Plant Pathology & Quarantine 14(1): 143–148 (2024) ISSN 2229-2217
www.ppqjournal.org

Article
Doi 10.5943/ppq/14/1/12

First record of *Fusarium cortaderiae* on climbing asparagus (*Asparagus scandens*): an invasive plant species in New Zealand

Tang T[1*], Blanchon DJ[1,2], Wells S[1], Fisher LKM[1], Cox H[3] and Waipara N[4]

[1]*Applied Molecular Solutions Research Centre, Unitec Institute of Technology – Te Pūkenga, Private Bag 92025, Auckland 1142, New Zealand*
[2]*Auckland War Memorial Museum Tāmaki Paenga Hira, Private Bag 92018, Victoria Street West, Auckland 1142, New Zealand*
[3]*Auckland Council, Private Bag 92300, Victoria Street West, Auckland 1142, New Zealand*
[4]*The New Zealand Institute for Plant and Food Research Limited, Private Bag 92169, Auckland Mail Centre, Auckland 1142, New Zealand.*

Tang T, Blanchon DJ, Wells S, Fisher LKM, Cox H, Waipara N 2024 – First record of *Fusarium cortaderiae* on climbing asparagus (*Asparagus scandens*): an invasive plant species in New Zealand. Plant Pathology & Quarantine 14(1), 143–148, Doi 10.5943/ppq/14/1/12

Abstract

Climbing asparagus (*Asparagus scandens*) is a harmful invasive plant in New Zealand, causing a reduction in native biodiversity. In a survey of *A. scandens* populations for potential fungal pathogens in New Zealand, one species, *Fusarium cortaderiae*, caused significant symptoms. The pathogenicity of *F. cortaderiae* to climbing asparagus was demonstrated through inoculations under controlled conditions, thus fulfilling Koch's postulates.

Keywords – Biocontrol – Internal transcribed spacer – mycoherbicide – pathogenicity – Translation elongation factor 1

Introduction

Climbing asparagus (*Asparagus scandens*) is a slender, scrambling or climbing perennial endemic to the Western Cape of South Africa (Timmins & Reid 2000). It has naturalised in both New Zealand and Australia and is considered to be a serious threat to biodiversity (Timmins & Reid 2000, Lawrie 2004). It is associated with declines in native plant abundance and species richness and is negatively correlated with native seedling recruitment (McAlpine et al. 2015). The development of a biological control agent or mycoherbicide is a potential method to control this species, reduce herbicide application and its negative effects on native plants (Wagner & Nelson, 2014, Dupont et al. 2018). With this in mind, a survey throughout the New Zealand range of *A. scandens* was carried out to look for plants with disease symptoms and to isolate and identify fungal isolates with the potential to be biocontrol agents or mycoherbicides (Berestetskiy & Sokornova 2018, Dalinova et al. 2020, Kumar et al. 2021).

Materials & Methods

Disease symptoms, sample collection, and fungal isolation

Twenty-four sites where *Asparagus scandens* had been recorded were visited between 2020 and 2022 (Fig. 1). Living plant samples were collected if plants were symptomatic (e.g., with

Submitted 13 August 2024, Accepted 10 October 2024, Published 01 November 2024
Corresponding Author: Tianyi Tang – e-mail – ttang@unitec.ac.nz
Accepted reviewer: Mahajabeen Padamsee

visible lesions or discolouration). Collections were placed in sealed bags and stored at 4°C in the fridge, and then surface sterilised with 1% Sodium hypochlorite solution for 1 minute and washed twice in distilled water. Symptomatic leaves and stem tissues were placed onto Potato Dextrose Agar (PDA) plates with chloramphenicol (Fort Richard, New Zealand) for fungal selective isolation. Isolated fungal cultures were placed at 18°C with 12h light per day and repeatedly sub-cultured until single isolate cultures were obtained. Colony observations were performed on 7-day post inoculation (dpi) and 14 dpi depending on growth status. Production of conidiospores was stimulated by culturing mycelium on water agar containing either autoclaved carnation leaves or wheat bran (Hassan & Bullerman 2009).

