First report of *Pestalotiopsis clavispora* from *Selaginella kraussiana* (African club moss): an invasive plant species in New Zealand

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Abstract *Pestalotiopsis clavispora* was detected for the first time on wild plants of *Selaginella kraussiana* in New Zealand.

Keywords *Pestalotiopsis* · *Selaginella kraussiana*

Introduction

Selaginella kraussiana (Kunze) A. Braun (African club moss) is an invasive ground cover, which readily establishes in shady damp conditions in New Zealand (Barton 2005), reducing the species richness of native plants (Barton et al. 2007; Nessia et al. 2013). This report forms part of a study investigating possible naturally occurring pathogens of *S. kraussiana*, and reports the first record of *Pestalotiopsis clavispora* Atk. from *S. kraussiana* in New Zealand. To our knowledge, *Pestalotiopsis clavispora* has not previously been reported from *Selaginella*. There is one previous record of *P. clavispora* from New Zealand from the leaves of *Rhododendron catawbiense* Michx. (Dick and Hopkins 2008).

A survey of *Selaginella kraussiana* patches was carried out in August 2011, in Spragg Bush, a 21 ha remnant of native forest in the Waitakere Ranges (36°54'S, 174°32'E) in the Auckland region of New Zealand. Areas of damage were

recorded (Fig. 1a) and marked for further investigation. Samples of leaves, stems and strobili were taken from these areas of dieback, surface sterilised with 0.5% sodium hypochlorite and placed onto plates of potato dextrose agar (PDA) with chloramphenicol. A range of fungal cultures developed, including several morphologically identified as isolates of *Pestalotiopsis*. These cultures were associated with general dieback of plants and root to tip browning off of stems and leaves in wild plants of *Selaginella kraussiana*.

To confirm the identification, the ribosomal DNA internal transcribed spacer region (ITS) was amplified and sequenced using primers ITS1f (Gardes and Bruns 1993) and ITS4 (White et al. 1990). The sequence was 100% identical to sequences on GenBank identified as *Pestalotiopsis clavispora* (AY924263 and AY924264) by Tejesvi et al. (2008) isolated from *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. The sequence from the New Zealand isolate from *Selaginella kraussiana* has been deposited on GenBank as accession KC683818. Conidia (Fig. 2) were 5-celled, 17–24×6–8 µm, slightly constricted at the septae, the upper two intermediate cells darker brown than the lower, with 3 unbranched setulae, 14–26 µm in length, matching the description for *Pestalotiopsis clavispora* (Guba 1961). A culture of this isolate has been deposited in the International Collection of Micro-organisms from Plants (ICMP19752).

Pathogenicity testing was carried out on greenhouse-grown plants of *Selaginella kraussiana*. Plants were inoculated with agar plugs containing the *Pestalotiopsis clavispora* isolate using 5mm sterilised plastic straws. This experiment was performed in two replicates, with two damaged and two undamaged plants being inoculated in each experiment. Damage was inflicted using a sterile needle, piercing six wounds into the stem and one wound

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Fig. 1 **a** Dieback symptoms in wild plants of *Selaginella kraussiana*, Spragg Bush, Auckland, New Zealand. **b** Symptoms from artificial inoculation of *Selaginella kraussiana* by *Pestalotiopsis clavispora* isolate. Arrows indicate inoculating agar plug (left) and leaves and stem showing dieback symptoms (right)

into 6 leaves of each plant being damaged. After inoculation, individual plants were placed in zip lock plastic bags and returned to the greenhouse. Plants were examined for signs of symptoms at weekly intervals for 3 weeks. Within 7 days, damaged plants exhibited extensive leaf and stem dieback and browning (Fig. 1b). After 3 weeks damaged plants were dead. Samples were again taken, surface-sterilised and inoculated onto plates of PDA with chloramphenicol. Cultures morphologically identified as *Pestalotiopsis* were consistently recovered and sequencing confirmed the presence of the initial *Pestalotiopsis clavispora* isolate. Further research is

continuing to determine if this isolate may be of use in the control of *Selaginella kraussiana*.

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Fig. 2 Conidia of *Pestalotiopsis clavispora*; bar=10 μ m