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Theme 4. Biodiversity, conservation and genetic improvement of range and forage species
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Routes of dicyandiamide uptake in pasture plants: a preliminary laboratory study

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Introduction

A consequence of intensification of New Zealand pastures is increased nitrogen (N) inputs to the soil in the form of urine, dung and mineral fertiliser. Dairy cow urine has a high N content that causes large N losses from the grazed system via nitrate (NO_3^-) leaching, nitrous oxide (N_2O) emissions and ammonia volatilization. Dicyandiamide (DCD) is a nitrification inhibitor that has been proven to reduce NO_3^- leaching and N_2O emissions, and increase pasture in New Zealand pastures (De Klein *et al*., 2014). DCD was commercially available for use in New Zealand pastures until 2013 when its use was suspended due to detection of traces of DCD in exported milk. Although DCD at high doses is relatively non-toxic there is no set maximum residue limit for its consumption. The contamination incident has highlighted the need to understand the pathway by which DCD entered the dairy cow.

Nutrients can be absorbed (or taken up) through the leaves via leaf cuticle and stomata of plants (Eichert and Fernández, 2012) and this phenomenon is used to fertilize golf courses and horticultural crops mainly using urea as a spray formulations. Because of the similarity between DCD and urea in terms of molecular weight and structure, we suspected that DCD could similarly be taken up in pasture plants. Few studies have shown the root uptake of DCD but none using pasture plant species.

Our objective was therefore to quantify foliar and root uptake of DCD in pasture plants following its application under glasshouse conditions. We hypothesized that DCD can be taken up by both foliar and root uptake pathways.

Materials and Methods

Experiment 1 - Foliar uptake of DCD: This experiment was conducted at constant temperature (15^{o}C), stable humidity (91% relative humidity) and a regular cycle of 16 h light/8 h of dark. Intact soil samples (10 cm diameter, 10 cm height) were collected using stainless steel (SS) cores from a permanent ryegrass-clover pasture managed for sheep grazing at Massey University Research Dairy Farm 1, Palmerston North, New Zealand. The sampled soil was an alluvial, well-drained, Manawatu sandy loam soil. The SS cores were placed on a 12 cm diameter glass saucer where 25 mL of deionised (DI) water was applied at two day intervals. Prior to spraying DCD, the shoots from all cores were cut to 5 cm height to simulate grazing. On the day of treatment application, DCD was sprayed on to the foliage of each core (n = 6 and a control that was sprayed with DI water) at 0.630 mL per core equivalent to 10 kg DCD ha^{-1} in 800 L water. Shoots were harvested at 7 h after the spray and on days 1, 2, 5, 8, 15, and 21 after DCD application. Immediately following each sampling, the shoot biomass was weighed and then extracted twice with water, filtered, and analysed to determine the surface residues of DCD using method of Kim *et al*. (2012). The same shoots were then pulverised using a mortar and pestle, extracted, filtered, and analyzed as described below.

Experiment 2 - Root uptake of DCD: This experiment was conducted under the same conditions as Experiment 1. Intact soil samples of two soils differing in texture and drainage were collected as above. One of the soils in this current study was the Manawatu soil described above, while the other soil was a poorly drained Tokomaru soil.

On the day of treatment application, the lower half of each collected soil core was removed from the core liners, sieved to 4 mm, treated with DCD (0.63 mL DCD mixed thoroughly with the soil) and repacked into the core. The treated cores were managed identically to that of Experiment 1. Shoots were harvested at 9, 15, 22, 30, and 37 days after treatment application and DCD concentrations were determined as above. At day 97 all the cores were destroyed and the roots, soil, and shoots were analyzed for DCD as above.

Quantification of the absorbed residues of DCD: The modified method of Schwarzer and Haselwandter (1996) was adopted where the pulverised extract was purified by eluting it through a Waters Sep-Pak® cartridge and analyzed on a

Shimadzu HPLC (Shimadzu Co., Kyoto, Japan) fitted with a Bio-Rad Aminex® organic acid column HPX-87H (300 × 7.80 mm ID) and the DCD peak detected using an UV detector at 220 nm.

