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Sporulation and spore viability in *Nephrolepis exaltata* (L.) Schott, Nephrolepidaceae, collected from a naturalised population (Oratia, Tāmaki Makaurau / Auckland, Aotearoa / New Zealand)

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Abstract

This paper examines fertile material of *Nephrolepis exaltata* (L.) Schott grown in cultivation, but originally obtained from a naturalised population in Oratia, Tāmaki Makaurau / Auckland, Aotearoa / New Zealand. Spore viability is assessed, with material showing c.40–50% spore abnormality. The presence of spores with a more regular morphology are recorded. However, viability assessed with fluorescein diacetate (FDA) is low to negligible.

Keywords

Nephrolepis exaltata (L.) Schott, Nephrolepidaceae, spore viability

Figure 1. *Nephrolepis cordifolia* AK 288143, collected by W. Stahel from Tauranga and determined by B. Parris as *N. cordifolia* cv 'Plumosa'. Photo: Auckland Museum Herbarium

Figure 2. Site of *Nephrolepis exaltata* populations originally growing along the stream bank at the confluence of Potter and Oratia Stream systems (where they meet at West Coast Road, Oratia, Tāmaki Makaurau / Auckland). Photo: Jaime Patmore, 27 January 2023

Introduction

Large and Farrington (2016) presented a preliminary study that reported the presence of members of the 'Boston fern' complex (including *Nephrolepis exaltata*) apparently naturalised in the Tāmaki Makaurau / Auckland region. The aggregate, as reported by them, was without bulbils and could superficially be confused with other native and non-native species.

This 2016 paper specifically identified two entities: a crested taxon without bulbils that compares well with specimen AK 288143 collected by W. Stahel from Tauranga and determined by B. Parris as *N. cordifolia* cv. Plumosa (Figure 1) (see also Hovenkamp and Miyamoto [2005], for an assessment of cultivar form); and a second entity with scales that were elongate, linear, long-attenuate, often with a dark basal spot. These were identical to those observed from herbarium material of *N. exaltata sensu stricto* (see vouchers UNITEC 9557 [determined by P.J. Brownsey, 25/07/2019]; UNITEC 9554). The original populations associated with this latter taxon were significantly affected by major flooding before and during the Cyclone Gabrielle weather event in the summer of 2023. The original site has now been lost to inundation (Figure 2).

At the time of the 2016 publication, the naturalised population of *N. exaltata* was not fertile. Consequently, plants were taken into cultivation with the aim of inducing sporulation. Eventually this cultivated material produced fertile fronds at irregular intervals from March to early May 2023. This current paper aims to assess this fertile material for spore morphology and possible viability.

Materials and methods

Material was originally collected from a population growing alongside Potter Stream and from either side of the bridge under West Coast Road where Potter Stream meets Oratia Stream (36.918326, 174.607674, Oratia, West Auckland) (UNITEC 9557; UNITEC 9554). These plants were potted in Garden Time Potting Mix (Bunnings) and grown on at Waiatarua, West Auckland at c. 370 m above sea level. Fertile fronds appeared

in early summer 2023 and continued to be produced from March to late May. Herbarium vouchers were made for all material collected and are held at the Unitec (UNITEC 14113; UNITEC 14114) and Auckland Museum (AK 386794) herbaria.

Mature fertile fronds were examined as fresh material and also gently dried in spore packets for a period of one week and the spores extracted. As spore size may vary depending on pre-treatment (Large & Braggins 1991), measurements were made under the same conditions with spores mounted and stained with cotton blue, after Large and Farrington (2016). Viability was also checked with fluorescein diacetate (FDA) (for example see Gabriel y Galán and Prada [2011]) with a fluorescent compound microscope Meiji FL–PWJ at 400× magnification. Spores and sporangia were also examined under a compound microscope (Olympus BH2) at 400× and 600× magnification. Sori were examined with a Leica Stereozoom S9i at 60× magnification.

Spores were also placed on nutrient agar (Fort Richard) plates to check for germination and gametophyte development. These were held in a culture room at 22° C in semi-shade, for eight weeks.

