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ORIGINAL RESEARCH ARTICLE

Pucciniastrum minimum is the causal agent of blueberry leaf rust on different *Vaccinium* species in Hawke's Bay, New Zealand

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Abstract
Blueberry leaf rust has become a prevalent disease in New Zealand blueberry production. To identify the pathogen responsible for this disease in the Hawke's Bay region of New Zealand, leaves showing signs or symptoms of rust infection were collected from three blueberry cultivars ('Centra Blue' [Rabbiteye], 'Georgia Dawn' [Southern Highbush] and 'Nui' [Northern Highbush]) and the pathogen subjected to morphological characterization using both scanning electron and bright-field microscopy. Meanwhile, genomic DNA was extracted from urediniospores of infected leaves collected from cultivar 'Rahi' (Rabbiteye) and the Internal Transcribed Spacer (ITS) region was sequenced and compared to the corresponding nucleotide sequence of known rust pathogens. Results from both experiments indicated that *Pucciniastrum minimum* (syn. *Thekopsora minima*) was the causal agent of blueberry leaf rust disease in Hawke's Bay. Next, the level of disease caused by *P. minimum* was quantified on 23 blueberry cultivars in this region during the 2019 blueberry production season. Here, a total of 20 leaves selected from each cultivar were continually monitored, and the lesion area was calculated using *ImageJ* based on images taken in the field. Based on this analysis, all leaves were found to be infected by the rust pathogen. However, disease intensity, as a function of the 'area under the disease progress curve' (AUDPC) value, was found to be different. This suggests that certain cultivars display a lower disease intensity during the harvest season. Further field assessment covering a whole growing cycle will give a better understanding about blueberry leaf rust infection on these cultivars.

Keywords *Pucciniastrum minimum* (syn. *Thekopsora minima*) · Blueberry leaf rust · *Vaccinium* spp. · Blueberry · Internal transcribed spacer (ITS) region · Area under the disease progress curve (AUDPC) · *ImageJ*

Introduction

Blueberries (*Vaccinium* spp.) are the main export berry fruit in New Zealand, contributing NZ$40.5 million to the country's economy in 2021 (Plant and Food Research Limited and Horticulture New Zealand Limited 2021). In line with this importance, blueberry plantations are widespread in New Zealand, being found in both the North Island (Northland, Waikato, Bay of Plenty and Hawke's Bay regions) and South Island (Ngatea, Ohaupo and Otautau) (Blueberry Country Limited n.d.; Blueberries New Zealand Inc. n.d.). However, blueberry leaf rust has become a prevalent disease, causing early defoliation and a reduction in fruit quality (Plant Biosecurity and Product Integrity 2016; Polashock et al. 2017). A typical sign of blueberry leaf rust disease is yellow to orange pustules (uredinia) on the abaxial leaf surface which are often associated with dark brown necrotic symptoms on the adaxial leaf surface (Plant Biosecurity and Product Integrity 2016). Although the fungus *Pucciniastrum minimum* (syn. *Thekopsora minima*) has been identified as the causal agent of blueberry leaf rust disease in the Auckland, Waikato, and Canterbury regions of New Zealand (Padamsee and McKenzie 2019), it is not yet clear whether the same pathogen is responsible for blueberry leaf rust disease in the Hawke's Bay region, which is one of the main blueberry production regions in the country.

Different methodologies have traditionally been used to assess blueberry leaf rust disease incidence or severity, as well as host susceptibility. One of the most commonly used

