Infection process of *Septocyta ruborum*, a coelomycetous fungus with potential for biological control of European blackberry in Australia

Janita Baguant[1], Robin J. Adair[2,3] and Ann C. Lawrie[1]
[1] RMIT University, School of Applied Sciences, PO Box 71, Bundoora West, Victoria 3083, Australia
[2] Department of Primary Industries, PO Box 48, Frankston, Victoria 3199, Australia
[3] CRC for Australian Weed Management
Email: robin.adair@dpi.vic.gov.au

Summary The coelomycetous stem canker pathogen *Septocyta ruborum* causes stem die-back of European blackberry (*Rubus fruticosus* agg.). The fungus infects its host by penetrating stomata, stem wounds and possibly crevices associated with hair bases. Invasion of vascular bundles by hyphae disrupted internal transportation processes and appears to be the main mechanism for stem decline. Multiple cutting of the stem surface increased infection in whole plants, but the epidermis was vital to infection. Host-testing and field evaluation of *S. ruborum* as a potential biological control agent for European blackberry will involve histological evidence of infection to assess the risks it poses to non-target plant species.

Keywords *Rubus fruticosus*, biocontrol, weed.

INTRODUCTION

In southern Australia, European blackberry (*Rubus fruticosus* L. agg.) causes substantial economic losses in agriculturally productive areas as well as declines in biodiversity values in native vegetation. Although herbicide-based control techniques can provide effective suppression, these have limited application (Bruzzese and Lane 1996). A classical biological control program for blackberry commenced in 1973 and, although issues associated with conflicts of interest delayed implementation, the defoliating rust fungus *Phragmidium violaceum* (Schultz) Winter was officially released in 1991. *Phragmidium violaceum* can cause severe defoliation of European blackberry taxa, but its impact is limited by climatic, genetic and taxonomic factors (Adair and Bruzzese 2006). Large areas of blackberry infestations remain without adequate biological control, particularly in native vegetation and regions where the annual rainfall is less than 750 mm per annum.

Additional biological control agents are required to provide more comprehensive suppression of blackberry. The coelomycetous stem pathogen, *Septocyta ruborum* (Lib.) Petrak (purple blotch disease), is one of several organisms identified with potential for biological control (Sagliocco and Bruzzese 2003). It is widespread on *R. fruticosus* in Europe and can cause significant declines in the health and productivity of infected plants (Punithalingam 1980). Wild populations of *R. fruticosus* and some commercial blackberry cultivars with strong *R. fruticosus* pedigrees are susceptible to infection (Koellreuter 1950, Kövics 1980).

In this paper we report on infection processes of *S. ruborum*, particularly the modes of penetration, the mechanisms responsible for cane dieback and the symptomatic host response to infection by this fungus. Understanding infection processes, particularly internal tissue sampling to determine invasion success, assisted in the design and evaluation of host-specificity tests.

MATERIALS AND METHODS

In studies using stem pieces, conidia of *S. ruborum* were harvested from cultures grown on PDA plates. *Rubus anglocandicans* A.Newton and *R. ulmifolius* Schott primocanes from laboratory-grown plants were cut into stem pieces (3–4 cm long and 2.0–2.5 mm diameter), surface-sterilised and dipped in a suspension containing 10^7 conidia mL^{-1} of *S. ruborum*. Stem ends were removed and stem pieces were placed in a sterile Petri dish with moist filter paper and cultured in a phytotron at 20–25°C with a 12 h photoperiod for up to 35 days. Every three days, stems were harvested and 0.5 cm stem lengths fixed in a solution of 4% glutaraldehyde. Uninfected controls were dipped in sterile distilled water and cultured under the same conditions. There were three replicates for each sampling time for each *Rubus* species.

In preparation for sectioning, stem pieces were rinsed with 0.05M phosphate buffer (pH 7.0) and dehydrated through increasing concentrations of ethanol before embedding in paraffin wax. Transverse and longitudinal sections were cut 4 µm thick with a Leica rotary microtome and stained with 1% acid fuchsin. In addition, samples of *R. anglocandicans* at 24 days and *R. ulmifolius* at 35 days after inoculation were stained with 0.5% toluidine blue. Stained sections were mounted in DPX.

