Protection of budding wounds in apple nursery trees from European canker

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Abstract The fungus *Neonectria ditissima* can cause European canker in apple nursery trees. In this study, ten treatments of 'EMLA 9' rootstocks budded with 'Royal Gala' were examined, 5 and 15 months after budding and inoculation with conidia of *N. ditissima* (2×10^5 conidia/ml). Treatments included combinations of five budding tapes, two budding methods and three fungicidal treatments. Fifteen months after inoculation, all T-budded trees had died. Chip-budded trees inoculated after tying with either Flexiband type C or plastic pre-cut strips displayed significantly more canker (21% and 22%, respectively) than those tied with Buddy tape (11%, $P < 0.001$). Of the uninoculated trees, 9% displayed canker symptoms, because either the budding wounds were infected by natural inoculum, or the infection was present in the scion or rootstock before budding. Treating buds with tebuconazole/carbendazim reduced canker symptoms significantly (2%, $P < 0.001$). Calcium hydroxide also reduced canker symptoms (5%). Pyraclostrobin/boscalid did not reduce canker incidence.

Keywords *Neonectria ditissima*, apple, propagation, nursery tree, budding, budding tapes, wound protection

INTRODUCTION

European canker, caused by the fungus *Neonectria ditissima* (formerly *N. galligena*), is a concern for apple growers in the Nelson/Tasman region. An extensive study in the UK (the Millennium Study) showed that nurseries can be one of the sources of canker in orchards (McCracken et al. 2003). The authors showed that trees that became infected at budding were of particular concern, as these trees did not develop any symptoms until after planting in an orchard. Therefore, the protection of budding wounds

from infection with *N. ditissima* should reduce the chance of apparently healthy nursery trees developing symptoms after planting in an orchard.

Scheper et al. (2008) showed that treating pruning wounds with a wound dressing amended with carbendazim was effective in preventing infection, and spray applications of calcium hydroxide (slaked lime) onto leaf scars have been shown to reduce canker incidence in New Zealand (R.W.A. Scheper, Plant & Food Research, unpublished data). Similarly, calcium hydroxide and thiophanate-methyl (a

benzimidazole fungicide similar to carbendazim) applied to apple orchards during leaf fall, reduced the number of newly formed cankers in 3 successive years in the Netherlands (Heijne et al. 2005). It is likely that these products would also provide wound protection from *N. ditissima* infections during propagation in nurseries.

In this study, different budding methods and tapes, as well as fungicidal treatments, were assessed for their efficacy in protecting budding wounds from infection with *N. ditissima*.

MATERIALS AND METHODS

Trial design and assessments

Approximately 1,800 'EMLA 9' rootstocks were planted in Motueka, New Zealand, in October 2008, in 17 rows of 100-105 rootstocks and one row of 70 rootstocks, with row spacing of 85 cm and plants 50 cm apart. The trees were budded with 'Royal Gala' in March 2009. Ten budding treatments (Table 1), including different budding tapes (Table 2), methods and fungicidal treatments were applied. The fungicidal treatments were 3.2% calcium hydroxide (slaked lime), 0.0192%

pyraclostrobin and 0.0378% boscalid (0.15% Pristine®) in 50% water-based acrylic paint (Home living basix fence paint for exterior timber (colour redwood 18696857), manufactured by Orica New Zealand Ltd, Lower Hutt, New Zealand), and a mixture of 1% tebuconazole and 2.5% carbendazim in 50% water-based acrylic paint (4% Folicur® WG and 5% Headland Addstem, respectively). The fungicidal treatments were applied only to the budded section of the rootstock, using a knapsack with a modified three-nozzle wand (Figure 1).

Within 24 h of the budding treatments being applied, buds were inoculated with a suspension of conidia of *N. ditissima* (2×10^5 conidia/ml), by spraying one puff (60 µl) of inoculum on each bud using a hand-pumped DeVilbiss® glass atomizer (Model 15-RD). Inoculum was made by transferring sporodochia from cankers collected in Motueka (Nelson) to sterile distilled water containing 0.005% Tween®20 (Tween solution), using a binocular dissecting microscope and a sterile scalpel. The positive controls were inoculated immediately after budding and before taping. The negative controls were sprayed with only Tween solution after taping.

Each treatment was replicated ten times in a randomised block design, except for the inoculated positive control, which was replicated five times. All treatments neighboured the positive control only once or twice. Each plot consisted of 20 trees along a row, except for the T-buds covered with Okulette O30. These buds were placed 10-20 cm above the chip-buds with Flexiband type C, on alternate trees, which meant there were 10 trees in each plot.

One month after budding, all treatments except the negative control, were treated again with either conidia of *N. ditissima* (2×10^5 conidia /ml), or a fungicidal treatment followed by inoculation.