Results and Discussion

Experiment 1 - Foliar uptake of DCD: A total of 56.9 ± 9.1% of the applied DCD was intercepted on the leaves (Fig. 1). The surface residues of DCD decreased ($P < 0.005$) over time and 36.5 ± 9.5% of the applied DCD could be recovered at the end of the experimental period. This is in accordance with the study of Kim *et al*. (2012) who reported that DCD persisted on the plant canopy for < 6 to 16 days that was mainly dependent on rainfall and pasture height. Pasture biomass in the cores remained constant during the experimental period.

The total amount of DCD in the plant leaves resulting from foliar uptake ranged from 13 to 90 mg DCD m^{-2} (mean, 36 ± 18 mg m^{-2}, $n = 42$) and did not change ($P = 0.295$) over the 21 day experimental period. These values translate to 2.7 to 5.2% of the DCD applied (Fig. 1). This current study demonstrates for the first time that a significant proportion of DCD can be taken up via foliage by ryegrass-clover plants, supporting our hypothesis. Vilsmeier (1991) suggested that plant cells are unable to metabolise the DCD once absorbed. This might be the reason for why the foliar-absorbed DCD did not degrade over time in the current study.

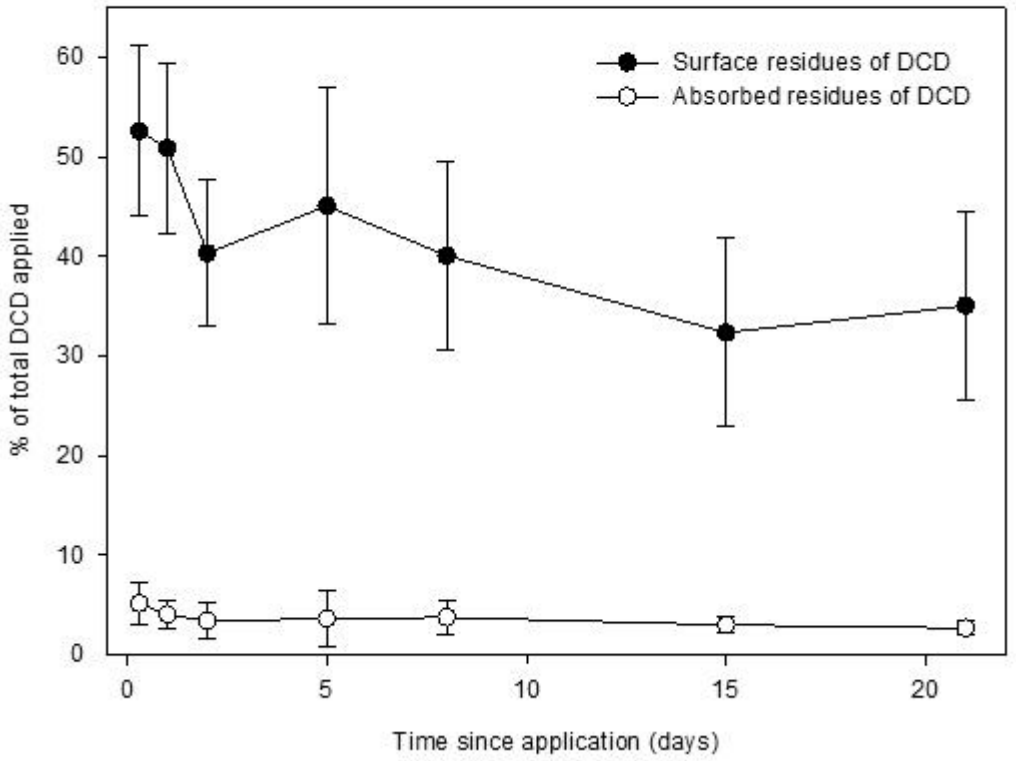

Fig. 1: Proportions of DCD on the leaf surface and within the leaf tissues via foliar uptake over time.

