



First Report of Shot Hole disease on Cherry Laurel (*Prunus laurocerasus*) Caused by *Micrococcus aloeverae* in Ireland

Journal:	<i>Plant Disease</i>
Manuscript ID	PDIS-03-20-0521-PDN
Manuscript Type:	Plant Disease Note
Date Submitted by the Author:	11-Mar-2020
Complete List of Authors:	Smith, Leighton; University College Dublin, School of Agriculture and Food Science Gibriel, Hesham; University College Dublin, School of Agriculture and Food Science Brennan, Ciaran; University College Dublin, School of Biology and Environmental Science Delpino-deelias, Mariluz; University College Dublin, School of Biology and Environmental Science Twamley, Tony; University College Dublin, School of Agriculture and Food Science Doohan, Fiona; University College Dublin, School of Biology and Environmental Science Grogan, Helen; Teagasc Food Research Centre Ashtown, Horticulture Development Department Feechan, Angela; University College Dublin, School of Agriculture and Food Science; University College Dublin, Earth Institute
Keywords:	Cherry Laurel, Bacteria, Shothole, Woody ornamental, <i>Micrococcus aloeverae</i> , <i>Prunus laurocerasus</i>

First Report of Shot Hole disease on Cherry Laurel (*Prunus laurocerasus*) Caused by *Micrococcus aloeverae* in Ireland

L. Smith¹, H. Gibriel¹, C. Brennan², M. del Pino de Elias², A. Twamley¹, F. Doohan², H. Grogan³ and †A. Feechan¹

¹ School of Agriculture & Food Science and UCD Earth Institute, University College Dublin, Belfield, D4, Ireland

² School of Biology & Environmental Science and UCD Earth Institute, University College Dublin, Belfield, D4, Ireland

³ Horticulture Development Department, Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland

Cherry laurel (*Prunus laurocerasus* L.) is an evergreen shrub of the Rosaceae family (Kolayli et al. 2003). It is native to the Black Sea coast and South-west Asia where it grows on moist soils in forest understoreys or as a subdominant tree (Hättenschwiler and Körner 2003). In Ireland it is used for hedging and is cultivated in plantations for evergreen foliage and stems which are harvested annually (Whelton 2013).

Shot hole disease of cherry laurel is characterised by brown lesions, red margins and abscissions. These symptoms reduce the quality of leaves and yield of marketable stems. Since the 1980's the disease has been reported in Europe and North America (Roberts 2011). Current opinion is that shot-hole is caused by the bacterial pathogen *Pseudomonas syringae* pv. *syringae* (*Pss*). However, *Xanthomonas arboricola* pv. *pruni* is also known to infect cherry laurel (Roberts 2011; Tjou-Tam-Sin et al. 2012).

During an epidemiological study, shot hole symptoms were observed on cherry laurel hedgerows cv. Rotundifolia at Belfield in Dublin, Ireland (53°18'33.7"N 6°12'58.6"W), on September 28th, 2017. Sampled leaves (5) were washed, surface sterilized (3.5% NaOCl, followed by 70% ethanol and rinsed 3 times with sterile water), and incubated on water agar at room temperature (25°C) for 7 days to allow lesion development. Tissue developing lesions were ground with a mortar and pestle and the solution plated on KB agar (King et al. 1948) for 2 days at 25°C. Isolated colonies were circular, pale yellow/cream, shiny, smooth and raised. Examination with a light microscope revealed spherical, tetrad forming bacteria.

Koch's postulates were fulfilled by inoculation of two plants of cv. Novita, 6 leaves per plant, with 5x10⁶ cfu/ml of a bacterial suspension using infiltration with a blunt 1ml syringe (300 µl). Water was infiltrated into control leaves. At 7 dpi necrotic areas were observed surrounded by a halo of water soaking. By 14 dpi an abscission layer formed around areas of water soaking on inoculated leaves and by 21 dpi these areas had undergone abscission. The symptoms were similar to lesions and abscissions observed on the hedgerow with the original shot hole symptoms. Koch's postulate was also carried out on 3 sterile detached leaves cv. Rotundifolia with 5x10⁶ cfu/ml of a bacterial suspension as above with water controls. Leaves were placed on water agar inside sealed plastic bags to maintain humidity and incubated at 20°C. An abscission layer formed by 21 dpi and by 28 dpi water soaked areas abscised. This was repeated three times independently. Bacteria re-isolated from the infected leaves had the same spherical tetrad forming morphology.

DNA was extracted from the initial and the re-isolated bacterial colonies. The conserved region of 16S rRNA genes were amplified using the primers, BAC338F and BAC805R (Yu et al. 2005). Sequences were submitted to GenBank under Accession Nos. MN630641 and MN630642. Blast analysis of these 16S rRNA fragments (410 bp and 399bp) shows 99% identity with *Micrococcus aloeverae* (NR_134088.1). The genome of the re-isolated colony was sequenced using an Illumina platform and submitted to GenBank (PRJNA603651). Phylogenetic analysis revealed 98% identity with *M. aloeverae* (PRJNA526783).

