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Sensitivity of *Peronospora destructor* (onion downy mildew) to different fungicides under controlled conditions

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EXECUTIVE SUMMARY

The New Zealand onion industry is currently concerned that fungicide resistant strains of *Peronospora destructor*, the causal agent of downy mildew, may be present in local onions. The aim of this study was to carry out a preliminary examination of the effectiveness of nine systemic and protectant fungicides for onion downy mildew control in New Zealand. Because *P. destructor* is an obligate pathogen (requires a living host to grow on) and cannot be grown on artificial growth media, an artificial inoculation method was used in this study. This method worked well, with 66% of the plants in the control–spores treatment (no fungicide + inoculation with *P. destructor* spores) having sporulating downy mildew lesions 12 days after inoculation with the pathogen. For the fungicide treatments, fungicides were applied on three occasions at 7-day intervals – two pre- *P. destructor* inoculation and one post-inoculation. For two of the nine fungicides tested, treated plants had downy mildew infection 12 days after inoculation; for the other seven fungicides, treated plants had no symptoms of downy mildew. Eleven percent of the 100 plants treated with cymoxanil (Concord 300 SC) and 7% of plants treated with mandestrobin (Intuity) had sporulating downy mildew infection, indicating that some strains of *P. destructor* may be resistant to these fungicides.

INTRODUCTION

Peronospora destructor, the pathogen that causes downy mildew of onion, is not a true fungus – it is part of the kingdom Chromista, subphylum Oomycota (commonly oomycetes), within the family Peronosporaceae. The New Zealand onion industry is currently concerned that fungicide-resistant strains of *P. destructor* may be present in local onions. The aim of this study was to carry out a preliminary examination of the effectiveness of nine fungicides – some systemic and some protectant – for onion downy mildew control in New Zealand. The nine fungicides are all currently registered in New Zealand for control of onion downy mildew.

Because *P. destructor* is an obligate pathogen (requires a living host to grow on) and cannot be grown on artificial growth media, onion downy mildew fungicide resistance testing can be difficult. Successful inoculation of onion plants with *P. destructor* depends on several factors. The fungicide resistance test needs be scheduled when there is plentiful downy mildew in the field and fine, clear weather is forecast for the next few days. It is important that there is a good dew formation on downy mildew-diseased onion leaves in the field on the morning of *P. destructor* inoculum collection to promote fresh sporulation of the pathogen – for the spore suspension used for inoculating the test plants. Collection of *P. destructor* spores and inoculation of plants needs to be done as early in the day as possible (before 10.00 a.m.) because spores are fragile and thin-walled and remain infective for less than 1 day.

METHOD

Inoculated plants

On 5 November 2018, 1100 young onion plants (4–5 leaf stage) of the downy mildew susceptible variety T400 were transplanted into polystyrene seed trays (595 x 420 x 190 mm) containing cultivated Patumahoe clay loam soil. There were 25 plants per tray. The trays were placed in an unheated greenhouse and watered daily via drip-tape (Photo 1). Four trays (replications) were done for each chemical treatment (i.e., 100 plants per treatment). Nitrogen (as urea), at a rate of 100 kg/ha, was applied to plants 1 week after transplanting to encourage growth of succulent leaf tissue. All plants were inoculated on 29 November 2018.

Fungicide treatments

Fungicide treatments are shown in Table 1. The fungicides used were as requested by Onions NZ and the products supplied by local growers. Each fungicide was applied at the manufacturer's recommended field rate. The number of treatments was limited to eleven (44 trays, 1100 plants) due to cool room space constraints. Fungicides were applied on three occasions, 7 days apart, twice before inoculation and once after inoculation, as follows:

1. On 19 November, 10 days before *P. destructor* inoculation
2. On 26 November, three days before *P. destructor* inoculation
3. On 3 December, four days after *P. destructor* inoculation.

Pre- and post-inoculation fungicide treatments were carried out because different fungicide modes of action act against the pathogen at different times – some fungicides affect spores on the leaves before or during infection and others affect mycelium growing inside the leaves (post-infection). The fungicides were prepared to a water rate of 700 L water per hectare and were applied to the point of runoff using hand-held sprayers. Care was taken to ensure good coverage on all sides of the onion plants whilst avoiding runoff (Photo 2). Contact™ Xcel nonionic surfactant at a rate of 50 mL/100 L was added to the spray mix where a surfactant was recommended by the manufacturer on the product container label.

Table 1. Fungicide treatments. Fungicide active ingredient and Fungicide Resistance Action Committee (FRAC) code in brackets. * = surfactant added.