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approaches involves a direct field assessment. For example, blueberry cultivar 'O'Neill' (may refer to 'O'Neal') was reported to be resistant to blueberry leaf rust disease based on a lack of pathogen signs associated with disease symptoms in field observations (Zheng et al. 2017). A number of blueberry cultivars, including 'Biloxi', 'Bluecrisp', 'Bluecrop', 'Bluegold', 'Brightwell', 'Brigitta', 'Climax', 'Elliott', 'Emerald', 'Gulfcoast', 'Jelly Bean', 'Jersey', 'Jewel', 'Liberty', 'Misty', 'Peach Sorbet', 'Powderblue', 'Premier', 'Rubel', 'Sapphire', 'Sharpblue', 'Snowchaser', 'Star' and 'Tifblue', were found to be susceptible to blueberry leaf rust disease (Barrau et al. 2002; Dal Bello and Perelló 1998; Huarhua et al. 2020; Keith et al. 2008; Mostert et al. 2010; Padamsee and McKenzie 2019; Pazdiora et al. 2018; Scherm et al. 2008; Schilder and Miles 2011; Shands et al. 2018; Wiseman et al. 2016; Zheng et al. 2017). The susceptibility of cultivars to blueberry leaf rust disease has also been assessed under nursery conditions, using a three-class scale (no disease symptoms; intermediate disease symptoms; and severe disease symptoms). More specifically, in a study by Wichura et al. (2020), five cultivars ('Aurora', 'Blueberry Glaze', 'Duke', 'Jelly Bean' and 'Peach Sorbet') were found to have no disease symptoms, while another nine cultivars ('Blue Bayou', 'Bluecrop', 'Cipria', 'Denise Blue', 'Goldtraube', 'Liberty', 'Pink Icing', 'Poppins' and 'Reka') were found to have either intermediate or severe disease symptoms.

The susceptibility of blueberry cultivars has also been assessed following artificial inoculation in two different protocols. One method involves spraying urediniospores onto healthy blueberry leaves, while the other involves directly brushing urediniospores from infected leaves onto healthy leaves. For instance, using a spray-based infection assay and a disease rating scale of 0–4, two (MS1718, PI638745) out of 15 Southern Highbush (SHB) blueberry accessions tested in a study by Babiker et al. (2018) were found to be resistant to blueberry leaf rust disease, while 11 commercial cultivars ('Biloxi', 'Bobolink', 'O'Neal', 'Pearl', 'Sharpblue', 'Snowchaser', 'Springhigh', 'Star', 'Suziblue', 'Ventura', and 'Windsor') were shown to exhibit different levels of susceptibility. Pustules with urediniospores and necrosis were also observed on cultivars 'Biloxi' and, 'Sharpblue' when inoculated by different spore suspensions (Huarhua et al. 2020; Keith et al. 2008; Mostert et al. 2010). With regards to the brushing method, where fresh urediniospores are directly brushed onto the abaxial leaf surface of healthy seedlings or detached leaves (Latham et al. 2022; Rebollar-Alviter et al. 2011; Zheng et al. 2017), two-year old seedlings of three SHB cultivars, namely 'Sharpblue', 'Misty' and 'Bluegold', were shown to be susceptible to blueberry leaf rust disease based on the presence of pathogen signs and symptoms, while 'O'Neal' was found without any disease signs and symptoms (Zheng et al. 2017). Yellow uredinia and symptoms of blueberry leaf rust disease were also observed on detached leaves of 'Biloxi' and 'Sharpblue', following inoculation of fresh urediniospores onto the abaxial leaf surface using the brushing method (Rebollar-Alviter et al. 2011).

