Phytopathologia Mediterranea (2015) 54, 1, 124–127
DOI: 10.14601/Phytopathol_Mediterr-14047

NEW OR UNUSUAL DISEASE REPORT

A new disease of *Erica arborea* in Italy caused by *Neofusicoccum luteum*

Benedetto T. LINALDEDDU, Bruno SCANU, Salvatore SEDDAIU, Antonio DEIDDA, Lucia MADDAU and Antonio FRANCESCHINI

Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università degli Studi di Sassari, Viale Italia 39, 07100 Sassari, Italy

Summary. Shoot blight was observed on *Erica arborea* L., in a natural growing area on Caprera Island (Italy), during 2011 and 2012. Fungal isolates obtained from 324 symptomatic shoots were identified as *Neofusicoccum luteum* by analysis of morphological and cultural characteristics, as well as DNA sequence data of the Internal Transcribed Spacer (ITS) of the ribosomal DNA and part of the translation Elongation Factor 1-alpha (EF-1α) gene. Pathogenicity of the fungus was verified by stem inoculation of 3-year-old seedlings of *E. arborea*. This is the first report of *N. luteum* as a pathogen of *E. arborea*.

Key words: *Botryosphaeriaceae*, shoot blight, Caprera Island.

Introduction

Tree heath (*Erica arborea* L.) in the family *Ericaceae*, is one of the main components of the Mediterranean "maquis" biome, and is a valuable ornamental plant for gardening and landscaping that is increasingly cultivated in commercial nurseries. In Sardinia (Italy) and in the surrounding minor islands of the La Maddalena Archipelago, tree heath grows preferably on siliceous substrates and in mixed stands with cork oak, holm oak and strawberry tree (Camarda and Valsecchi, 2008). Since 2011, an unusual disease on tree heath has been observed in a natural area located in the centre of Caprera Island. Given that there was no information about this disease, a survey was carried out to establish the causal agent and characterize the disease.

Materials and methods

Field surveys were carried out in autumn 2011 and spring 2012 at the major tree heath growing areas on Caprera Island [41°12′N, 9°28′E]. A network of six monitoring plots (MPs, diameter 10 m) was established inside the tree heath stands, and the geographic coordinates recorded by a portable GPS (eTrex, Garmin). The plots were randomly selected throughout the centre of the island where the disease was observed for the first time. At each MP the number of tree heath shrubs present was recorded and incidence of the disease estimated on the basis of number of symptomatic plants compared to the total number of plants. At each plot nine symptomatic shoots were collected from three tree heath shrubs chosen at random. Collected samples were processed within 48 h. After a preliminary microscopic examination aimed at checking the presence and nature of fungal reproductive structures, the samples were surface disinfected with 70% ethanol for 30 s, rinsed in sterile water and then placed to dry in aseptic conditions. Fungal isolations were made from fragments of inner bark and xylem tissues measuring approx. 3–5 mm, aseptically cut from the margin of necrotic lesions. All samples were cultured in Petri dishes containing potato dextrose agar (PDA, Oxoid Ltd). After incubation at 25°C for 1 week, fungal colonies

Corresponding author: B.T. Linaldeddu
E-mail: ben@uniss.it

ISSN (print): 0031-9465
ISSN (online): 1593-2095
www.fupress.com/pm
© Firenze University Press

were sub-cultured onto PDA and kept on the laboratory bench in natural daylight at room temperature (20–26°C). To induce sporulation, fungal isolates were also sub-cultured on PDA with sterile twigs of holm oak. Conidia oozing from pycnidia in resulting cultures were mounted in lactophenol for microscopic examination. Dimensions of conidia were recorded using an Optika™ Vision Pro version 2.7 digital camera connected to a Leitz Diaplan (Leitz, Wetzelar, Germany) microscope.

The colony appearance of cultures growing on PDA at 25°C in the dark for 1 week was recorded. Given that only a botryosphaeriaceous fungus was consistently isolated, six isolates were used as representative cultures for further molecular studies. These isolates were also stored on PDA slants under oil in the culture collection of the "Sez. di Patologia Vegetale ed Entomologia, Dipartimento di Agraria" at the University of Sassari, as catalogue numbers BL141 to BL146.