1
2
3 Species from the genus *Micrococcus* have been reported as endophytes (Prakash et al. 2014) and
4 pathogens of horse chestnut (Iakovleva et al. 2013) and mango (Rakhashiya et al. 2015). This is the first
5 report of *Micrococcus* causing shot hole disease on cherry laurel.
6
7

8
9 Acknowledgments. We thank DAFM for funding 15/S/759 .

10 The authors declare no conflict of interest.

11 12 References

13
14 Hättenschwiler, S., and Körner, C. 2003. Functional Ecology. 17:778. <https://doi.org/10.1111/j.1365-2435.2003.00785.x>

15
16
17 King, J.V., et al. 1948. Canadian Journal of Research. 26:514.

18
19 Kolayli, S., et al. 2003. Journal of Agricultural and Food Chemistry. 51:7489-7494.
20 <https://doi.org/10.1021/jf0344486>

21
22 Iakovleva, L.M., et al. 2013. Mikrobiologichnyi zhurnal (Kiev, Ukraine: 1993). 75:62.

23
24 Prakash, O., et al. 2014. International Journal of Systematic and Evolutionary Microbiology. 64:3427.
25 <https://doi.org/10.1099/ijs.0.063339-0>

26
27 Rakhashiya, P.M., et al. 2015. Plant Disease. 99:1640. <https://doi.org/10.1094/PDIS-12-14-1359-PDN>

28
29 Roberts, S.J. 2011. HDC Factsheet 04/10. Stoneleigh, UK.
30 <https://horticulture.ahdb.org.uk/download/431/file>

31
32 Tjou-Tam-Sin, N. N. A., et al. 2012. Plant Disease. 96:759. <https://doi.org/10.1094/PDIS-04-11-0265-PDN>

33
34
35 Whelton, A. 2013. Laurel: Technical Note.
36 <https://www.teagasc.ie/media/website/publications/2013/Laurel.pdf>

37
38 Yu, Y., et al. 2005. Biotechnology and Bioengineering. 89:670. DOI:10.1002/bit.20347
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41