Fungicide active ingredient and FRAC code	Product
Control-Water	No fungicide + no *P. destructor* inoculation
Control-Spores	No fungicide + inoculated with *P. destructor* spores
Dimethomorph (40) plus ametoctradin (45)	Zampro® @ 800 mL/ha *
Dimethomorph (40) plus mancozeb (M3)	Acrobat® MZ690 @ 2 kg/ha *
Metalaxyl (4)	Ventura® @ 640 mL/ha *
Cymoxanil (27)	Concord® 300 SC @ 550 mL/ha
Oxathiapiprolin (49)	Zorvec® Enicade® @ 350 mL/ha
Fenamidone (11)	Reason™ @ 300 mL/ha
Mandestrobin (11)	Intuity® @ 1.2 L/ha
Cyazofamid (21)	Ranman® @ 200 mL/ha *
Azoxystrobin (11)	Amistar® SC @ 500 mL/ha *

Peronospora destructor inoculation

Early in the morning of 29 November 2018, 3 days after the second 'round' of fungicide applications, leaves with fresh symptoms of downy mildew showing active sporulation were collected from non-fungicide sprayed onions in three fields near Plant and Food's Pukekohe Research Centre. Sporangia (spores) were washed from the surfaces of the collected downy mildew-infected leaves, and suspended in sterile distilled water. A spore suspension of 1 x 10^5 spores/mL in distilled water was prepared. Percent germination of sporangia was checked by spreading some spore suspension onto 1% water agar and examining spore germination under the microscope after 24 h incubation at 12° C (Photo 3).

A 1-L capacity hand-held sprayer was used to spray spore suspension onto the leaves. Care was taken to apply an even coverage of small spray droplets on all sides of the foliage - but not to the point of run-off. Immediately after inoculation, the 44 trays of inoculated plants were covered with black polythene bags (to create moist incubation chambers), then incubated for 48 h at 13–14°C in a cool room (Photo 4). The polythene bags were placed over three curved wire supports that had been inserted into each polystyrene box, creating an enclosed cloche or tent structure. The plastic bag openings were tightly gathered around the sides of the polystyrene trays and clamped with bulldog clips to maintain a closed moist chamber. After 48 h in the cool room, the polythene bags were removed and the trays of plants moved to a 'lean-to' shed, which provided the plants with cover from direct sunlight, for 8 days. During this time the plants were watered once, with care taken not to wet the leaves.

On 3 December, 4 days after *P. destructor* inoculation, the onion plants received their third fungicide application. After 8 days in the lean-to, the trays were moved outside to a position alongside a shelterbelt that sheltered the plants from direct sunlight after 1 pm. Twelve days after inoculation, the onions were moved to the cool room shed where the plants were lightly misted with sterile distilled water (Photo 5), covered with polythene bags and placed in the 13–14°C cool room to induce *P. destructor* sporulation on infected leaves. The next morning the incidence of *P. destructor* sporulation on each plant was assessed.

Results

Approximately 50% of the spores in the spore suspension used for the inoculation germinated after 24 h incubation at 12° C (Photo 3). The percent of plants with sporulating lesions for the 11 experimental treatments 12 days after inoculation with *P. destructor* spores is shown in Figure 1. The artificial inoculation technique worked well, with 66% of the control–spores (no fungicide + *P. destructor* inoculation) plants having sporulating downy mildew lesions (Photo 6). Only two fungicide treatments had downy mildew infection – cymoxanil (Concord 300 SC) with 11% of the 100 inoculated onion plants showing sporulating downy mildew infection, and mandestrobin (Intuity) with 7% infection.

Discussion

The presence of downy mildew infection in the cymoxanil- and mandestrobin-treated onions indicates fungicide resistance or a lack of activity against downy mildew. In this experiment, high resistance risk fungicides metalaxyl, fenamidone, mandestrobin and azoxystrobin, and the low to medium risk cymoxanil and oxathiapiprolin were applied on their own. This would not commonly occur in normal commercial practice because the container labels on all but one of these products state that that these fungicides should/must not be applied on their own, but instead be tank-mixed with protectant fungicides or fungicides from a different FRAC group. The

Intuity (mandestrobin) label does not have a recommendation to tank mix, but to be applied in a programme with other fungicides. The resistance risk of the fungicides tested in this study, and the cross resistance relationships between them, are shown in Table 2.

