infection, despite the fact that *A. candida* was detected in the leaves of 17 of the 20 randomly selected plants assayed (Table 3). The putative white rust symptoms observed in five plants consisted of pale green dots on some leaves and/or isolated necrotic lesions on the leaves and/or stems. These symptoms are not typical of *A. candida*, and therefore may have been caused by another pathogen.

4.3.1 Mortality

Albugo candida inoculation (plus no fungicide treatment) had a slight but statistically insignificant effect on plant mortality in the experiment: 16% of plants in the inoculated treatment died during the course of the pot trial versus 10% of plants in the uninoculated, fungicide treated controls.

4.3.2 Growth

Albugo candida infection had a slight but insignificant ($P = 0.054$) effect on plant growth. Inoculated plants attained a slightly lower average weight over 3 months (4.2 g dry weight) than control plants (5.2 g dry weight).

5. Discussion and conclusions

5.1 OVERALL FINDINGS

A PCR-based assay for the detection of ITS 1 of the ribosomal DNA (rDNA) of *Albugo candida* has been developed in this study. Similar PCR-based approaches have become widely used to detect *Albugo* spp. and other fungi within plant tissue (e.g. Tham et al. 1994; Lovic et al. 1995; Jacobson et al. 1998). Primers were designed based on sequence differences in the ITS 1 sequences between *A. candida* and *Lepidium oleraceum*. The primers preferentially amplified the *A. candida* ITS 1 from tissue samples where the plant ITS 1 template predominated. *Albugo candida* ITS 1 DNA was detected in a number of asymptomatic plants of *L. oleraceum*, as well as in surface-sterilised seeds collected from plants grown at ARBG. These findings suggest that in *L. oleraceum*, *A. candida* can be transmitted vertically through seed and can also exist as a latent asymptomatic infection. With further development, this technique could have the ability to detect *Albugo* in wild and *ex situ* populations, and could assist in efforts to ensure that wild stocks are not placed at risk by contamination from restoration plantings. However, because the possibility of false negatives has not been eliminated, further research is required to ensure unambiguous detection.

5.2 THE LIKELIHOOD OF FALSE NEGATIVE AND FALSE POSITIVE RESULTS

One problem with the technique developed in this study is that false negative and false positive results cannot be ruled out. Indeed, some of the results appear to be anomalous and call into question the stringency and reliability of the primers used to detect *A. candida*.

In 2 out of 20 cases, *A. candida* DNA was not detected in heavily infected, apparently sporulating tissue collected from fruiting adult plants at ARBG. Such false negatives, i.e. a failure to detect *A. candida* within the host when the pathogen is in fact present, may be due to a number of factors. PCR failure, which is a ubiquitous phenomenon, could explain these apparent false negatives, but insufficient time was available to re-extract and amplify these tissue samples with fresh reagents to determine whether this was responsible for these results. The likelihood of obtaining false negatives may also depend on the amount of *A. candida* DNA in the host plant, which may explain the failure to detect *A. candida* in 3 of 20 asymptomatic juvenile plants from the inoculated treatment. Even though none of these plants displayed obvious symptoms of the disease, they had all been inoculated with sporangia and were growing close to symptomatic plants. However, it is also possible that some plants had not been successfully inoculated or, alternatively, that the level of *A. candida* DNA in their tissues was below the PCR detection threshold. The quantitative limit of detecting *A. candida* cells in the host could not be determined and

requires further study. However, the fact that a large proportion of samples from both asymptomatic and symptomatic tissue assayed positively for *A. candida* (Table 2) indicates that this detection strategy can be reasonably sensitive. Furthermore, the PCR assay did not detect *A. candida* in any of 15 DNA samples from the leaves of a range of non-brassicaceous species not known to host the pathogen. In addition, by choosing the rDNA ITS 1 region as the PCR target, theoretically, fewer nuclei should be required to supply enough templates for PCR, because this gene is present in multiple copies per nucleus (White et al. 1990; Bruns et al. 1991). It is clear, therefore, that some uncertainty exists about why false negatives were generated in this study. More research is necessary to rule out the possibility of false negatives before this screening method can be used reliably.

The positive identification of *A. candida* in the PCR product was based on its correlation with a same-sized PCR product from tissue that was known to be infected with *A. candida*; this was then verified by sequence analysis. Despite this, it is possible that some false positive results may have been generated. This situation may result either from detecting a PCR product that is not from *A. candida* or from accidental contamination of samples with *A. candida*. The latter scenario is extremely unlikely given the stringent protocols used to avoid contamination in this study. Alternatively, the presence of other oomycetes within the tissue samples cannot be ruled out. One such candidate that could have led to false positives is the pathogen *Peronospora parasitica*, the cause of downy mildew in brassicas. Dual infections of *Peronospora* spp. and *Albugo* spp. are common in some species (e.g. Alexander & Burdon 1984). However, the ITS 1 sequences of *A. candida* and *Peronospora* are very different, and it is unlikely the ACAN-1 and ACAN-2 primers would amplify *Peronospora* species, although this needs to be verified experimentally. In addition, the symptoms of downy mildew are distinct from those of *A. candida* and were not seen on any of the plants assayed.