Conidial germination rates were determined by placing six 60-µl droplets of the conidial suspension on three glass microscopy slides, covered with cover slips. The slides were incubated at 20°C for 24 h at 100% relative humidity, and the numbers of germinated and non-germinated spores were counted using a compound microscope at $\times 100$ magnification. The germination rate of the first conidial suspension

Figure 1 Fungicidal treatments were applied using a knapsack and modified three-nozzle wand to chip-budded wounds of 'EMLA 9' apple rootstocks.

Table 1 Budding treatments applied to 'EMLA 9' apple rootstocks, using 'Royal Gala' budwood. T = T-budded; C positive = positive control; C negative = negative control.

Treatment	Method	Tape	Fungicidal treatment
1	Chip	Buddy tape	-
2	Chip	Plastic strips	-
3	T	Flexiband type A	-
4	Chip	Flexiband type C	-
5	T	Okulette O30	-
6	Chip	Buddy tape	Calcium hydroxide
7	Chip	Buddy tape	Tebuconazole/carbendazim
8	Chip	Buddy tape	Pyraclostrobin/boscalid
C positive	Chip	Buddy tape	-
C negative	Chip	Buddy tape	-

Table 2 Taping products tested for their efficacy in protecting apple budding wounds from canker infection when applied before inoculation with a conidial suspension of *Neonectria ditissima*.

Tape	Manufacturer	Description, mm
Buddy tape	Aglis Co. Ltd, Fukuoka, Japan	With perforations, 25×70
Plastic strips	Plastrip, Gardenview, South Africa	Pre-cut flexible PVC strips, 12×300
Flexiband type A	Fleischhauer KG, Ahrensburg, Germany	Pre-cut rubber strips, 3.5×140
Flexiband type C	Fleischhauer KG, Ahrensburg, Germany	Pre-cut rubber strips, 15×200
Okulette O30	Fleischhauer KG, Ahrensburg, Germany	Pre-cut rubber ties, 30×35

was 42% and that of the second was 86%.

Six weeks after budding, the plastic strips and Buddy tape were cut off using a craft knife, by gently cutting from the bottom to the top of the tape at the back of the rootstock. Okulettes and Flexiband that had not degraded were also removed.

All trees were headed back 5 months after budding, in August 2009, using secateurs disinfected with methylated spirits, and T-buds that had been covered with Okulettes were removed. The trees were cut 2–5 mm above the budding wound. Secateurs were disinfected after each budding treatment using methylated spirits and paper towels. Heading-back wounds were treated with a wound dressing.

Budding wounds were assessed for the presence or absence of canker symptoms 3, 5, 9 and 15 months after budding, in June, August, December 2009 and June 2010 respectively.

Statistical analysis

The proportion of buds or surrounding rootstock with canker symptoms was determined for each plot of 20 trees, except for the T-buds covered with Okulettes, where this was determined for each plot of 10 trees. Data were analysed using binomial generalized linear models, with a logit link and replicate and treatment as factors (categorical variables). Pairwise likelihood ratio tests ($\alpha=0.05$) were used to determine statistical differences in disease incidence of budding wounds among treatments. Analyses were carried out using GenStat (version 17, 2014, VSNi Ltd, Hemel Hempstead, UK) and the Biometris GenStat procedure library (2014, Biometris, Wageningen UR, Netherlands).

Figure 2 Budded ‘EMLA 9’ apple rootstocks, 3 months after budding with ‘Royal Gala’ and inoculation with a conidial suspension (2×10^5 conidia /ml) of *Neonectria ditissima*. (a) Chip-bud taped with Buddy tape that has healed without symptoms; (b) T-bud taped with Flexiband A that shows canker symptoms.

RESULTS

Three months after budding, canker symptoms were clearly visible on 53% of the positive controls, while approximately 11% of T-budded rootstocks tied with Flexiband type A displayed canker symptoms (Figure 2). Only 0–1% of rootstocks treated with the remaining budding treatments displayed canker symptoms. No canker symptoms were visible on the un-inoculated controls.

Five months after budding, significantly more positive control rootstocks (100%) and T-budded rootstocks tied with Flexiband A (98%) displayed canker symptoms than in all other treatments ($P < 0.001$). T-budded rootstocks tied with Okulettes displayed significantly more canker symptoms (40%) than chip-budded rootstocks (0–3%, Table 3).

Nine months after budding, lesions and dieback were found in all treatments (Table 3). Dieback, the description given to buds that did not take, was not always caused by European canker but, in this assessment, all lesions and dieback were included in the disease incidence score, as it was difficult to

determine those that were caused by *N. ditissima*. All T-budded trees tied with Flexiband A showed severe symptoms of European canker and had died. Chip-budded trees protected with Buddy tape had significantly fewer lesions and dieback (18%) than trees tied with plastic strips ($P < 0.001$). Calcium hydroxide treatment reduced this to 11%.