Notably, consistent results have been recorded between field assessments and inoculation tests for cultivars 'Biloxi', 'Bluecrop', 'Bluegold', 'Misty', 'Sharpblue', 'Snowchaser', and 'Star' (Babiker et al. 2018; Dal Bello and Perelló 1998; Huarhua et al. 2020; Keith et al. 2008; Latham et al. 2022; Mostert et al. 2010; Padamsee and McKenzie 2019; Pazdiora et al. 2018; Rebollar-Alviter et al. 2011; Shands et al. 2018; Wichura et al. 2020; Wiseman et al. 2016; Zheng et al. 2017). However, different results have also been found in some studies. For instance, 'Jelly Bean' and 'Peach Sorbet' were found to be susceptible to blueberry leaf rust disease in a field assessment by Wiseman et al. (2016), while these cultivars were shown to be resistant to leaf rust disease in a nursery assessment (Wichura et al. 2020). Likewise, cultivar 'O'Neal' demonstrated a different result between two studies, where different inoculation methods were used. More specifically, Zheng et al. (2017), but not Babiker et al. (2018), failed to observe any rust symptoms on 'O'Neal following inoculation with the leaf rust pathogen. The thirty cultivars mentioned above, including 'Aurora', 'Blue Bayou', 'Blueberry Glaze', 'Bluecrisp', 'Bobolink', 'Brightwell', 'Brigitta', 'Cipria', 'Climax', 'Denise Blue', 'Duke', 'Elliott', 'Emerald', 'Goldtraube', 'Gulfcoast', 'Jersey', 'Jewel', 'Liberty', 'Pearl', 'Pink Icing', 'Poppins', 'Powderblue', 'Premier', 'Reka', 'Rubel', 'Sapphire', 'Springhigh', 'Suziblue', 'Ventura' and 'Windsor', were assessed for susceptibility or resistance to blueberry leaf rust disease with either field assessments or direct inoculation, but not both.

In this study, the causal agent of blueberry leaf rust disease in the Hawke's Bay region of New Zealand is identified. Furthermore, the severity of disease caused by this agent is quantified across 23 blueberry cultivars.

Materials and methods

Plant material used in this study

All blueberry leaves used in this study were collected from plants naturally infected with the blueberry leaf rust pathogen in the field (plantation net houses) of Gourmet Blueberries. Ltd. in Hastings, Hawke's Bay, New Zealand.

Morphological characterization of the blueberry leaf rust pathogen

In June 2018, blueberry leaves infected with the blueberry leaf rust pathogen (i.e. showing evidence of urediniospore-containing pustules on their abaxial leaf surface), were randomly picked from the Rabbiteye (RE) cultivar 'Centra Blue', the SHB cultivar 'Georgia Dawn', and the Northern

Highbush (NHB) cultivar 'Nui'. These leaf samples were used for a morphological identification experiment involving scanning electron microscopy (SEM) or bright-field microscopy at the Manawatū Microscopy and Imaging Centre (MMIC; Palmerston North, New Zealand). The size of urediniospores was calculated with minimum 8 individual spores from clear SEM images of each cultivar.

For SEM, a small amount of leaf tissue harbouring rust pustules was excised, placed in a modified version of Karnovsky's fixative (3% glutaraldehyde, 2% formaldehyde in 0.1 M phosphate buffer, pH 7.2) with Triton X-100, and vacuum-infiltrated until wet. The fixative was replaced and the samples were allowed to fix for at least 8 h at room temperature. Fixed samples were then washed three times (10–15 min each) in phosphate buffer (0.1 M, pH 7.2), dehydrated using a graded ethanol series (25%, 50%, 75%, 95%, 100%) for 10–15 min each, and subsequently washed for 1 h in 100% ethanol (Alves et al. 2013). Next, samples were critical point-dried using liquid CO_2 as the critical point fluid and 100% ethanol as the intermediary (Polaron E3000 series II critical point drying apparatus). Samples were mounted onto aluminium stubs using double-sided tape and sputter coated with approximately 100 nm of gold (Baltec SCD 050 sputter coater) and viewed under the FEI Quanta 200 Environmental Scanning Electron Microscope at an accelerating voltage of 20 kV.

