

# Dose-response relationship between mancozeb fungicide application in the field and sugarcane growth response

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## Abstract

Based on research in glasshouse experiments, dithiocarbamate fungicides promote growth responses when applied to soil obtained from cane fields affected by sugarcane yield decline. A consistent dose-response relationship has been linked to biological factors. Research was therefore conducted to determine if the same relationship could be obtained from field applications and in a crop grown for the normal cropping period of 12 months. Prolonged effects of the fungicide were also examined by investigating carry over effects in the first ratoon crop. A similar dose-response relationship between glasshouse and field was found with large responses to high concentrations of the fungicide mancozeb, and to fumigation with methyl bromide. Carry-over crop responses in the first ratoon crop were minimal, confirming a biological basis to the response.

## Introduction

Sugarcane yield decline has been defined as the 'loss in productive capacity of sugarcane soils under continuous monoculture' (Garside *et al.* 1997). Wherever sugarcane is grown for a prolonged period in monoculture, a growth limitation develops. This phenomenon is not new and has been recognized in the sugarcane industry for nearly 70 years (Bell 1938). Even in other industries, such a yield limitation is well researched and has been observed in such crops as apples, pears, peaches, grapevines, asparagus, potatoes and even in natural ecosystems (Magarey 1999). Sugarcane yield decline has been intensively researched in the Australian sugar industry for over 20 years. The initial focus was poor root growth in sugarcane growing in northern Queensland (Egan *et al.* 1984, Croft *et al.* 1984, Croft and Magarey 1984) but later involved yield limitations throughout the state (Magarey and Croft 1995, Magarey 1996, Magarey and Bull 1996, Garside *et al.* 2002).

In the late 1980s, application of the fungicide mancozeb to yield decline-affected soils in the glasshouse resulted in markedly improved root health and shoot growth (Magarey and Bull 1994a). Field experiments were planned to determine if similar responses could be obtained

under commercial cropping conditions. This paper reports on a field experiment in northern Queensland investigating the dose-response relationship between mancozeb and sugarcane growth and the comparison of field results with glasshouse growth responses using soils from the same field experiment.

## Materials and methods

### Location

A suitable experimental site was located in the El Arish district of northern Queensland (Lat 17.5°S, long 145.6°E) where sugarcane had been grown for over 40 years. Other yield decline experiments have been conducted on the same farm (Magarey *et al.* 1997a, Garside *et al.* 1997). The soil type in the trial area was a Thorpe series soil (Cannon *et al.* 1992). A randomized complete block experimental design was used with two replicates. Plot size was four rows (1.5 m row spacing) by 10 metres. The cultivar used was Q117.

### Application of treatments

The experimental site was prepared for planting according to normal district practices. Preparation of the field site consisted of removal of the previous sugarcane crop through offset discs and several passes with a rotary hoe. The trial area and individual plots were then marked. Mancozeb and methyl bromide treatments were applied just before planting.

**Fumigant.** Crop response to soil fumigation with methyl bromide has been used as a comparison in previous research since this treatment leads to excellent root health and greatly improved sugarcane growth at yield decline affected sites (Croft *et al.* 1984, Garside *et al.* 2002). Methyl bromide (Dow-fume – methyl bromide 98% and chloropicrin 2%) was released as a gas under black plastic sheeting buried around the edges of the treated plots. Application dose was 1000 kg ha<sup>-1</sup>. After 24 hours, the plastic sheeting was removed and the plots were allowed to air for a minimum of 48 hours before sugarcane was planted.

**Fungicide.** Mancozeb was applied at seven different doses (Table 1). Comparisons between mg kg<sup>-1</sup> and kg ha<sup>-1</sup> doses were based on the assumption that mancozeb

was mixed evenly into the top 20 cm of the soil profile by the rotary hoe after field application. The fungicide (80% wettable powder) was applied as a slurry to the plot surface. This was achieved by mixing the required amount of fungicide for each plot with around 50 litres of water in a 90-litre garbage bin. Constant mixing of the slurry kept the fungicide suspended and this was then evenly distributed over the plot surface using watering cans. When all mancozeb was distributed, the fungicide treated plots were again rotary-hoed to ensure fungicide incorporation to plough depth (around 15–20 cm).

### Planting

Plots were planted using a whole stick 'trash' planter. Fertilizer was also applied using standard recommended district doses of nitrogen, phosphorus and potassium. The experiment was planted on the 17th August 1990.

