



NEW DISEASE REPORT

First report of *Calonectria henricotiae* causing box blight in Spain

P. Kong¹ | R. loos² | C. X. Hong¹¹Virginia Tech, Hampton Roads Agricultural Research and Extension Center, Virginia Beach, Virginia, USA²Agence Nationale de Sécurité Sanitaire de l'Alimentation (ANSES), Laboratoire de la Santé des Végétaux - Unité de Mycologie, Malzéville, France**Correspondence**P. Kong, Virginia Tech, Hampton Roads Agricultural Research and Extension Center, 1444 Diamond Springs Road, Virginia Beach, Virginia, USA. Email: pkong@vt.edu**Funding information**

the USDA National Institute of Food and Agriculture - Specialty Crop Research Initiative, Grant/Award Number: 2020-51181-32135

KEYWORDSboxwood blight, *Buxus sempervirens*, *Calonectria pseudonaviculata*, *Cylindrocladium*

Box blight is caused by two closely related fungi, *Calonectria pseudonaviculata* (*Cps*) and/or *C. henricotiae* (*Che*) (Gehesquière et al., 2016). *Cps* is widespread globally (Daughtrey, 2019) while *Che* is limited to eight central European countries (Hong, 2023). To determine whether *Che* is present in southern Europe, we visited ten large boxwood gardens and street plantings in Madrid and Toledo, Spain and in Bordeaux, Lyon and Sainte-Foy-la-Grande, France.

Boxwood (*Buxus* spp.) twigs with black streaks (Figure 1) were only observed in Madrid, Toledo, and Sainte-Foy-la-Grande. Diseased twigs were collected in sealed triple bags and brought to the laboratory under the USDA permit (P526P-21-05960). After surface sterilization with 10% bleach for two minutes and three rinses in deionised water, twigs were placed on a mesh with wet paper towels underneath in closed plastic containers at 23°C and wetted with sterile distilled water every two days until white mould appeared on leaves (Figure 2). The mould was suspended in 15 µL sterile distilled water then transferred onto a glass slide for microscopy. Following observations of rod-shaped conidia, typical of *Calonectria* spp., 1-µL drops of the suspensions were pipetted from slides onto acidified potato dextrose agar (PDA) for isolation (Kong et al., 2023). Clean colonies were only obtained from the Madrid and Sainte-Foy-la-Grande samples (Figure 3) and were subcultured on regular PDA.

Colony PCR (Kong et al., 2005) was performed from a total of 159 colonies grown from three-day PDA cultures, using *Cps*- and *Che*-specific primers (Guo & Pooler, 2021). Also included in the PCR were three French isolates from the ANSES collection: LSVM0960 collected by Department Île-de-France in 2013, LSVM1007 by Department



FIGURE 1 Blight symptoms observed in Madrid, Spain in 2023, the ellipse highlights the black streaks observed on the stems.

Meurthe-et-Moselle in 2014, and LSVM1235 by Department Finistère in 2015. All 99 isolates from Spain were identified as *Che* while 63 isolates from France were *Cps*. Seven Spanish and nine French isolates were subcultured in potato dextrose broth for DNA extraction. Their identities were confirmed by PCR of the DNA (Figure 2) and PCR product sequencing (GenBank Accession Nos. PP632113-19 for *Che* isolates and PP632120-28 for *Cps* isolates) with the same primer pairs. The same isolates were tested for temperature tolerance. The Spanish isolates had an average colony diameter of 1.6 cm in PDA at 28.5°C for

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Authors. *New Disease Reports* published by John Wiley & Sons Ltd on behalf of British Society for Plant Pathology.



FIGURE 2 Disease signs appeared on the French samples two weeks after incubation under humid conditions at 23°C, the ellipse highlights the appearance of white mould.

