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Effects of calcium and nitrogen on the incidence of bacterial soft rot in the field and store – 12-month progress report for the period to June 2005

P J Wright

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A report prepared for
New Zealand Onion Exporters Association

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New Zealand Institute for Crop & Food Research Limited
PO Box 383, Pukekohe,, New Zealand

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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 that was initiated by the New Zealand Onion Exporters Association. Project 10 (comprised of parts 10A and 10B) of the MAF SFF project is titled “Bacterial soft rot in onions”. This report is the first annual report for project 10A, which investigates the effects of calcium (Ca) and nitrogen (N) on the incidence of bacterial soft rot in the field and in store.

In the 2004-05 growing season, a field experiment was carried out at the Crop & Food Research field station at Pukekohe to test three N and Ca applications at the 7-8 leaf growth stage, two Ca foliar fertiliser treatments, 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. Prior to experimental treatments, N and Ca contents of foliage from each trial plot were determined.

Nitrogen, as either urea or calcium nitrate at the 7-8 true leaf stage, increased the N concentration in the foliage compared to the foliage of onions not given N. Onions given calcium nitrate at the 7-8 true leaf stage had higher Ca levels in foliage than onions that received either urea or nothing on that date. Seven foliar applications of 15% Ca (as calcium chloride) at weekly intervals, starting at the 4-leaf stage, did not significantly increase the Ca content of onion leaves.

Wounding of plants using the sandblaster had a significant effect on the incidence of foliar rots. Plants that were not wounded had 0.8–1.3% soft rot infection, whereas wounded plants had between 14 and 21.5% infection. In the wounded treatments, onions that were given N, as either urea or calcium nitrate at the 7-8 true leaf stage, had higher levels of foliar rots than onions not given N. Seven foliar applications of 15% Ca (as calcium chloride) starting at the 4-leaf stage did not affect levels of foliar soft rot. There were no differences in onion yields between Ca and N treatments – bulb sizes and mean weights were similar across all treatments. Levels of rots of onion bulbs after storage for 4 months was low (<2.3% total rots) for all treatments. Different Ca and N treatments and wounding of plants did not affect the incidence of soft rot in onion plants 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. Project10A: 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 10A - Effects of calcium and nitrogen on incidence of bacterial soft rot in the field and in store.

3 Introduction

The value of plant nutrition in reducing the incidence and severity of plant pathogens has been recognised for many years. Although most metabolic or physiological mechanisms involved in host-pathogen interactions are not clearly understood, specific nutrients are known to reduce disease severity by affecting the virulence of the pathogen, enhancing resistance of the plant, and activating plant defences against infection.

Onions require a good supply of available nitrogen (N). However, excess N applications have been reported to cause late maturity of onions, large necks that are difficult to cure, soft bulbs, and poor storage quality. Nitrogen has been intensively studied in relation to host nutrition and disease severity for many years on many crops. Some foliar pathogens can penetrate, multiply, and develop more rapidly in succulent tissues promoted by abundant N. Research findings from overseas report that increased levels of N increased the severity of diseases caused by 15 phytopathogenic bacteria including *Pseudomonas viridiflava*, *P. marginalis* and *Erwinia carotovora*.

Several scientific papers also report the role of calcium (Ca) in plant nutrition and a consequent reduction in the impact of several fungal and bacterial plant diseases. It is generally believed that Ca increases the resistance of plant tissue by enhancing the structural integrity of cell walls and membranes, making them more resistant to degradation by pectolytic enzymes produced by pathogens.

Project 10A is part of Project 10 - Bacterial soft rot in onions. Project 10A examines the effects of Ca and N on the incidence of bacterial soft rot in the field and in store.

3.1 *Aims of Project 10A*

1. To determine the effects of Ca and N on bacterial soft rot of onion.
2. To transfer results of research on effects of Ca and N on onion soft rot to growers via the IPM/best practice manual.

3.2 *Goals of Project 10A*

Years 1 and 2

- To determine the effects of Ca and N on the 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.