### Harvest

The field experiment was harvested on the 6th and 8th August 1991. Counts were made of all the mature sugarcane stalks in each plot. Any small shoots that were not of 'millable' size (that is less than 0.5 m in height) were not included. On the 8th August, 60 mature stalks from each plot were cut by hand and weighed to determine the average weight of stalks in each treatment. Six-stalk samples were also collected to determine commercial cane sugar (CCS) content; CCS determinations were undertaken using standard industry practice.

Following harvest, a ratoon crop was grown according to normal industry practices. No further biocides were added in any of the plots during this period. The ratoon crop was harvested on 22nd June 1992 using a commercial harvester and associated weighing equipment routinely used in BSES research experiments. Results from the 1991 field harvest were compared with the glasshouse results.

**Table 1. Doses of mancozeb applied to field plots, and the comparative dose in the soil, at the farm of Costanzo, El Arish.**

Treatment	Total fungicide (kg ha <sup>-1</sup> )	Glasshouse experiment equivalent (mg kg <sup>-1</sup> soil)
1	0	0
2	150	50
3	300	100
4	600	200
5	1200	400
6	1800	600
7	2400	800

*Glasshouse experiment*

As the plant crop was growing (in December 1990), soil samples were collected from each plot for a glasshouse experiment so that responses obtained in the field could be compared with those obtained in glasshouse trials. Soil samples were collected to a depth of 20 cm and sub-samples from each replicate of the same treatment were bulked. After sieving (0.5 cm diameter aperture) to remove rocks and large pieces of organic matter, the soils were weighed into clay pots (15 cm top diameter) with 1.40 kg dry weight-equivalent of moist soil added per pot. Pre-germinated plantlets of the cultivar Q117 were placed one per pot and the pots transferred to an air-conditioned bench (Reghenzani 1984) in a glasshouse at Tully Sugar Experiment Station, Tully. Additional quantities of untreated soil were obtained and these were pasteurized (100°C for 90 minutes) to provide a 'no root disease' control treatment. In this soil, there was no opportunity for field re-infection by root pathogens (there may have been opportunity for reinvasion of soil in field fumigated plots). This treatment was used to gauge the extent of re-invasion of fumigated plots by pathogens. No additional fungicide application was made to the soils in pots other than that applied in the field four months earlier. Plants were irrigated using clay saucers that were regularly filled with water using an automatic watering system.

After six weeks, the plants were harvested by washing soil gently away from the roots and measurements were then made of the oven dry weight of both shoots and roots.

**Statistical analyses**

Data were analysed using Minitab Statistical Software, Release 13.1 (2000) Minitab Inc. The purpose of the analyses was to statistically assess the extent and nature of relationships between mancozeb dose and physical characteristics of sugarcane as described in the previous section. As some of the relationships are intrinsically non-linear both linear and 2nd order polynomial regressions were fitted, with the quadratic term being retained only where it was significant.

As the field data and ratoon data were replicated, Analysis of Variance was carried out as an initial step, and Bartlett's test applied to check for homogeneity of variances. In no case was the assumption of homogeneity of variance rejected. Glasshouse data were not replicated so this step was not necessary.

With the replicated data, the block effect was removed, via analysis of variance and the treatment effect partitioned into linear trend and deviations-from-linearity (Zar 1999, Clewer and Scarisbrick 2001). The ratio of deviation-from-linearity mean square and error mean square

(within-groups MS) was used to test the hypothesis that the regression was linear. In all cases the hypothesis of linearity was accepted. The significance of the regression was then tested via an F test with the variance ratio calculated as the regression mean square over the residual MS (pooled deviation-from-linearity MS and within-groups MS). These F values and their associated P-values are presented below. The coefficient of determination ( $R^2$ ) was calculated as the ratio of Regression sum of squares and Treatment sum of squares.