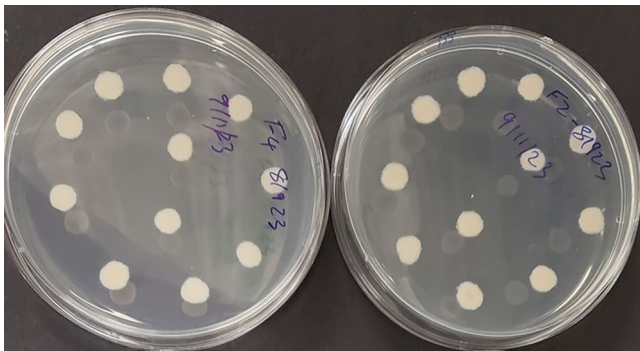


FIGURE 3 Resultant colonies from 1-µL drops of the white mould suspension on acidified potato dextrose agar after two-days' incubation at 23°C.

10 days, while the French isolates did not grow under the same conditions (Figure 4), further confirming their identities (Gehesquière et al., 2016).

To our knowledge, this is the first report of *Calonectria henricotiae* causing box blight in Spain. Knowing the actual distribution of the fungus is critical to preventing its further spreading to other countries and areas currently not affected by this pathogen, promoting safe plant trade.

ACKNOWLEDGEMENTS

The work was funded by the USDA National Institute of Food and Agriculture - Specialty Crop Research Initiative (2020-51181-32135).

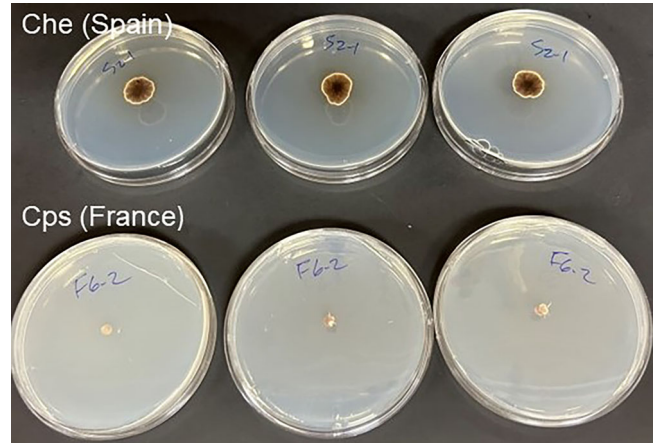


FIGURE 4 Growth of the Spanish and French isolates of the box blight pathogen on potato dextrose agar after ten-days' incubation at 28.5°C.

ORCID

P. Kong <https://orcid.org/0000-0002-7752-6997>

R. loos <https://orcid.org/0000-0001-9359-5098>

REFERENCES

- Daughtrey, M.L. (2019) Boxwood blight: threat to ornamentals. *Annual Review of Phytopathology*, 57, 189–209. <https://doi.org/10.1146/annurev-phyto-082718-100156>
- Gehesquière, B., Crouch, J.A., Marra, R.E., Van Poucke, K., Rys, F., Maes, M., et al. (2016) Characterization and taxonomic reassessment of the box blight pathogen *Calonectria pseudonaviculata*, introducing *Calonectria henricotiae* sp. nov. *Plant Pathology*, 65, 37–52. <https://doi.org/10.1111/ppa.12401>
- Guo, Y. & Pooler, M. (2021) Real-time and conventional PCR tools for detection and discrimination of *Calonectria pseudonaviculata* and *C. henricotiae* causing boxwood blight. *Plant Disease*, 105, 164–168. <https://doi.org/10.1094/PDIS-09-19-2053-RE>
- Hong, C.X. (2023) BBIG Team met in boxwood country – Oregon. *Phytopathology News*, 57, 05. <https://doi.org/10.3390/jof9020181>
- Kong, P., Daughtrey, M.L. and Hong, C.X. (2023) Differential adaptation has resulted in aggressiveness variation of *Calonectria pseudonaviculata* on hosts *Buxus*, *Pachysandra* and *Sarcococca*. *Journal of Fungi*, 9, 181.
- Kong, P., Richardson, P.A. and Hong, C.X. (2005) Direct colony PCR-SSCP for detection of multiple pythiaceae oomycetes in environmental samples. *Journal of Microbiological Methods*, 61, 25–32. <https://doi.org/10.1016/j.mimet.2004.10.019>

How to cite this article: Kong, P., loos, R. & Hong, C.X. (2024)

First report of *Calonectria henricotiae* causing box blight in

Spain. *New Disease Reports*, 49, e12278.

<https://doi.org/10.1002/ndr2.12278>