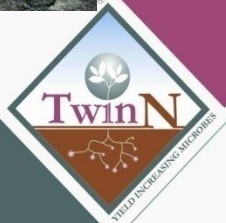




# TwinN in tree and vines crops

## October 2012



# Topics

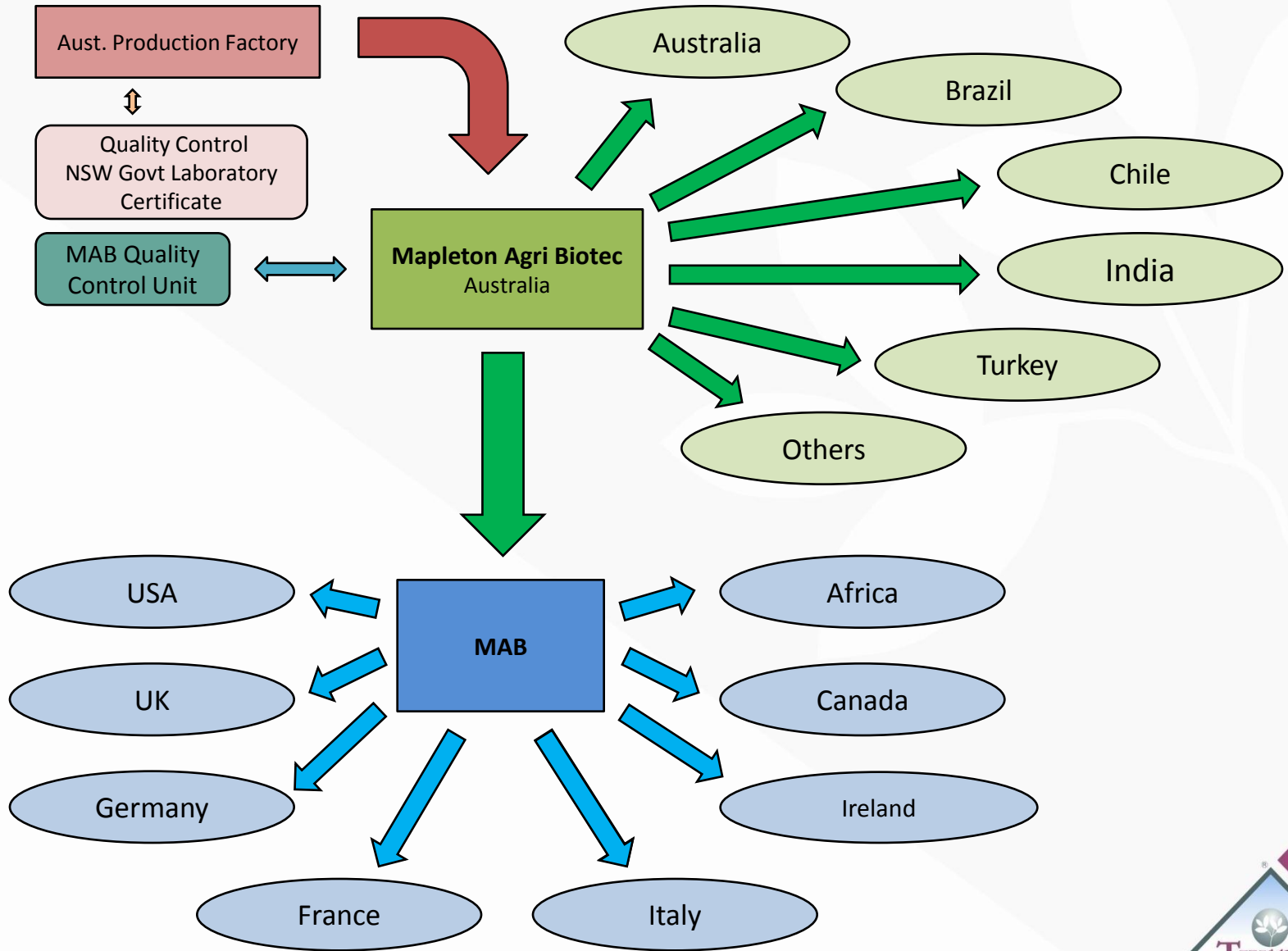
- What is TwinN?
- How to apply TwinN
- How TwinN works in crops – mechanisms and examples
- Results in grapes, citrus, apple,
- Discussion of how to integrate TwinN into your crop systems