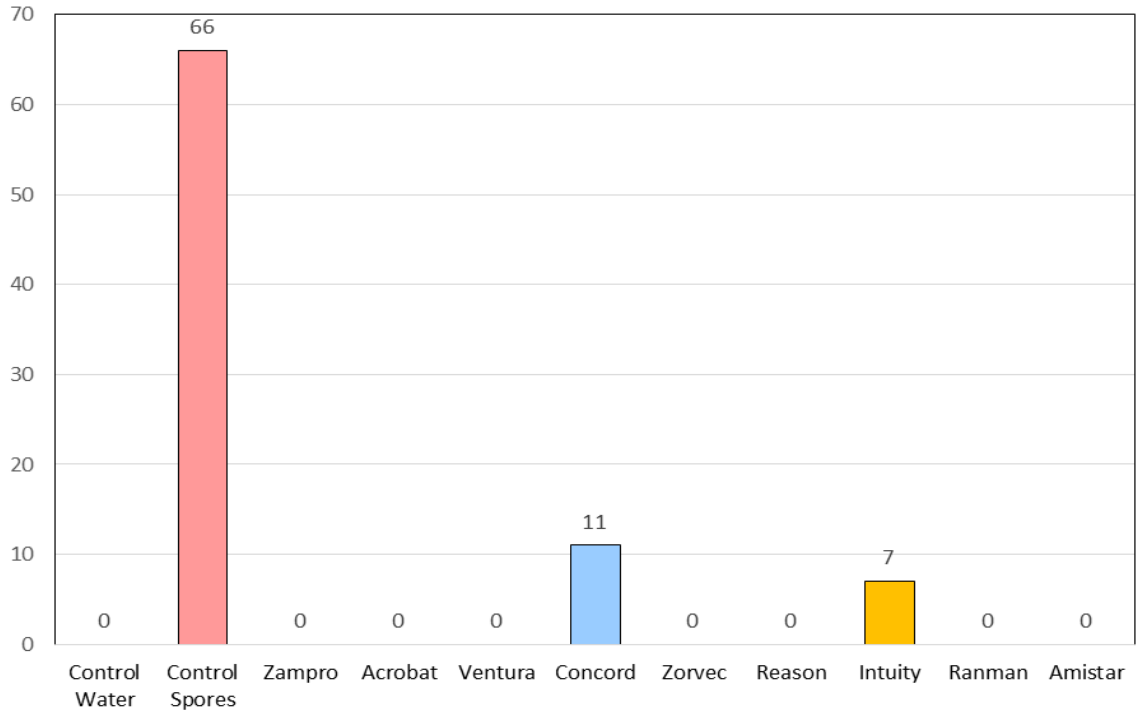

Figure 1. Percent plants with sporulating lesions for the 11 treatments 12 days after inoculation with *Peronospora destructor* spores.

Table 2. Fungicide Resistance Action Committee (FRAC) cross resistance groupings for the fungicides used in the study that require resistance management (FRAC 2018).

Group name	FRAC code	Active ingredient	Product	Resistance risk
Phenyl amide (PA)	4	Metalaxyl	Ventura	High
[1]Quinone outside inhibitor (QoI)	11	Fenamidone Mandestrobin Azoxystrobin	Reason Intuity Amistar	High
Quinone inside inhibitor (QiI)	21	Cyazofamid	Ranman	Medium to high
[1]Quinone outside inhibitor, stigmatellin binding type (QoSI)	45	Ametoctradin	Zampro (with dimethomorph)	Medium to high
Oxysterol binding protein homologue inhibition (OSBI)	49	Oxathiapiprolin	Zorvec	Medium to high
Carboxylic Acid Amide (CAA)	40	Dimethomorph	Zampro (with ametoctradin) Acrobat MZ	Low to medium
Cyanoacetamideoxime	27	Cymoxanil	Concord	Low to medium

[1]There is no cross resistance between QoIs and QoSIs

To delay fungicide resistance problems, it is normally recommended to alternate the use of fungicides with different modes of action (FRAC codes) that control the same pathogen (*P. destructor*) and to use tank mixtures of fungicides with different codes where possible/practicable. Fungicide container labels indicate the FRAC code of each active ingredient in the product. This makes it easy to avoid using products with the same code consecutively or in mixtures. Labels must be read carefully and completely, noting the maximum number of applications permitted per season in a crop. It is important for the grower to 'walk the crop' to monitor fungicide 'failures' and the occurrence of unexplained/unexpected disease that may be caused by fungicide-resistant pathogen populations.

Future work should focus on encouraging growers to routinely monitor their onion crops to identify resistance issues when they observe a lack of downy mildew control from previously effective products. Fungicide resistance tests, such as the one used in this study, can be undertaken to confirm a reduction in sensitivity of *P. destructor* strains to particular fungicides under controlled conditions. This confirmation step is crucial as there are many reasons why previously efficacious compounds are not efficacious in any given field, such as intense disease pressure, incomplete application coverage, inaccurate dosing or application timing (FRAC 2018). Routine disease monitoring by growers is important as it allows scientists to identify fungicide resistance issues and take appropriate disease resistance management measures before resistant *P. destructor* strains become widespread in the pathogen population.

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Photos

Photo 1. Trays, each containing 25 plants in an unheated greenhouse.

Photo 2. Fungicides applied to point of runoff.

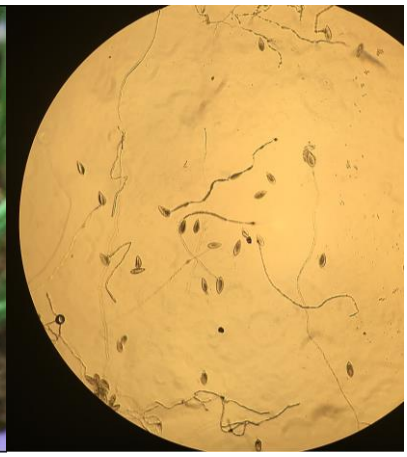

Photo 3. Germinating *Peronospora destructor* sporangia under the microscope

Photo 4. Trays of inoculated plants covered with black polythene bags in cool room

Photo 5. Leaves lightly misted with sterile distilled water

Photo 6. Sporulating downy mildew lesion on inoculated onion leaf

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