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Effects of copper sprays on bacterial soft rot of onion plants in the field

12 month progress report for the period to June 2007

P J Wright and C Triggs

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Contact: Paul Munro, PO Box 383, Pukekohe

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1 Executive summary

"Developing an Integrated Pest and Disease Production System for Onions and other *Allium* Crops" is a MAF SSF project instigated by the New Zealand Onion Exporters Association. Project 10 of the MAF SFF project is titled "bacterial soft rot in onions". This report is the third annual report for project 10, and reports the effects of the timing copper sprays and the concentration of soft-rotting bacteria on soft rot of onion plants in the field.

The incidence and severity of bacterial soft rot of onion during the growing season varies from year to year at Pukekohe. The most severe outbreaks of foliar soft rot have generally followed hail damage to crops and extended periods of wet weather. The objective of this work was to determine the effects of different copper formulations and timing of copper applications on incidence and severity of foliar bacterial soft rot of onion.

A small plot (3.5 m) field experiment, laid out in randomized blocks with 4 treatment replications, was carried out at Pukekohe. Eighteen treatments, comprising combinations of six copper treatments (Cu applied at different times before and after wounding and inoculation of foliage with soft-rotting bacteria), and three bacterial inoculation treatments (no bacteria, 'low' numbers of bacteria (10^5 cfu/ml), and 'high' numbers of bacteria (10^8 cfu/ml) were carried out in December 2006. Copper sprays were copper hydroxide (Kocide 2000) at 150 g product/100 l water at a water rate of 500 l water/ha. Foliage was wounded less than 2 min before bacterial inoculation using a motor-blower (to simulate strong wind).

Bacterial inoculation had the biggest effect (p-value < 0.001) on foliar soft rot with a mean 10.9 % of non-inoculated ('no bacteria') onions with foliar rots compared to a mean 31.8% and 41.0% for onions that had been inoculated with soft-rotting bacteria ('low' and 'high' concentrations respectively). Copper also had a big effect (p-value < 0.001), with copper applied on the same day (1 hour after) as wounding and inoculation significantly (P<0.05) reducing foliar soft rot compared to not applying copper. In all three bacterial concentration treatments copper sprays applied on the same day as inoculation reduced the incidence of soft rot compared to the other treatments.

Project Background

In 2004, the New Zealand Onion Exporters Association initiated a project titled: “To develop an Integrated Pest and Disease Production System for Onions and other *Allium* Crops”. Over a 3-year period, a “best practice” manual is going to be developed for New Zealand *Allium* growers. The manual will document integrated pest and disease management (IPM) strategies from current knowledge and from contracted research over this period.

The project team includes growers and exporters from the New Zealand onion industry. A science team comprising agronomists, consultants and scientists from Crop & Food Research and HortResearch, is working on 12 individual projects identified as areas where there are gaps in IPM knowledge. Bacterial soft rot in onions is Project 10 of the Integrated Pest and Disease Management Programme for onions and other *Allium* crops in New Zealand.

This report is the third annual report for project 10 - Effects of calcium fertiliser and copper bactericide applications on incidence of bacterial soft rot of onion plants in the field and bulbs in store.

3 Introduction

Bacterial soft rot is an economically important disease of onions (*Allium cepa* L.) grown in New Zealand and throughout the world. All parts of onion plants can be affected by the disease, which can occur at any stage of plant growth. Several species of bacteria cause soft rot of onion in New Zealand, including *Pseudomonas marginalis*, *Pseudomonas viridiflava*, *Burkholderia gladioli* pv. *alliicola*, and *Erwinia carotovora* subsp. *carotovora*.

The incidence and severity of bacterial soft rot onion in the field varies from year to year at Pukekohe. The most severe outbreaks of foliar soft rot have generally followed hail damage to crops and extended periods of wet weather.

Some local onion growers spray fixed copper on to their crops as 'protectant' applications to help prevent foliar bacterial soft rot. Copper is used widely worldwide to control bacterial pathogens on many horticultural crops. However, there are few published reports of copper for control of bacterial diseases of onion foliage. Bacterial streak of onion, caused by *Pseudomonas viridiflava*, and Xanthomonas leaf blight of onion caused by *Xanthomonas axonopodis* pv. *allii* have been controlled by copper sprays overseas.

The objective of this work was to determine the effects of timing of copper applications and levels of pathogenic bacteria on incidence and severity of bacterial soft rot of onion foliage at Pukekohe.

Aims of Project 10:

1. To determine the effects of Ca and N on bacterial soft rot of onion.
2. To determine if copper sprays control onion soft rot in the field and in storage.
3. To transfer results of the research on copper to control bacterial soft rot to growers via the IPM/best practice manual.
4. To transfer results of research on effects of Ca and N nutrition on onion soft rot to growers via the IPM/best practice manual.

Goals of Project 10:

Year 1 and 2

- To determine the effects of Ca and N on incidence and severity of bacterial soft rot in wounded and non-wounded onions that were artificially inoculated in the field.
- To determine the effects of Ca and N on bulb yield, bulb skin quality characteristics, and rots in storage.
- To investigate the effects of different copper formulations for control of bacterial soft rot of onion.
- To investigate the effects of timing of different copper formulations for control of onion soft rot.
- To develop a method to artificially wound and inoculate onions with soft-rotting bacteria to be able to fully test the copper treatments.
- To determine any phytotoxicity effects of different copper formulations.