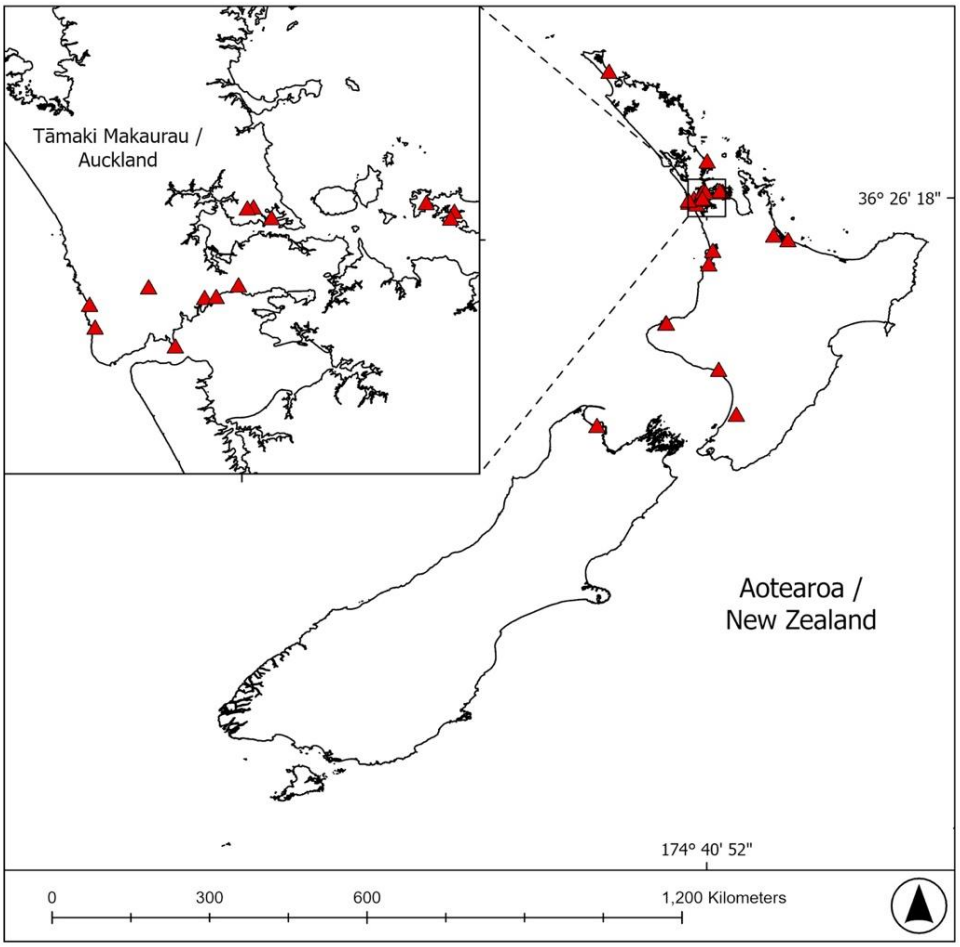

Fig. 1 – Map of collection sites for symptomatic *A. scandens*. Each triangle represents one collection site.

DNA extraction, PCR amplification, sequencing, and Phylogenetic analyses

Fungal DNA was extracted using DNeasy Plant kits (Qiagen, USA) and amplified by PCR with ITS1F and ITS4 primers (Manici & Caputo 2020) for the ITS (internal transcribed spacer) region (Larena et al. 1999) to identify samples at genus level. PCR protocols were applied as follows: Hot start at 98°C for 2 min; with 40 reactions of denaturation at 98°C for 15 seconds, annealing and extension at 60°C for 30 seconds, following Invitrogen Platinum II Hot-Start Green PCR Master Mix instructions. Amplified PCR products were sequenced by Massey University (New Zealand) and the ITS sequence was uploaded onto the US National Center for Biotechnology Information (NCBI) for the Basic Local Alignment Search Tool (BLAST) via Geneious Prime® 2023.1.2. The closest match (Percent Identity) was recorded for each isolate. *Fusarium* spp. were also amplified with the primers Fa and Ra, as well as Fa+7 and Ra+6 for the TEF1 (Translation elongation factor 1 alpha) region (Karlsson et al. 2016) and their sequences were compared with the

Fusarioid-ID database (https://fusarium.org) for further identification. RAxML-GTR GAMMA analysis was carried out on TEF1 sequences from *Fusarium* 12385-5/ICMP 25017 and 23 closely related isolates from Genbank using Geneious Prime® 2023.1.2 (Fig. 3) (Laraba et al. 2021).

