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Searching for fungal mycoherbicides effective against climbing asparagus (*Asparagus scandens*) in New Zealand

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Background

Climbing asparagus (*Asparagus scandens*) is a widespread invasive species in New Zealand and is harmful to biodiversity due to its coverage of the forest floor[1] (Figure 1). Conventional chemical herbicides such as glyphosate can affect cause disturbance to nearby native plant species. New control approaches against *A. scandens* are essential.

Fungal bioherbicides can control weeds and minimize toxicity to native plants[2]. Some fungal phytopathogens lead to severe symptoms on *Asparagus* weeds[3]. This study surveyed fungal pathogens presence and their pathogenicity on *A. scandens*.

Project Aims:

- **Survey for the presence of fungal pathogens of *A. scandens* from selected New Zealand sites.**
- **Demonstrate the pathogenicity of isolated fungal pathogens against *A. scandens in vitro* and *in planta*.**
- **Establish baselines for future fungal mycoherbicide application *in planta.***

Methods:

- **Site visits and sample collection:** Twenty-four sites have been visited across New Zealand (Figure 2). Symptomatic *A. scandens* were collected and surface sterilized with 1% NaClO solution
- **Fungal identification:** Fungal strains were isolated from surface-sterilized *A. scandens* leaves and stems, cultured then purified on Potato Dextrose Agar (PDA) at 18°C. Fungal DNA was extracted and amplified for sequencing; results were logged onto NCBI for identification.
- ***In vitro* test:** Pure PDA fungal plugs were placed onto asymptomatic *A. scandens* tissues (Figure 3). Experiment groups included needle-wounded and unwounded. Samples were kept at 18°C, symptoms were recorded after 14 days.
- ***In planta* test:** Fungal strains with potentials were inoculated by placing on stem close to soil (Figure 4) with needle wounded and unwounded. Foliage symptoms were recorded after 14 days.

Figure 1: *A. scandens* cultured at Unitec lab (left) and collected from an Auckland sampling site (right).

Figure 2: Collection sites across New Zealand until June 2022

Figure 3: *In vitro* test of fungal plugs inoculation on *A. scandens* tissue.

Figure 4: *In planta inoculation* test of fungal pathogenicity on whole *A. scandens* plant.

Results

- There are 20 different genera (51 unique isolates) identified across 19 sites (Figure 5). *Fusarium* spp. and *Penicillium* spp. Were identified from the highest number of sites.
- *Pestalotiopsis spp., Colletotrichum spp. and Fusarium spp.* have the largest number of unique isolates identified in our sites across New Zealand.
- *In vitro* test: Plant samples showed a variety of symptoms from slightly yellow on leaves and/or stem to entirely death (Figure 6). Ten isolates resulted in moderate to severe symptoms in both wounded and unwounded plant samples (Table 1).
- *In planta* test- Four fungal isolates (*Fusarium graminearum* (12385-5), *Colletotrichum* D (12379-6), *Neofusicoccum spp.*(12378-5 and 12382-1, Table 2) out of 17 tested isolates resulted in moderate to severe results in both setups.

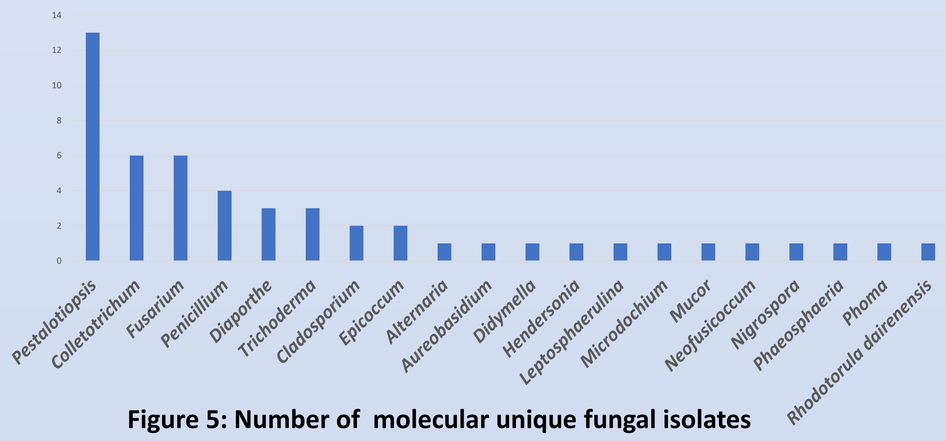

Figure 5: Number of molecular unique fungal isolates identified.

Fungal isolates	Sample code
Alternaria alternata	12380-3
Colletotrichum A	12379-1
Colletotrichum F	12383-4
Cladosporium A	13344-2
Fusarium acuminatum	12381-1
Fusarium acuminatum	12912-3
Fusarium fujikuroi	12881-9
Fusarium E.	12900-4
Hendersonia culmiseda	12379-8
Neofusicoccum parvum	12382-1
Nigrospora muase	13040-3
Pestalotiopsis E	12380-2

Table 1. *In vitro* results: fungal strains showed severe results in both needle-wounded and unwounded plants.
Plant symptoms were recorded as severe when: Plant completely dead, and/or more than 95% leaves were yellow, and/or stem completely discoloured.

Figure 6: *In vitro* inoculation of *Pestalotiopsis* E (12380-2) with severe symptoms.

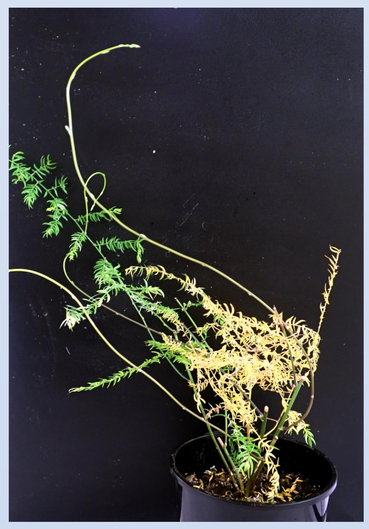

Figure 7: *In planta* inoculation of *Pestalotiopsis* E (12380-2) with severe symptoms.

Fungal isolates	Sample code
Colletotrichum D	12379-6
Fusarium graminearum	12385-5
Neofusicoccum sp.	12378-5
Neofusicoccum parvum	12382-1

Table 2. *In planta* results: Fungal isolates showed moderate or above in either needle-wounded or unwounded plants. Plant symptoms were recorded as severe when: Plant completely dead, and/or more than 95% leaves were yellow, and/or stem completely discoloured.

Discussion

- In our study, *Colletotrichum*, *Fusarium graminearum* and *Neofusicoccum parvum* are three of the most effective fungal isolates inducing symptoms both *in vitro* and *in planta* to date.
- A relatively low temperature (18°C) was applied to reduce fungal growth and test their tolerance under unideal conditions.
- Inoculation of two or three promising fungal isolates on the same *in planta* trail is planned in the future.
- Effective range tests are required to evaluate their impacts on the commercial asparagus industry.

References

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