Year 3

- To develop a Ca and N application strategy and evaluate it in growers' fields.
- To transfer the results of the research on effects of Ca and N on 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 60 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 12 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*

Twelve treatments consisting of combinations of three N and Ca applications at 7-8 leaf stage, two Ca foliar fertiliser treatments, and two wounding treatments were tested (Table 1).

Table 1: Experimental treatments.

Treatment number	7-8 true leaf	4-leaf onwards	Wounding of plants
1	Nothing	Nothing	No
2	Nothing	$CaCl_2$	No
3	Urea	Nothing	No
4	Urea	$CaCl_2$	No
5	Calcium nitrate	Nothing	No
6	Calcium nitrate	$CaCl_2$	No
7	Nothing	Nothing	Yes
8	Nothing	$CaCl_2$	Yes
9	Urea	Nothing	Yes
10	Urea	$CaCl_2$	Yes
11	Calcium nitrate	Nothing	Yes
12	Calcium nitrate	$CaCl_2$	Yes

A. Additional N at 7-8 true leaf stage on 11 November 2004

1. No N application.
2. Urea (46% N) at 65 kg N/ha (0 kg Ca/ha).
3. Calcium nitrate (15% N; 21% Ca) at 65 kg N/ha (91 kg Ca/ha).

B. Ca foliar applications from 4-leaf stage 20 October 2004.

1. No Ca applications.
2. Wuxal Aminocal (15% calcium as calcium chloride) at 4 litres in 500 litres water/ha. Applied at 7-day intervals, commencing at 4-leaf stage. Seven applications, the final one on 1 December.

C. Wounding

1. No wounding of foliage before bacterial inoculation.

2. Foliage wounded before bacterial inoculation.

On 9 December 2004 10 fully-expanded, mature leaves were randomly taken from 10 plants (1 leaf per plant) for each plot and sent to Gribbles Analytical Laboratories for analyses of N and Ca content.

After 3.30 pm on 10 December 2004 a sandblaster was used to damage the foliage of onions 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, was added to the bacterial suspension at a concentration of 0.05%.

On 20 December 2004, ten days after bacterial inoculation, the 100 plants between the locator tags in each plot were assessed individually for foliar rot. A plant with one or more rotting leaves was deemed to have bacterial soft rot disease.

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, 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

Nitrogen applied at the 7-8 true leaf stage on 11 November 2004 increased the N concentration in the foliage on 9 December 2004 from 2.95% to 3.06% for calcium nitrate and to 3.16% for urea (LSD = 0.11, $P = 0.002$). This effect was consistent in the presence and absence of calcium chloride ($P = 0.41$). Addition of calcium chloride had no effect on N concentration of foliage (no $CaCl_2$ 3.06%; $CaCl_2$ 3.06%, LSD = 0.091, $P = 0.96$).

Onions given calcium nitrate on 11 November had higher levels of foliar Ca (1.94%) than onions that received either urea or nothing on that date (1.78%) (LSD = 0.12, $P = 0.009$). This effect was consistent in both the presence and absence of $CaCl_2$ ($P = 0.34$). Seven foliar applications of 15% Ca (as $CaCl_2$)

starting at the 4-leaf stage did not significantly increase the calcium content of onion foliage (no $CaCl_2$ 1.81%; $CaCl_2$ 1.86%, LSD = 0.098, $P = 0.29$).

Table 2: Ca and N content of foliage on 9 December 2004 (prior to wounding).

7-8 true leaf	4 leaf onwards	Ca (%)	N (%)
Nothing	Nothing	1.75	2.95
Nothing	$CaCl_2$	1.80	2.95
Urea	Nothing	1.71	3.13
Urea	$CaCl_2$	1.85	3.20
Calcium nitrate	Nothing	1.96	3.10
Calcium nitrate	$CaCl_2$	1.93	3.02
	$LSD_{0.05}$ (df = 39)	0.17	0.16

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 variance before analysis of variance was carried out (Table 3). Plants wounded using the sandblaster had a significantly ($P < 0.001$) greater incidence of foliar rots than non-wounded plants (17.2 and 0.5% respectively). Addition of Ca did not significantly affect the incidence of foliar rots in wounded onions ($P = 0.88$). Addition of N significantly increased the incidence of foliar rots in wounded onions ($P = 0.07$). Seven foliar applications of 15% Ca (as $CaCl_2$) starting at the 4-leaf stage had no effect on the incidence of foliar soft rot.