**Results***Shoot counts*

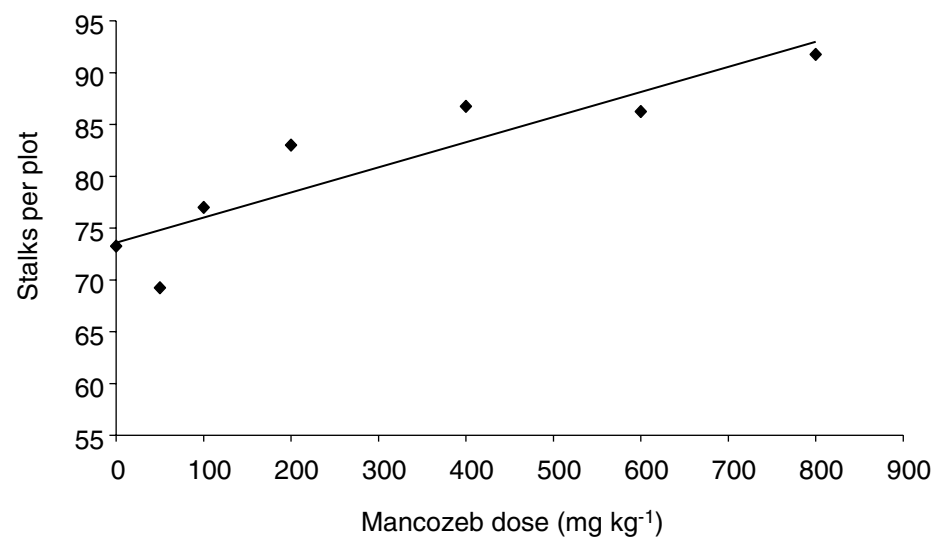
Based on shoot counts, undertaken twice during growth of the plant crop, a significant relationship was found between

mancozeb dose and stalk number (Figures 1 and 2). Responses to mancozeb increased up to 800 mg kg<sup>-1</sup>. Stalk counts in August were slightly higher (98 stalks per plot) in methyl bromide, compared to mancozeb treated plots.

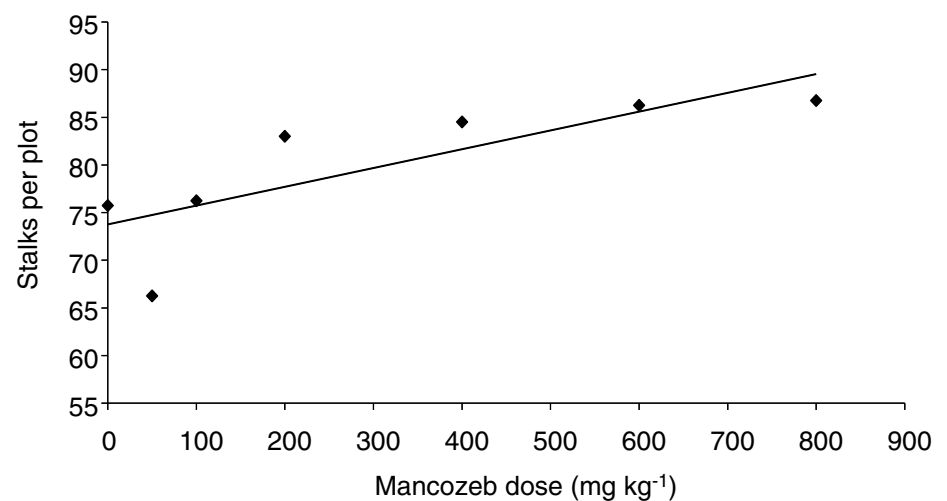
*Stalk weight*

The response of sugarcane stalk weight to increasing mancozeb dose was not strong; the resultant  $R^2$  was only 0.39 (Figure 3). Responses in stalk weight were higher in soils treated at high dose with mancozeb than in methyl bromide treated plots (average per 60 stalks was 77 kg for methyl bromide).

There was a strong response in total yield to increasing mancozeb dose, reflected in an  $R^2$  value of 0.81 (Figure 4).



**Figure 1.** Response of sugarcane stalk numbers in April 1991 (235 days after planting) to mancozeb dose at Costanzo, El Arish ( $y = 0.0242x + 73.59$ ,  $R^2 = 0.82$ ,  $F = 16.48$ ,  $P < 0.01$ ).



**Figure 2.** Response of sugarcane stalk numbers in August 1991 (354 days after planting) to mancozeb dose at Costanzo, El Arish ( $y = 0.0197x + 73.77$ ,  $R^2 = 0.64$ ,  $F = 8.67$ ,  $P < 0.05$ ).

Yield response to the maximum mancozeb dose was 37% greater than yield of the untreated control. By comparison, there was a 32% yield increase in response to soil fumigation (methyl bromide).

#### Glasshouse experiment

**Shoot weight.** There was a significant response in shoot weight to increasing mancozeb dose in the glasshouse experiment also. Maximum responses were obtained at doses up to 400 mg kg<sup>-1</sup> (Figure 5).

**Root growth.** There was no significant regression between root weight and mancozeb dose.

#### Correlation between glasshouse and field responses (plant cane)

There was a good correlation between the results obtained from the glasshouse (shoot weight) and field (total yield) experiments ( $r = 0.81$ ).

#### First ratoon growth responses

An analysis of results from the harvest of the first ratoon (re-growth from the original trial planting) crop showed no significant relationship between mancozeb dose and either stalk number or total yield parameters.

