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Client Report No. 12217

Optimising spray application efficiency for Dothistroma control – persistence of different formulations

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EXECUTIVE SUMMARY

The formulations and application volumes currently in use for the operational control of Dothistroma are based on research undertaken 20-25 years ago. The aim of this investigation is to determine if the methods currently in use can be improved.

Objective

Experiments were undertaken to measure the persistence of copper on pine needles when it was applied using three different spray oils (standard Dothi oil, another mineral oil and a vegetable oil) and with different oil concentrations and total spray volumes.

Key results

- Cuprous oxide at 0.86 kg/ha metallic equivalent copper produced uniform suspensions with all three of the test oils.
- The oils tested had no effect on copper persistence. Consequently, the choice of oil for operational spraying can be dictated by other factors such as cost, handling properties, and environmental considerations.
- There was an indication of a slight improvement in copper persistence from increased oil concentration.
- Reducing the application volume from 5 L/ha to 3 L/ha did not adversely affect the persistence of copper on pine needles.

Application of results

- Results support the concept that there is scope to improve Dothistroma control efficiency by reducing total spray volumes.

Further work

- Operational field trials should be undertaken to validate results from these experiments.
- If water is omitted from the spray mixture, oils could be reformulated to omit the expensive emulsifiers.
- Laboratory tests are required to determine the effect of new formulations on droplet sizes produced by Micronair atomisers.

Keywords: Dothistroma, copper fungicides, spray oils, aerial application

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Introduction

In 1962 *Dothistroma septosporum (syn. Dothistroma pini*) an ascomycete fungus was discovered in New Zealand, near Tokoroa and identified by Gilmour in 1964 (Gilmour 1967; Ridley and Dick, 2001). Asexual, non-motile spores (vegetative spores) called conidia are released from fruiting bodies and germinate on the needle surface. Hyphae then penetrate the needles of *Pinus radiata* through the stomata (Gadgil, 1967). Dothistromin, a mycotoxin is released by the fungus into the needle which stimulates a plant response causing red necrotic lesions, needle cast, and subsequently tree growth loss (Jones *et al*., 1995; Bassett *et al*., 1970).

Pinus radiata D. Don (radiata pine) plantations are treated from October to November, with an aerial application of copper fungicides, once crown infection level reaches 25%, (Bulman *et al*., 2004). From 1965 to 2006, an average of 70 000 ha of plantation forest were annually treated against Dothistroma in New Zealand. A second spray might be required during summer (January – February) in severely infected plantations.

Copper fungicides are non-systemic and form a protective barrier on the needle surface, thereby preventing the fungi from entering the plant host (Ridley and Dick, 2001). Fungicides also reduce inoculum by stopping the release of conidia from fruiting bodies (Bulman *et al*., 2004). Therefore, the effectiveness of the Dothistroma control sprays depends on a range of application characteristics, particularly:

- The amount of copper landing on needles within the forest canopy. For any copper spray application, it is desirable to maximise the amount of copper (the active ingredient in the spray) landing on the pine needle surfaces.
- Once spray droplets containing copper are deposited on pine needle surfaces, their distribution over the needle surface is important because the greater the degree of coverage with copper, the more effective the application.
- After copper is deposited on the needle surfaces it is eroded by rainfall, wind, and by abrasion (the needles rubbing against each other). However, the longer the copper persists on the needle surface the more effective the application.

The standard formulated spray mixture for Dothistroma needle blight control consists of three components; a copper fungicide (0.86 kg/ha metallic equivalent), a mineral spray oil (2 L/ha or 40% v/v) and enough water to apply the mix at 5 L/ha. Application costs contribute from 60% to 80% of the total cost of Dothistroma control. Therefore there is a cost incentive to improve the efficiency of the application by, for example, reducing total spray volumes. Similarly, there may be opportunities to reduce rates of both copper and mineral spray oil. In addition, substituting mineral oil for vegetable oil might have perceived environmental benefits.

Two copper products (cuprous oxide and copper oxychloride) and two spray oils (Caltex winter spray oil and BP Dothi oil) have been used in operational Dothistroma control. A recent study (Gous and Richardson, 2006) evaluated the application characteristic of a range of alternative products and overall spray mixes. The study showed that sprays containing cuprous oxide and either BP Dothi oil or Syntol mineral oil had the most desirable spraying characteristics. Also, increasing the oil percentage in the spray mixture resulted in larger spread on the leaf surface.

The purpose of the study was to quantify the persistence of copper on the foliage and to seek opportunities to increase application efficiency, using the most promising spray mixtures identified by Gous and Richardson (2006).