# What is TwinN?

TwinN is a unique product that reduces the amount of N fertiliser needed for high yields

- Freeze-dried microbial product - *Diazotrophs*
- Produced in modern, sterile fermentation facility
- **Every batch quality control tested by NSW Govt lab**
  - $> 10^{11}$  cfu/ha - very high concentration
  - All strains present
- Reliable shelf life 12 mo – cool (4°C) storage
- 1, 5, 10, 100 ha packs





Schematic of supply and distribution of TwinN

# Who uses TwinN?

- Conventional farmers wanting to reduce N costs and increase profits
- Biological farmers wanting a reliable microbial N fixer and soil improver
- Organic farmers wanting improved N nutrition and better crop growth

# Which crops?

- Broadacre/field crops – cereals, corn, cotton
- Sugarcane
- Intensive temperate and tropical pastures/forage/lucerne
- **Temperate fruits – apples, stone fruits, blueberries**
- Tropical tree fruits, avocado, bananas, citrus, coffee, tea
- **Macadamia, pecan, almonds**
- **Wine and table grapes**
- Vegetables
- Others



# Where do the TwinN microbes act after application?

- **If applied via soil application** they colonise the rhizosphere – the zone of soil very close to roots. They also move up into the plant tissues and end up throughout the plant.
- If applied to bare soil they do not survive long – **they need a plant host**
- TwinN microbes get carbohydrates and nutrients as root exudates and from plant tissues. In return they provide N, increase root efficiency and root/soil health – a true symbiosis

# How to apply TwinN?

TwinN is applied to the roots where the microbes colonise, multiply and move all through the plant

## **Common application methods include:**

- Drip, micro sprinklers or overhead irrigation systems – very convenient
- Trickle tape for vegetable crops
- Knifing into the root zone as for liquid fertilisers - common in cotton and sugarcane
- Spraying onto moist soil under trees – use a streaming nozzle and apply easily down the rows
- Boomspray onto wet soil or pasture

**Application must deliver the microbes into a moist root zone for them to thrive**

# How does TwinN work?

## TwinN improves crop performance by three main mechanisms

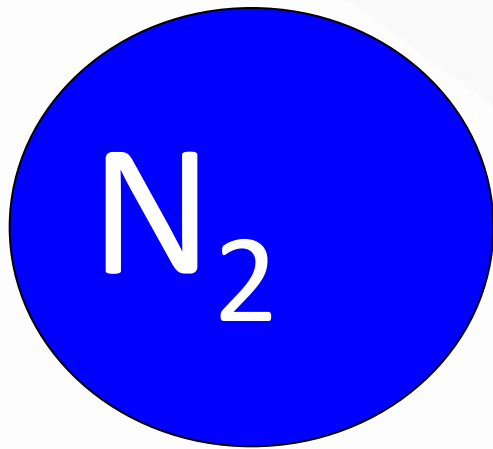
1. Converts **N<sub>2</sub> from the air** into a **steady supply of plant available N** through the entire crop season
2. Produces **larger, more efficient root system** due to production of Plant Growth Factors (PGFs) gives improved capture of applied N fertilisers (= **improved N use efficiency**)
3. **Improved soil health and structure.** Longer term use of TwinN lowers soil disease pressure and **builds soil carbon.** TwinN microbes assist in mobilising bound P





# 1<sup>st</sup> Mechanism of Action – N fixation

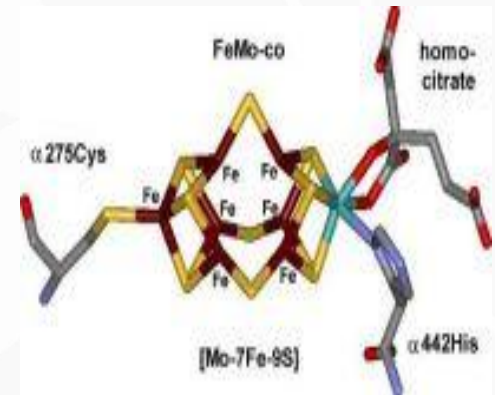
- NH<sub>3</sub> is supplied steadily right through the season
- This helps keep total leaf N at optimum levels without high nitrates which reduce fruit quality and shelflife



