Infection of Tree Stems by Zoospores of *Phytophthora ramorum* and *P. kernoviae*[1]

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Abstract

The invasive *Phytophthora ramorum*, *P. kernoviae,* and other aerial *Phytophthoras* are causing bleeding lesions on the trunks of mature trees, especially beech (*Fagus sylvatica*), in Cornwall, southwest England. The relationship between the results of host susceptibility tests using wound inoculation of excised logs in the laboratory and field observations in Cornwall has generally been good. However there have been some discrepancies. For example European sycamore (*Acer pseudoplatanus*) is moderately to highly susceptible to *Phytophthora ramorum* and *P. kernoviae* in the lab tests but is rarely susceptible in the field, although frequently exposed to natural inoculum. This suggests the possibility that host resistance is operating at two levels: at the bark surface-resistance to initial zoospore penetration; and in the phloem once the pathogen has gained entry. Little is known about the ability of zoospore inoculum to directly penetrate intact tree bark, although this is the presumed mode of entry of *Phytophthoras* above ground level. We are investigating this issue by examining the ability of zoospores to penetrate unwounded stems using both laboratory generated and natural zoospore inoculum. We summarize here research in progress.

In a preliminary laboratory test, *P. ramorum* zoospore suspensions were placed in plastic tubes attached to unwounded surfaces of freshly cut 50 cm long x 12 cm diameter stems of several tree species using modelling clay. After two weeks the inoculum source and outer bark were removed. Lesions of up to 5 cm were observed in the phloem of *F. sylvatica*, *Castanea sativa*, *Quercus rubra,* and *Picea sitchensis* and the pathogen was re-isolated, confirming zoospore penetration of unwounded bark. Lesions were absent in *Q. robur* but the pathogen could be re-isolated from visually healthy phloem beneath the inoculum points, in other words the bark was again penetrated although no lesions developed.

In a similar experiment, zoospores of *P. kernoviae*, *P. citricola,* and *P. cambivora* also caused phloem lesions in logs of *F. sylvatica.* Data from a large experiment, involving logs of *F. sylvatica, Q. robur,* and *A. pseudoplatanus* and laboratory produced zoospores of *P. ramorum*, *P. kernoviae*, *P. citricola,* and *P. cambivora*, could not be utilised owing to an adverse host response to the commercial adhesive used for attaching the plastic tubes. It is hoped to repeat the experiment in future with modelling clay or another benign adhesive.

To examine the comparative potential for infection in the field, freshly cut sealed logs 60 cm long x 15cm diameter of *F. sylvatica, Q. robur,* and *A. pseudoplatanus* were placed under a canopy of 2 to 4 m tall rhododendrons heavy infected with either *P. ramorum* or *P. kernoviae* at two woodland sites. Potential inoculum was assumed to be of zoospore origin coming from

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[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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splash dispersed sporangia. Twelve logs of each species were used at each site, half being examined for lesion development after 6 weeks and half after 11 weeks.

The experiment was carried out twice. In the first experiment, established in early July 2006, hot dry conditions prevailed from July to mid August and no lesions were found. In the second, established in early August, rain occurred from late August onwards. Lesions caused by both *Phytophthora* species were found on the upper surfaces of the *F. sylvatica* logs only after 6 weeks. By 11 weeks the *P. kernoviae* exposed *F. sylvatica* logs averaged 2.7 lesions per log of mean lesion area about. 23 cm^2; and the *P. ramorum* logs 6.2 lesions per log of mean lesion area only about 8 cm^2. Lesions were therefore more numerous with *P. ramorum* but larger with *P. kernoviae*. The *Phytophthora* species were confirmed by isolation. External bleeds developed in association with a number of the lesions. For both *Phytopthoras*, 36 to 55 percent of the lesions were associated with cut ends of the logs; 33 percent showed no obvious association with a stem feature; and the remainder were associated with fully healed branch stub positions or with live epicormic shoots.

Four lesions (but no bleeds) developed on the six *Q. robur* logs exposed to *P. ramorum* and the pathogen was reisolated from the lesions. No lesions occurred on the *Q. robur* logs exposed to *P. kernoviae*. No lesions developed on any of the logs of *A. pseudoplatanus*. These results are broadly consistent with the relative susceptibility of standing trees of the same host species at these field sites, although this is the first evidence of 'natural' infection of *Q. robur* by *P. ramorum*. It is therefore possible that some *Q. robur* trees become infected by *P. ramorum* in the field without exhibiting external bleeding.

The above data refer to the upper surfaces of the logs only. Destructive sampling revealed that similar numbers of lesions also developed on the bottom surfaces of the *F. sylvatica* logs, probably because the logs were in contact with the litter layer. However the lesions on the bottoms of the logs were much larger than those on the tops, averaging 119 cm^2 for the *P. kernoviae* exposed logs and 62 cm^2 for *P. ramorum*. This difference could reflect the different microclimate at the underside of the logs, such as greater surface moisture levels and a higher phloem water content. Further tests using logs exposed to natural inoculum are planned at these sites, including tests with logs placed on plastic sheeting to prevent contact with inoculum from the litter layer.

Key words: *Phytophthora ramorum*, *Phytophthora kernoviae*, zoospores, bark penetration, phloem lesions.