

Crop & Food Research Confidential Report No. 1356

***Effects of different formulations, rates and
timing of copper applications on bacterial
soft rot in the field and store – 12-month
progress report for the period to June 2005***

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*A report prepared for
New Zealand Onion Exporters Association*

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1 *Executive summary*

Project 10B is part of the MAF SSF project titled “Developing an Integrated Pest and Disease Production System for Onions and other *Allium* Crops” that was initiated by the New Zealand Onion Exporters Association. Project 10B investigates the effects of different formulations, rates, and timing of copper applications on onion soft rot in the field and in store. This report is the first annual report for project 10B.

In the 2004-05 growing season a field experiment was carried out at the Crop & Food Research field station at Pukekohe to test fourteen treatments consisting of combinations of four copper formulations, two application times, and two wounding treatments. A sandblaster (to simulate hail damage) was used to wound the foliage of onions in the ‘wounded’ treatments. A suspension of soft-rotting bacteria was sprayed on to all plants in the trial.

Wounding plants using the sandblaster had a significant effect on the incidence of foliar rots. Plants that were not wounded had 0.5–1% soft rot infection, whereas wounded plants had between 13 and 25.3% infection. Wounded onion plants that were sprayed with copper after bacterial inoculation had lower disease levels than plants that were sprayed with copper before bacterial inoculation. Onions sprayed with copper before the bacterial suspension had similar (or higher) levels of soft rot than plants that did not receive copper applications. Levels of rots of onion bulbs in store on 20 May 2005 were low (<3%) for all treatments, and different copper formulations, timing of copper applications, and wounding of plants did not affect the incidence of bulb rots in storage.

2 *Project background*

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”. During the next three years, a “best practice” manual will 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 will work on 12 individual projects that address 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. For convenience of research and reporting, the bacterial soft rot project has been split into two parts:

1. Project 10A: Effects of calcium and nitrogen on incidence of bacterial soft rot in the field and in store;
2. Project 10B: Effects of different formulations, rates, and timing of copper applications for control of onion soft rot in the field and in store.

This report is the first annual report for project 10B - Effects of different formulations, rates, and timing of copper applications for control of onion soft rot in the field and in store bacterial soft rot in onions.

3 *Introduction*

Bacterial soft rot can cause significant losses in the field, in storage, and during transit to export destinations. Soft rot infection in the field is favoured by cool, wet conditions and damage to the foliage (e.g. hail, severe rainstorms and wind). At present there are few chemicals available to control bacterial diseases in horticultural crops. Copper is the most widely used chemical. However, some negative aspects of copper applications including phytotoxicity, reduced copper sensitivity by some bacterial strains, and environmental impacts have been reported. These aspects have been reduced through time of applications, tank mixes, use rate reductions, and formulation used.

Project 10B is part of Project 10 - Bacterial soft rot in onions. Project 10B examines the effects of different formulations, rates, and timing of copper applications for control of onion soft rot in the field and in store.

3.1 *Aims of Project 10B*

1. To determine if applications of copper control onion soft rot in the field and in store.
2. To transfer results of the research on copper to control bacterial soft rot to growers via the IPM/best practice manual.

3.2 *Goals of Project 10B*

Years 1 and 2

- 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 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.

4 *Materials and methods*

4.1 *Trial layout*

A field experiment was carried out at the Crop & Food Research field station at Pukekohe on a soil type described as a Patumahoe mottled clay loam (pH 6.5). Seed of the onion cultivar Kiwigold were direct-seeded on 15 June 2004 using a Stanhay precision seed planter in nine eight-row beds, each 70 m long and 1.5 m wide. Plant density 1 month after sowing was 55-65 plants/m of bed. Base fertiliser application of 15% potassic superphosphate (1 t/ha) was applied to the experimental site six weeks prior to sowing. Nitrogen was applied at 130 kg N/ha (as urea), with half applied at flag stage and the remainder 8 weeks later. Irrigation and the control of weeds, insect pests, and fungal diseases during the growing seasons were managed as is local commercial practice.

