The role of TwinN, a microbial bio-fertiliser, in avocado production.

La función del TwinN, un fertilizante biológico, en la producción de aguacate

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Abstract

As avocado producers around the world face rising nitrogen costs, an increased need for sustainable production and ongoing pressures from soil disease organisms, many growers are looking for new technologies to assist them. TwinN® is a freeze dried microbial inoculum that is used commercially by avocado growers and is also in trials with Avocado Australia, the national industry body. Trial results will be presented on the capacity of the product to enable significant reductions in nitrogen fertiliser applications in tree crops without loss of yield, or decreased leaf nitrogen levels. Commercial trials in citrus in South Africa and Australia showed that a reduction of 25% (~35kgN/ha) in annual nitrogen program levels was possible, in combination with TwinN, with leaf nitrogen levels remaining in the adequate range.

Results from the USDA show that application of Roundup herbicide to GM soya beans increased root infection from *Fusarium solani* by 58% with an associated 76% decrease in beneficial fluorescent pseudomonads. The same treatment plus TwinN resulted in a reduction of *Fusarium* infection level back to the levels prior to Roundup application and a restoration of fluorescent pseudomonads to 68% of the initial levels. Preliminary work with soil collected from underneath TwinN + urea treated pineapples has shown that infectivity by *Phytophthora cinnamomi* (*Pc*), as determined by the lupin baiting technique, decreased with reduced N fertiliser applied, irrespective of whether the source of N was urea or TwinN. TwinN application enhanced growth of avocado feeder roots in late winter/early spring at a time critical for overcoming effects of *Pc* infection. Increased root growth and vitality are important factors in determining tree crop nutritional status and yield.

Debido al aumento de los costos del nitrógeno para los productores de aguacate de todo el mundo, a la creciente necesidad de producción sostenible y a la presión continua de los organismos patógenos del suelo, muchos productores están buscando nuevas tecnologías que los asistan. TwinN es un inóculo microbiano liofilizado que utilizan comercialmente los productores de aguacate y también se están realizando pruebas en Avocado Australia, el organismo industrial de ese país. Se presentarán los resultados de los ensayos sobre la capacidad del producto para permitir reducciones significativas en las aplicaciones de fertilizantes nitrogenados en cultivos arbóreos sin pérdida de rendimiento ni disminución de los niveles de nitrógeno en las hojas. Pruebas comerciales de cítricos de Sudáfrica y Australia mostraron que una reducción del 25% (~ 35kgN/ha) en los niveles anuales de nitrógeno programa fue posible, en combinación con TwinN, con los niveles de nitrógeno de la hoja que queda en el rango adecuado.

Los resultados del USDA muestran que la aplicación del herbicida Roundup en soja GM aumentó la infección de las raíces por *Fusarium solani* en un 58%, con una disminución asociada del 76% en las pseudomonas fluorescens beneficiosas. El mismo tratamiento más TwinN resultó en una reducción del nivel de la infección con *Fusarium* a los niveles originales previo a la aplicación de Roundup y una recuperación de pseudomonas fluorescens al 68% de los niveles iniciales. El trabajo preliminar con suelo recolectado debajo de piñas tratadas con TwinN + urea ha mostrado que la infectividad por *Phytophthora cinnamomi* (*Pc*), de acuerdo a lo determinado por la técnica de cebo con lupino, disminuyó al reducir la aplicación de fertilizante nitrogenado, independientemente de la fuente de N, tanto si esta fue urea o TwinN. La aplicación de TwinN promovió el crecimiento de raíces absorbentes en aguacate a fines del invierno/comienzo de la primavera en un momento crítico para superar los efectos de la infección con *Pc*. El aumento del crecimiento de las raíces y la vitalidad son factores importantes para la determinación del estado nutricional del cultivo arbóreo y el rendimiento.

Key words Avocado, nitrogen, fertiliser, *Phytophthora*, bio-fertiliser, TwinN, roots

Introduction

Avocado production is dependent on supply of adequate nutrients, particularly nitrogen, to the tree in sufficient amounts and in the correct timings. Three main factors can affect how effectively this is achieved and this paper will examine the role of microbial bio-fertilisers in avocado production systems in relation to these factors.

