

Control of bacterial soft rot of calla (*Zantedeschia* spp.) by pathogen exclusion, elimination and removal

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Abstract The incidence and severity of bacterial soft rot of calla (*Zantedeschia* spp.) tubers at harvest were substantially reduced by planting method, chemical control measures, and removal of infected plant material. Callas grown using a sawdust-shadecloth method had fewer soft-rotted plants and tubers than callas grown in soil. Dipping tubers before planting in an aqueous solution of 0.08% copper hydroxide, 0.12% thiram, plus 0.03% benomyl reduced plant and tuber losses from bacterial soft rot. Drenching plants during the growing season with an aqueous solution of 0.12% thiram, 0.08% copper hydroxide, plus 0.15% quitozene gave further control against rots. Weekly removal of rotting foliage and tubers during the growing season also reduced levels of plant and tuber soft rots. This study has demonstrated that effective control of calla soft rot can be achieved using disease management that combines appropriate pathogen exclusion, elimination, and removal methods.

Keywords *Zantedeschia* spp.; calla; soft rot; *Erwinia carotovora*; agronomic methods; chemical control; sawdust growing media

INTRODUCTION

Calla (*Zantedeschia* spp.), a tuberous plant native to areas of south-central Africa, is grown in New Zealand and several other countries, for export both as tubers and cut flowers. The origin, botanical classification, morphology, and culture of calla has been described in detail by Funnell (1993). Calla has become an increasingly important crop in New Zealand, with export revenue earnings of NZ\$9.2 million in the year ending June 1999, up from NZ\$1.9 million in 1990 and NZ\$6.6 million in 1995 (Statistics NZ 1999). The major markets for calla exports are Japan, Europe, and North America (New Zealand Calla Council pers. comm.). Tubers are mainly exported from June to September each year, and flowers between November and February, although growers have successfully extended the flowering season to October–May by greenhouse production (Welsh & Clemens 1992; Clemens & Welsh 1993).

Bacterial soft rot caused by *Erwinia carotovora* subsp. *carotovora* is the most important disease of calla in New Zealand (Wright 1998), and is a major factor affecting the commercial viability of this crop (Funnell 1993). The disease results in the death of plants and decay of tubers. Infection can occur at all stages of the calla life cycle from young plants to tubers in store (Wright 1998). The severity of soft rot depends chiefly on soil and weather conditions, with warm, wet weather and poor-draining soils favouring infection (Kamerman 1975; Wright 1998). *E. carotovora* subsp. *carotovora* enters plants through natural openings, growth cracks, wounds, and lesions caused by fungal pathogens (Chupp & Sherf 1960; Agrios 1969; Beckman & Lukens 1997). The pathogen can reside in an inactive state inside calla tubers as latent infections, until conditions occur that stress the tubers and favour growth and multiplication of the bacteria, giving rise to soft rot

(Blom & Brown 1999). Chemical control is not effective against *E. carotovora* subsp. *carotovora* once plant or tuber infection has occurred (Beckman & Lukens 1997).

There has been little published research on control of calla soft rot. Control measures including discarding diseased tubers before planting, growing callas in well-drained growing media at moderate temperatures (<24°C), careful management of irrigation, and use of chemicals as tuber dips before planting or as drenches during crop growth have been recommended (Kuehny et al. 1998; Wright 1999). Sawdust mulch was found to reduce the incidence of soft rot in irrigated, field-grown callas (Wright & Burge 2000). New Zealand calla growers generally use control measures based on anecdotal advice, past experience, and recommendations for other crops.

Control of plant diseases is primarily aimed at protecting plants from becoming diseased rather than curing them after disease has developed (Agrios 1969). Control measures for soil-borne plant diseases involve chemical and cultural methods and use of resistant varieties. Cultural methods include those that are aimed at: (1) excluding the disease from new plantings (exclusion); (2) eliminating the pathogen from the plant or area in which the plant is growing (eradication); and (3) reducing the amount of inoculum that may spread to healthy plants (sanitation) (Forsberg 1963; Agrios 1969; Pirone 1978).

Chemical control is often an integral part of any plant disease control strategy (Agrios 1969). Dipping tubers in fungicide and/or bactericide and drenching the soil around infected plants with fungicides are recognised measures for control of soil-borne fungal and bacterial diseases of flowering bulb crops including tulip (*Tulipa* sp.), lily (*Lilium* spp.), *Narcissus* spp., *Gladiolus* spp., and *Iris* spp. (Moore 1979). Pre-planting chemical dips have been shown to be effective against bacterial soft rot of calla in some studies (Kuehny et al. 1998; Blom & Brown 1999; Wright 1999), but not in others (Wright & Burge 2000). Beckman & Lukens (1997) recommended drenching calla tubers in pots with broad spectrum fungicide within 1–3 days after planting as a method for control of soil-borne diseases.

