CSIRO PUBLISHING

www.publish.csiro.au/journals/apdn *Australasian Plant Disease Notes*, 2007, **2**, 109–110

In vitro sensitivity of *Cryphonectria parasitica* to six agrochemicals

G. González-Varela[A] *and A. J. González*[A,B]

[A]Laboratorio de Fitopatología, Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA), Apdo. 13, Villaviciosa 33300, Spain.

[B]Corresponding author. Email: anagf@serida.org

Abstract. The *in vitro* effectiveness of six agrochemicals (captan, epoxiconazole, azoxistrobin, folpet in combination with cymoxanil and ofurace, carbendazim plus flutriafol, and flusilazole plus carbendazim) was tested against *Cryphonectria parasitica*, the causal agent of chestnut blight. Epoxiconazol was the most effective product since it inhibited fungal growth even at the lowest concentration tested.

Additional keywords: chemical control, fungicides.

Cryphonectria parasitica (Murrill) Barr, formerly called *Endothia parasitica* (Murr) And & And, is the causal agent of chestnut bark disease or chestnut blight. *Cryphonectria parasitica* is the most important pathogen of chestnut. It has nearly eliminated the American chestnut tree (*Castanea dentata* (Marsh.) Borkh.) from its natural range and has heavily affected the European chestnut tree (*Castanea sativa* (P.) Mill) (Anagnostakis 1982, 2001; Aksoy and Serdar 2004). In the Principality of Asturias, a region in the North of Spain with over 59 000 ha of chestnut (Anonymous 2003), this disease has spread rapidly, from five districts in 1982 to 60 out of the 78 regions in 2000 (Valdezate *et al*. 2001).

Many fungicidal and fungistatic chemicals, such as methyl-2-benzimidazolecarbamate, carbendazim, copper oxychlorite, benomyl and azaconazole plus imazalil, have been applied to blight cankers over the years but some of them have proven not to be useful for long-term therapeutic treatment (Jaynes and Van Alfen 1977; Anagnostakis 1982; Canciani *et al*. 1995; Aksoy and Serdar 2004). Others (such as benzimidazoles) are now banned in Spain (Anonymous 2007). *In vitro* tests are fast and easy to perform and can give an indication of the effectiveness of the product. This effect will depend on a variety of factors such as: degradability, persistence, mode of action and interaction with other compounds.

In the present work, six agrochemicals were tested *in vitro* with regard to their activity on *C. parasitica*. Their active elements were captan (85%), epoxiconazole (12.5%), azoxistrobin (25%), folpet (32%) in combination with cymoxanil (3%) and ofurace (6%), carbendazim (20%) plus flutriafol (9.4%), and flusilazole (0.5%) plus carbendazim (1%).

Each product was incorporated, before autoclaving, into potato dextrose agar medium (PDA) (Gams *et al*. 1980) in a geometric progression, ranging from 1 to 1024 µg/mL, related to their active ingredients.

Six isolates of *C. parasitica*, collected in four different districts in the Principality of Asturias, were chosen for the study. Four were from the central area of Asturias: *LPPAF-1*, *LPPAF-14.1*, *LPPAF-23* (Aller district) and *LPPAF-150* (Lena district); one from the east: *LPPAF-140* (Amieva district); and one from the west: *LPPAF-147* (Coaña district). Two strains from culture collections were also used. They were ATCC-52571 (American Type Culture Collection) and CCP-52 (from Canada, provided by the Laboratorio de Sanidad Vegetal del Principado de Asturias).

Plugs of 5-mm diameter were cut from the margins of 5-day-old mycelia of the different strains, actively growing on PDA. The plugs were positioned in the centre of Petri dishes containing either amended or non-amended PDA, with the mycelium in contact with the medium. Culture dishes containing non-amended PDA were used as a control. The experiment was repeated three times. After 5 days of incubation in darkness at 25°C, the plugs were removed from those culture dishes in which growth inhibition was observed. These plugs were then placed, with the mycelium up, on new culture dishes containing non-amended PDA, and incubated as previously described. Subsequent growth of the fungus was taken as an indicator of fungistatic activity of the agrochemical which was present in the medium from which the plug derived. On the contrary, when the fungus was unable to grow, the agrochemical was regarded as fungicidal.

The antifungal effect of the six agrochemicals tested varied considerably. Azoxistrobin (25%) and the mixture of folpet (32%) plus cymoxanil (3%) plus ofurace (6%) was unable to inhibit fungal growth at any of the concentrations tested. Captan (85%) was a product with a variable response depending on the concentration and isolate. It caused total inhibition of *LPPAF-140* at 128 µg/mL, of *LPPAF-1*, *LPPAF-14.1*, *LPPAF-23* and *LPPAF-150* at 512 µg/mL, and of *LPPAF-147*, ATCC-52571 and CCP-52 at 1024 µg/mL. Fungal growth was scarce and reculture of the inoculum indicated fungistatic activity of the agrochemical at all concentrations for all strains.