Experiment 2 - Root uptake of DCD: DCD was taken up by the roots and translocated to the shoots and these amounts increased significantly ($P < 0.001$) over time with maximum amounts of 63 ± 29 mg m^{-2} (equivalent to 6.3% of the total DCD applied) on day 37 in the Tokomaru soil. The uptake rates did not differ between the two soil types ($P > 0.05$) and uptake rates ranged between 2.6 and 6.3% of the applied DCD on day 37 (Fig. 2). Vilsmeier (1991) suggested that DCD can be absorbed by the plant roots via mass-flow and translocated aboveground where it can accumulate and crystallise at the hydathodes due to plant cells being unable to metabolise DCD. The author suggested that the relatively higher uptake rate was probably due higher transpiration rates and higher DCD application rates.

Destructive analysis of randomly selected cores after 97 days of DCD application showed no residues in the roots and soil. However, 0.49-2.03% and 1.26-3.72% of the applied DCD was detected in the shoots of the Manawatu and Tokomaru soils, respectively. Our study also shows that the washed off DCD from the pasture leaves on to soil surface are subject to root uptake.

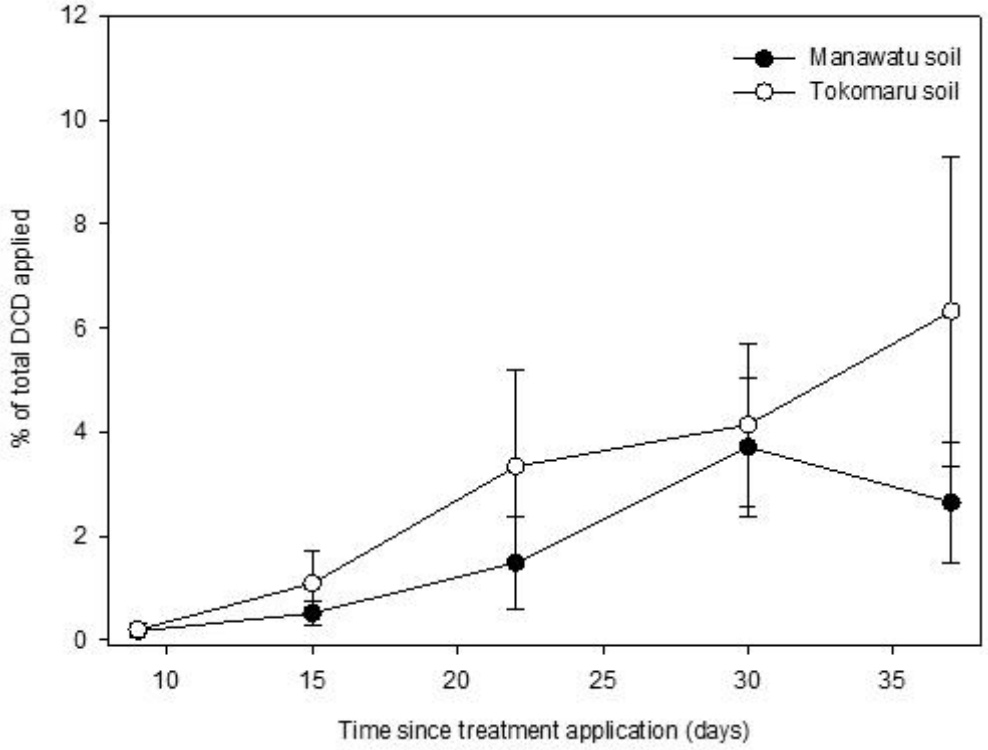

Fig. 2: Fraction of applied DCD found in the shoots of clover-ryegrass plants *via* root uptake in two different soil types over time.

Conclusion

Our glasshouse experiments showed that a significant proportion of DCD can be taken up by ryegrass-clover pastures via both foliar uptake (2.7-5.2% of the applied within 21 days of application) and root uptake (2.6-6.3% of the applied within 37 days of application) pathways, despite little pasture growth. We observed that once the DCD is taken up by either of the foliar or root uptake pathways, it is not prone to degradation probably due to plants' inability to metabolise the absorbed DCD.

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