Genomic DNA was extracted from cultures grown on PDA for 5 d at 25°C following the CTAB method (Doyle and Doyle, 1987). The Internal Transcribed Spacer (ITS) of the ribosomal DNA was amplified and sequenced with primers ITS1 and ITS4 (White *et al*., 1990), while the primers EF446f and EF1035r (Inderbitzin *et al*., 2010) were used to amplify and sequence part of the translation Elongation Factor 1-alpha (EF-1α) gene. Polymerase chain reaction (PCR) mixtures and amplification conditions were conducted as described by Linaldeddu et al. (2013). The PCR products were purified using the EUROGOLD gel extraction kit (EuroClone S.p.A.) following the manufacturer's instructions. Both strands were sequenced by the BMR Genomics DNA sequencing service (www.bmr-genomics.it). The nucleotide sequences were read and edited with FinchTV 1.4.0 (Geospiza, Inc.; http://www.geospiza.com/finchtv) and compared with sequences deposited in GenBank using the BLAST software (http://blast.ncbi.nlm.nih.gov).

Pathogenicity was verified by shoot and stem inoculation on 3-year-old seedlings of tree heath. Shoots of seven seedlings were each inoculated by placing a mycelial plug (3–4 mm^2) taken from the margin of an actively growing colony on PDA into a shallow wound (≈3 mm) made by a sterile scalpel. A further seven seedlings were inoculated at the base of the stem using the technique described above. The inoculation point was covered with cotton wool soaked in sterile water and wrapped with Parafilm®. Fourteen control seedlings were inoculated (seven on shoots and seven on the stems) with a sterile PDA plug. Inoculated seedlings were kept in the laboratory at 18–26°C in natural daylight for 2 months.

Results and discussion

Symptomatology

Typical symptoms of this unusual disease appear in late spring with the sudden wilting of new shoots (Figure 1a). The infection spreads within the shoot tissues, rarely into the secondary xylem tissues of the branches, causing the shoot tips to curl and form crooks (Figure 1b). An abnormal mass of new epicormic shoots grows under infected shoots, with the resulting structures resembling witch's brooms (Figure 1c). Leaves on affected shoots turn yellow, then dull red and finally brown. They often remain attached for some time after the death of the shoots. All diseased plants showed these symptoms.

Disease incidence

Field surveys conducted on 120 tree heath shrubs occurring inside the six MPs showed that 112 shrubs were symptomatic and that the disease affected all areas of tree heath located in the central part of the island. In particular, disease incidence among the six MPs plots ranged from 45 to 100%.

Aetiology

All of the 324 symptomatic shoot samples yielded colonies of a single species of Botryosphaeriaceae. On PDA at 25°C, all isolates developed moderate aerial mycelium, with a light yellow pigment diffusing into the medium that became pale grey on the reverse side of Petri dishes after 5–6 d (Figure 1d). Conidiomata were formed from the centre of colonies within 3–4 weeks. All isolates produced hyaline, aseptate and fusiform to elliptical conidia (average of 50 conidia: 18.1 × 6.3 μm, length:width ratio = 2.9) (Figure 3e), and sporadically elliptical microconidia. On the basis of morphological and cultural features, the strains were identified as *Neofusicoccum luteum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips. Identification was confirmed by analysis of the ITS and EF1-α sequences. BLAST searches in GenBank showed 100% similarity with reference

Figure 1. a: Early dieback symptoms on shoots of tree heath. b: Shoots wilted with brown leaves still attached during the summer. c: Extensive twig and branch dieback. d: Colonies of *Neofusicoccum luteum* on PDA after 7 d at 25°C (left, upper view; right, reverse). e: Hyaline and aseptate conidia.

sequences of *N. luteum* including the ex-type isolate (CBS110299). New sequences were deposited in GenBank (ITS accession numbers: KJ406187-KJ406192; EF1-α accession numbers: KJ943360-KJ943365).

Pathogenicity

Two months after inoculation, all seedlings inoculated with the strain BL142 obtained in this study showed dark brown necrosis at the inoculation points. However, on stems the mean lesion length produced by *N. luteum* was not significantly different from that of the control (Figure 2). On inoculated shoots *N. luteum* caused extensive necrotic lesions significantly different from those on controls, and the symptoms were identical to those observed in the field. The pathogen was successfully re-isolated from all shoots and stem samples processed, thus fulfilling Koch's postulates.

Neofusicoccum luteum was first isolated from lesions on ripe fruit of *Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson in New Zealand (Pennycook and Samuels, 1985), and more recently has been found to be pathogenic to a wide range of woody hosts of agricultural and forestry importance in different countries around the world. These include *Cra-*

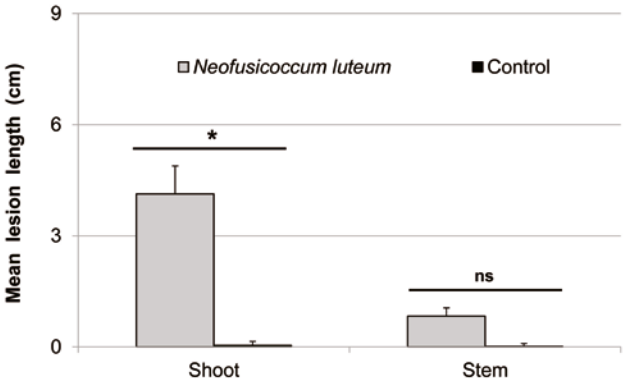

Figure 2. Mean lesion length caused by *Neofusicoccum luteum* on tree heath seedlings. Error bars represent the standard deviations from the means. Significant differences among mean values were determined with the Student's *t* test, using XLSTAT software (Addinsoft, France). * = $P<0.05$, ns = not significant.

