EFFECT OF MYCLOBUTANIL AND PROPICONAZOLE ON ENDOPHYTE AND RUST FUNGI IN RYEGRASS

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SUMMARY

Perennial ryegrass plants infected with the endophyte fungus (*Acremonium lolii*) were grown in a greenhouse, and sprayed either once or twice with myclobutanil or propiconazole at rates up to 250g/ha. The number of endophyte hyphae in leaf sheaths was unaffected by the fungicides, and when seed harvested from these plants was grown, the number of hyphae in seedlings was also unaffected. Field plots of perennial ryegrass attacked by stem rust (*Puccinia graminis*) were treated with fungicides. Seed yields were significantly higher in fungicide sprayed plots than in untreated controls. Plots sprayed twice with myclobutanil at 250g/ha outyielded the controls five-fold.

INTRODUCTION

Timely applications of systemic fungicides to control rust diseases in ryegrass seed crops can greatly increase yields. However, Latch and Christensen (1982) showed that some systemic fungicides can kill the endophyte fungus (*Acremonium lolii*) in perennial ryegrass (*Lolium perenne*). Consequently there is some concern that seed from fungicide treated crops may not contain viable endophyte. Presence of *A. lolii* gives protection from Argentine stem weevil (*Listronotus bonariensis*) attack (Prestidge *et al* 1982).

This paper reports an investigation of the effects of two ergosterol biosynthesis inhibiting fungicides on *A. lolii* in plants and seeds of perennial ryegrass, in greenhouse and field trials. The efficacy of these systemic fungicides for controlling stem rust (*Puccinia graminis*) was also studied in the field trial.

MATERIALS AND METHODS

Glasshouse trial

Two seedlings of perennial ryegrass (Grasslands Ruanui), which had been inoculated with the same strain of *A. lolii* 18 months previously, were split into ramets. Twenty four ramets from each of the two plants were transplanted separately into 10 cm diameter pots containing a composted bark potting mix and grown in a greenhouse (10-24 °C). Eight weeks later, plants to be sprayed were placed in a 10 m² area marked out on the ground. Water and fungicide sufficient to treat 10 m² at the required rate in 375 litres water/ha were mixed in a gas operated knapsack sprayer. Six clones from each of the two plants were sprayed with myclobutanil (Systhane 125 EC) at 125g/ha and six clones at 250g/ha. A further six clones from each plant were sprayed with propiconazole (Tilt 250 EC) at 125g/ha, and six clones were sprayed with water. The sprays were repeated on three plants from each treatment 23 days later. After treatment the plants were returned to the greenhouse for further growth.

The density of endophyte mycelium was determined by removing three tillers from each plant 29 days after application of the second spray. The third oldest leaf and sheath was peeled from each tiller and the basal 1cm of each sheath removed. The sheath segments were cleared for 2 hours at room temperature in a chloroform-methanol (2:1 v/v) mixture, rinsed with 95% ethanol, and immersed for 3 days in a clearing and staining solution (Skipp pers. comm. modified from Bruzzese and Hasan 1983). This solution consisted of Trypan blue 0.005g, chloral hydrate 45g, phenol 15g, 90% lactic acid 12.5ml, 95% ethanol 30ml, water 15ml. Stained sheath segments were rinsed in 50% ethanol, destained in aqueous chloral hydrate (2.5g/ml) for 2 hours, and mounted on microscope slides in lactoglycerol (80% lactic acid, glycerol, water 1:1:1).
Proc. 41st N.Z. Weed and Pest Control Conf.

Transects of sheath tissue (1mm wide) were examined at 400 x magnification and the number of endophyte hyphae present was recorded.

Seed was collected later from plants which had been sprayed twice with the higher rate of myclobutanil, twice with propiconazole, and with water. This seed was sown singly in 10 cm pots of potting mix. Ten weeks later the numbers of endophyte hyphae in the leaf sheath base of three tillers from each of three plants in each treatment were counted as described above.

Field trial

Experimental plots were laid out at the Grasslands Division farm 20 km west of Palmerston North in a field of 'Grasslands Nui' perennial ryegrass which had been drilled in rows, 70cm apart, in March 1985. Plots measured 4m x 3m and the 10 treatments were randomised within each of three replicate blocks. Myclobutanil was sprayed on appropriate plots at rates of 62.5, 125, and 250g/ha, and propiconazole at 125g/ha, both in 375 litres water/ha. Control plots were sprayed with water. Plots received either one or two applications of fungicide. The first was applied on 13 November 1987, when pustules of stem rust were present on most leaves, and on some panicles. The second spray was applied to half the plots 3 weeks later.