Methods

Seedlings

Three month old, open rooted radiata pine seedlings (GF26) were planted in 2 litre plastic pots using “Bloom” potting mix. Trees were then placed in a shade house for one month where after they were placed outside in full sunlight for an additional month prior to treatment.

Spray formulations

During recent years, the benchmark formulation for Dothistroma control consisted of 2 L/ha BP Dothi oil, mixed with 1.14 kg/ha cuprous oxide, made up to volume (5 L/ha) with water (L. Bulman, 2007 *Pers. Comm.*). In this benchmark formulation, the respective volume of each component is: 40% oil, 8% cuprous oxide and 52% water.

For the new persistence tests, three spray oils, BP Dothi (BP), Syntol Mineral (SM) and Syntol Vegetable (SV) oil, were each applied in mixes with three different oil concentrations (40%, 43% and 86%) equating to two application volumes and a total of 9 treatments (Table 1). However, the rate of copper was maintained at 1.1 kg/ha cuprous oxide (i.e. 0.86 kg/ha metallic copper equivalent). All three of the test oils produced uniform copper suspensions that were easy to handle.

Table 1: Spray oil, concentration and total volume per hectare applied.

	Spray Oil	Oil concentration	Volume applied
1	BP Dothi Oil (BP)	40% volume / volume	5 L/ha
2	BP Dothi Oil (BP)	43% volume / volume	3 L/ha
3	BP Dothi Oil (BP)	86% volume / volume	3 L/ha
4	Syntol Mineral Oil (SM)	40% volume / volume	5 L/ha
5	Syntol Mineral Oil (SM)	43% volume / volume	3 L/ha
6	Syntol Mineral Oil (SM)	86% volume / volume	3 L/ha
7	Syntol Vegetable Oil (SV)	40% volume / volume	5 L/ha
8	Syntol Vegetable Oil (SV)	43% volume / volume	3 L/ha
9	Syntol Vegetable Oil (SV)	86% volume / volume	3 L/ha

Spray applications

The copper/oil spray mixtures were applied to trees using an Ulva+ (Micron Sprayers Ltd.) controlled droplet applicator (Figure 2). For the 40% and 43% oil mixtures, the volume median diameter (VMD) of the applied droplet spectrum was approximately 65 μm.

However, due to a higher viscosity the VMD of the 86% oil mixture was approximately 80 μm. VMD's were calculated by measuring the crater diameter, created by the impaction of droplets onto a magnesium oxide covered microscope slide. Before and after each batch of 10 seedlings a mylar sheet of 160cm^2 was sprayed to determine the applied copper rate.

Figure 1: Ensis track sprayer

Figure 2: Ulva+ applicator

Treatments

The basic approach was to apply each of the above nine spray mixtures to 60 radiata pine seedlings, under standard conditions in the Ensis track spraying facility (Figure 1). Each of the nine combinations of oil type and concentration were applied to 60 individual trees.

Following spraying, 10 randomly selected trees from each treatment were grouped (i.e. 90 trees total) and were placed outside where they were exposed to natural weathering conditions. In total six blocks of 90 trees were established at the start of the experiment.

Heights of all seedlings were measured prior to treatment.

Copper persistence

At 0, 4, 8, 10, 13 and 17 weeks post treatment, individual blocks of 90 seedlings were randomly selected and harvested. At each harvest, individual seedling height was recorded and a side-on photographed taken. Image analysis software (V++, Digital Optics Ltd.), allowed estimation of the projected total seedling foliage area. Each seedling sample was placed in a zip lock plastic bag. Copper was digested for 48 hours with 100 ml of 20% HCl in methanol (K. Steele, *Pers. Comm.*). The copper content of the solution was analysed by atomic absorption spectrophotometer (Varian SpectrAA 220FS, Varian Ltd.). Seedling samples were washed with water and a 1 molar KOH solution to neutralise the acid, and were then dried at 70 deg C. Needle and total dry weights were measured for each sample.

Statistical analysis

An analysis of covariance (ANOCOVAR) was used to analyse residual copper levels on each plant (mg/l) over time. Covariance analysis accounted for the effect of different at the time of spraying, given that larger plant sizes intercept more spray. This analysis compared the effects of oil concentration (40%, 43%, 86%), oil type (BP, SM, SV), and time since application (weeks).

Results and Discussion

Plant height was the most effective variable influencing initial deposition. Projected leaf area divided by height was also highly significant. Including leaf area directly in the covariate analysis was less effective because of its strong correlation with height. Both covariates (height and area/height) were therefore included in the analysis. This analysis allowed differences between treatments to be compared over time for effectively identical sized plants. The ANOCOVAR table showing the importance of the experimental factors and covariates is given in Table 2.