#### Relative responses

A comparison of the growth responses to mancozeb dose in each experiment is presented in Table 2.

#### Discussion

Based on these data, significant sugarcane growth responses may be gained by field application of mancozeb; this confirms other reported data (Magarey and Bull 1998, Garside *et al.* 2002, Pankhurst *et al.* 2002). There was a strong correlation between field and glasshouse yield data confirming that glasshouse responses are consistent with field observations. This provides additional credibility to the substantial amount of glasshouse data reporting increased sugarcane growth associated with the application of mancozeb to yield decline-affected soils (Magarey and Bull 1994a, Magarey and Bull 1994b; Magarey *et al.* 1995, Magarey *et al.* 1997ab, Magarey and Bull 1996). Root growth responses to mancozeb application were not as pronounced in this experiment as in other glasshouse work reported previously (Magarey and Bull 1994).

Other mancozeb dose-response experiments conducted in the glasshouse suggest a similar relationship between fungicide dose and sugarcane growth (Magarey *et al.* 1997b). In previous research, most of the associated growth response was achieved at doses of 100 mg kg<sup>-1</sup> or less. In the research reported here, large responses were obtained up to 200 mg kg<sup>-1</sup> or greater. In this experiment, higher doses of mancozeb

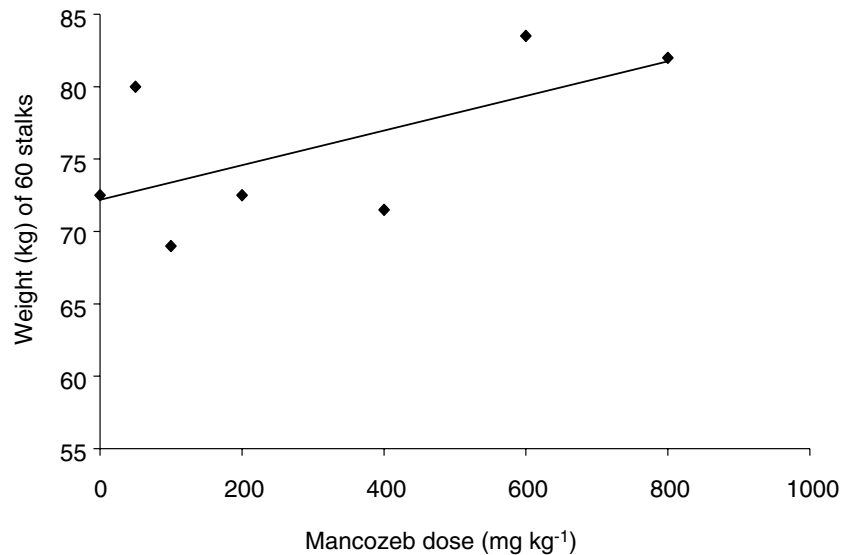


Figure 3. Relationship between mancozeb dose and stalk weight (356 days after planting) at Costanzo, El Arish ( $y = 0.012x + 72.18$ ,  $R^2 = 0.39$ ,  $F = 5.09$ ,  $P$  not significant).

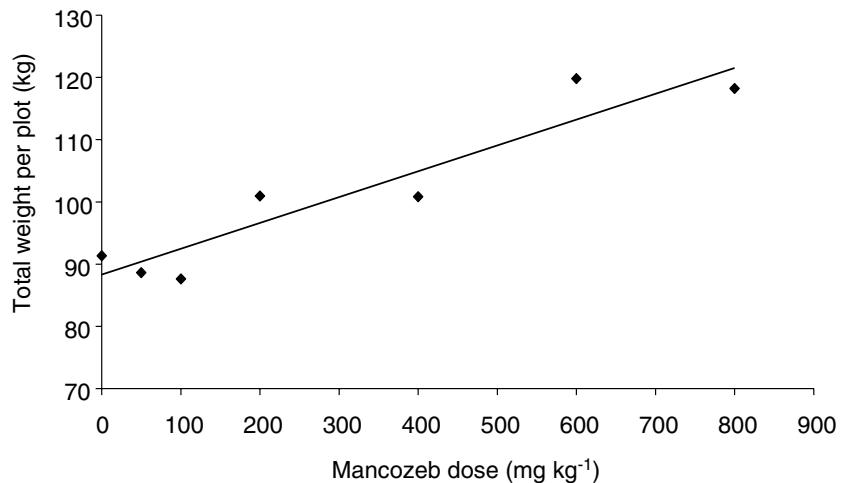


Figure 4. Response of sugarcane yield (weight per plot 356 days after planting) to mancozeb dose at Costanzo, El Arish ( $y = 0.0415x + 88.324$ ,  $R^2 = 0.88$ ,  $F = 32.81$ ,  $P < 0.01$ ).