1. Nitrogen supply is usually achieved via application of nitrogen fertiliser and this has been quite effective. However nitrogen fertilisers in high amounts have negative effects including, decreasing soil pH, increasing production costs, increasing carbon footprint and various environmental impacts. Use of an effective microbial bio-fertiliser has been shown to decrease the amounts of synthetic nitrogen fertiliser needed to maintain correct nitrogen levels in the tree. One mechanism by which this is achieved is by fixation of atmospheric nitrogen, N_2, into plant available NH_3 (Kennedy et al., 1997; reviewed

in Sakia and Jain, 2007) and the results presented here show that use of TwinN enabled maintenance of leaf nitrogen levels in tree crops with reduced nitrogen fertiliser applications.

2. In addition to supplying nitrogen via fixation of atmospheric nitrogen, use of microbial biofertilisers has been shown to increase nitrogen use efficiency by increasing capture of applied nitrogen which increases nitrogen use efficiency. This occurs due to production of plant growth factors (PGFs), particularly auxins, by the microbes in the root zone after application. These beneficial effects of TwinN microbes on root growth directly influence the proportion of applied nitrogen fertiliser that is captured by the crop. This means that products like TwinN are very effective in conventional production systems that use nitrogen fertilisers. Photographs presented in this paper show effects of TwinN on root growth in avocados.

3. Results presented in this paper show suppression of numbers of some soil pathogens after use of TwinN. Avocado growers in most countries face a challenge controlling *Phytophthora cinnamomi*, which severely affects root function and ability to take up water and nutrients. Root vigour and health is vital for consistent, high production in avocado and use of biofertilisers can be a useful strategy to achieve this goal.

Microbial bio-fertilisers are a useful tool for both conventional and organic avocado growers to supply nitrogen, increase nitrogen use efficiency and improve the soil and crop health. This paper will present data from both commercial tree crop growers and research institutions to demonstrate efficacy of TwinN, a microbial bio-fertiliser, in several different cropping systems.

Results

The results in Table 1 and Table 2 show the leaf analysis from seven commercial citrus and mango producers in South Africa and Australia. The leaf nitrogen values in 2009 resulted from standard fertigation applications of nitrogen while those from 2010 resulted from a 25% (~35kgN/ha) reduction in nitrogen fertiliser rates on the same blocks, plus a single application of TwinN applied via fertigation.

Table 1 Leaf analysis results from seven commercial citrus and mango producers in South Africa after a 25% reduction in nitrogen fertiliser plus a single application of TwinN.

Citrus in South Africa:			**Leaf nitrogen analyses:**	
Client	**Ha**	**Block Name**	**2009**	**2010 + TwinN**
Piet Engelbrecht Drip	49	24 Nawels	2.4	2.57
PLM	9	PLM 36 Midknigh Drup	1.92	2.5
Schoonbee	13	Schoonbee SL1 Eureka	1.82	1.97
Petrus Berg Groep 1	20	4Jong Nova	2.34	2.55
		Mid 1 & 2	1.87	1.73
Bosveld Midknights	27	2C Mid	2.56	2.4
		14 Mid	2.35	2.4
		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	2.1
		Rivierplaas 15 Autumn Gold	1.83	1.91
		Average leaf N	2.2	2.22
Mangoes in South Africa:				
Bavaria Mango Grovedale	6	Grovedale Groep 5 H3	0.87	0.97

The underlined values in 2009 in Tables 1 & 2 are blocks where a larger reduction than 25% was made in nitrogen to reduce excess leaf nitrogen levels.

Table 2 Leaf analysis results from three commercial citrus producers in Australia after a 25% reduction in nitrogen fertiliser plus a single application of TwinN.