Infected stem sections were also prepared for scanning electron microscopy (Jeol JSM-35CF). Stems were rinsed with 0.05M phosphate buffer, dehydrated

in an ethanol series, critical-point dried and sputter-coated with gold before examination. Specimens were also prepared for examination by transmission electron microscopy (Jeol 1010). Stems were rinsed with 0.05M phosphate buffer, refixed with 1% osmium tetroxide, dehydrated in an ethanol series, infiltrated with London White resin and baked at 70°C for 48 h. Sections 60–90 nm thick were cut and sections stained with uranyl acetate followed by lead citrate before observation.

In studies using whole potted plants, healthy *R. ulmifolius* were inoculated with a 5–10 µL droplet of a 10^6 conidia mL^{-1} suspension of *S. ruborum* following three types of stem wounding: leaf removal, stem scraping, and multiple transverse cuts to the epidermal layer. Control plants consisted of (1) inoculated but unwounded plants and (2) plants that received no wounding or inoculation. All plants were kept at 4°C for 10 days with 80–90% humidity and then cultured at 20°C until infection was apparent (Baguant 2007).

RESULTS

In stem pieces, pycnidia of *S. ruborum* became apparent 15–18 days post-inoculation (dpi) in *R. anglocandicans* and 28 dpi in *R. ulmifolius*. The region around pycnidia was brown and necrotic. The stem was completely necrotic 24 and 35 dpi in *R. anglocandicans* and *R. ulmifolius,* respectively. Leaves grew during that period on stem sections where nodes were present. Pink lesions developed on leaves and infected leaves became necrotic and died. Control stem sections remained completely green with healthy leaf growth on stems where nodes were present.

Conidia commonly collected around hair bases after inoculation and germination frequency was very high on both *Rubus* species. Although hyphae often crossed or grew over the stomata without any attempt to penetrate the stomatal aperture, the main means of penetration was through the stomata. Direct piercing through the epidermis was not observed. Either a single germ tube or a cluster of hyphae entered each of the stomata in multiple penetrations of the stem. Hyphal penetration through a wounded section of a stem of *R. anglocandicans* was also observed.

Once inside the stomatal cavity, *S. ruborum* hyphae moved between the mesophyll cells and penetrated only some cells in the parenchyma. Invaded cells became fragile and their contents collapsed. The mycelium remained intercellular and gradually increased in density. As stems became more invaded, chloroplasts degenerated in all cells. Hyphae were located both between and inside the cells in the vascular bundle (phloem, xylem vessels and tracheids) and in the pith. Hyphae moved intracellularly in the vascular tissues through pitted walls connecting the cells. Non-lignified invaded cells were fragile and collapsed readily. No appressoria or haustoria were apparent in those regions (Baguant 2007). Pycnidia burst through the epidermis of *R. anglocandicans*, often close to the base of stem hairs.

In whole plants, only plants wounded by multiple transverse stem cuts and inoculated with *S. ruborum* conidia developed infection symptoms. By contrast, spore germination was detected in other inoculation treatments, but no pycnidia developed within six months. *Septocyta ruborum* was re-isolated from stems receiving multiple transverse cuts.

DISCUSSION

The initial attachment process of a fungal pathogen to the host surface is essential for successful pathogenesis (Agrios 2005). High densities of *S. ruborum* conidia attached to hairs and the close proximity of pycnidia to hairs suggest that pathogenesis could be directly related to the density of hairs on stem surfaces of *R. fruticosus*.

The main method of host invasion by *S. ruborum* is through stomata or stem wounds and our observations support earlier reports on the biology of this pathogen (Koellreuter 1950). We did not detect direct epidermal penetration, but the epidermis is vital to invasion, as its removal resulted in no infection. Both topographic and chemical signals play an important role in hyphal penetration and germ tube orientation of fungal pathogens (Hoch and Staples 1991). *Septocyta ruborum* mycelium migrates through the host cortex between mesophyll cells, with few parenchyma cells subject to intracellular invasion, resulting in chloroplast disintegration and the characteristic purple blotch. Hyphae move between and inside porous lignified cells in the vascular bundles, blocking or reducing the translocation of water in xylem vessels, presumably the cause of the dieback symptom in floricanes. The fungus also destroys phloem elements, preventing the downward translocation of nutrients to the roots. Infected stems gradually decline as infection progresses, resulting in the wilting and eventual death of both primocanes and floricanes. Similar invasion processes and impacts are reported for canker diseases in poplars, grapes and sweet cherry (Biggs *et al.* 1983, Munkvold 1994).