Table 2: Importance of experimental factors and covariates.

Source of variation	Degrees of Freedom	F-ratio	P-value
Height	1	183.3	<0.0001
Area/Height	1	103.3	<0.0001
Time	5	466.6	<0.0001
Oil	2	36.5	<0.0001
Concentration	2	163.2	<0.0001
Oil x Conc	4	3.85	<0.0043
Time x Oil	10	6.08	<0.0001
Time x Conc	10	5.67	<0.0001
Time x Oil x Conc	20	2.84	<0.0001
Residual	484		

As expected, the most significant factor is time, with a highly significant reduction in copper over time. Both oil concentration and oil type had highly significant effects also, although these were in part a reflection of differences in initial dose of copper at the beginning of the

trial (time zero). There were significant interactions with time for both oil type and concentration indicating both factors affected the rate of decline in copper.
Figures 3 to 6 illustrate the trends for the different key variables. All charts show means adjusted to a common plant size by the ANOCOVAR. The LSD bars in these charts indicate the least significant difference between means that is statistically significant (α=0.05). There were slightly different trends over time for the oils (Figure 3) and concentrations (Figure 4). However, there were also initial differences between treatments (especially for the 40%) so these results are hard to interpret.

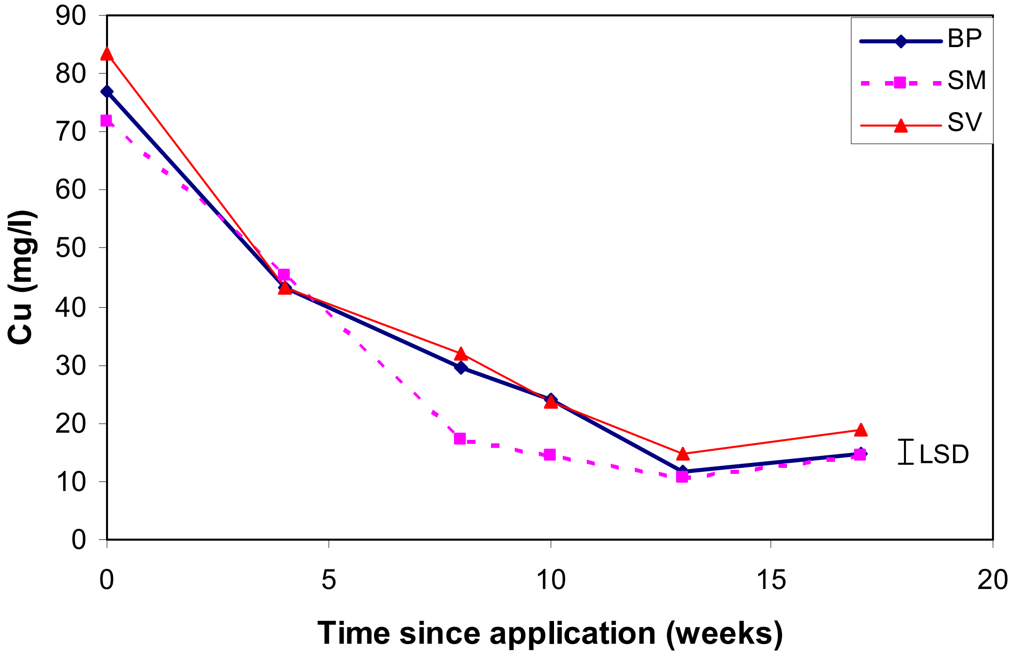

Figure 3: Persistence of copper for three different oil types.