For bright-field microscopy involving cross-sections, a small amount of leaf tissue harbouring rust pustules was again excised, fixed, vacuum-infiltrated and washed as above. Samples were then post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2) for 1 h and washed three times in 0.1 M phosphate buffer (pH 7.2). Following this step, samples were dehydrated using a graded acetone series (25%, 50%, 75%, 95%, 100%) for 10–15 min each, followed by two rounds of 100% acetone for 1 h each. Next, samples were placed in 50:50 resin:acetone and stirred overnight, and then fresh 100% resin (Procure 812, ProSciTech Australia) was replaced and stirred for 8 h. Finally, samples were embedded in moulds with fresh resin and cured in a 60 °C oven for 48 h. To visualise the samples, sections were first cut at 1 micron on an ultramicrotome (Leica EM UC7, Germany) and heat-fixed onto glass slides. These were then stained with 0.05% toluidine blue for approximately 12 s and viewed by bright-field microscopy (Zeiss Axiophot compound light microscope), with images taken using a DFC320 camera. The same microscopy and camera were also used to visualise non-cross-sectioned samples.

Molecular characterization of the blueberry leaf rust pathogen

Using a pipette tip, approximately 100–200 urediniospores from the blueberry leaf rust pathogen were brushed onto a glass slide from pustules (uredinia) of disease lesions formed on the abaxial surface of five leaves from the RE blueberry cultivar 'Rahi'. Collected urediniospores were then crushed between two glass slides and suspended in a 20 µl buffer (10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% sodium dodecyl sulfate [SDS], and 0.01% Proteinase K) (Virtudazo et al. 2001) for crude genomic DNA extraction. Crude genomic DNA extraction was carried out by incubation at 37 °C for 60 min, followed by 95 °C for 10 min, and the resulting genomic DNA stored at − 20 °C until required (Yang et al. 2015).

A polymerase chain reaction (PCR) experiment was carried out to amplify the Internal Transcribed Spacer (ITS) region from the genomic DNA of the blueberry leaf rust pathogen. This was achieved using Phusion Flash High-Fidelity PCR Master Mix (Thermo Fisher Scientific), according to the manufacturer's instructions, in conjunction with 3 µl (10- to 100-fold) genomic DNA template, and one of two primer pairs. The first primer pair, made up of Rust2inv (5'-GATGAAGAACACAGT GAAA-3') (Aime 2006) and LR6 (5'-CGCCAGTTCTGC TTACC-3') (Vilgalys and Hester 1990), generates a PCR amplicon of approximately 1,400 bp, covering the 5.8 S subunit, the ITS-2 region, and part of the large 28 S subunit. The second primer pair, made up of LROR (5'-ACC CGCTGAACTTAAGC-3') (Bunyard et al. 1994) and LR3 (5'-GGTCCGTGTTTCAAGAC-3') (Vilgalys and Hester 1990), produces a PCR amplicon of around 600 bp, overlapping part of the large 28 S subunit amplified by the first primer pair. PCR amplicons were subsequently resolved by 1% Tris-acetate-EDTA (TAE) agarose gel electrophoresis, purified from the gel using an E.Z.N.A. Gel Extraction Kit (Omega Bio-tek), and sequenced in both the forward and reverse directions using the same primers mentioned above at the Massey Genome Service sequencing facility (Palmerston North, New Zealand).

The resulting nucleotide sequences were aligned using the ClustalW tool in Geneious v9.0.5 (Kearse et al. 2012) to generate a single contiguous sequence and deposited in the National Centre for Biotechnology Information (NCBI) GenBank under accession number MK604179 (https://www.ncbi.nlm.nih.gov/nuccore/MK604179.1/). Next, a BLASTn analysis was performed against the non-redundant/nucleotide sequence (nr/nt) database at NCBI using the MK604179 sequence as a template to identify the most closely related nucleotide sequence from previous studies.

Field assessment and data analysis

Field assessments were performed from January to March in 2019, which overlapped within the main yearly blueberry harvesting period of December to April. In total, 23 cultivars were used to quantify blueberry leaf rust disease. These were

