



First report of *Cryphonectria parasitica* on abandoned galls of *Dryocosmus kuriphilus* on sweet chestnut in the United Kingdom

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There are two quarantine pests affecting sweet chestnut (*Castanea sativa*) in the United Kingdom, chestnut blight caused by the fungus *Cryphonectria parasitica* and the Oriental chestnut gall wasp (OCGW), *Dryocosmus kuriphilus*. Chestnut blight is one of the most important diseases on chestnut and it was detected in planted orchards in England in 2011 (Hunter *et al.*, 2013). The disease was eradicated and it was not detected again until 2016 when it was reported in the wider environment. Since then, it has been detected in woodlands, parks and street trees in different parts of England (Pérez-Sierra *et al.*, 2019). The OCGW is one of the most important pests of *Castanea* species in the world and was detected in the UK for the first time in 2015 (Morath *et al.*, 2015).

There have been reports in other countries in Europe indicating that the exit hole produced by the female wasp could potentially be an entry point for *C. parasitica* (Prospero & Forster, 2011). Initially no OCGW was detected in chestnut blight-infected areas in England. However, during surveys for *C. parasitica* in 2018 and 2019, both insect and pathogen were detected on 26 sites in London and West Sussex. Twigs bearing abandoned galls of OCGW (Fig. 1) were collected to confirm the presence or absence of *C. parasitica*. Abandoned galls were initially surface disinfected with 70% ethanol, then cut open and the inner gall tissues, or tissue from the leading edge of the lesions (Fig. 2) selected for isolation and DNA extraction. Necrotic tissues were plated onto 2% malt extract agar amended with 0.25 g/l of streptomycin sulphate salt. Plates were incubated for at least four days in the dark at 20°C and resulting colonies were transferred to PDA agar (39 g/l potato dextrose) and incubated at 25°C. DNA was extracted from gall tissues and from the isolated fungal colonies using the DNeasy Plant Pro DNA extraction kit (Qiagen, Germany) following the manufacturer's protocol. Up to 100 µg of gall or fungal tissue was added to a tissue disruption tube. The samples were homogenised on a PowerLyzer24. A real-time PCR assay developed by Chandelier *et al.* (2019) was used to test for the presence of *C. parasitica* in each individual sample, using the 18S-specific primers (Ioos *et al.*, 2009) as an internal control. The real-time PCR was performed on a LightCycler 480 (Roche, Switzerland) system.

In total, 17 twigs containing several OCGW from five different infected sites were processed. Of those, ten tested positive for the presence of *C. parasitica* by real-time PCR and seven tested negative. The positives were detected from the five sites surveyed in London. Only in three cases was it possible to isolate *C. parasitica* from the galls in pure culture and they corresponded to three of the sites surveyed. In one of the samples the twig infested with OCGW galls showed a typical canker of *C. parasitica*.

To our knowledge, this is the first report of the colonisation of abandoned galls of OCGW by *C. parasitica* in United Kingdom. Further studies are

needed to determine the interaction between the OCGW and *C. parasitica* and how this relates to the incidence of disease. Moreover, as the number of new sites which are co-infected/infested increases their interaction could imply a higher incidence of the disease in areas where both insect and pathogen occur.

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References

- Chandelier A, Massot M, Fabreguettes O, Gischer F, Teng F, Robin C, 2018. Early detection of *Cryphonectria parasitica* by real-time PCR. *European Journal of Plant Pathology* **153**, 29–46. <http://dx.doi.org/10.1007/s10658-018-1538-0>
- Hunter GC, Wylder B, Jones B, Webber JF, 2013. First finding of *Cryphonectria parasitica* causing chestnut blight on *Castanea sativa* trees in England. *New Disease Reports* **27**, 1. <http://dx.doi.org/10.5197/j.2044-0588.2013.027.001>
- Ioos R, Fourrier C, Iancu G, Gordon TR, 2009. Sensitive detection of *Fusarium circinatum* in pine seed by combining an enrichment procedure with a real-time polymerase chain reaction using dual-labeled probe chemistry. *Phytopathology* **99**, 582–590. <http://dx.doi.org/10.1094/PHYTO-99-5-0582>
- Morath S, Fielding N, Tilbury C, Jones B, 2015. Oriental chestnut gall wasp. News of a recent unwelcome discovery and how 'citizen science' can play an important role in surveying and identification. *Quarterly Journal of Forestry* **109**, 253–258.
- Pérez-Sierra A, Romón-Ochoa P, Gorton C, Lewis A, Rees H, Van Der Linde S, Webber J, 2019. High vegetative compatibility diversity of *Cryphonectria parasitica* infecting sweet chestnut (*Castanea sativa*) in Britain indicates multiple pathogen introductions. *Plant Pathology* **68**, 727–737. <http://dx.doi.org/10.1111/ppa.12981>
- Prospero S, Forster B, 2011. Chestnut gall wasp (*Dryocosmus kuriphilus*) infestations: new opportunities for the chestnut blight fungus *Cryphonectria parasitica*? *New Disease Reports* **23**, 35. <http://dx.doi.org/10.5197/j.2044-0588.2011.023.035>



Figure 1



Figure 2

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