Removal of infected plant material in the field to prevent the spread of several diseases of ornamental crops has been recommended by Forsberg (1963) and Moore (1979). Diseases controlled by this method include soft rot of iris (caused by *E. carotovora* subsp. *carotovora*), black slime of hyacinth (*Hyacinthus orientalis*) caused by *Sclerotinia*

bulborum, fusarium scale rot of lily caused by *Fusarium oxysporum*, and grey mould of snowdrop (*Galanthus nivalis*) caused by *Botrytis galanthina*.

Because it is often easier to exclude diseases from plants than it is to control them after they appear, many New Zealand calla growers plant their crops in one or a mixture of soil-less growing media, including sawdust, bark, pumice, and/or peat. One planting method, the “sawdust-shadecloth method”, is becoming increasingly popular. This involves placing tubers on thin (c. 20 mm) layers of sawdust on nylon mesh shadecloth which has first been laid on the soil. Tubers are then covered with sawdust to a depth of 80–100 mm. The calla roots grow through the shadecloth into the soil to obtain nutrients and water, and the tubers in the sawdust stay out of direct contact with the soil. The rationale behind this method is that two of the main factors that favour calla soft rot—high numbers of *E. carotovora* subsp. *carotovora* and wet, anaerobic conditions—commonly occur in waterlogged soil, and that these conditions can be avoided by placing tubers above the soil in free-draining, *Erwinia*-free sawdust. Wet weather often delays the harvesting of tubers in the field (Clemens & Welsh 1993), and an advantage of the sawdust-shadecloth method is that tuber harvest can be carried out regardless of weather or soil conditions. The effectiveness of the sawdust-shadecloth planting method as a control measure for calla soft rot has not yet been determined.

The present paper reports the effects of the sawdust-shadecloth planting method, pre-planting chemical dips and drenches during the growing season, and removal of soft rotted plant material on the incidence and severity of bacterial soft rot of calla grown at Pukekohe, New Zealand.

MATERIALS AND METHODS

The experimental site consisted of six beds 30 m long. Treatment plots were 1.2 m long × 1.0 m wide and separated by buffer zones of 0.5 m. Five blocks (replications) of 12 plots, comprising two parallel sets of six plots (at right angles to the direction of the rows) were set out. Callas were grown in soil in the first set of six plots, and in sawdust-shadecloth in the other set of six plots. The split-plot experimental design was necessary because the two planting methods required different irrigation regimes. Treatment plots were randomly located within each set of six plots.

The 12 treatments tested comprised a complete factorial design of all combinations of: two planting

methods—planting tubers in (1) soil-only or (2) sawdust-shadecloth method; three chemical control treatments—(1) no chemicals, (2) pre-plant tuber bactericide/fungicide dip, or (3) pre-plant dip plus fungicide drenches; and two “sanitation” measures—(1) removal and (2) non-removal of infected foliage and rotting tubers.

Planting method

Calla tubers, size 30–40 mm, of the cultivar ‘Black Magic’ were planted on 20 October 1999 at the Crop and Food Research Centre at Pukekohe, New Zealand. The soil type was a Patumahoe mottled clay loam. Base fertiliser application of 15% potassic superphosphate (equivalent of 1 t/ha) was applied to the experimental site 6 weeks before planting to bring the soil levels of potassium (K) and phosphorus (P) within the range recommended for callas (K: 1.4–2.1 meq/100 g; P: 40–80 µg/ml) (Burge et al. 1994).

Twenty tubers were planted in each plot with tuber spacing 200 mm × 200 mm. Tubers in the soil-only treatment were hand-planted 100 mm deep using a trowel. For the sawdust-shadecloth treatments, 1.5 m × 1.5 m pieces of woven nylon windbreak cloth (38–45% shading) were placed on the soil. The cloth was covered with c. 20 mm of untreated pine sawdust onto which tubers were placed. A further 80 mm of sawdust was then placed on top of the tubers. Calcium ammonium nitrate (100 g/m²) was spread over the sawdust to counteract nitrogen absorption by the sawdust as it decomposed. The sawdust-shadecloth plots were irrigated to fully wet the sawdust immediately after planting, whereas the soil plots were not watered at that time.