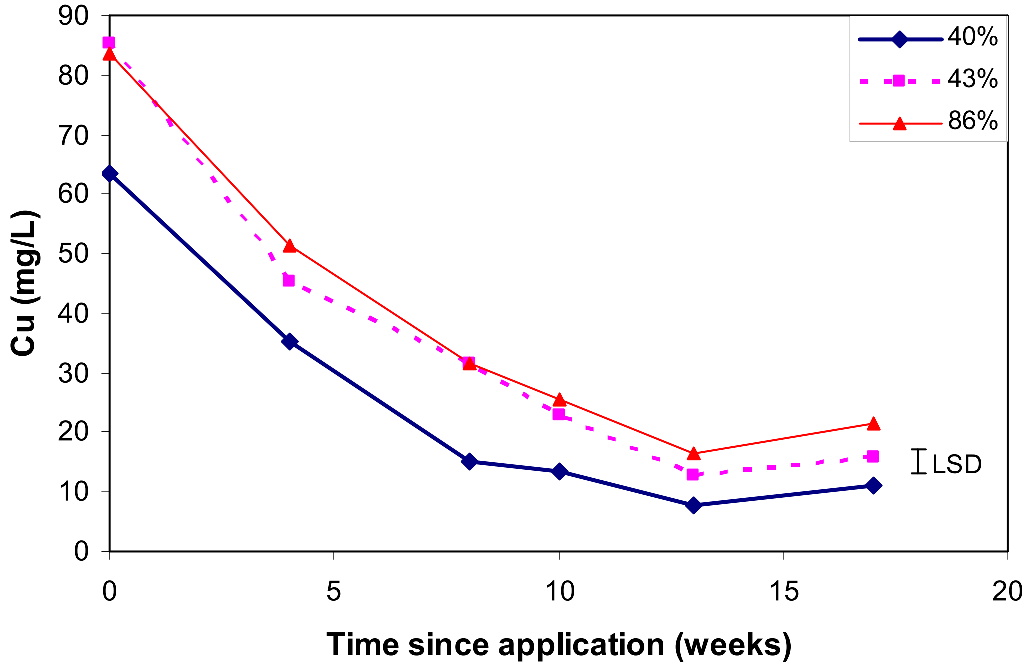

Figure 4: Persistence of copper for the three oil concentrations.

Figures 5 and 6 show the same trends after eliminating the initial differences in copper deposition by normalising deposition at time zero (i.e. the figures show residual copper levels as a percentage of initial concentration).

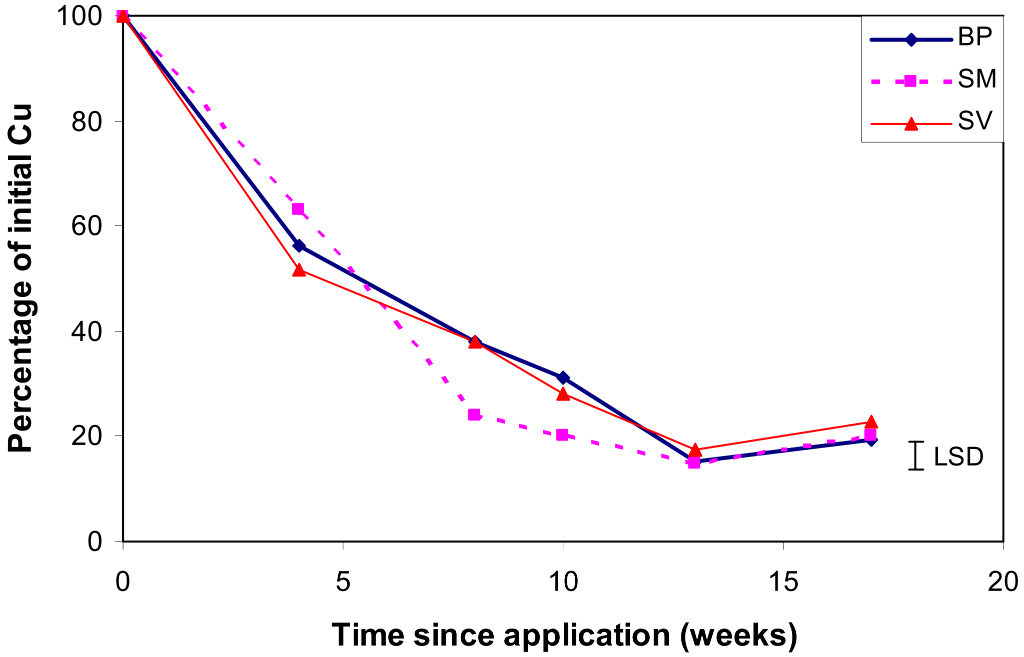

Figure 5: Persistence of copper for three different oil types expressed as a percentage of initial deposition.

