TWINN IN TREE CROPS

INTRODUCTION

TwinN is used in many tree crops in Australia and internationally. TwinN is a freeze dried microbial bio-fertiliser and it contains species of beneficial microbes selected to provide nitrogen to tree crops. As well as supplying nitrogen TwinN provides several other important benefits that help producers farm more profitably and sustainably. The following sections provide a summary of TwinN's benefits in tree crops and more information is available at **www.mabiotec.com**. Here are three mechanisms of action for TwinN in tree crops:

1. Nitrogen Fixation

TwinN microbes fix N_2 from the atmosphere into plant available nitrogen that is provided in a steady supply through the growing season. Conventional growers reduce their nitrogen fertiliser rates and use TwinN to make up the difference, while organic growers use TwinN in combination with their normal sources of organic nitrogen. The following trials show:

- TwinN in a wheat trial (Figure 1) that shows accurately how TwinN was able to replace 50% of the normal nitrogen rate with no loss of yield.
- TwinN in a long term organic citrus crop (Figure 2) showing TwinN supplying adequate to luxury levels of nitrogen to the citrus trees.

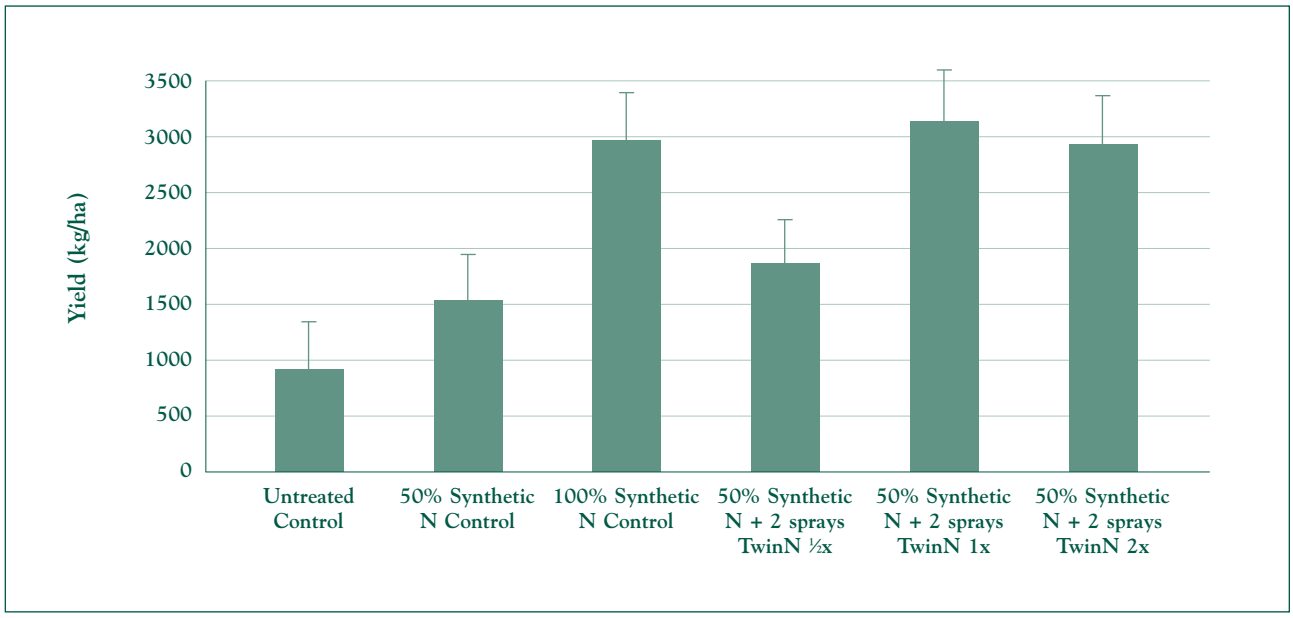

Figure 1: Registration trial performed by an independent agronomist in South Africa

100% synthetic control has 112 kgN. 50% synthetic control plus 2 sprays of TwinN 1x is the recommended TwinN treatment. It replaced 56 kgN with no yield loss.