Plots were harvested for seed on 30 December by cutting all the plants which grew in a 2m length, in each of the two centre rows. The herbage was placed in jute bags and dried for 2 weeks before threshing with a belt thresher, followed by two passages through a 2-screen scalper seed cleaner. All seed samples were given a final clean using a Westrup indent cylinder. Seed from all treatments was weighed, and the weights of four groups of 50 seeds from each treatment were also recorded.

One hundred seeds from control plots, from plots sprayed twice with myclobutanil at 125g or 250g/ha, and from plots sprayed twice with propiconazole at 125g/ha, were examined for the presence of endophyte using the method of Latch *et al* (1987).

RESULTS

Greenhouse trial

Neither myclobutanil nor propiconazole had any significant effect on the amount of endophyte mycelium in the plants (Table 1). The density of mycelium in the sheaths of 10 week old plants grown from seed of fungicide sprayed plants was also not significantly different from that of seedlings grown from seed of control plants.

TABLE 1: Effect of fungicides on the mean number of endophyte hyphae visible at 400 x magnification in 1mm transects of basal leaf sheath tissue of perennial ryegrass plants treated with fungicides.

Fungicide	Rate gai/ha	Number of applications	No.hyphae/mm leaf sheath
myclobutanil	125	1	53.3
myclobutanil	125	2	54.1
myclobutanil	250	1	45.9
myclobutanil	250	2	55.3
propiconazole	125	1	43.6
propiconazole	125	2	56.6
control, water only			45.5
LSD (5%)			19.5

Field trial

There was a severe outbreak of stem rust on the crop and control plots became heavily infected, causing damage to leaves and flowering stems. All fungicide treatments increased seed yield significantly when compared with yields from control plots (Table 2). Both fungicides sprayed twice at 125g/ha increased yields threefold when compared with controls. Highest yields, almost five times those from control plots, came from plots sprayed twice with myclobutanil at 250g/ha.

Eighty six percent of seeds harvested from control plots contained endophyte, not

significantly different from that in seeds from plots sprayed with fungicides.

Seed weight (mg) from each of the treatments was generally greatest for the highest rates of fungicides applied (Table 2).

TABLE 2: Effect of fungicides on seed yields and mean weight of 50 seeds harvested from Nui ryegrass plots attacked by stem rust and treated with fungicides.

Fungicide	Rate g ai/ha	Number of applications	Seed yield kg/ha	Wt. of 50 seeds mg
myclobutanil	62.5	1	493	95
myclobutanil	62.5	2	516	99
myclobutanil	125	1	673	96
myclobutanil	125	2	598	99
myclobutanil	250	1	626	106
myclobutanil	250	2	937	108
propiconazole	125	1	370	92
propiconazole	125	2	589	95
control, water only		1	191	83
control, water only		2	183	89
LSD (5%)			128	7

DISCUSSION

Results from these experiments suggest that farmers can apply either myclobutanil or propiconazole to their ryegrass seed crops confident that neither fungicide will affect the incidence, or amount, of endophyte mycelium in the plants or the seed. Even when myclobutanil was used at twice the manufacturer's recommended rate the incidence of *A. lolii* was unaffected. This result is not unexpected because the rate of propiconazole required to kill the endophyte in seedlings (1g/kg seed) is phytotoxic (Latch and Christensen 1982).

The field trial showed the benefits of systemic fungicides for controlling rust in ryegrass seed crops, because both seed yield and with some treatments, seed weight, were increased in treated plots. Nui is particularly susceptible to stem rust, and the outbreak was both early and severe. The first application of fungicides should probably have been made 1-2 weeks earlier to prevent the initial damage. However, the eradication action of the systemic fungicides was apparent as pustules on sprayed plants dried up and died within a week of the first fungicide application. In most cases, yields from fungicide sprayed plots were higher when a second application was made.

We recommend that farmers apply either of these systemic fungicides to their ryegrass seed crops at the first sign of rust pustules on leaves. A second application should be made if the attack is severe.

ACKNOWLEDGEMENTS

We wish to thank Rohm and Haas New Zealand Ltd for financial support for these trials.

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