Pathogenicity tests

Seed-grown *A. scandens* plants with no disease symptoms were grown in pots under 12 hours LED light (Fig. 2A) for pathogenicity testing. Plant tissues were cut from the main stem (Fig. 2B), and separated branches were placed in sterile glass petri dishes with filter paper at the bottom at 18°C, with 1 mL distilled water, and an additional 1 mL distilled water at 3 dpi, for *in vitro* testing. Seed-grown whole plants were used for *in planta* pathogenicity testing.

Isolated fungal strains on PDA culture plugs were inoculated onto *A. scandens* plant material for *in vitro* and *in planta* tests, with each group containing three replicates of needle-wounded and unwounded plant samples. Symptoms were monitored at 7, 10 and 14 dpi. Fungal isolations were performed on inoculated symptomatic whole plants and identified by sequencing as outlined above, to satisfy Koch's postulates.

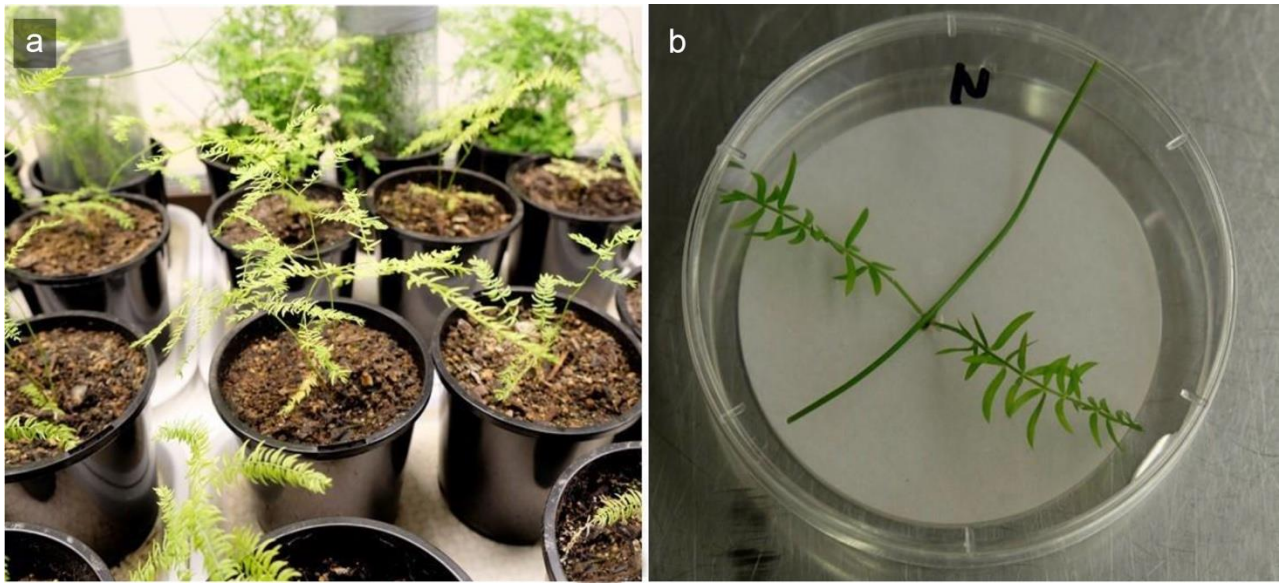

Fig. 2 – a Cultured *A. scandens* plants in pots. b The plant sample was cut from the main stem and placed in a petri dish.

Results

One hundred and forty-four colonies were initially recovered, and 91 were successfully sequenced using the ITS region (morphologically identical colonies isolated from the same plant samples were discarded), comprising 20 different genera and 51 unique fungal strains with some genetically identical colonies. Of these, one isolate of *Fusarium* sp. (12385-5, from Waiheke Island, Auckland) was found to cause significant symptoms (yellowing and wilting) in plants of *A. scandens* in both *in vitro* and *in planta* tests. This isolate was deposited in the culture collection at Landcare Research, New Zealand (ICMP 25017).