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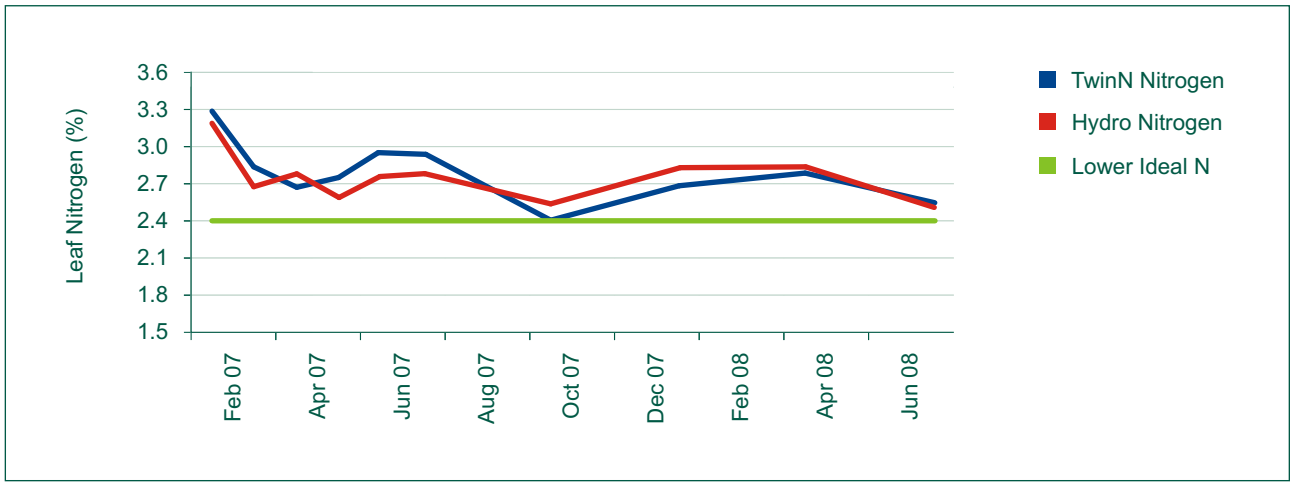


Figure 2: Citrus Nitrogen Farm Trial of TwinN vs Open Hydroponics

A comparison of TwinN as a nitrogen supply in an organic citrus system versus a standard hydroponics system in Forbes, NSW. Both systems maintained good leaf nitrogen levels over 16 months.

TwinN in Blueberries, Chile

On the left, no TwinN and nitrogen deficiency is evident.

On the right, 21 days after TwinN application and healthy leaf colour is regained.

2. More Effective Root Systems

The microbes in TwinN produce Plant Growth Factors (including auxins) that drive the development of larger and more effective root systems. This can be seen in the examples below, with both larger root systems, and also with much greater secondary and finer roots. At a microscopic level the root hair density is also increased. The end result is that capture of any applied nitrogen is improved and uptake of other nutrients is also more efficient.

More vigorous root growth from TwinN is used in some crops to combat effects from root pathogens (see below for effect of TwinN on soil pathogen pressures). For example, in Ecuador, TwinN is used to help banana crops cope with root nematode damage. Below are examples of root growth effects from TwinN in tree crops:

Avocado: Blackbutt, Qld

On the left, no TwinN.

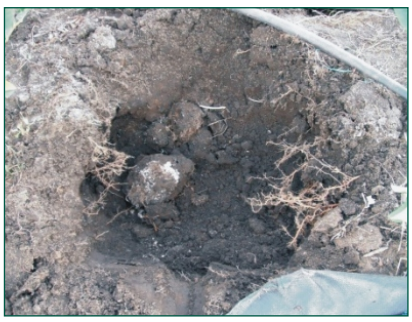
On the right, 2 months after a soil application of TwinN.

These photos are from an Avocado Australia trial of TwinN.

Cherry: Chile

On the left, no TwinN.

On the right, 120 days after TwinN application via fertigation. Roots showed much greater secondary root development.



Peach: Chile

On the left, no TwinN.

On the right, 100 days after TwinN application via fertigation, and root extension in between trees is much greater in the TwinN crop.

A healthy, vigorous root system is vital to support tree crop nutrition and water use efficiency. TwinN promotes root growth and increases the growth of fine roots that are responsible for nutrient uptake. Vigorous root growth can enable trees to recover from root damage from different sources.

3. Reduced Soil Pathogen Pressure

A number of studies have confirmed what TwinN users have reported to us on the effects of TwinN in reducing the effects of soil pathogens in different crops. Mapleton Agri Biotec does not claim the use of TwinN as a disease control agent. However we have sufficient data to indicate that it has a place as part of an integrated management program to assist with control of some soil pathogens. At this stage our official data comes from studies in *Phytophthora* and *Fusarium*.