The development of infection symptoms and pycnidia at the bases of blackberry canes in the field suggests that this region is more susceptible to invasion than more distal regions of the stem, possibly due to humid conditions around the base of blackberry plants.

This information will allow better monitoring of the disease both in the field and in host-specificity

experiments. Variation in virulence of the fungus and susceptibility of certain *Rubus* species and cultivars can be also be related to level of invasion in the vascular bundles, hence the ability to cause dieback in affected stems.

Transverse cuts as a wounding treatment accelerates infection and pycnidial development of *S. ruborum* on *R. ulmifolius,* possibly by providing more infection courts on the plant for hyphal penetration. While this technique may expedite development of disease symptoms, it may also increase apparent pathogenicity and may decrease differences in infection among isolates of *S. ruborum*. Host-testing of *S. ruborum* will require a tiered experimental approach using both wounded and unwounded test species.

Septocyta ruborum has the potential to compliment the action of *P. violaceum* in Australia and deliver improved biocontrol outcomes, particularly in shaded and moist riparian areas.

Future development of *S. ruborum* as a potential biological control agent for *R. fruticosus* will focus on susceptibility of Australian native *Rubus* and commercial brambleberry cultivars, diversity in DNA phenotypes using a range of molecular tools, and the identification and selection of specific virulent pathotypes.

ACKNOWLEDGMENTS

The National Defeating the Weed Menace program provided funding support for this project.

REFERENCES

Adair, R.J. and Bruzzese, E. (2006). Blackberry: treading a prickly path to effective biological control in Australia. Proceedings of the 15th Australian Weeds Conference, eds C. Preston, J.H. Watts and N.D. Crossman, pp. 557-60. (Weed Management Society of South Australia, Adelaide).

Agrios, G.N. (2005). Plant Pathology, 4th edn. (Academic Press, London).

Baguant, J. (2007) A new approach to biological control of blackberry. Honours thesis, RMIT University, Australia.

Biggs, A.R., Davis, D.D. and Merrill, W. (1983). Histology of cankers on *Populus* caused by *Cytospora chrysosperma*. *Canadian Journal of Botany* 61, 563-74.

Bruzzese, E. and Lane, M. (1996). The blackberry management handbook. Department of Conservation and Natural Resources, Victoria, 50 pp.

Hoch, H.C. and Staples, R.C. (1991). Signalling for infection structure formation in fungi. *In* 'The fungal spore and disease initiation in plants and animals', eds G.T. Cole and H.C. Hoch, pp. 25-46 (Plenum Press, New York and London).

Koellreuter, J. (1950). Morphologie und biologie von *Rhabdospora ramealis* (Desm. et Rob.) Sacc. *Phytopathologische Zeitschrift* 17, 129-60.

Kövics, G.J. (1997). New record of *Septocyta ruborum* (Lib.) Petrak a pathogen of blackberry in Eastern Hungary. Proceedings of the 2nd International Seminar on Soil Plant-Environment Relationships, pp. 270-8.

Munkvold, G.P. (1994). Eutypa dieback of sweet cherry and occurrence of *Eutypa lata* perithecia in the Central Valley of California. *Plant Diseases* 78, 200-7.

Punithalingam, E. (1980). *Septocyta ruborum*. CMI Descr. Pathogenic Fungi and Bacteria No. 667. (CMI, Kew).

Sagliocco, J.L. and Bruzzese, E. (2004). Biological control of *Rubus fruticosus* agg. (blackberry): is the leaf rust the only option for Australia? Proceedings of the XIth International Symposium on the Biological Control of Weeds, eds H. Spafford Jacob, J. Dodd and J.H. Moore, pp. 141-144. (CSIRO Entomology, ACT).