Comparison of the ITS sequence (OR251369) with published sequences on Genbank indicated *Fusarium* sp. 12385-5 belongs to the *F. graminearum* clade, whereas TEF1 sequence (PQ165126) indicated a 100% match to a *Fusarium cortaderiae* isolate from carnation (*Dianthus caryophyllus*) in Auckland (ICMP 5238, MG857235.1). Examination of colonies of *Fusarium* sp. (12385-5) on PDA found them to be pinkish white with dense aerial mycelia, and on the reverse side light pink at the margins and dark pink in the centre at 18°C, 14 dpi (Fig. 4A). Conidiospores had 3 to 5-septa (usually with five septa) (Fig. 4B) with a size range of 30 – (39.04) – 47.5 µm × 3.75 – (4.96) – 5.0 µm, n=30.

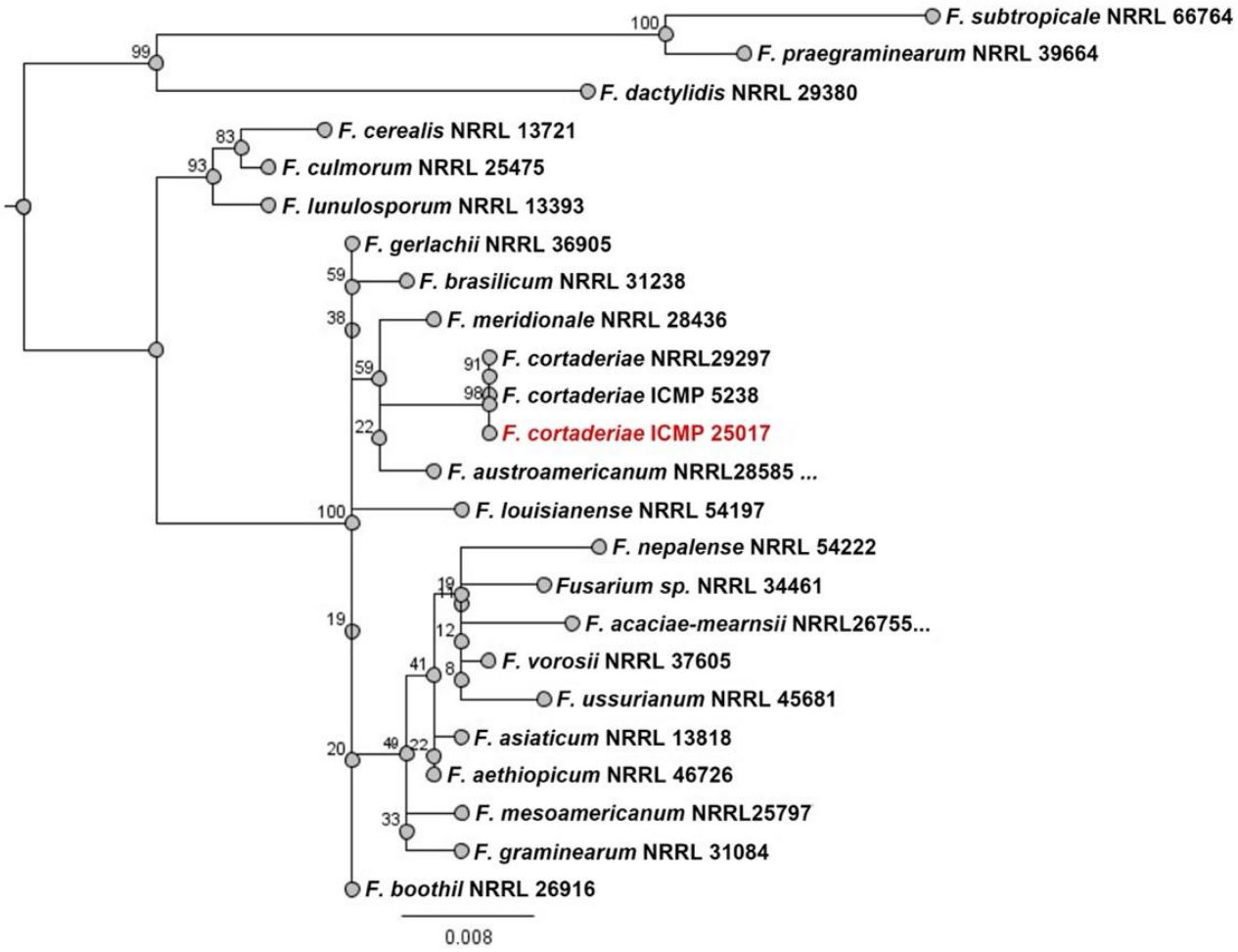

Fig. 3 – Phylogenetic tree generated from maximum likelihood analysis based on TEF1 sequences of *Fusarium cortaderiae* (ICMP 25017) with other species from the *Fusarium graminearum* clade.