made up of five RE ('Rahi', 'Centra Blue', 'Centurion', 'Titan' and 'Sky Blue'), three NHB ('Nui', 'Blue Moon' and 'Duke'), and 15 SHB ('Camellia', 'Palmetto', 'Misty', 'O'Neal', 'Springhigh', 'Scintilla', 'Snowchaser', 'Miss Jackie', 'Miss Lilly', 'Georgia Dawn', 'Southern Splendour', 'Suziblue', 'Kestrel', 'Flicker' and 'Sweetcrisp') cultivars. Ten mature blueberry plants of each cultivar were selected randomly in different production blocks in the field and, on each plant, a one-year-old shoot was labelled. Two fully-expanded leaves of each labelled shoot were then selected and used to monitor rust symptom development. During the monitoring period, a photo of the adaxial surface from each labelled leaf was taken once per week for eight weeks to record rust symptom development. A ruler was placed horizontally next to the leaf as a standard scale for calculating the lesions and leaf area using *ImageJ* (*Fiji* v3.0) (Schindelin et al. 2015). The lesion area used for calculations in this study was a reddish to purple pustule on the adaxial leaf surface (Supplemental Fig. 1).

Appling the Region of Interest (ROI) Manager function in *Fiji*, lesion and leaf area were calculated from each leaf image. An 'area under disease progress curve' (AUDPC) value for each leaf was then calculated and the average AUDPC value for each cultivar was used for statistical analysis (Campbell 1990):

$$AUDPC = \sum_{i=1}^{n=1} \left[\frac{(t_{i+1} - t_i)(y_i + y_{i+1})}{2} \right]$$

where t is time in days of each measurement, y is the lesion area at each measurement, and n is the number of measurements.

An Analysis of Variance (ANOVA) was performed in RStudio v4.0.5 (Racine 2012) to assess whether differences in AUDPC value were significant between SHB, NHB and RE blueberry cultivars. The Tukey Honestly Significant Differences (TukeyHSD) test was used to quantify the severity of leaf rust disease by comparing the mean AUDPC value between SHB, NHB and RE cultivars. The AUDPC value was also used to compare between these 23 commercial cultivars.

Results

Morphological identification of the blueberry leaf rust pathogen

As a starting point for identification of the blueberry leaf rust pathogen, the morphology of the fungus was examined using SEM and bright-field microscopy. Fresh yellow-orange colored pustules were found on the abaxial leaf surface of blueberry cultivars 'Centra Blue' and 'Georgia Dawn', while light yellow to grey colored pustules, sometimes surrounded by a halo of red-purple plant tissue, were observed on the abaxial leaf surface of blueberry cultivar 'Nui', using bright-field microscopy (Fig. 1a1–a3). On all of these leaf samples, both uredinia and urediniospores, but not telia or teliospores, were observed using SEM and bright-field microscopy. The fungal specimens had the following morphology. Urediniospores were obovoid to elliptical in shape and their surface was echinulate. The average size of the urediniospore varied from 10.3 to 12.7 µm in width and 13.9 to 16.2 µm in length (Fig. 1b1–b3). The uredinia were up to 180 µm in diameter and mostly clustered with a dome shape and protruded from stomata (Fig. 1c1–c3). The peridia were grown within the epidermal cells of the host and lacked conspicuous ostiolar cells (Fig. 1d1–d3).

Sequence analyses of the blueberry leaf rust pathogen

To conclusively identify the causal agent of the blueberry leaf rust disease, the ITS region was sequenced from genomic DNA of urediniospores isolated from cultivar 'Rahi' (deposited in NCBI under Accession MK604179.1) and screened against sequences from known organisms in NCBI using BLASTn. Based on this analysis, the top hit, with 99.79% nucleotide sequence identity (GU355675.1), was found to be from a *Pucciniastrum. minimum* isolate of diseased blueberry (*Vaccinium corymbosum*) in South Africa. The rust isolate collected in the Hawke's Bay region, however, did contain three mismatched nucleotides in the aligned sequence with GU355675 (Supplemental Fig. 2). Through the alignment results, it can be concluded that *P. minimum* is the rust species found in the blueberry plantation of Gourmet Blueberries. Ltd, in Hastings of the Hawke's Bay region of New Zealand.