Irrigation was by drip tape (holes 100 mm apart) between each row of plants. Irrigation began at early crop emergence on 15 November 1999, and was not applied after 13 March 2000. The evapotranspiration rate for the Pukekohe region in summer is c. 5 mm per day (30–35 mm over 7 days). For the soil-only treatment, 30 mm of water was applied when <25 mm of rain had been recorded over a 7-day period. The sawdust-shadecloth method required a different irrigation regime because the free-draining sawdust dried out more rapidly than the heavy clay loam soil beds. For the sawdust-shadecloth method, 30 mm of water was applied at 7-day intervals regardless of quantity of rainfall.

Chemical treatments

The tuber dip treatment was applied by immersing tubers for 15 min in an aqueous solution of 0.08%

copper hydroxide (Kocide DF), 0.12% thiram, and 0.03% benomyl (Benlate). The tubers were left to dry overnight in single layers in vented plastic produce crates before planting the next day. The soil drench treatment was an aqueous solution of 0.12% thiram, 0.08% copper hydroxide, and 0.15% quinterozone (Terraclor 75 WP). Drench solution (250 ml) was applied to the soil around the base of each plant at 14-day intervals on eight occasions between early crop emergence (17 November 1999) and 1 March 2000.

“Sanitation” method

For the treatment where infected plant tissue was removed, leaves showing symptoms of soft rot (yellowing and collapsing) were removed from plots at weekly intervals between 17 November 1999 and 1 March 2000. Tubers of plants that had completely collapsed during the same period were carefully dug from the ground using a garden trowel. Care was taken to minimise disturbance to neighbouring plants.

Disease assessments

At 21 weeks after planting (13 March 2000), each plant was assessed for symptoms of soft rot using a four category scale: 0 = no soft rot symptoms; 1 = <50% of the plant had symptoms; 2 = >50% of the plant had symptoms; and 3 = plant completely dead.

The first symptom of soft rot was usually a collapsed leaf (Wright 1998). Twenty-four soft-rotted petioles (two from each treatment) were selected at random on 13 March for isolation of causal organisms.

Flowers were not picked. Tubers were harvested on 17–18 July 2000 when the crop had fully senesced (foliage brown and shrivelled). Tubers were removed from the soil. Those from the sawdust-shadecloth plots were gathered from the sawdust after the shadecloth was pulled up. Many of the tubers were still attached to the shadecloth by their roots. After lifting, all tubers were thoroughly washed to remove adhering dirt, sawdust, and rotted tissue. The total weight of tubers and the number of large (>20 g) tubers with no symptoms of soft rot were determined for each plot.

Statistical analyses

The experiment was analysed as a split-plot design with planting method as the whole plot factor and chemical and removal treatments as the within plot factors. Analysis of variance was carried out on the percentages of large healthy tubers after applying the

arcsine transformation to stabilise variance. Analysis of variance was also carried out on the tuber weight data from the split-plot design after a log transformation to stabilise variance. Since the extent of soft rot was assessed on an ordinal and not quantitative scale, the distributions of the counts of bulbs in each disease category for each combination of planting and chemical treatments were compared using ordinal regression (Dobson 2002). The method of Hannah & Quigley (1996) was used to display the results of the ordinal regression analysis on the original categorical disease assessment scale. All analyses were performed using the GENSTAT 6.1 statistical package (Genstat Committee 2002).

RESULTS

Rainfall and temperature conditions

Total rainfall and mean air temperatures during each month of the trial growing season were: 180 mm and 15.8°C (November); 35 mm and 16.2°C (December); 134 mm and 18.3°C (January); 12 mm and 18.3°C (February); 58 mm and 17.2°C (March); and 115 mm and 15.8°C (April). The soil plots were irrigated on eight occasions.

Soft rot of plants

The most commonly isolated micro-organism from the healthy-diseased margin of infected petioles was *E. carotovora* subsp. *carotovora*, which was found in all samples.

Growing plants using the sawdust-shadecloth method, dipping tubers before planting, drenching plants during the growing season, and removal of infected foliage, all reduced the incidence and severity of soft rot. The distribution of disease scores from the 100 plants from the five replicate plots 21 weeks after planting was summarised using ordinal regression (Fig. 1).

The sawdust-shadecloth planting method reduced the mean disease score compared with plants grown in soil ($P < 0.001$). This effect was consistent for all combinations of chemical treatments and removal of diseased tissue.

