# Fungicide Interactions with Australian isolates of *Leptosphaeria maculans*

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*Leptosphaeria maculans* (anamorph= *Phoma lingum*) which causes blackleg in oilseed Brassicas, is the greatest disease threat to the Australian canola (*Brassica napus)* industry. The main source of infection is the sexual ascospores, which are released from fungal fruiting bodies housed on crop debris. Ascospore release is induced by rain, and distribution by wind coincides with emergence of vulnerable seedlings between May and August. Measures to control the disease currently include sowing resistant cultivars, removing crop debris, rotating crops and applying the triazole fungicide, Impact® (a.i. flutriafol) in-furrow (Salisbury *et al.*,1995). Flutriafol is taken up by plant roots and rapidly translocated to the leaf tips. It has three-fold activity: protective, curative (ceases development of growing mycelia) and eradicative (destroys sexual and asexual propagation units) (Berg and Plempel, 1988).

Triazole chemicals exert fungicidal activity by inhibiting the biosynthesis of ergosterol, which is the major product of sterol biosynthesis in most fungi and functions as an architectural membrane component and stimulant in cell cycle regulation (Nes, 1989). Ergosterol biosynthesis involves between 10 and 15 enzymic reactions, beginning with squalene (Goosey and Moore, 1991). Triazoles act by binding to 14a -demethylase, one of the early enzymes in the pathway, and a member of the cytochrome P450 monooxygenase family (Joseph-Horne *et al.*, 1995).

Extensive use of chemicals with precise modes of inhibitory activity can induce the build up of resistance in target organism populations. Common traits conferring fungicide resistance include increased expression of 14 a- demethylase or alteration of its active site, increased fungicide efflux through ABC-transporters, mutation of sterol D5,6 desaturase accompanied with mutation of 14a-demethylase or intracellular fungicide sequestering (Marichal and Vanden Bossche, 1995). Reduced response to triazole treatment has already been observed in powdery mildew of wheat, barley and cucurbit, barley net blotch and *Penicillium digitatum* infections of citrus fruit (Berg and Plempel, 1988). Impact® is presently the only fungicide

registered in Australia for control of blackleg disease. As the canola industry continues to expand, increased use of this chemical imposes greater pressure on *L. maculans* to develop resistance. Nothing is known about the biochemical interaction of flutriafol with Australian isolates of *L. maculans* or the propensity and mechanisms for azole resistance to develop. Greater knowledge of these aspects will lead to better-informed decisions regarding blackleg control.

We are examining the effects of flutriafol on ergosterol biosynthesis in a well-characterized Australian *L maculans* isolate (M1), which is sensitive to the fungicide. Reverse phase high performance liquid chromatography (RP-HPLC) has resolved a peak for ergosterol in ethanol extracts of mycelia. We are developing thin layer chromatography (TLC) and gas liquid chromatography - mass spectrometry (GLC-MS) techniques to separate and identify as many ergosterol precursors as possible. The effect of flutriafol on the sterol profile of *L. maculans* will be determined.

Fungicide-resistant *L. maculans* cultures have been produced in the laboratory by insertional and UV mutagenesis and by *in vitro* conditioning on flutriafol-amended media. Seven mutants with resistance have been identified. The sterol composition of these mutants will be studied by TLC and GLC-MS following growth in the presence and absence of flutriafol. Additionally the expression of genes involved in fungicide resistance in other fungi will be examined in these mutants.

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