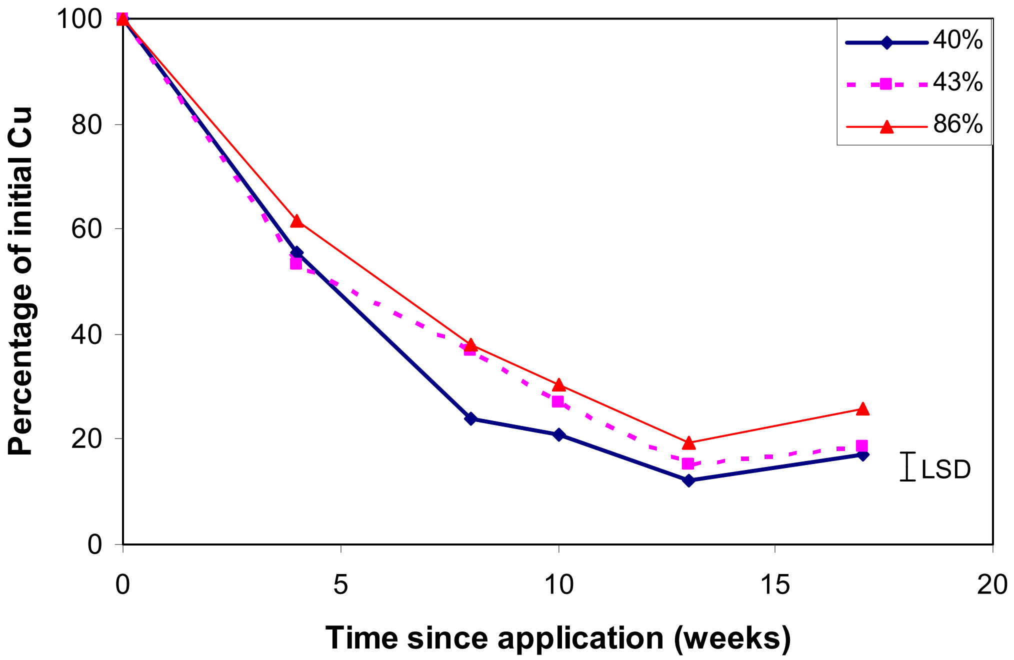

Figure 6: Persistence of copper for three oil concentrations expressed as a percentage of initial deposition.

The trend over time for SM oil was slightly different to that for BP and SV, although by the end of the trial, all oils show similar levels. From these results it would be hard to confidently state that any of the oils outperformed the others. Therefore, as copper persistence with all oils was similar, the choice of oil for operational spraying should be dictated by other factors such as cost, handling properties, and environmental considerations.

Results for the analysis of oil concentration indicate two important findings:

1. Copper persistence can be maximised by increasing the concentration of oil in the spray mix.
2. Copper persistence with high oil concentrations is improved by reducing total spray volumes from 5 L/ha to 3 L/ha.

With a larger droplet size produced for the high oil concentration (80 μm VMD versus 65 μm VMD), it is possible that the observed results reflect a droplet size effect rather than an oil type effect. However, the fact is that persistence was not adversely affected by reduced spray volumes.

The implications of these findings are that there is potential for reducing total spray volumes for Dothistroma control because as concentration increases, overall copper persistence increased. This is an important result because it suggests that reducing total spray volumes from 5 L/ha to 3 L/ha is worth evaluating in field studies. Such a reduction in total spray volumes would produce significant cost savings.

Conclusions

- Cuprous oxide at 0.86 kg/ha metallic equivalent copper produced uniform suspensions with all three of the test oils.
- The oils tested had no effect on copper persistence. Consequently, the choice of oil for operational spraying can be dictated by other factors such as cost, handling properties, and environmental considerations.
- There was an indication of a slight improvement in copper persistence from increased oil concentration. Although, the cause of this improvement may have been due to a droplet size effect, field studies using high oil concentrations are warranted.
- Reducing the application volume from 5 L/ha to 3 L/ha did not adversely affect the persistence of copper on pine needles.

Future research

- **Operational efficacy trials:** Results from this project suggest that there is scope for reducing total spray volumes by reducing water content without having a negative effect on copper persistence and possibly having a positive effect. It is recommended that larger operational trials are undertaken to test this finding in the field. Choice of oils to compare to the standard should be based on costs and environmental considerations. If the vegetable oil (SV) is cost-competitive, it is probably worth testing in a field trial.

- **Formulation issues:**
    - If water is to be potentially omitted from the spray mixture, then the possibility of producing a new oil formulation that does not contain expensive emulsifiers should be evaluated.
    - New spray mixtures need to be tested for signs of phyto-toxicity on radiata pine.
    - Formulation technology has made considerable advances over the last 20 years. Slow release formulations are common features of many applications such as slow release boron for timber protection. It is possible that smarter, slow release copper formulations could have some advantage for Dothistroma control. This is a potential area for future research.

- **Viscosity and droplet size:** Work should be undertaken to clarify:
    - The effect on droplet size of the new spray mixes chosen for field trials. Micronair settings may need to be adjusted to maintain a target droplet size with the more viscous, concentrated copper spray mixes.
    - Whether the improved persistence of concentrated copper spray mixes was a droplet size effect or a copper concentration effect.

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