Fifteen months after budding, chip-budded trees tied with either plastic strips or Flexiband C displayed significantly more canker symptoms than those tied with Buddy tape ($P < 0.001$). Of the uninoculated trees, 9% displayed canker symptoms. Treatment with tebuconazole/carbendazim reduced canker symptoms significantly (2%, $P < 0.001$). Calcium hydroxide also reduced canker symptoms (5%), but pyraclostrobin/boscalid did not (Table 3).

DISCUSSION

Budding wounds were shown to be susceptible to European canker, despite being covered with different tapes. However, there were significant differences among taping treatments, with T-budded trees being least protected from infection with *N. ditissima* and chip-budded trees treated with Buddy tape most protected from infection. The high canker incidence in T-budded trees was probably because Flexiband type A did not protect the budding wounds, and Okulettes did not provide an effective seal against infection. If T-buds were taped with Buddy tape, they might be more protected from infection. Of all tapes used in this trial, Buddy tape provided the best protection against canker and also against other causes of bud death and lesions. Buddy tape prevented infection of chip-buds with *N. ditissima* inoculum applied immediately after budding, as well as 1 month after budding when the tape had been exposed to the elements for a month.

Walter et al. (2016) showed that only 3 to 30 conidia were required to infect wounds of various sizes. In this study, approximately 12,000 conidia were sprayed towards each bud, which ensured that even if most of the inoculum missed the wound, the taped buds were still exposed to a very high

Table 3 Mean disease incidence 5, 9 and 15 months after budding ‘EMLA 9’ rootstocks with ‘Royal Gala’ and inoculation with conidia of *Neonectria ditissima*. Disease incidence data were analysed with binomial generalized linear models; back-transformed data are presented. Pairwise likelihood ratio tests ($\alpha=0.05$) were used to determine differences among treatments. Means with the same letter within each disease assessment period are not significantly different.

Treatment	Description	Disease incidence (%)		
		5 months	9 months ¹	15 months ²
7	Buddy tape, T/C	0 a	16 abc	2 (15) a (a)
6	Buddy tape, CH	0 a	11 a	5 (15) ab (a)
C negative	Buddy tape	0 a	14 ab	9 (17) b (a)
8	Buddy tape, P/B	0 a	21 bc	11 (22) b (ab)
1	Buddy tape	1 b	18 abc	11 (22) b (ab)
4	Flexiband C	1 b	26 cd	21 (32) c (bc)
2	Plastic strips	3 b	33 d	22 (34) c (c)
5	Okulette O30	40 c	-	-
3	Flexiband A	98 d	100 e	100 (100) d (d)
C positive	Buddy tape	100 e	100 e	100 (100) d (d)

¹ Disease incidence from lesions by *N. ditissima* as well as lesions and bud death from other causes.
² In brackets, the disease incidence that includes lesions caused by *N. ditissima* as well as lesions and bud death from other causes.
T/C tebuconazole/carbendazim; CH calcium hydroxide; P/B pyraclostrobin/boscalid.

inoculum pressure. At an inoculum pressure normally present in a commercial orchard, Buddy tape should be able to protect budding wounds from all external canker infections. Both uninoculated and inoculated chip-budded trees tied with Buddy tape displayed 9-11% canker incidence after 15 months. It is possible that these infections were already present in either the scion wood or the rootstock before budding, highlighting the necessity to disinfect propagation material before budding and grafting (Amponsah et al. 2016). The rootstocks in this trial had been in the ground for 5 months before budding, and were adjacent to a block of apple trees heavily infected with *N. ditissima*. It is, therefore, likely that symptomless infections were present in the rootstocks before budding. Alternately, natural inoculum could have caused these infections after budding. However, neither inoculation immediately after budding nor 1 month after budding caused canker in buds taped with Buddy tape. It is also possible that natural infection occurred after the tape was removed, when the wound appeared healed. Further research is needed to determine

whether apparently healed budding wounds can become infected after the tape is removed. Spray painting of the budding wounds with tebuconazole/carbendazim significantly reduced canker incidence to 2%. Calcium hydroxide also reduced canker symptoms compared with the uninoculated control, but pyraclostrobin/boscalid did not. Both calcium hydroxide and carbendazim have been shown to be effective in reducing European canker in Dutch orchards with both high and normal inoculum pressure (Heijne et al. 2005), and also in inoculated leaf scars in New Zealand orchards (R.W.A. Scheper, Plant & Food Research, unpublished data). In addition, carbendazim reduced canker incidence in inoculated pruning cuts when added to pruning paint (Scheper et al. 2008). Nevertheless, it was surprising that sprays applied to taped wounds, followed by inoculation, resulted in a reduced canker incidence compared with that in the uninoculated control. This implies that the fungicides may permeate the Buddy tape and protect the wound from infections that were already sealed in.

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