Field assessment of the blueberry leaf rust pathogen

To better understand how the severity of blueberry leaf rust disease differs between the commercial cultivars, a field assessment was carried out for eight weeks in 2019. However, no cultivar was observed without signs of blueberry leaf rust infection during this observation period. A difference in AUDPC value was found among SHB, RE and NHB cultivars ($p = 0.00223$), whereby the RE cultivars had a lower AUDPC value than the NHB and SHB cultivars (Fig. 2A). In addition, a significant difference was found among these commercial cultivars ($p < 2e\text{-}16$). Based on TukeyHSD test results, the AUDPC value (< 0.0147) of 19 cultivars ('Rahi', 'Centra Blue', 'Centurion', 'Sky Blue', 'Duke', 'Titan' 'Camellia', 'Palmetto', 'Misty', 'O'Neal', 'Springhigh', 'Snowchaser','Miss Jackie', 'Miss Lilly', 'Georgia Dawn', 'Suziblue', 'Kestrel' and 'Flicker') was significant lower than 'Blue Moon', 'Scintilla' and 'Southern Splendour' (Fig. 2B). 'O'Neal' (SHB) had the lowest AUDPC value (0.00064), while both 'Scintilla' (SHB) and 'Southern Splendour' (SHB) had a higher AUDPC value (> 0.061).

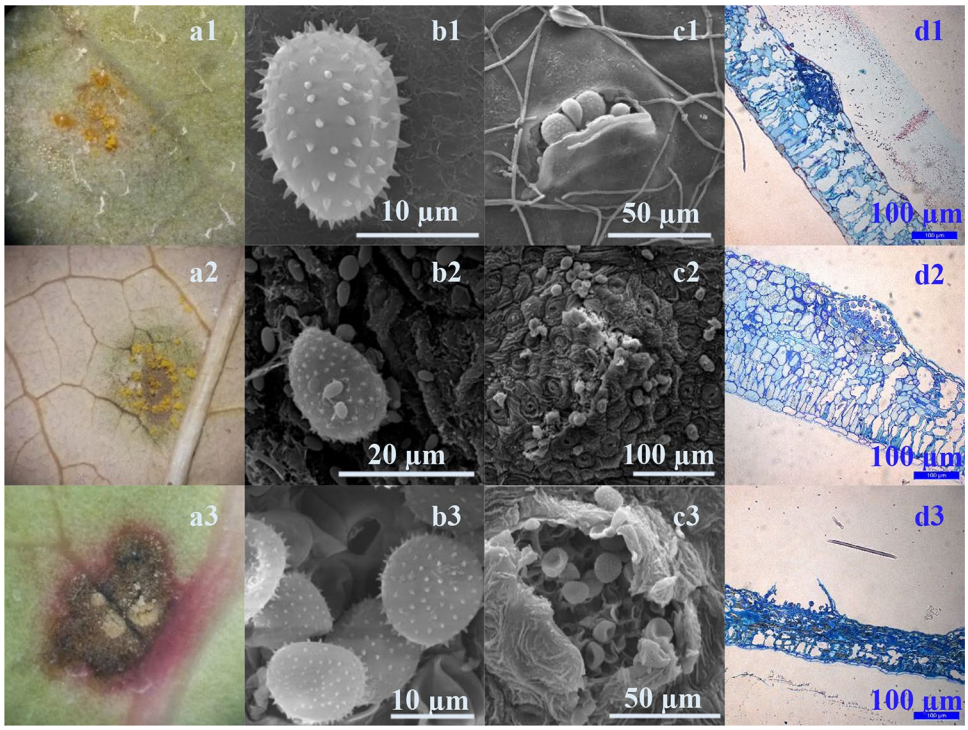

Fig. 1 Rust pustules, urediniospores and uredinia on rust-infected leaves of blueberry cultivars 'Centra Blue' (**a1**–**d1**), 'Georgia Dawn' **(a2–d2)**, and 'Nui' (**a3–d3**). **a1–a3** Rust pustules on the abaxial leaf surface observed by bright-field microscopy; **b1–b3** Urediniospores observed by scanning electron microscopy (SEM); **c1–c3** Uredinia observed by SEM; **d1–d3** Cross-sections of uredinia observed by bright-field microscopy