The experiment was laid out in randomised blocks with four treatment replications along four beds. Each datum bed was flanked on both sides by a guard bed. Plot size was 5 m long x 1.5 m wide and treatments were randomly allocated to plots. There were 14 plots in each datum bed. In the centre 4 rows of each plot 100 healthy onion plants were selected for subsequent soft rot assessments – a 'locator' tag was placed in the ground at each end of a line of 25 consecutive onions in each of the 4 rows.

4.2 Treatments

Fourteen treatments consisting of combinations of four copper formulations, two application times, and two wounding treatments were tested (Table 1).

Table 1: Experimental treatments.

Treatment number	Copper formulation	Timing of copper	Wounding of plants
1	No copper	-	No
2	Cu-hydroxide	Before inoculation	No
3	Cu-hydroxide	After inoculation	No
4	Cu-oxide	Before inoculation	No
5	Cu-oxide	After inoculation	No
6	Cu-sulfate	Before inoculation	No
7	Cu-sulfate	After inoculation	No

8	No copper	-	Yes
9	Cu-hydroxide	Before inoculation	Yes
10	Cu-hydroxide	After inoculation	Yes
11	Cu-oxide	Before inoculation	Yes
12	Cu-oxide	After inoculation	Yes
13	Cu-sulfate	Before inoculation	Yes
14	Cu-sulfate	After inoculation	Yes

A. Copper formulations

1. No copper.
2. Copper hydroxide (Kocide 2000) at 150 g/100 L water.
3. Copper oxide (Nordox 75WG) at 100 g/100 L water.
4. Copper sulfate (e.g. Cuprofix at 400 g/100 L water.

The surfactant, Actiwett, at a rate of 25 ml/100 L, was added to all fungicide applications, including the no-copper treatment. Spray applications were at 500 L/ha, using standard hollow cone nozzles.

B. Timing of copper applications:

1. Applied 24 h **before** inoculation.
2. Applied 16 h **after** inoculation.

C. Wounding

1. No wounding of foliage before bacterial inoculation.
2. Foliage wounded before bacterial inoculation.

After 3.30 pm on 10 December 2004 a sandblaster was used to damage the foliage of onions with impacting sand in the 'wounded' treatments (simulated hail damage). The nozzle of the sandblaster was moved in a gentle sweeping motion 0.5-1 m above foliage for approximately 30 seconds per plot. Within 2 minutes of completion of sandblasting, a knapsack was used to spray a bacterial suspension on to the plants to the point of run-off.

The bacterial suspension used for inoculation consisted of 24-hour-old cultures (grown on nutrient agar) of *Pseudomonas viridiflava* (ICMP 8132), *P. marginalis* (ICMP 8129), and *Erwinia carotovora* subsp. *carotovora* (ICMP 3915) in sterile water adjusted to a concentration of c. 10^8 colony forming units per ml (cfu/ml) for each bacterium strain. The wetting agent, Tween 80, at a concentration of 0.05% was added to the bacterial suspension.

On 20 December 2004, 10 days after bacterial inoculation, the 100 plants between the locator tags in each plot were individually assessed for foliar rot. A plant with one or more rotting leaves was deemed to have bacterial soft rot disease. Observations of any symptoms of plant phytotoxicity (plant stunting, discoloration, leaf scorch, unusual appearance, etc.) caused by any of the copper formulations were made at weekly intervals for four weeks following inoculation.

The 100 onion bulbs within the locator tags were lifted by hand on 10 January 2005 when the foliage had collapsed (top-down) for >90% of the plants. Bulbs were left on the ground to field cure on the ground for 10 days, then mechanically topped. Bulbs were graded according to bulb size and a random sample of 100 bulbs 70-90 cm diameter per replicate were placed in nylon string bags and stored in a ventilated shed at ambient temperatures (range 12-27°C) and humidity (range 70-85% RH).

On 20 May 2005, all onion bulbs were cut in half longitudinally and examined for soft rot. Incidence of bulb rotting was determined; no attempt was made to measure the degree of rotting.

5 *Results*

No symptoms of plant phytotoxicity were observed in any of the plots within four weeks of copper applications.

Sand from the sandblaster, upon striking onion leaves, left small puncture wounds <1 mm in diameter (Fig. 1). A few seconds after being damaged, small droplets of moisture exuded from most wounds.