Citrus in Australia				Leaf nitrogen analyses:	
Client	Ha 2009	Block Name:		2009	2010
Shane Kay	10.4	Kay Farm 1	1.4 A Late Lane	3.06	2.62
			1.4 B Late Lane	3.07	2.62
			1.6 A Murcott	3.05	2.5
			1.6 B Murcott	2.78	2.41
	8.6	Kay Farm 2	Late	3.31	ND
			Late	3.53	ND
			2.3 A Murcott	2.95	2.32
			2.3 B Murcott	2.78	2.4
	8.1	Kay Farm 3	Clementine	2.36	ND
			Clementine	2.42	ND
			3.1A Navel	ND	2.87
			3.1B Navel	ND	2.81
Twynam	8.34	Stage 1	Stage 1 South	2.97	2.87
	47.9	Stage 2	Stage 2	2.79	2.67
	53.2	Stage 3	Stage 3	2.9	2.61
John Davidson	55	Farm 484	Salistiana	2.6	2.9
			Hamlin	2.5	2.9
			Average leaf N	2.87	2.65

ND No Data

The results in Table 1 and Table 2 show that use of a bio-fertiliser was effective in allowing reductions of ~35kgN/ha in nitrogen fertiliser while maintaining leaf nitrogen levels in the optimum range in citrus and mango in commercial farms in South Africa and Australia.

Table 3 Leaf analysis results from tea treated with standard nitrogen rates or 50% nitrogen plus TwinN

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

Three blocks in Nchima Tea Estate, Malawi, received either 144 kgN/ha (Standard Fert.) or 75 kgN/ha plus TwinN. These treatments were continued for two years prior to the leaf analyses shown in Table 3. In combination with the results shown in Tables 1 & 2 these results show that use of TwinN can enable significant reductions in nitrogen fertiliser application with no loss of tree nitrogen status. This has positive implications for cost of production in avocado, as well as assisting in maintenance of tree and soil health.

The positive effects of PGPR microbes on root growth and improved capture nitrogen fertiliser from soil are reviewed in Adesemoye et al (2009). The images below show the effects of TwinN on avocado root growth in a trial performed by Avocado Australia.

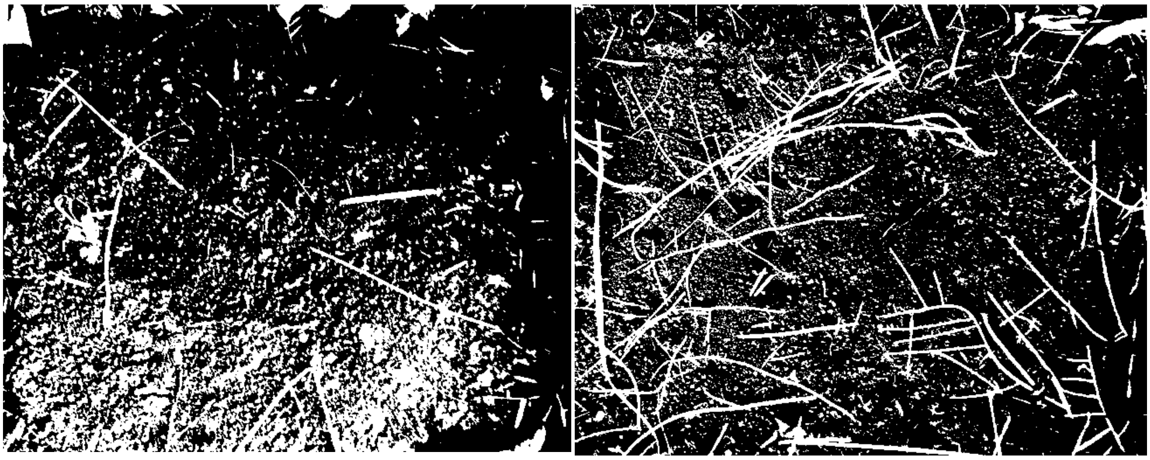

Figure 1 Photograph of root growth assessment of avocado root growth from an Avocado Australia trial. The image on the left shows root growth under a Perspex window from a non-TwinN treated tree while the image on the right shows root growth from a tree that received a soil application of TwinN two months previously.

A soil application of TwinN was made and Perspex plates were put in place to enable observations and root tracings after two months. Soil was replaced over the Perspex plates between observations. Tracings of roots were taken to enable quantification of root growth (in progress). Figure 2 shows a clear effect of stimulation of avocado root growth after a soil application of TwinN compared to non-treated trees.