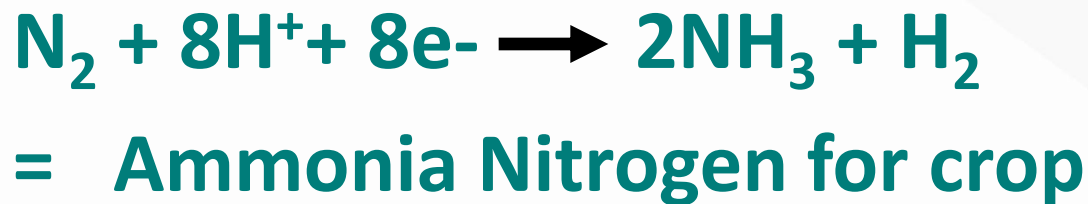
From the atmosphere



N Fixing Bacteria

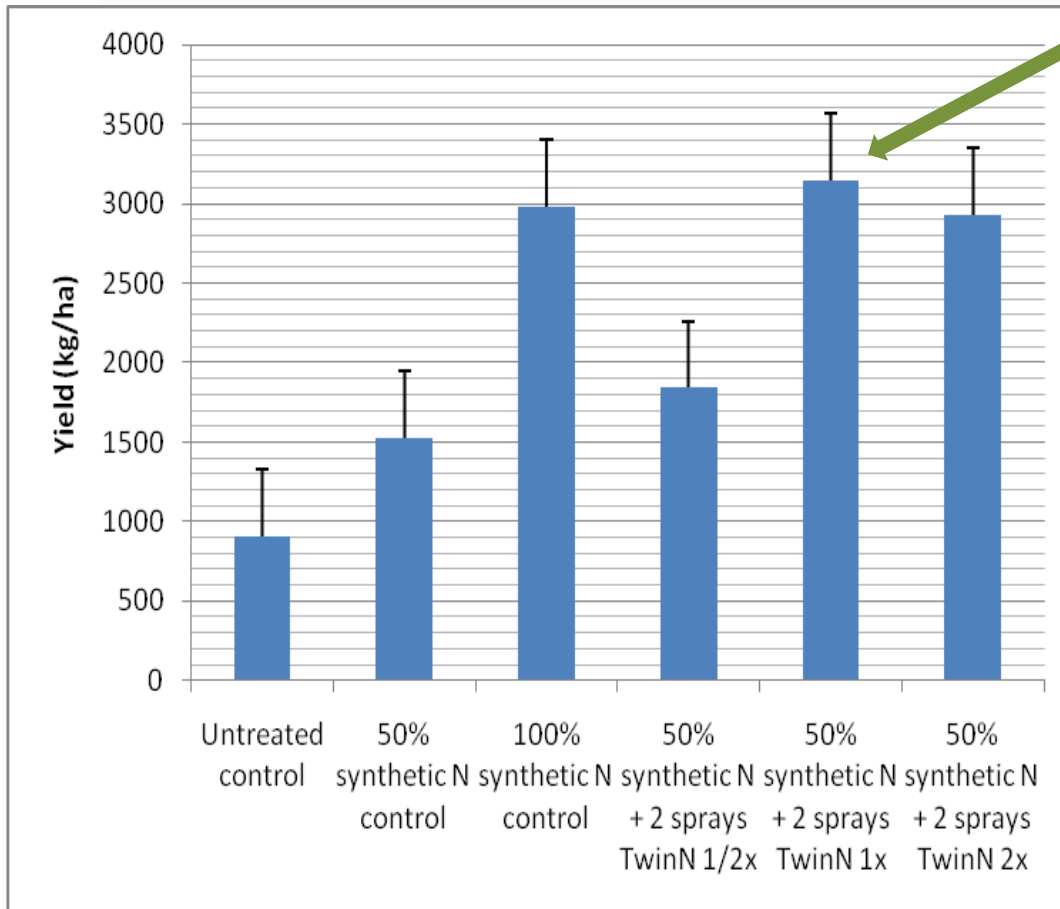


Nitrogenase enzyme



# 1<sup>st</sup> Mechanism of Action – N fixation

## Wheat – South Africa – 2010 – Independent Registration Trial



- Full rate TwinN with 50% N gave the highest yield and was statistically equal to 100% N.