Results

In total, five fertile fronds were produced. These were c. 400–500 mm long and 60–90 mm wide, with alternate to sub-alternate pinnae, that were deltoid in shape, glabrous and 23(41)70 mm in length, (n = 20).

Sori (Figures 3A–3C) numbered 20–30 on each of the adaxial and abaxial margins. Indusia were rounded-cordate to horseshoe shaped. A narrow band of tissue attaching indusium, constricting growth, created the cordate form. Size: base width 0.30–0.53 mm; length 0.30–0.53 mm. These covered mostly immature sporangia and were largely retracted when sporangia were mature measuring 0.50–0.81 × 0.46–0.92 mm. No pattern of soral maturity was observed, with dark and lighter sporangia scattered within sori along the pinna. Sporangia were leptosporangiate, with 10–18 annulus cells, which were dark brown in colour when mature. The sporangia contained c. 50–64 spores (Figure 4).

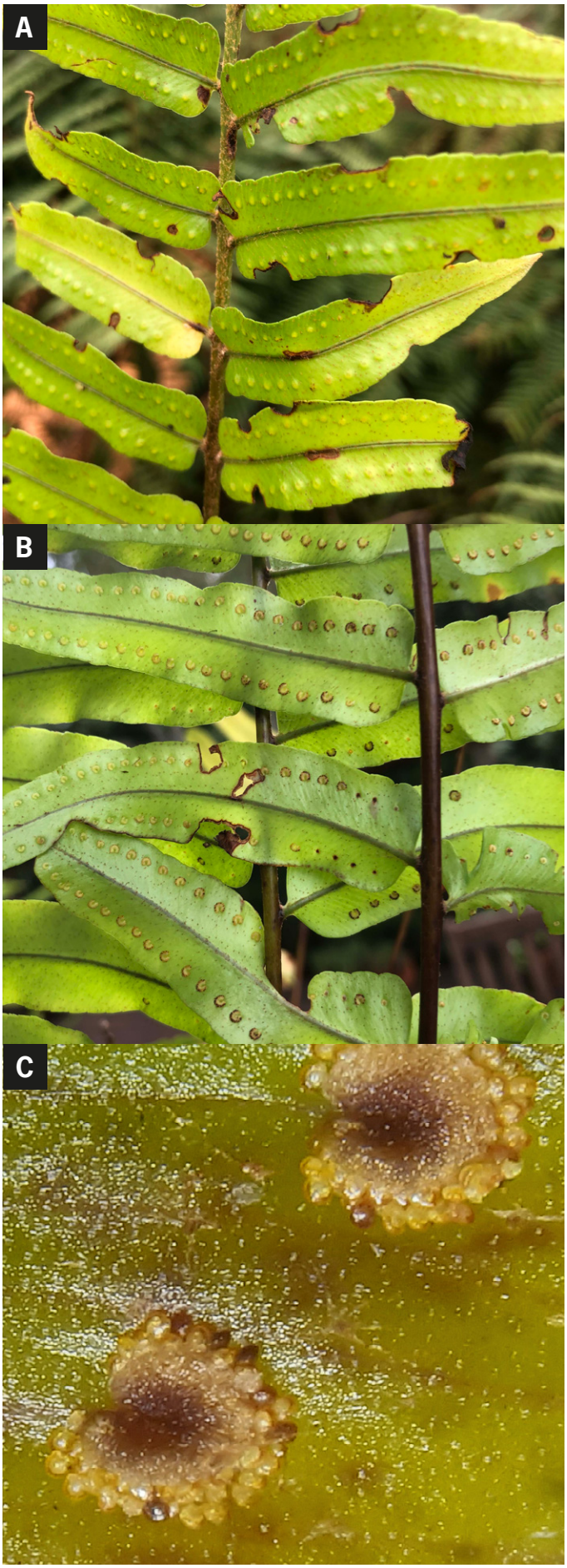

Figure 3A–3C. Lower surface of fertile frond *Nephrolepis exaltata* showing sori along both edges of pinnules (3A upper, 3B lower). Close up of sori (3C) with indusium (60× magnification). Photo: Mark Large