The tuber dip treatment reduced the mean disease score ($P < 0.001$), and the additional drench treatment further reduced soft rot ($P < 0.001$). The reduction as a result of dipping was the same for both planting methods ($P = 0.54$), but for bulbs grown in sawdust, drenching after dipping had a synergistic effect giving an additional reduction in mean disease score ($P = 0.002$).

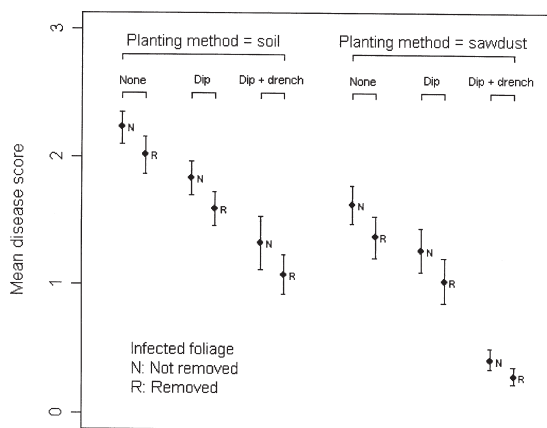


Fig. 1 Mean soft rot score of plants 21 weeks after planting, calculated from distributions fitted to disease category counts. Arrows represent \pm LSD for each backtransformed mean disease score.

Removal of infected foliage systematically reduced mean disease score for every combination of planting method and chemical treatment ($P < 0.001$). The effect of removal was consistent for all combinations of the other two factors.

Proportion of large healthy tubers

Growing plants using the sawdust-shadecloth method, dipping tubers before planting, drenching plants during the growing season, and removal of infected foliage, all increased the proportion of large healthy tubers at the end of the crop growing period (Table 1).

The effect of dip and drench chemical treatments on percentage of large healthy tubers differed for plants from the two planting method treatments (Table 1). Dipping and drenching had much larger effects on tubers grown in soil than for tubers grown in sawdust ($P < 0.001$), even though the tubers grown in sawdust had a much higher proportion of healthy bulbs ($P < 0.001$).

Where tubers were either not dipped, or dipped and not drenched, removal of infected plant material increased proportion of healthy tubers both in soil and sawdust growing media ($P = 0.01$). In soil, removal of infected plant material did not increase the proportion of healthy tubers where both dipping and drenching were applied ($P = 0.97$). For tubers grown using the sawdust-shadecloth method, removal of infected plant material had an effect when no chemical treatment was applied or when only the dip treatment was used ($P = 0.004$).

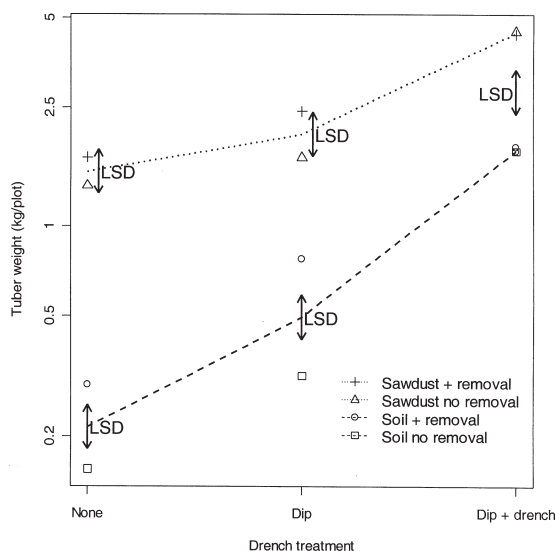


Fig. 2 Mean weight of healthy calla (*Zantedeschia* spp.) tubers harvested from plots (each planted with 20 tubers) to which treatments of chemicals, planting method, and removal of diseased plant material had been applied. LSDs ($P < 0.05$) to compare means for the different treatments are indicated. LSDs are constant for all pairs of treatments.

Weight of healthy tuber tissue of harvested tubers

Growing plants using the sawdust-shadecloth method, dipping tubers before planting, drenching plants during the growing season, and removal of infected foliage, all reduced the severity of soft rot of harvested calla tubers (Fig. 2).

The effect of dip and drench chemical treatments on the weight of healthy tuber tissue differed for

plants grown with the two methods. Dipping and drenching had a much larger effect on tubers grown in soil than for tubers grown in sawdust ($P < 0.001$), even though the tubers grown in sawdust had much greater healthy tuber weights ($P < 0.001$) (Fig. 2).

Where tubers were either not dipped, or dipped and not drenched, removal of infected plant material increased the weight of healthy tuber tissue in both soil and sawdust growing media ($P < 0.001$). For both planting methods, removal of infected plant material did not increase the weight of tubers where both dipping and drenching were carried out ($P = 0.31$) (Fig. 2).