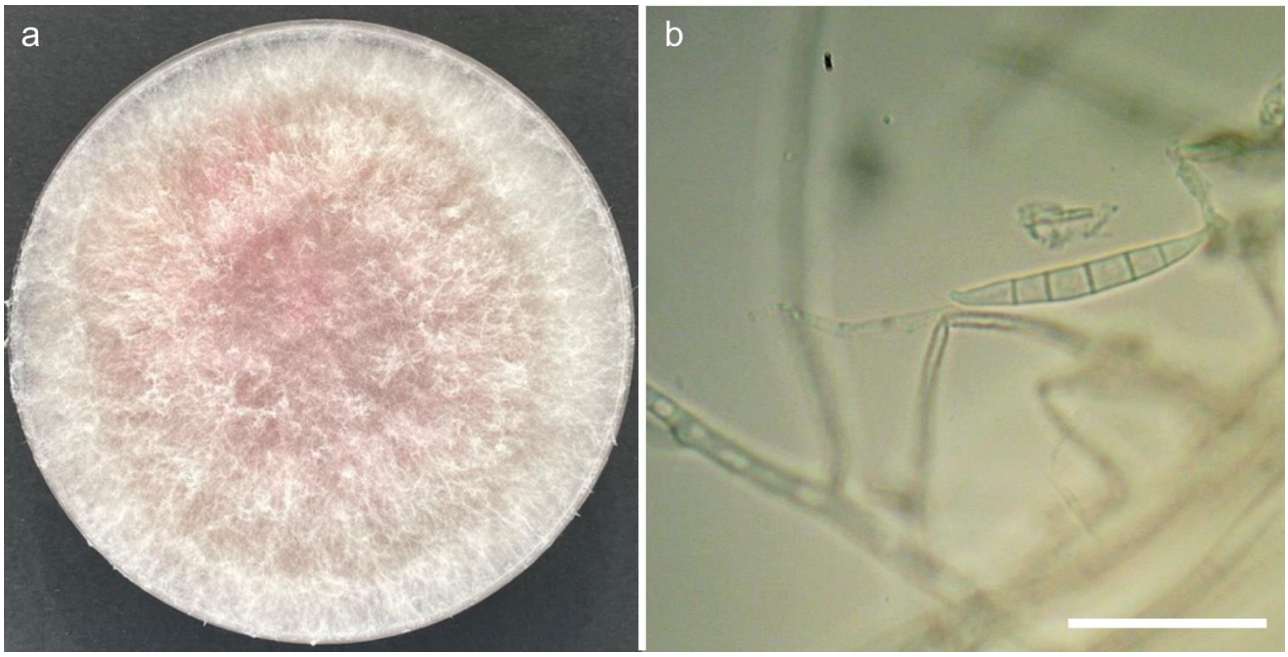

Fig. 4 – *Fusarium cortaderiae* (ICMP 25017). a Fungal on PDA plate with chloramphenicol on 14 dpi, 18°C. b Conidiospore and conidia. Scale bar: b = 20µm.

Inoculated plant samples of *A. scandens* showed moderate to significant symptoms when treated with *F. cortaderiae* 12385-5 ICMP 25017 (Fig. 5) both *in vitro* and *in planta*. Re-isolation from symptomatic inoculated test plants showed identical colony morphologies and their ITS and TEF1 sequences were identical to the original *F. cortaderiae* isolate.

Fig. 5 – *Fusarium cortaderiae* (ICMP 25017) inoculated *Asparagus scandens* on 14 dpi. Yellow discolouration and wilted tissues are visible on some parts of the plant.

Discussion

Colony characteristics and conidiospore morphology and size are consistent with the published morphology of *F. cortaderiae* (O'Donnell et al. 2004). The species has previously been reported from maize crops in Europe and New Zealand (Valverde-Bogantes et al. 2020). The holotype culture material of *F. cortaderiae* was isolated from pampas grass (*Cortaderia selloana*) in Auckland, New Zealand, but pampas grass is not native to New Zealand, and *F. cortaderiae* is likely to have originated in South America, where *C. selloana* is native (O'Donnell et al. 2004).

