Mapleton Agri Biotec Pty Ltd

CALIFORNIA 2018: Reduction of nitrate leaching rates via use of TwinN

An independent consultant company conducted a trial to test the capacity of TwinN to improve nitrogen fertiliser capture by roots and to reduce leaching of environmentally damaging nitrates.

SUMMARY OF TRIAL AND RESULTS

The trial used replicated pot grown celery, lettuce and broccoli plants. Calcium ammonium nitrate (CAN) was applied twice to plants, either treated or untreated with TwinN. The leachate from pots was collected at two time periods and the nitrate levels were measured. After 50 days growth plants were harvested and fresh weights were measured and statistically analysed.

- The first set of measurements showed an 18% reduction in nitrates in leachates from TwinN treated plants compared to untreated plants
- The second set of measurements showed an 35% reduction in nitrates in leachates from TwinN treated plants compared to untreated plants
- Despite the short 50 day growth period the TwinN treated lettuce and broccoli plants were statistically heavier than the untreated plants, while the celery plants showed no statistically significant difference. The pooled weights for all three crop species were significantly heavier for TwinN treated plants.

INTRODUCTION

This study was performed by the independent consultant company, Holden Research and Consulting, in Camarillo, California. The trial was designed primarily to test the capacity of a microbial bio-fertiliser, TwinN, to improve the capture of applied nitrogen fertiliser by three species of vegetable crops and therefore to reduce the amount of nitrates that are leached out of the root zone. In field situations these would enter aquifers and waterways. In addition, the effects of TwinN on vegetative growth were measured. This summary of the study findings does not attempt to provide references for all statements as for an academic journal. Rather the summary confines itself to what are regarded as commonly known facts and scientific understandings.

Nitrates (NO_3^-) are released from nitrogen fertilisers and because they are negatively charged they are not efficiently bound by soil particles and are subject to leaching out of the root zone. From an agricultural perspective this reduces nitrogen use efficiency and therefore yield and profitability. From an environmental perspective the increase of nitrate levels in rivers, lakes and estuaries has a range of negative effects including promoting algal blooms and eutrophication problems (see https://water.usgs.gov/edu/nitrogen.html for a summary of this problem). Excess nitrate levels in drinking water results in a number of well documented negative health effects in humans and livestock, with particular damage to young humans and livestock.

TwinN and a related product, NitroGuard, have been shown to promote the growth of root systems with improved secondary root structure and studies of the effects of microbe species similar to those in TwinN have been shown to increase root hair density. Molecular and plant physiology studies have shown that many of this class of microbes contain the gene for auxins such as indole acetic acid (a major plant auxin) and the microbes produce auxins that stimulate the plants in whose root zones they are found, to produce root systems with improved nitrogen capture capacity. Thus, the relationship between these microorganisms and the plants they colonise is symbiotic.

In general, the capacity to demonstrate improved nitrogen capture and increased yield from TwinN is best observed in soil-based trials, as opposed to pot trials, because the capacity of the TwinN microbes to drive improved root development is constrained in a pot. However, the use of pots for this trial allowed efficient capture of leachate and accurate measurement of total nitrates in leachates.

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TRIAL PROCEDURES

Eight replicate plants each of lettuce (*Lactuca sativa var. romana*) and broccoli (*Brassica oleracea italica*) and celery (*Apium graveolens*) were transplanted into pots and grown for 50 days as described in the timeline.

Timeline

Day 0 Plants transplanted into pots (eight replicates per species per treatment)

Day 14 T1 (Control) received no TwinN. T2 received TwinN at standard field rate as a root drench

Day 28 Apply CAN17 to all pots at 21.5 lb N/ac (10 gall/ac @ 2.15 lbN/gall)

Day 32, 33, 34 Collect leachate on three consecutive days after standardised watering

Day 42 Re-apply CAN17 to all plants. Same dose.