Discussion

In a recent study, *P. minimum* (syn. *T. minima*) was identified as the causal agent of blueberry leaf rust disease in the Auckland, Waikato, and Canterbury regions of New Zealand (Padamsee and McKenzie 2019). In our study, we set out to determine whether this pathogen was also responsible for causing leaf rust disease in the blueberry plantations of Hawke's Bay region in New Zealand, as it remained possible that other rust species, specifically *Naohidemyces vaccinii*, which has been reported to be the causal agent of blueberry leaf rust disease in other parts of the world, such as Argentina, Hawaii, Japan and Spain (Barrau et al. 2002; Dal Bello and Perelló 1998; Keith et al. 2008; Sato et al. 1993), may instead be to blame.

The morphological assessment of urediniospores collected from infected NHB, SHB and RE blueberry cultivars, combined with the ITS sequencing of urediniospores collected from an infected RE cultivar, 'Rahi', confirmed that the pathogen responsible for blueberry leaf rust disease in the Hawke's Bay region is indeed *P. minimum*. This finding is consistent with previous studies from the USA (California, Oregon and Michigan), Australia (Bundaberg, Queensland and New South Wales), Brazil, China (Sichuan), Europe (Belgium, Germany,

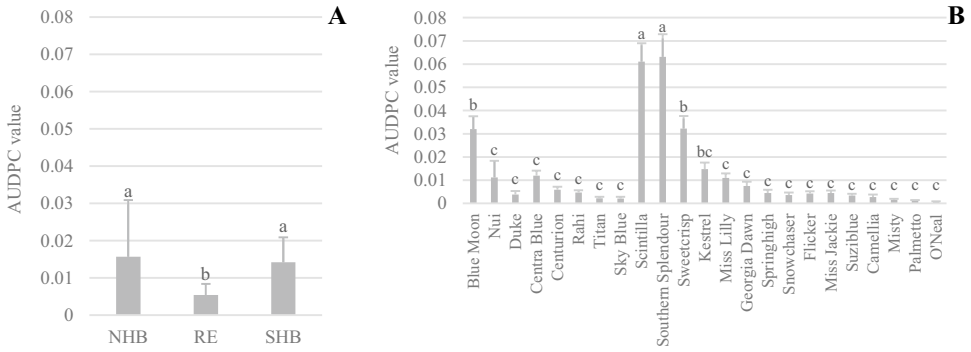

Fig. 2 The Tukey Honestly Significant Differences test for 23 commercial cultivars that belong to Northern Highbush (NHB), Rabbiteye (RE) and Southern Highbush (SHB): results are presented for cultivar group/type (**A**) and individual cultivars (**B**) based on the 'area under disease progress curve' (AUDPC) value, which was calculated from the blueberry leaf rust lesion area using 20 leaf samples from each cultivar. Values are shown as the mean ± standard error

Portugal, the Netherlands, Sweden and the United Kingdom), Mexico, Peru and South Africa (EPPO Global Database 2022; McTaggart et al. 2013; Mostert et al. 2010; Pazdiora et al. 2018; Rebollar-Alviter et al. 2011; Schilder and Miles 2011; Shands et al. 2018; Wiseman et al. 2016; Zheng et al. 2017), where *P. minimum* has also been identified as the causal agent. Furthermore, this finding also supports the previous conclusion made by Padamsee and McKenzie (2019) that the pathogen responsible for blueberry leaf rust disease in New Zealand is *P. minimum*.

The 23 commercial production cultivars used in this study were found without any immunity to the leaf rust disease. Until now, sixteen of these cultivars had not been reported to be susceptible to blueberry leaf rust disease, including four RE ('Rahi', 'Centra Blue', 'Centurion' and 'Titan'), two NHB ('Duke' and 'Nui'), and ten SHB ('Camellia', 'Palmetto', 'Scintilla', 'Miss Jackie', 'Miss Lilly', 'Georgia Dawn', 'Southern Splendour', 'Kestrel', 'Flicker' and 'Sweetcrisp') cultivars.