Year 3

- To develop a Ca and N application strategy and evaluate it in grower fields.
- To transfer results of the research on effects of Ca and N on bacterial soft rot to growers via the IPM/best practice manual.
- To develop a copper application strategy and evaluate it in grower fields.
- To transfer results of the research on copper to control bacterial soft rot to growers via the IPM/best practice manual.

Change to year 3 goals: At the Onion SFF project leaders meeting on 28 August 2006 it was decided that in Year 3 the onion soft rot project should focus on the timing of applications of copper for control of foliar soft rot, and that the importance of the soft rot bacterial inoculum load should be investigated. The treatments for a field experiment to evaluate the timing of copper applications and the concentration of soft rotting bacteria were decided by the participants at this meeting. To keep within budget constraints the field trial proceeded, and the Ca and Cu evaluations on grower fields were not carried out.

2 Materials and Methods

A field experiment was carried out at the Crop & Food Research Centre at Pukekohe on a soil type described as a Patumahoe mottled clay loam (pH 6.5). Seed of the onion cultivar Early Longkeeper was direct-seeded on 27 June 2006 using a Stanhay precision seed planter in nine six-row beds, each 70 m long and 1.5 m wide. The experiment was laid out in four randomised blocks with one treatment replication along each of four beds. Each datum bed was flanked on both sides by a guard bed. Plots were 3.5 m long x 1.5 m wide, and treatments were randomly allocated to plots. There were 18 plots in each datum bed.

Eighteen treatments, comprising combinations of six copper treatments (applied at various times before and after wounding) and three bacterial inoculation treatments (no bacteria, ‘low’ numbers of bacteria (10^5 colony forming units per ml (cfu/ml)), and ‘high’ numbers of bacteria (10^8 cfu/ml) were carried out in December 2006. Copper sprays were copper hydroxide (Kocide 2000) at 150 g product/100 L water at a water rate of 500 L water/ha.

Foliage was wounded using a motor-blower to simulate strong wind (Fig. 1). Within 5 minutes of wounding, a 3-nozzle knapsack was used to spray bacterial suspensions (at rate of 300 ml suspension/m^2) on to plants to the point of run-off. The bacterial suspensions used for inoculations consisted of 24-hour-old cultures (grown on nutrient agar) of *P. viridiflava* (ICMP 8132), *P. marginalis* (ICMP 8127), and *E. carotovora* subsp. *carotovora* (ICMP 3915) in sterile water adjusted to concentrations of 10^5 cfu/ml and 10^8 cfu/ml for each bacterium strain.

On 22 December, 10 days after bacterial inoculation, 50 consecutive plants per plot (25 from the 2 inner rows of plants (out of 6 rows) were examined. Disease incidence was calculated as the percentage of plants out of 50 with disease symptoms.

The mean foliage soft rot scores were compared using analysis of variance (ANOVA). The experiment was analysed as a randomised block complete two factor factorial. Data were transformed to arcsine scale to stabilise variance before analysis of variance.

3 Results

Bacterial inoculation had the biggest effect ($P < 0.001$) on foliar soft rot with a mean 10.9 % of non-inoculated ('no bacteria') onions with foliar rots compared to a mean 31.8% and 41.0% for onions that had been inoculated with soft-rotting bacteria ('low' and 'high' concentrations respectively) (Table 1). Copper also had a big effect ($P < 0.001$), with copper applied on the same day (1 hour after) as wounding and inoculation significantly ($P<0.05$) reducing foliar soft rot compared to not applying copper. In all three bacterial concentration treatments copper sprays applied on the same day as inoculation reduced the incidence of soft rot compared to the other treatments. In the 'no bacteria' treatment, applying copper before inoculation and 1 day after inoculation significantly ($P<0.05$) reduced soft rot incidence. In the 'no bacteria' treatment, applying copper either before or after inoculation significantly ($P<0.05$) reduced soft rot.

Table 1: Effect of timing of copper applications and levels of soft-rotting bacteria on incidence (%) plants with foliar soft rot (fitted means).

Copper applications	'No bacteria'	'Low'	'High'	Mean
None	16.9	43.3	45.9	35.3
15+8+1 days before inoculation	9.7	31.4	38.3	26.4
1 day before inoculation	9.3	32.4	40.7	27.5
Same day (1 hour after inoculation)	7.8	23.8	31.3	21.0
1 day after inoculation	9.7	30.2	45.3	28.4
2 days after inoculation	11.9	29.5	44.7	28.7
Mean	10.9	31.8	41.0	

$LSD_{0.05}$ (df = 54) to compare mean levels of Bacterial Inoculation 2.7

$LSD_{0.05}$ (df = 54) to compare mean levels of Copper Treatments 3.8

$LSD_{0.05}$ (df = 54) to compare mean levels of Inoculation x Copper means 6.6

4 Discussion

The field experiment demonstrated that incidence of foliar rot is related to the numbers of pathogenic bacteria on the foliage (inoculum load). Applying copper sprays during the growing season reduced the incidence of foliar soft rot, especially when copper was applied soon after an infection event, which can occur during a heavy rain or hail storm when onion leaves are damaged and bacteria are splashed from the soil on to the plants. The numbers of soft rot bacteria in the 'high' bacteria treatment in this experiment would not normally occur in a commercial onion field. Applying protective copper sprays regularly (e.g. every 7 days) should reduce the incidence of bacterial soft of onion in the field, and this procedure is recommended to growers of onions in regions where foliar soft rot is problematic.

Figure 1 Using motor blower to blow onion leaves.

Figure 2 Onion plants showing symptoms of foliar soft rot 10 days after water blaster and bacterial inoculation.