5.3 DETECTION OF LATENT SYSTEMIC ASYMPTOMATIC *A. candida* INFECTION

Albugo candida was frequently detected in the leaves of field and glasshouse-grown plants that showed no symptoms of disease, as well as in seeds stored at ARBG (Table 3). From these observations it is concluded that *A. candida* does exist as a systemic asymptomatic infection in *L. oleraceum*, supporting the hypothesis that *A. candida* forms latent infections for indeterminate time periods (Alexander & Burdon 1984; Edwards & Williams 1987; Liu & Rimmer 1990; Saharan & Verma 1992; Jacobson et al. 1998). This conclusion also accords with observations of the latent nature of the disease in the field and in *ex situ* cultivation (Norton & deLange 1999).

Positive assays of surfaced-sterilised seed from ARBG also support the hypothesis of vertical transmission of the pathogen through seed, a finding that has particular implications for the management of *Lepidium* spp. in *ex situ* conservation management and seed storage.

The possibility of latent asymptomatic infection may also explain why, by the end of the growth trial period, very few of the inoculated plants (5 of 60) displayed any obvious signs of infection, despite the fact that the pathogen was detected in the leaves of 17 of 20 asymptomatic juveniles assayed. The shift from asymptomatic to symptomatic infection is probably due to environmental triggers that were not present in the glasshouse situation, or perhaps to seasonally related factors (the glasshouse trial was conducted in winter). It is also possible that if the trial had been continued for a few more months, stagheads and pustules would have appeared on emerging inflorescences, as observed in adult plants at ARBG. Insufficient time and resources were available to continue the growth trial, and further work is required.

5.4 FITNESS CONSEQUENCES OF *A. candida* INFECTION ON *L. oleraceum*

Firm conclusions regarding the consequences of *A. candida* infection on the fitness of *L. oleraceum* cannot be drawn from the results reported in this study. Although *A. candida* infection had a negative effect on both plant growth and survival, the magnitude of this effect was slight and, in both cases, not statistically significant. However, although fitness costs imposed by *Albugo* infection on *L. oleraceum* do not appear to be substantial under glasshouse conditions, they may be limiting in situations where plants experience environmental stresses, competition from weed species or heavy browsing.

Further studies are required to ascertain whether asymptomatic *A. candida* infection may reduce the fitness of *L. oleraceum* in the field, as demonstrated in other taxa (e.g. Alexander & Burdon 1984). It is possible that *A. candida* infection in both symptomatic and asymptomatic forms is contributing to the observed rapid decline in abundance of native *Lepidium* species in New Zealand (Norton & de Lange 1999). Further research is required to establish and quantify the effect of *Albugo* infection on plant reproductive output. For *L. oleraceum*—an annual (sometimes weakly perennial), weed-like, r-strategist—seed production may be much more critical to population viability than plant growth itself, although the two are likely to be strongly positively correlated.

5.5 SOURCES OF INFECTION AND TRIGGERS FOR SYMPTOMS OF *A. candida* INFECTION

In other published studies, seedling infection of brassicaceous hosts has been shown to be through primary inocula, such as oospores present in the soil at germination (Petrie 1975; Verma & Petrie 1980) or sporangia disseminated in the autumn or spring (Alexander & Burdon 1984). In this study, the presence of *A. candida* in glasshouse-reared seedlings, including uninoculated plants, suggests that there is seed-borne infection from vertical transmission through a systemically infected mother plant (as suggested in Petrie 1975).

This hypothesis is also supported by the detection of the pathogen in seed stored at ARBG.

The parameters affecting symptom development of *A. candida* are not fully understood; systemic symptoms and staghead development are not easily predicted and are variable in other brassicaceous taxa (Verma & Petrie 1980; Saharan & Verma 1992). Apparently, the systemic presence of *A. candida* is not sufficient for symptoms to be expressed systemically (Alexander & Burdon 1984). It is clear that further research is required to establish how pathogenicity is triggered and how its effects can be minimised.

6. Recommendations

The author recommends:

- Conducting further research to continue to eliminate the generation of false positives and false negatives. Once the technique's accuracy has been improved, it could be applied to the management of other threatened *Lepidium* spp. as well as other genera affected by *Albugo* (e.g. *Rorippa divaricata*). The information obtained may help to ensure the safety of wild populations undergoing population enhancement through *ex situ* cultivation and restoration. The importance of this is highlighted by the fact that *ex situ* cultivation is considered crucial for the continued survival of many New Zealand native brassicas (Norton & de Lange 1999). The ability to monitor this pathogen may help ensure the survival of these species.
- Screening of *ex situ* populations for the presence of *A. candida* in both vegetative material and seed stored for propagation.
- Screening for the presence of the pathogen in key wild populations to prioritise sites for pathogen control.
- Research into more effective chemical control measures to reduce the severity of the disease in both cultivated and wild populations, if further research supports the initial findings of this study that the pathogen is widespread, systemic and present in seed. It is unclear, and seems unlikely from the research presented here, that this latent systemic, vertically transmitted pathogen can be eradicated entirely from *Lepidium* species in New Zealand. Consequently, despite its unpopularity, chemical control may be the only effective method currently available to secure the status of some *Lepidium oleraceum* populations and those of other highly threatened native species.
- Further research into pathogenicity and disease progression, with particular focus on identifying environmental triggers leading to symptom formation and sporulation. This could make chemical control measures more efficient and effective by targetting vulnerable populations for control at key times to ensure minimum plant loss and maximum seed production.

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