Phytophthora

Phytophthora is a serious disease of many fruit tree crops and other horticultural crops. It can be partially controlled by chemical treatments, but a valuable role exists for any technology that can reduce disease pressure. The following results are from a nutrition trial in pineapple where TwinN was used to reduce nitrogen fertiliser application rates. Since the field suffered from *Phytophthora*, the agronomist sent soil samples from TwinN and non-TwinN plots to DEEDI for testing. Lupin baiting tests provide a crude indicator of the amount of *Phytophthora* present in the soil sample; the results show a decreased mortality from TwinN-plot samples. Chlamydospores were counted to give a more accurate estimate of *Phytophthora* pressure; there was a clear decrease in spores. TwinN effects on *Phytophthora* disease pressure will be one factor examined in current Avocado Australia trials.

Treatment	Lupin Baiting Tests: Mortality %	Chlamydospore counts
Standard Farm Practice	66.7	2.34
Twin N every 3 months + 50% N	46.7	Not tested
Twin every 6 weeks + 25% N	40.0	0.56

Fusarium

Fusarium is also a serious soil pathogen in some crops. The table below shows data collected by researchers with US Dept of Agriculture from soy bean. Similar results were collected in corn and the trial was repeated in a subsequent year with similar beneficial effects of TwinN on *Fusarium* numbers. The purple cell shows a normal level of *Fusarium* in roots in soy bean and the cell below shows the high infection levels (106.4) after application of Roundup herbicide. Roundup reduced numbers of root pseudomonads (beneficial microbes) drastically and since these are partially responsible for controlling pathogens the *Fusarium* numbers increased greatly. Application of TwinN was able to recover the population of beneficial root pseudomonads and reduce the number of *Fusarium* infections (see green cell) back to the level in a healthy soil.

Treatment	Fusarium root colonisation	Fluorescent pseudomonads	Mn-reducing bacteria	Mn-oxidising bacteria
No herbicide	67.5 a	116.9 a	73.25 a	104.75 a
+ Roundup	106.4 b	28.2 b	35.12 a	169.5 b
+ TwinN + Roundup	64.0 a	80.0 a	56.25 a	101.5 a

TwinN acts to decrease soil pathogen levels by enabling development of a healthy microflora (we call this 'bio-balancing'). Our results show this is a strong effect and valuable for tree crop growers.

RESULTS IN TREE CROPS

Tree crops grown with TwinN application include avocado, pecan, macadamia, apples, peach, cherry, nectarines, apricot and other stone fruit, blueberry, apple, citrus, mango and others. Growers use TwinN to enable decreased nitrogen fertiliser inputs, increased yield, and improved tree health and vigour. Longer term improvements in soil health and fertility are also important to TwinN users. The following results show some of the experiences of growers who use TwinN in tree crops.

TwinN used in organic avocado, Blackbutt, Qld

TwinN used in organic macadamia, NSW

Citrus and Mango

Typical application of TwinN in these crops is two applications with a reduction of between 25 - 50% in nitrogen fertiliser. The following data (overleaf) is leaf analyses from various South African growers using a combination of TwinN and a carbon product, CarboTech.

Citrus in RSA: N Leaf Analyses 2009 and 2010

Client	Ha 2009	Block Name:	2009	2010
Piet Engelbrecht Drip	49	24 Nawels	2.40	2.57
PLM	9	PLM 36 Midknigh Drup	1.92	2.50
Schoonbee	13	Schoonbee SL1 Eureka	1.82	1.97
Petrus Berg Groep 1	20	4 Jong Nova	2.34	2.55
		Mid 1 & 2	1.87	1.73
Bosveld Midknights	27	2C Mid	2.56	2.40
		14 Mid	2.35	2.40
		15 Mid	2.39	2.35
		16 Mid	2.62	2.35
		17 Mid	2.36	2.13
		Zero 3	2.32	2.36
Bruwer LRochelle Afourer	104	No. 1 Teerpad	3.07	2.41
		Tennisbane	2.01	2.16
		Groot Dam	2.49	2.45
		Agter Groot Dam	1.57	2.05
		Hemanus Huis	2.13	2.18
MEsterhuizen	16	Rivierplaas 14 Lina	2.28	2.10
		Rivierplaas 15 Autumn Gold	1.83	1.91
Total Average			2.20	2.22

Mangoes in RSA: N Leaf Analyses 2009 and 2010

Bavaria Mango Grovedale	6	Grovedale Groep 5 H3	0.87	0.97

Growers were aiming to either reduce leaf nitrogen levels to get back down to the optimal range (values in red) or to increase them to get up into the optimal range (values in blue).

Leaf analyses in the 2009 column were from their standard nutrient program, while those from 2010 were from the TwinN/CarboTech program and they included a minimum 20% reduction in nitrogen fertiliser and larger reductions in some blocks. Growers were very pleased with the results.