 10.1071/DN07045 1833-928X/07/010109

Table 1. Fungicidal and fungistatic activity of the indicated agrochemicals on eight isolates of *Cryphonectria parasitica*
■, fungicidal activity; □, fungistatic activity

Strains	Epoxiconazole (12.5%)					Carbendazim (20%) + Flutriafol (9.4%)					Flusilazole (0.5%) + Carbendazim (1%)				
	1	2	4	8	16	1	2	4	8	16	1	2	4	8	16
	(μg/ml)					(μg/ml)					(μg/ml)				
LPPAF-1	■	■	■	■	■	□	□	■	■	■	□	□	■	■	■
LPPAF-14.1	■	■	■	■	■	■	■	■	■	■	□	□	□	■	■
LPPAF-23	■	■	■	■	■	□	□	■	■	■	□	□	□	■	■
LPPAF-150	■	■	■	■	■	□	■	■	■	■	□	□	■	■	■
LPPAF-140	■	■	■	■	■	□	□	■	■	■	□	□	□	■	■
LPPAF-147	■	■	■	■	■	□	□	■	■	■	□	□	□	■	■
ATCC-52571	■	■	■	■	■	□	■	■	■	■	□	□	□	■	■
CCP-52	■	■	■	■	■	□	■	■	■	■	□	□	■	■	■

Only three products, epoxiconazole (12.5%), carbendazim (20%) plus flutriafol (9.4%), and flusilazole (0.5%) plus carbendazim (1%), were able to inhibit growth of all strains at the lowest concentration. Table 1 shows the fungicidal or fungistatic activity of these compounds at different concentrations.

The most efficient compound was epoxiconazole (12.5%), which totally inhibited growth of the eight strains at 1 μg/mL, and reculture of the inoculum in the absence of the agrochemical demonstrated a fungicidal action at this concentration. A combination of carbendazim (20%) plus flutriafol (9.4%) also caused total growth inhibition of the eight strains at 1 μg/mL. Reculture of the mycelium in non-amended medium showed that it was fungicidal at 1 μg/mL only for *LPPAF-14.1*, while higher concentrations were required for the remaining strains: ≥2 μg/mL in the case of *LPPAF-150*, *ATCC-52571* and *CCP-52*, and ≥4 μg/mL in the case of *LPPAF-1*, *LPPAF-23*, *LPPAF-140*, *LPPAF-147*. At lower concentrations the agrochemical was fungistatic.

As with the previous two agrochemicals, flusilazole (0.5%) plus carbendazim (1%) caused total growth inhibition of all strains at 1 μg/mL. Reculture of *LPPAF-1*, *LPPAF-150* and *CCP-52* revealed fungicidal activity at concentrations ≥4 μg/mL and fungistatic at lower concentrations. Killing of *LPPAF-14.1*, *LPPAF-23*, *LPPAF-140*, *LPPAF-147* and *ATCC-52571* was only achieved at ≥8 μg/mL, while lower concentrations displayed fungistatic activity.

These results suggest the possibility that epoxiconazol (12.5%) could be useful and in the future we are going to evaluate the *in vivo* effectiveness of this agrochemical.

Acknowledgements

We gratefully thank M. R. Rodicio for her critical reading of the manuscript. We thank Laboratorio de Sanidad Vegetal del Principado de Asturias for having given us two strains. This work was supported by grants from the Caja Rural de Asturias and the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA).

References

Aksoy HM, Serdar U (2004) A research on chemical control against chestnut blight (*Cryphonectria parasitica* (Murill) Barr). *The Plant Pathology Journal* **3**, 44–47.

Anagnostakis SL (1982) Biological control of chestnut blight. *Science* **215**, 466–471. doi: 10.1126/science.215.4532.466

Anagnostakis SL (2001) The effect of multiple importations of pests and pathogens on a native tree. *Biological Invasions* **3**, 245–254. doi: 10.1023/A:1015205005751

Anonymous (2003) Superficie forestal según especies. Available at http://www.sadei.es/datos/cuadros%20tematicos/capitulo%20K/2/K28201A2000a.xls [Verified 17 July 2007]

Anonymous (2007) Ministerio de Agricultura, Pesca y Alimentación. http://www.mapa.es/es/agricultura/pags/fitos/registro/menu.asp

Canciani L, Dallavalle E, Zambonelli A, D'Aulerio AZ (1995) Prove di protezione chimica su innesti di castagno. *Difesa delle Piante* **18**, 116–121.

Gams W, Van der AHA, Van der Plaats-Niterink AJ, Samson RA, Stalpers JA (1980) 'CBS Course of Mycology.' 2nd edn. (Institute of the Royal Netherlands Academy of Sciences and Letters: Baarn)

Jaynes RA, Van Alfen N (1977) Control of the chestnut blight fungus with injected methyl-2-benzimidazolecarbamate. *Plant Disease Reporter* **61**, 1032–1036.

Valdezate C, Alzugaray R, Landeras E, Braña M (2001) Situación actual de *Cryphonectria parasitica* (Murill) Anderson, cancro cortical, en los castañares asturianos. *Boletin de Sanidad Vegetal, Plagas* **27**, 401–410.

Manuscript received 9 July 2007, accepted 16 July 2007