Figure 1: Small puncture wounds on onion foliage caused by sandblasting.

Data were transformed using an arcsine transformation to stabilise variances before analysis of variance was carried out. Wounding of plants using the sandblaster had a significant effect on the incidence of foliar rots, with a substantial increase in the incidence of rots from 0.3 to 18% due to wounding ($P < 0.001$) (Table 2). There was no difference in the incidence of rots between any of the treatments before wounding ($P = 0.63$). Application of copper after wounding reduced the incidence of rots from 22.1 to 13.6% ($P < 0.001$). Addition of copper before wounding and inoculation had no effect. The incidence of foliar rots in untreated plots subjected to wounding was 19.5%, and in copper-treated plots sprayed before wounding and inoculation it was 22.1%. There was no difference in the effect of the three copper compounds on the incidence of bacterial soft rot when applied after wounding and inoculation ($P = 0.65$).

Table 2: Effects of different copper formulations, timing of copper applications, and wounding of plants on incidence of bacterial soft rot in onion plants in the field. Mean percent of plants with foliar rot on 20 December 2004. Foliar rot (%) back-transformed from fitted means on transformed scale.

Trt	Copper formulation	Timing of copper application	Plants wounded	Foliar rot (%)	Fitted means
1	No copper	-	No	0.3	2.9
2	Cu-hydroxide	Before inoculation	No	0.5	4.1
3	Cu-hydroxide	After inoculation	No	0.3	2.9
4	Cu-oxide	Before inoculation	No	0.1	2.0
5	Cu-oxide	After inoculation	No	0.3	2.9
6	Cu-sulfate	Before inoculation	No	0.4	3.5
7	Cu-sulfate	After inoculation	No	0.3	2.9
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8	No copper	-	Yes	19.5	26.2
9	Cu-hydroxide	Before inoculation	Yes	21.1	27.3
10	Cu-hydroxide	After inoculation	Yes	12.8	21.0
11	Cu-oxide	Before inoculation	Yes	20.4	26.8
12	Cu-oxide	After inoculation	Yes	13.5	21.6
13	Cu-sulfate	Before inoculation	Yes	25.0	30.0
14	Cu-sulfate	After inoculation	Yes	14.4	22.3
				LSD _{0.05} (df = 39)	4.70
				<i>P</i> -value	<0.001

The proportion of onion bulbs with rots on 20 May 2005 was low (<3.1% total rots) for all treatments (Table 3). Different copper formulations, timing of copper applications, and wounding of plants did not affect the incidence of soft rot in onion plants in storage.

Table 3: Effects of different copper formulations, timing of copper applications, and wounding of plants on incidence of soft rot in onion bulbs in storage. Mean percent of bulbs with rots on 20 May 2005.

Trt	Copper formulation	Timing of copper application	Plants wounded	Rots (%)
1	No copper	-	No	1.0
2	Cu-hydroxide	Before inoculation	No	1.0
3	Cu-hydroxide	After inoculation	No	1.0
4	Cu-oxide	Before inoculation	No	0.5
5	Cu-oxide	After inoculation	No	0.5
6	Cu-sulfate	Before inoculation	No	0.5
7	Cu-sulfate	After inoculation	No	0.0
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8	No copper	-	Yes	3.0
9	Cu-hydroxide	Before inoculation	Yes	1.0
10	Cu-hydroxide	After inoculation	Yes	2.0
11	Cu-oxide	Before inoculation	Yes	1.5
12	Cu-oxide	After inoculation	Yes	1.0
13	Cu-sulfate	Before inoculation	Yes	2.5
14	Cu-sulfate	After inoculation	Yes	2.5

6 *Discussion*

The field experiment clearly demonstrated that wounding of plant leaves is necessary for severe bacterial soft rot of onion foliage to occur. The experiment also showed that applying copper sprays to recently damaged onion leaves may help reduce the incidence of rot. For valid comparisons of products for control of foliar soft rot of onion, it is important that the results of more than one experiments are compared, so this experiment will be repeated next season, with some minor alterations. The sandblaster caused varying amounts of damage between plants and plots so other wounding methods will be also be used next season.