Leaf Analyses Norms (RSA)	N
Delta (small fruit)	2.1-2.3
Lemon (oil)	2.2-2.6
Midknight Val	2.3-2.6
Navel	2.6-2.8
Delta (large fruit)	2.3-2.6
Grapefruit	2.1-2.4
Midseasons	2.1-2.3
Young trees	2.3-2.6
Soft Citrus	2.2-2.5
Lemon	2.2-2.6
Mango	1.1-1.3

Tea

TwinN is used on large tea estates in Malawi and other African countries. Tea is a very hungry crop with heavy applications of nitrogen fertiliser often applied. In these heavy nitrogen situations nitrogen is reduced by ~25% with TwinN while other estates use lower nitrogen rates. In the example below, Nchima Estate, they have been using TwinN for several years with a reduction from 144 kgN/ha to 75 kgN/ha and have seen an average yield increase of ~9%. In addition they report improved resilience to short term water stress and improved tree health.

Yield data from eight tea blocks on Nchima Estate over four months in 2009

Division	Total block size (ha)	TwinN + 75 kg N/ha	144 kg N/ha	% Yield increase
Nchima	173.4	1928	1832	5.3
Chiwale	169.3	2413	2001	20.6
Mango	176.7	2500	2004	24.7
Bandanga	162.0	1899	1705	10.8
Pemba	161.1	2196	2055	6.9
Namitete	160.9	2010	1971	2.0
Nabomba	146.5	2101	1833	14.6
Mankhamba	133.4	1379	1691	-18.5
Total Area	1283	2052	1866	8.8

Yields are mean cumulative four month harvests. The -18.5% yield result for Mankhamba is aberrant and is believed to be due to non-application of TwinN.

Leaf analysis data from Nchima blocks in 2010

Trial plot	N %	P %	K %	Mg %	Ca %
Standard Fert: Central Divn	0.68	0.63	0.14	0.24	0.47
Twin N: Central Divn	0.89	1.16	0.89	0.26	0.43
Standard Fert: Bandanga	0.84	0.87	1.05	0.2	0.45
Twin N: Bandanga	0.91	1.04	0.77	0.16	0.38
Standard Fert: Nabomba	1.47	1.93	0.39	0.2	0.47
Twin N: Nabomba	1.03	0.41	0.56	0.22	0.54

These leaf analyses show that use of TwinN over several years has enabled a reduction from 144 kgN/ha to 75 kgN/ha with good leaf analysis results for key nutrients. This underpins the yield shown in the previous table.

RECOMMENDATIONS FOR USE OF TwinN IN TREE CROPS

1. Conventional Fertiliser Systems

TwinN application

- Use two applications of TwinN per year. Applications should be in autumn and spring.
- Rehydrate the TwinN microbes as per the instructions.
- Apply the microbes via fertigation if available. Run the system to pre-wet the soil then mix the TwinN solution into the mixing tank and apply with sufficient water to wash the microbes into the root zone. Do not co-apply with agrochemicals.
- **If fertigation is not available, apply TwinN to soil via this method:** Calculate the total volume of TwinN solution to be applied per ha by multiplying the number of trees per ha by an application of several litres per tree onto the root zone. Then, apply using a coarse nozzle or any system to apply the TwinN solution into the root zone under each tree. Make sure the TwinN solution goes into a moist or wet soil so the microbes can get into the root zone.

Nitrogen fertiliser reduction

- Cut nitrogen fertiliser application by up to 50% up to a maximum of 60 U of N less than standard. Monitor leaf analysis results and adjust as necessary.
- Reduce the nitrogen fertiliser applications proportionately through the season so that if there are key periods of demand for N the N applications reflect that need.
- Do not reduce other nutrients. If growers are using potassium nitrate or calcium nitrate then they should consult their agronomist to tailor a blend that supplies adequate K or Ca via other sources. This is not difficult and MAB can supply examples where this has been done (eg. see Tomato Trial report on **www.mabiotec.com**).

2. Organic Production Systems

- For organic systems use the same application suggestions to apply TwinN as described above, with an application in autumn and spring.
- Do not reduce applications of organic sources of nitrogen eg. manures, composts etc. TwinN microbes thrive in a well composted soil.
- If your crop is looking nitrogen deficient then co-apply a nitrogen source such as manure, plus TwinN.

3. Planting and Establishment

Mix a 1 ha dose of TwinN into sufficient water to enable application of 1L of TwinN solution per tree at planting. For example, at 250 trees per ha, mix a 1 ha dose of TwinN into 250L and add a litre into the planting hole at planting. This will speed growth of a strong root system and promote development of a vigorous, well balanced young tree.

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