DISCUSSION

This experiment has clearly demonstrated that the incidence and severity of bacterial soft rot of calla plants and tubers can be substantially reduced by planting tubers in sawdust, using the sawdust-shadecloth method, rather than by planting in soil. The experiment also showed that dipping tubers before planting in bactericide/fungicides can reduce plant and tuber losses as a result of bacterial soft rot. It must be noted that the effectiveness of chemical dips can vary from one year to the next (Kuehny et al. 1998). Wright & Burge (2000) found that dipping tubers in Enhance® (250 g/litre quaternary hydroxybiphenyl), before planting did not markedly reduce soft rots in a field experiment carried out at Pukekohe in 1996/97. Drenching plants during the growing season with bactericide/fungicides gave further control against rots. Removal of rotting foliage and tubers during the growing season reduced

Table 1 Percentage of large (>20 g) healthy tubers harvested from plots (planted with 20 tubers) to which different treatments of chemicals, planting method, and removal of diseased plant material had been applied. Analysis carried out on arcsine transformed data, and means are back transformed from fitted means. LSD ($P < 0.05$, d.f. = 40) to compare planting method × chemical treatment × removal of infected plant material means = 7.1 (7.1 for means in same planting method). LSD ($P < 0.05$, d.f. = 40) to compare planting method × chemical treatment of infected plant material means = 5.1 (5.0 for means in same planting method). LSD to be applied to arcsine transformed means only.

Planting method	Removal	Chemical treatment					
		Nil		Dip only		Dip + drench	
		Back-transformed % healthy	Mean (arcsine scale)	Back-transformed % healthy	Mean (arcsine scale)	Back-transformed % healthy	Mean (arcsine scale)
Soil	No	0.0	0.0	3.9	11	21	28
	Yes	0.8	5.2	6.8	15	23	29
	Mean	0.2	2.6	5.3	13	22	28
Sawdust	No	17	24	26	31	76	61
	Yes	25	30	37	37	78	62
	Mean	21	27	32	34	77	61

levels of plant and tuber soft rots. When all four control methods were applied (plants grown in sawdust, pre-planting tuber dips, drenches during the growing season, and removal of infected plant material), the highest incidence of completely healthy tubers at lifting (78%) and highest weight of healthy tuber tissue (4.5 kg per plot) resulted. On the other hand, when none of the three control measures were applied (plants grown in soil, no chemical treatments, and no removal of infected plant material), none of the tubers were completely healthy at lifting, and the lowest weight of healthy tuber tissue (0.16 kg per plot) was recorded. These two treatment combinations also displayed the lowest and highest proportions of completely dead plants, 2% and 47% respectively (data not shown).

Field-grown calla tubers are normally lifted in April–June, which are among the wetter months of the year at Pukekohe, when the mean rainfall is 120–150 mm per month. The wet weather in combination with the local heavy clay loam soils makes machine-lifting of calla tubers difficult, and often impossible. Digging soil-grown tubers by hand in our trial was a laborious, time-consuming task and most tubers were damaged in the process. On the other hand, lifting tubers that were grown by the sawdust-shadecloth method was quick, easy, and clean, and very few tubers were damaged during the lifting process. Although some growers using the sawdust-shadecloth method have found that the contractile roots of callas can pull the tubers downwards and through the cloth, increasing the risk of tuber damage and making harvesting of tubers time-consuming, this did not happen in our trial. The experiment demonstrated that the sawdust-shadecloth planting method is a practical option for growers who have ongoing problems with soft rot, who grow calla crops on heavy soils, and/or who normally experience wet weather during tuber lifting. The sawdust-shadecloth method provides good aeration around tubers, reduces the incidence and severity of soft rot, eliminates the need to wash tubers after lifting, and allows efficient management of tuber harvest regardless of the moisture status of the soil.

Our research has identified a planting method, chemical control measures, and a sanitary practice that greatly reduces the incidence and severity of calla soft rot. The use of the disease prevention procedures described here, which incorporated pathogen elimination, exclusion, and removal, can provide good levels of control of soft rot. The application of good grower practices with the emphasis on temperature and water management aimed at producing

strong, healthy plants, is also recommended to help ameliorate the soft rot problem in callas. We have demonstrated that the use of a combination of control methods, incorporating appropriate cultural and chemical approaches, is likely to provide successful management of a disease that can cause severe economic losses for calla growers.

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