Table 3: Effects of calcium (Ca) and nitrogen (N) on the 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	7-8 true leaf applications	4 leaf onwards applications	Plants wounded	Foliar rot (%)	Fitted means
1	Nothing	Nothing	No	0.4	3.5
2	Nothing	$CaCl_2$	No	0.4	3.5
3	Urea	Nothing	No	0.4	3.5
4	Urea	$CaCl_2$	No	0.3	2.9
5	Calcium nitrate	Nothing	No	0.9	5.5
6	Calcium nitrate	$CaCl_2$	No	0.7	4.9
7	Nothing	Nothing	Yes	13.8	21.8
8	Nothing	$CaCl_2$	Yes	12.4	20.6
9	Urea	Nothing	Yes	19.7	26.4
10	Urea	$CaCl_2$	Yes	21.5	27.6
11	Calcium nitrate	Nothing	Yes	16.8	24.2
12	Calcium nitrate	$CaCl_2$	Yes	19.7	26.3
				$LSD_{0.05}$ (df = 33)	5.5
				P-value	<0.001

There were no statistically significant differences in onion yields between Ca and N treatments. Bulb sizes and mean weights were similar across all treatments (Table 4).

Table 4: Effects of Ca and N on onion yields – percent bulbs in different size grades and mean bulb weights (wounded and non-wounded treatments combined) on 2 February 2005.

7-8 true leaf applications	4 leaf onwards applications	Small bulbs	Medium bulbs	Large bulbs	Mean bulb weight (g)
Nothing	Nothing	23	77	0	122
Nothing	$CaCl_2$	24	76	1	119
Urea	Nothing	23	77	1	117
Urea	$CaCl_2$	24	76	0	121
Calcium nitrate	Nothing	23	77	0	123
Calcium nitrate	$CaCl_2$	22	78	0	125
			$LSD_{0.05}$ (df = 36)		11
				P-value	0.69

The incidence of storage rots was unaffected by Ca or N treatments (Table 5).

Table 5: Effects of Ca and N on the incidence of bacterial soft rot in onion plants in store. Data transformed using arcsine transformation to stabilise variance before analysis of variance carried out.

Trt	7-8 true leaf applications	4 leaf onwards applications	Wounding	Rots (%)	Fitted means
1	Nothing	Nothing	No	0.3	2.9
2	Nothing	$CaCl_2$	No	0.3	2.9
3	Urea	Nothing	No	0.4	3.5
4	Urea	$CaCl_2$	No	0.4	3.5
5	Calcium nitrate	Nothing	No	0.3	2.9
6	Calcium nitrate	$CaCl_2$	No	0.4	3.5
7	Nothing	Nothing	Yes	0.7	4.7
8	Nothing	$CaCl_2$	Yes	0.3	2.9
9	Urea	Nothing	Yes	0.4	3.5
10	Urea	$CaCl_2$	Yes	2.4	8.8
11	Calcium nitrate	Nothing	Yes	1.1	6.1
12	Calcium nitrate	$CaCl_2$	Yes	0.4	3.5
				$LSD_{0.05}$ (df = 36)	5.7
				P-value	0.64

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 ‘late’ applications of N can increase the susceptibility of damaged onion foliage to infection by soft-rotting bacteria.

This experiment will be repeated next season, with some minor alterations, to obtain more data because more than one experiment must be carried out under different conditions to enable a valid comparison of the effects of N and Ca applications for the control of foliar soft rot of onion to be made.

Because the sandblaster caused varying amounts of damage between plants and plots, other wounding methods will also be used next season.