TwinN treated trees | TwinN treated trees | Untreated trees

Figure 2 Photographs of root tracings from trees treated with TwinN (left and centre rows) and untreated trees (right row)

The nutritional status of tree crops can be adversely affected by a build up of soil pathogenic microbes and in avocado *Phytophthora cinnamomi* is a serious problem. A number of agrochemicals provide partial protection but additional management techniques are being researched by Avocado Australia and other organisations to develop an integrated management strategy for control of this disease. Kloepper (1993) reviews use of plant growth-promoting rhizobacteria (PGPR) as biological control agents. We show here two results of the effects of addition of RGPR bacteria on levels of *Fusarium solani* and *Phytophthora cinnamomi* in soil under crops treated with TwinN.

A trial was conducted in soybean at the USDA/University of Illinois (Kremer, unpublished data) to examine the effects of TwinN on levels of *Fusarium solani* root colonisation, numbers of beneficial root pseudomonads and the proportions of manganese oxidising and reducing bacteria. A high ratio of Mn reducing to oxidising bacteria favours availability of some micronutrients including Mn. The amount of *Fusarium* infection was quantified by counting numbers of infection sites per length of root and the numbers of bacterial in the three classes was determined by plating of root zone samples.

Table 4 The effects of application of TwinN and Roundup herbicide on *Fusarium* infection and three bacterial classes

Treatment	*Fusarium* root colonisation	Root fluorescent pseudomonads	Mn-reducing bacteria	Mn-oxidising bacteria
1. No herbicide	67.5 a	116.9 a	73.25 a	104.75 a
2. + Roundup	106.4 b	28.2 b	35.12 a	169.5 b
3. + TwinN + Roundup	64.0 a	80.0 a	56.25 a	101.5 a

Means followed by the same letter are not statistically different at p=0.05

Fusarium root colonisation was increased after application of Roundup herbicide and decreased back to the levels in T1 when TwinN was applied after the Roundup treatment. The increases in *Fusarium* colonisation corresponded to substantial decreases in root fluorescent pseudomonads in T2 and an increase in their numbers in T3 after TwinN application. Fluorescent pseudomonads have been used as a biocontrol method for some diseases and it is likely that the effect of TwinN on reducing *Fusarium* colonisation was enabled by the effect of TwinN on increasing the fluorescent pseudomonads. Further research to investigate this link would be useful.

In a separate trial conducted by an independent agronomist (Col Scott) in Queensland, Australia, TwinN was applied to pineapples in combination with reduced nitrogen fertiliser rates. Soil samples were taken from under trial plots and sent to a Queensland Government laboratory (DEEDI) for lupin mortality and *Phytophthora* chlamydospore counts. Lupin mortality provides a crude approximation of the amount of infective *Phytophthora* spores present in the soil, while chlamydospore counts provide a more accurate estimation. The results show that treatments receiving TwinN and reduced nitrogen fertiliser had lower spore counts, although these were not statistically analysed. The trial did not allow a conclusion on whether the decrease in spore count was due to TwinN, or reduced nitrogen rates, or both. However, the results provide an indication that a combination of TwinN and reduced nitrogen rates may be of value as part of an integrated management program to combine a different approach to nitrogen nutrition and soil pathogen management in avocado.

Table 5 The effects of different TwinN application frequencies and nitrogen fertiliser levels on *Phytophthora cinnamomi* spore levels in soil under pineapples

Treatment	Lupin baiting tests (% Mortality)	Chlamydospore counts
Standard Farm Practice	66.7	2.34
TwinN every 3 months + 50% N	46.7	Not tested
TwinN every 6 weeks + 25% N	40.0	0.56

Conclusion

Avocado producers face challenges of rising costs for nitrogen fertiliser, increasing need to reduce the environmental impacts of production, and increasing demands from consumers for avocados produced sustainable farming systems. Use of an effective microbial bio-fertiliser can provide a partial solution to each of these demands. When selecting a microbial bio-fertiliser growers should consider the following four factors:

- Consistency of product. The product should be produced in a modern fermentation facility and batches should be certified by an independent Government laboratory for microbe counts and lack of contaminating organisms.
- Demonstrated capacity to supplement nitrogen needs. The product should be backed by independent replicated trials showing capacity to enable reduced nitrogen requirements without loss of yield.
- Convenience of application. The product should be convenient to apply and easy to integrate into conventional or organic production systems.
- Cost effective. The product should provide an increase in profitability by reducing nitrogen costs or increasing production.

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