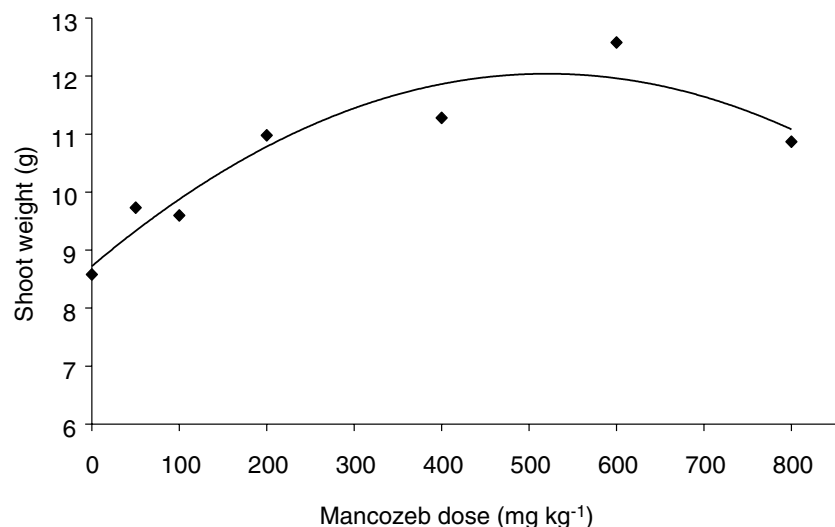


Figure 5. Response in sugarcane weight to mancozeb dose in a glasshouse experiment in soils collected from the field experiment ( $y = -1E-05x^2 + 0.0128x + 8.7232$ ,  $R^2 = 0.90$ ,  $F = 17.58$ ,  $P = 0.01$ ).

**Table 2. A comparison of the maximum per cent growth response to fungicide application in the plant and first ratoon field crops and in the glasshouse.**

Crop	Stalk number (% increase)	Stalk weight (% increase)	Total yield (% increase)
Field – Plant crop	22	13	37.5
Field – First ratoon	7	n/a	6
Glasshouse	n/a	n/a	38 (Sh*) 27 (Ro*)

\* Sh = shoot weight; Ro = root weight.

(up to 800 mg kg<sup>-1</sup>) were applied than in previous studies. There was a trend to reduced root growth at high mancozeb doses (though not significant) and this could be associated with phytotoxicity.

Reasons for the mancozeb-associated sugarcane growth responses have been investigated by Magarey *et al.* (1997b). Obvious improvements in root health have been associated with mancozeb application in experiments conducted since the late 1980s. This is also seen with methyl bromide fumigation. In a specific study of root pathogens, fungal species were isolated from root systems and soil particles in soils treated with a range of mancozeb doses (Magarey *et al.* 1997b). The authors found an association between dematiaceous fungi and mancozeb dose. This was consistent with other isolation studies and associated glasshouse pathogenicity tests where dematiaceous fungi reduced root and shoot growth (Magarey *et al.* 1995). In general isolation studies correlating fungal, bacterial and actinomycete populations in soils exposed to various treatments with shoot and root growth, total fungi were associated with reduced yield (Magarey *et al.* 1995). Recent research by Pankhurst *et al.* (2002) resulted in similar findings. A suite of fungal pathogens, and dematiaceous fungi in particular, may be significant biological factors associated with sugarcane yield decline. The influence of nutrition in mancozeb responses was investigated by Magarey *et al.* (1995), Garside *et al.* (2002) and Pankhurst *et al.* (2002). All three authors suggested soil biology was likely to be a major factor involved, although some nutritional influence was not ruled out (Pankhurst *et al.* 2002).

Similar improvements in yield parameters between methyl bromide fumigation and mancozeb treatments were found in this study. Both treatments have a similar effect in improving root health and reducing signs of root disease. In other studies not reported here, methyl bromide fumigation appears to lead to a slightly higher yield response. The failure of mancozeb to routinely affect some soil pathogens (Pachymetra root rot and nematodes; R.C. Magarey, unpublished data), may explain this yield difference.

The treatments applied here are not economical and were used for research

purposes. Sugarcane responses to lower doses were investigated by Magarey and Bull (1998); although reduced strategic application was possible, economic treatments were considered unlikely.

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