Day 46, 47, 48 Collect leachate on three consecutive days after standardised watering

Day 50 Measure above ground plant mass from eight replicates per species per treatment

Leachates were pooled from T1 and T2 and analysed for nitrate levels by A & L Western Agricultural Laboratories.

RESULTS

Table 1 Nitrate levels in irrigation water and leachates from TwinN treated and untreated plant pots

Sample	Nitrate levels (ppm)
Background nitrate level in irrigation water	< 2
Day 32, 33, 34 Control plant pots	79
Day 32, 33, 34 TwinN treated plant pots	65 (-18%)
Day 46, 47, 48 Control plant pots	31
Day 46, 47, 48 TwinN treated plant pots	20 (-35%)

TwinN treated plants developed a more extensive root system in each of the tree crop species by two weeks after TwinN inoculation (see images on page 4) and captured a larger proportion of applied CAN, resulting in a 18% reduction in NO_3 levels in leachate over Days 32, 33, 34. On Day 42 a second CAN application was made and by Days 46, 47, 48 the effects of the original TwinN application on root structure were more fully developed and a 35% reduction in NO_3 levels compared to control pots was measured in leachates. These are very substantial reductions.

It should be noted that two factors suggest that under normal field production conditions this benefit can be expected to be at least reproducible and probably enhanced compared to pot trials. Firstly, as mentioned, the effects of TwinN on nitrogen capture (and yield) is generally expected to be more evident when the soil mass able to be occupied by roots is larger than that found in a pot, where root development is constrained. Secondly, in normal use of TwinN in field production systems some reduction in nitrogen fertiliser rates are recommended. This can reasonably be expected to result in further reductions in nitrate leachates into water ways and aquifers with use of TwinN in commercial cropping.

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Table 2 Above ground average fresh weights for celery, lettuce and broccoli (each 8 reps, TwinN treated and untreated)

Crop	Standard CAN, No TwinN	Standard CAN plus TwinN
Celery	74.4 a	63.8 a
Lettuce	213.1 a	261.3 b
Broccoli	69.4 a	86.9 b
Mean of three crops	119.0 a	137.3 b

Despite the short (50 days) growth period for the trial and the growth constraints associated with use of pots for the trial the TwinN treated lettuce and broccoli showed statistically significant vegetative yield increases while celery showed no statistically significant yield difference. Mean above ground fresh weight for all three species averaged was 15% higher in TwinN treated plants.

CONCLUSIONS

1. TwinN use in crops can be used to reduce nitrate pollution of waterways and aquifers via improved capture of applied nitrogen fertiliser. This benefit will be enhanced when reductions in nitrogen fertiliser rates are used as recommended with TwinN. For situations where nitrate pollution is an issue, use of TwinN allows a partial solution with no yield penalty.

2. Use of TwinN increased vegetative yields in two of three crop species tested. Yield correlates with profit.

Product summary

TwinN is a microbial bio-fertiliser produced by Mapleton Agri Biotec Pty Ltd (MAB) in Australia. It contains microbe species from the Diazotroph group. The species have been selected for capacity to fix N_2 from the atmosphere into NH_3 which is used by plants. In addition, the microbe species produce plant growth regulators, including auxins, which drive development of fine secondary root structure, allowing improved capture of applied nitrogen fertiliser.

TwinN is supplied as a freeze-dried product which allows reliable supply of the necessary very high microbe numbers needed to be effective in field situations. Every batch of TwinN is tested by an Australian Government laboratory to guarantee the correct species and microbe counts are present. This is part of MAB's quality control program.

TwinN and other MAB products have been trialled by many organisations (including USDA) in many countries and trial summaries can be downloaded from www.mabiotec.com.

For information on TwinN in USA contact:

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Images of root growth for celery, broccoli and lettuce at 50 days post-transplant. TwinN treated plants are on the right in each Image. Note extensive fine root Development in TwinN plants.

Celery

Broccoli

Lettuce