taegus mexicana Moc. & Sessé ex DC., *Olea europaea* L., *Persea americana* Mill., *Quercus robur* L., *Rhododendron* spp., *Syzygium cordatum* Hochst. ex C. Krauss and *Vitis vinifera* L. (Pavlic *et al.*, 2007; McDonald *et al.*, 2009; Sergeeva *et al.*, 2009; Pintos Varela *et al.*, 2011; Amponsah *et al.*, 2012; Adesemoye *et al.*, 2013; Barradas *et al.*, 2013). To our knowledge, this is the first report of *N. luteum* as a pathogen of tree heath. Despite the ecological importance of Mediterranean "maquis", the diseases affecting these plants have not yet been adequately studied. Given the high ecological relevance of this outbreak and the abundance of Mediterranean "maquis" ecosystems in Sardinia, further investigations are underway to monitor their health status.

Acknowledgments

This work was supported by grants from Parco Nazionale dell'Arcipelago di La Maddalena: projet "Linee guida per la bonifica fitosanitaria dei popolamenti di leccio nell'isola di Caprera". Antonio Deidda gratefully acknowledges Sardinia Regional Government for the financial support of his PhD scholarship (P.O.R. Sardegna F.S.E. Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2007–2013 - Axis IV Human Resources, Objective l.3, Line of Activity l.3.1.).

Literature cited

Adesemoye A.O., J.S. Mayorquin and A. Eskalen, 2013. *Neofusicoccum luteum* as a pathogen on Tejocote (*Crataegus mexicana*). *Phytopathologia Mediterranea* 52(1), 123–129.

Amponsah N.T., E.E. Jones, H.J. Ridgway and M.V. Jaspers, 2012. Susceptibility of grapevine tissues to *Neofusicoccum luteum* conidial infection. *Plant Pathology* 61, 719–729.

Barradas C., A. Correia and A. Alves, 2013. First report of *Neofusicoccum australe* and *N. luteum* associated with canker and dieback of *Quercus robur* in Portugal. *Plant Disease* 97, 560.

Camarda I. and F. Valsecchi, 2008. Alberi e Arbusti Spontanei della Sardegna. Carlo Delfino Editore, Sassari, Italy.

Doyle J.J. and J.L. Doyle, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19, 11–15.

Inderbitzin P., Bostock R.M., Trouillas F.P. and T.J. Michailides, 2010. A six-locus phylogeny reveals high species diversity in *Botryosphaeriaceae* from California almond. *Mycologia* 102, 1350–1368.

Linaldeddu B.T., A. Franceschini, A. Alves and A.J.L. Phillips, 2013. *Diplodia quercivora* sp. nov.: a new species of *Diplodia* found on declining *Quercus canariensis* trees in Tunisia. *Mycologia* 105(5), 1266–1274.

McDonald V., S. Lynch and A. Eskalen, 2009. First report of *Neofusicoccum australe*, *N. luteum*, and *N. parvum* associated with avocado branch canker in California. *Plant Disease* 93, 967.

Pavlic D., B. Slippers, T.A. Coutinho and M.J. Wingfield, 2007. *Botryosphaeriaceae* occurring on native *Syzygium cordatum* in South Africa and their potential threat to *Eucalyptus*. *Plant Pathology*, 56, 624–636.

Pennycook S.R. and G.J. Samuels, 1985. *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (kiwifruit) in New Zealand. *Mycotaxon* 24, 445–458.

Pintos Varela C., V. Redondo Fernández, J.P. Mansilla Vázquez and O. Aguín Casal, 2011. First report of dieback on hybrid rhododendrons caused by *Neofusicoccum luteum* and *N. parvum* in Spain. *Plant Disease* 95, 221.

Sergeeva V., A. Alves and A.J.L. Phillips, 2009. *Neofusicoccum luteum* associated with leaf necrosis and fruit rot of olives in New South Wales, Australia. *Phytopathologia Mediterranea* 48, 294–298.

White T.J., T. Bruns, S. Lee and J. Taylor, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: *PCR protocols: A guide to methods and applications* (M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White, ed.). Academic Press, San Diego, CA, USA, 315–322.

Accepted for publication: July 7, 2014
Published online: September 19, 2014