Figure 4. Leptosporangium of *Nephrolepis exaltata* with annulus of c. 14 cells containing 50–64 spores. 'Normal' ornamented spores are dark brown (100× magnification). Photo: Campbell James

Figure 5. Mature and abnormal spores of *Nephrolepis exaltata*. Mature normal spore central (brown), abnormal spores, top and bottom (600× magnification). Spores were from the same sporangium. Photo: Campbell James

All material examined showed between 40–50% abnormal spores, which were highly irregular in shape and apparently empty of living material (Figure 5), testing negative with fluorescein diacetate (FDA). Sizes: P10(14)20; E16(25)30 µm; P:E 14:25; n = 50. These contrasted with spores that were well formed, dark brown in colour, monolete, rounded to ellipsoid, with irregularly tuberculate surface. Again, fluorescein diacetate results were largely inconclusive, with little fluorescence noted. Sizes: P10(15)25±3; E20(26)38±4 µm; P:E 7:13; n = 50.

An attempt to germinate spores using nutrient agar was not successful, with no gametophyte development visible after eight weeks.

Discussion

Tryon and Lugardon (1991) record spore morphology for *Nephrolepis exaltata* (L.) Schott, collected in the Dominican Republic. These are described as monolete, ellipsoid to almost spherical, with irregularly tuberculate surface with globules, and are illustrated in Figure 148.6. The latter image of two spores suggests that this surface ornamentation is actually very small and variable, which the authors comment may be "in part related to the extent of perine deposition" (Tryon & Lugardon 1991: 383). These spores compare well with the brown 'mature' spores seen and described in this current study.

Nauman (1981) indicates uniformity in spore form across the genus, and certainly images (for example, see Tryon and Lugardon [1991], Figure 148.1–11) show very little differentiation between species. Nauman does note that hybridity seems to increase irregularity in form, which consequently increases irregularity in surface ornamentation. He specifically identifies a hybrid of *Nephrolepis biserrata* × *N. exaltata* as having large and spherical spores, albeit being somewhat irregular.

Spore morphology derived from the Oratia material compares well with the description given by Nauman (1981), and Tryon and Lugardon (1991), who also give equatorial measurements of E 25(30)34 (unfortunately P values are not available). Material examined in this study has a smaller overall average equatorial size (E20(26)38±4 µm), but is encompassed within the range of their measurements.

The irregular spore morphology, size and abnormality/abortion rate seen in this study may indicate hybridity or may be associated with environmental conditions. Hybridity is common amongst 'Boston ferns'

with *N. exaltata* a supposed source of the common cultivar and house plant, *Nephrolepis* cv. Bostoniensis (Hovenkamp & Miyamoto 2005). Indeed, it is reported that *N. exaltata sens str* is of hybrid origin (Hennequin et al. 2010; Yahaya et al. 2016), with *N. biserrata* and *N. cordifolia* as suspected parents.

Although germination experiments carried out in this study were inconclusive, the potential for future sporulation, viability and germination should not be entirely dismissed. Although Hovenkamp and Miyamoto (2005) suggest that *N. exaltata* did not naturalise outside of its native range, reports of naturalisation from the Canary Islands, Africa, Asia, India and Polynesia contradict this, as does gametophyte production as reported in India (Javalgekar & Mahabale 1959; Muthukumar & Prabha 2012). Given the probability of environmental change due to global warming / climate variation, the chance of fertile material appearing in Aotearoa / New Zealand is likely to increase and, along with it, the chance of spread.

A relatively high degree of abnormal spores c. 40–50% as recorded in this study, low viability, and the lack of germination indicate that spread by spores is unlikely under current conditions. The primary means of dispersal is still likely to be vegetative. Given the recent flooding of the Potter–Oratia Stream complex, it is possible that the vegetative dispersal of this species will occur further in this catchment, consequently future surveillance of this stream system will be useful.

Data Accessibility Statement

No additional database

Author Contributions

Campbell James: Conceptualisation (5%); data curation (90%); validation (50%); visualisation (50%): writing – review and editing (50%)
Mark Large: Conceptualisation (95%); data curation (10%); validation (50%); visualisation (50%): writing – review and editing (50%)

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