In agreement with our field assessments, previous studies that used spraying of *T. minima* inoculum onto fully expanded leaves have shown that the SHB cultivars 'O'Neal', 'Snowchaser', 'Springhigh', and 'Suziblue' are susceptible to blueberry leaf rust disease (Babiker et al. 2018). Likewise, the SHB cultivars, 'Misty' and 'Snowchaser', have also been shown to be susceptible to blueberry leaf rust disease caused by this pathogen using field and inoculation assessments (Barrau et al. 2002; Keith et al. 2008; Padamsee and McKenzie 2019; Pazdiora et al. 2018; Shands et al. 2018; Zheng et al. 2017). However, in contrast to our field assessments, the study performed by Zheng et al. (2017) did not find 'O'Neal' to be susceptible to blueberry leaf rust disease caused by *T. minima*. Furthermore, the NHB cultivar 'Duke' was reported to be without infection symptoms in a nursery under natural infection conditions (Wichura et al. 2020). In our field study, symptomatic leaves were observed from both 'Duke' and 'O'Neal'. It is possible that the contrasting results may be due to variation in the inoculation assay and the natural infection under different growing and climate conditions.

In our study, blueberry leaf rust disease in the field was quantified using AUDPC values based on the area of lesions with a reddish to purple pustule/lesion, providing a simplified means of quantitatively assessing disease intensity over time and giving clearly quantified data for each blueberry cultivar to compare with other studies in the future. Of the 23 blueberry cultivars mentioned above, differences were observed in disease intensity over time caused by *P. minimum*. Both 'Scintilla' and 'Southern Splendour' had a higher AUDPC value than other blueberry cultivars, indicating they were more susceptible to leaf rust disease, with much quicker lesion development. Comparatively, the remaining 19 cultivars ('Rahi', 'Centra Blue', 'Centurion', 'Sky Blue', 'Duke', 'Titan', 'Camellia', 'Palmetto', 'Misty', 'O'Neal', 'Springhigh', 'Snowchaser', 'Miss Jackie', 'Miss Lilly', 'Georgia Dawn', 'Suziblue', 'Kestrel' and 'Flicker') had much slower lesion development, showing a higher tolerance to blueberry leaf rust disease in the fruit harvesting period, suggesting that they may be a better choice for present commercial blueberry production. As no resistant cultivars to blueberry leaf rust disease were observed in this study, it was impossible to establish a complete disease rating scale by using the data in this study.

Notably, infection symptoms of blueberry leaf rust disease were observed on all monitored blueberry cultivars. To enable the sustainable production of blueberries in New Zealand, it is important to develop and use new cultivars with resistance to leaf rust disease. This is essential, because the disease not only reduces the yield and quality of the fruit (Plant Biosecurity and Product Integrity 2016), but it is also one of the diseases under fresh fruit import regulation in Australia (Australian Government Department of Agriculture, Fisheries and Forestry 2021); a country which made up 89% of the New Zealand blueberry export value in 2021 (Plant and Food Research Limited and Horticulture New Zealand Limited 2021).

In conclusion, we have identified *P. minimum* as the causal agent of blueberry leaf rust disease in the Hawke's Bay region of New Zealand. Furthermore, based on lesion development in the field, we suggest that the cultivars with a slower lesion development may be more suitable for commercial blueberry production in New Zealand as they appeared to be less infected by the pathogen of blueberry leaf rust disease. The quantification method used in this study provided a comparable data format on blueberry leaf rust disease of different cultivars. To establish a quantification scale for blueberry leaf rust disease, more blueberry cultivars and accessions need to be assessed across a whole growing cycle for their disease level in further studies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13313-023-00907-x.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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