Fusarium cortaderiae cultures showed pathogenicity against *Asparagus scandens* during *in vitro* and *in planta* tests and have potential to be applied as biocontrol agents or mycoherbicides to suppress the growth of invasive *A. scandens*. Research is underway to understand the pathogenicity of *F. cortaderiae* in field situations and against non-target plant species.

Acknowledgements

We would like to thank Auckland Council for funding this research programme and Andrew Marshall for creating Fig. 1.

References

Berestetskiy A, Sokornova S. 2018 – Production and Stabilization of Mycoherbicides. In R. Radhakrishnan (Ed.), Biological Approaches for Controlling Weeds. InTech. Doi 10.5772/intechopen.76936

Dalinova AA, Salimova DR, Berestetskiy AO. 2020 – Fungi of the genera *Alternaria* as producers of biological active compounds and mycoherbicides. Applied Biochemistry and Microbiology, 56(3), 256–272. Doi 10.1134/S0003683820030023

Dupont YL, Strandberg B, Damgaard C. 2018 – Effects of herbicide and nitrogen fertilizer on non-target plant reproduction and indirect effects on pollination in *Tanacetum vulgare* (Asteraceae). Agriculture, Ecosystems & Environment 262, 76–82. Doi 10.1016/j.agee.2018.04.014

Hassan YI, Bullerman LB. 2009 – Wheat bran as an alternative substrate for macroconidia formation by some *Fusarium* species. Journal of Microbiological Methods 77(1), 134–136.

Karlsson I, Edel-Hermann V, Gautheron N, Durling MB et al. 2016 – Genus-Specific Primers for Study of *Fusarium* Communities in Field Samples. Applied and Environmental Microbiology, 82(2), 491–501. Doi 10.1128/AEM.02748-15

Kumar V, Singh, M, Sehrawat N, Atri N et al. 2021 – Mycoherbicide Control Strategy: Concept, Constraints, and Advancements. Biopesticides International 17(1), 13.

Laraba I, McCormick SP, Vaughan MM, Geiser DM et al. 2021 – Phylogenetic diversity, trichothecene potential, and pathogenicity within *Fusarium sambucinum* species complex. PLOS ONE, 16(1), e0245037. Doi 10.1371/journal.pone.0245037

Larena I, Salazar O, González V, Julián MC et al. 1999 – Design of a primer for ribosomal DNA internal transcribed spacer with enhanced specificity for ascomycetes. Journal of Biotechnology, 75(2–3), 187–194. Doi 10.1016/S0168-1656(99)00154-6

Lawrie SL. 2004 – Asparagus weeds research and management in South Australia. Proceedings of the 14th Australian Weeds Conference. BM Sindel & SB Johnson (eds.). Published by Weed Society of New South Wales. Pp. 203–206.

Manici LM, Caputo F. 2020 – Growth promotion of apple plants is the net effect of binucleate *Rhizoctonia* sp. as rhizosphere-colonizing fungus. Rhizosphere 13 (2020) 100185. Doi 10.1016/j.rhisph.2020.100185

McAlpine KG, Lamoureaux SL, Westbrooke I. 2015 – Ecological impacts of ground cover weeds in New Zealand lowland forests. New Zealand Journal of Ecology 39(1), 50–60.

O'Donnell K, Ward TJ, Geiser DM, Kistler HC et al. 2004 – Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genetics and Biology 41(6), 600–623. Doi 10.1016/j.fgb.2004.03.003

Timmins SM, Reid V. 2000 – Climbing asparagus, *Asparagus scandens* Thunb.: a South African in your forest patch. Austral Ecology 25(5), 533–538. Doi 10.1046/j.1442-9993.2000.01077.x

Valverde-Bogantes E, Bianchini A, Herr JR, Rose DJ et al. 2020 – Recent population changes of *Fusarium* head blight pathogens: Drivers and implications. Canadian Journal of Plant Pathology 42(3), 315–329. Doi 10.1080/07060661.2019.1680442

Wagner V, Nelson CR. 2014 – Herbicides can negatively affect seed performance in native plants. Restoration Ecology 22(3), 288–291.