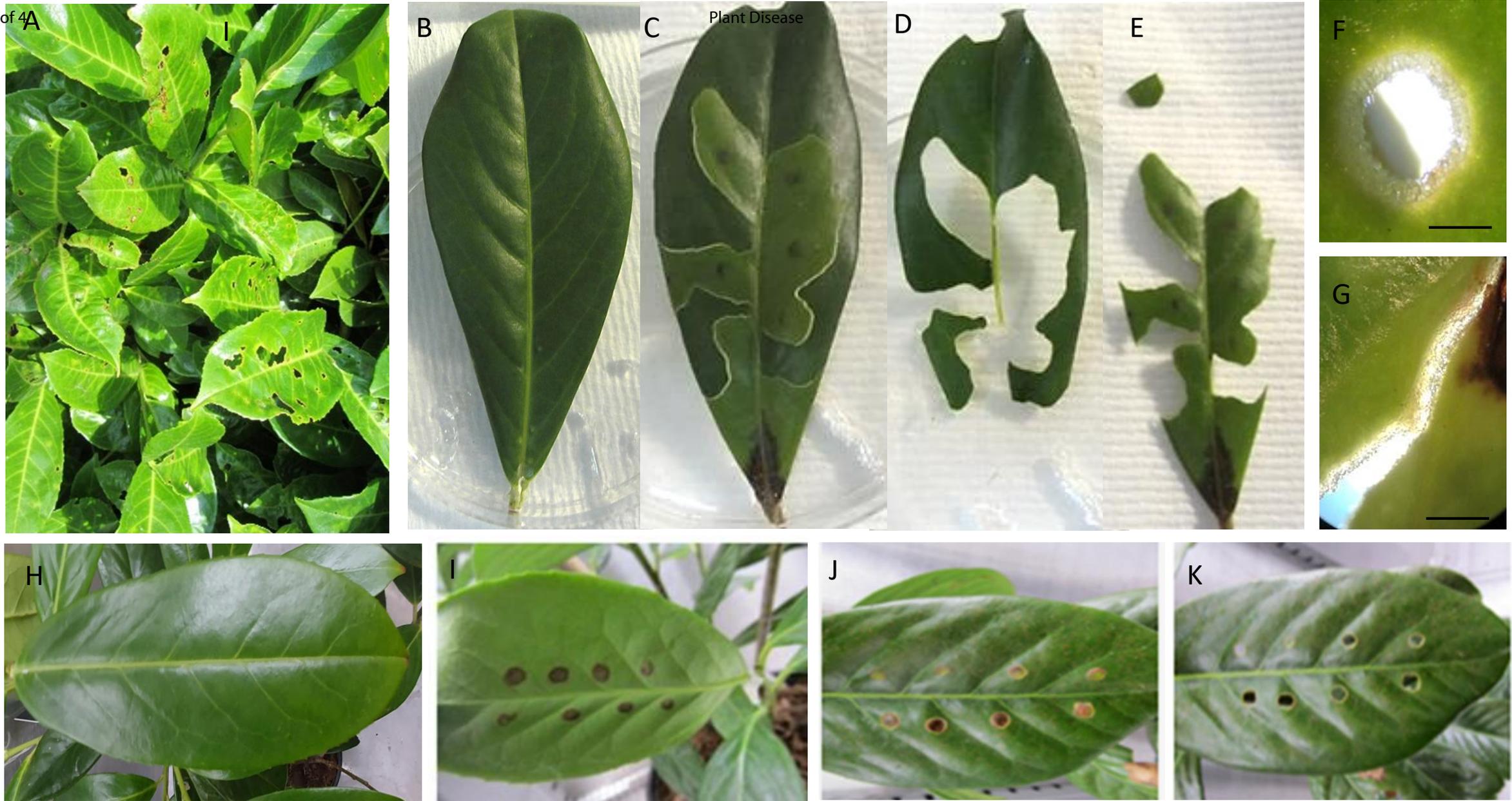


Figure S1. *Prunus laurocerasus* (cherry laurel) (A) hedgerow cv. Rotundifolia displaying shot hole symptoms (B) cv. Novita uninfected detached leaf (C) infected with *Micrococcus aloeverae* detached leaf 21 days post infection (dpi) (D) (E) 28 dpi abscission area (F) (G) abscissions, scale bar =2.5mm (H) uninfected cv. Rotundifolia plant (I) infected with *Micrococcus aloeverae* 7 dpi (J) 14 dpi and (K) 21 dpi.

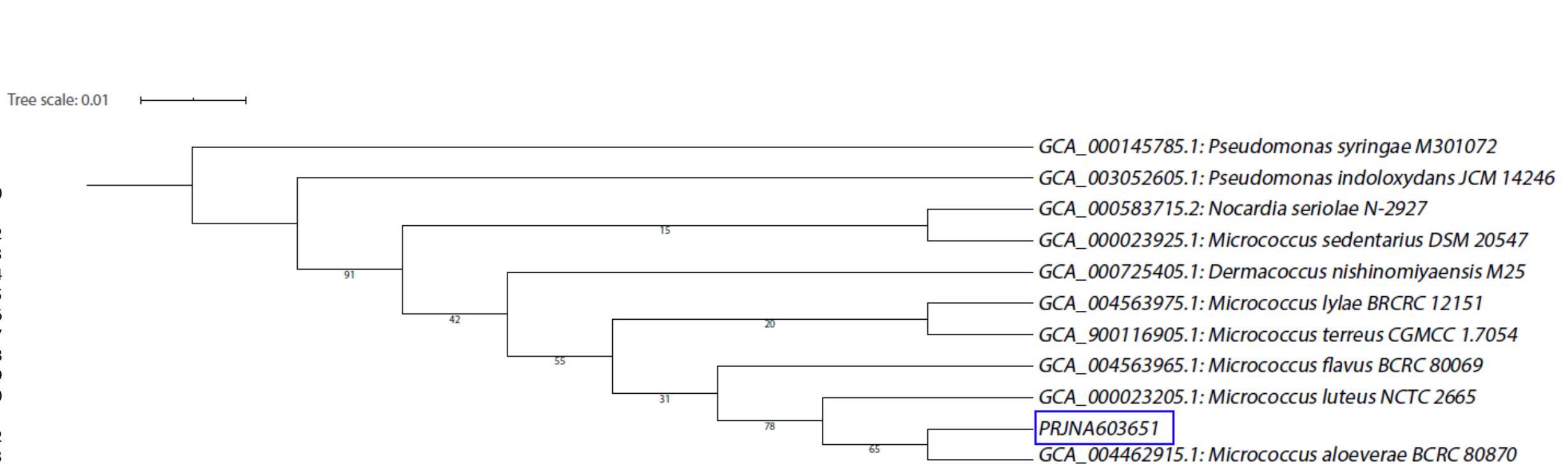


Figure S2. Phylogenetic tree using the whole genome sequencing of the isolated *Micrococcus* (box, PRJNA603651). Species are shown with accession numbers followed by strain information. *Pseudomonas syringae* was used as an outgroup. RAxML was used to construct a maximum-likelihood phylogeny with the GTRGAMMA substitution model (v8.2.4) (Stamatakis, 2014). The number of seeds for parsimony inferences and rapid bootstrap analysis was set to 12345 (-p 12345 -x 12345, respectively). The robustness of the inferred phylogeny was assessed by 1000 rapid bootstrap (-#1000).