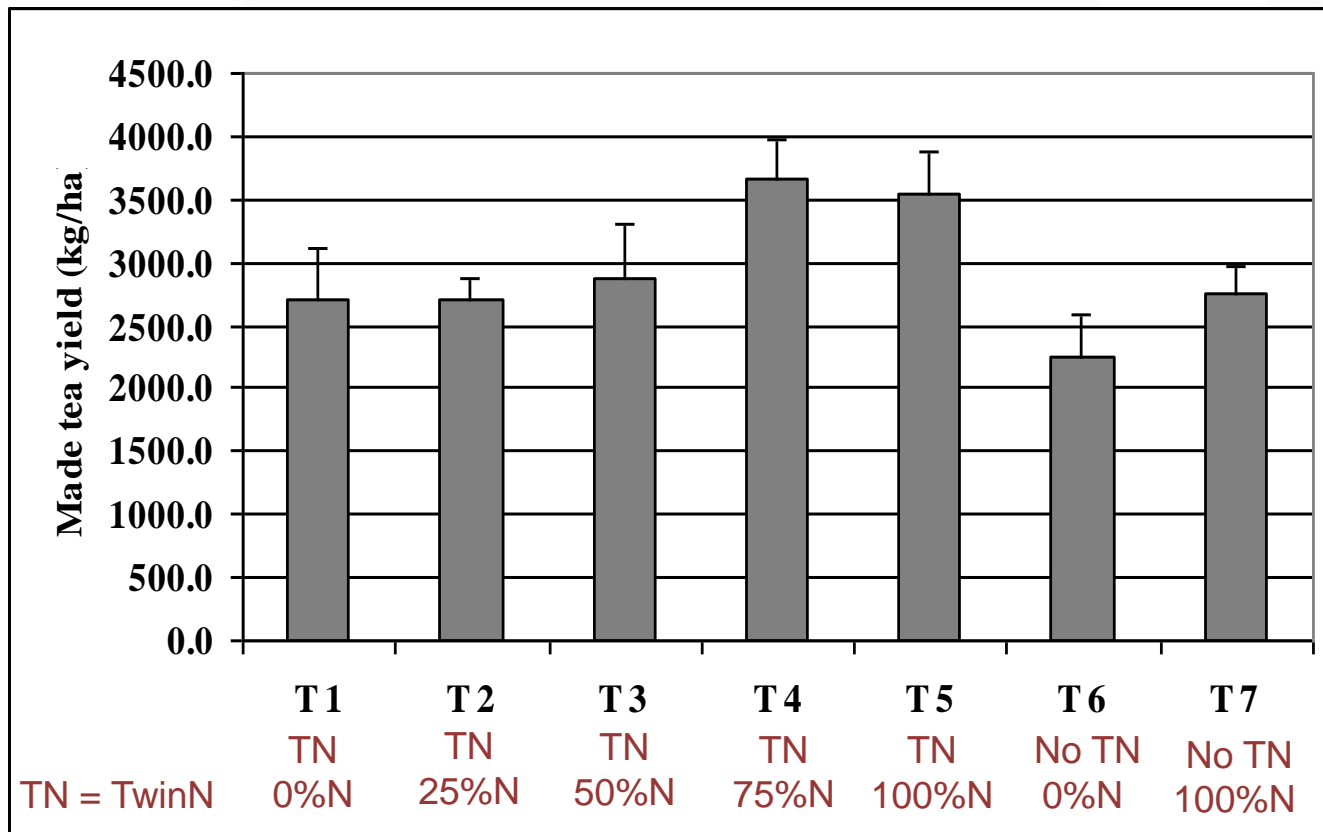
- 50% N with no TwinN delivered significantly lower yield (50% of control).

- 2X rate TwinN performed no better than 1X

- Half rate TwinN did not perform and is not recommended at all.

# Two year/ two site trial of TwinN by TRFCA in Malawi

Tea is a high N use crop and high crop value means yield is the main target



Yield from the second year of a trial in tea by TRFCA in Malawi. Results from a second site were similar. Standard 100%N was 275 kgN/ha. Because tea is a high value crop these yield increases were very profitable.

# Tea Industry Trial, Nchima Estate, Malawi, 2009

100% nitrogen (N) fertiliser application (Standard) versus two applications of TwinN plus 52% the normal rate of N.

Yield data for TwinN plus 52% N versus Standard 100% N over four months at eight sites					Mean cumulative 4 month yield (kg/ha)		
Division	Total trial size (ha)	No. TwinN plots	No. Standard plots	Average plot size (ha)	TwinN + 52% N	Standard 100% N	% inc. TwinN versus Standard
NCHIMA 5600	173.4	2	9	15.8	1928	1832	5.3
CHIWALE	169.3	2	13	11.3	2413	2001	20.6
MANGO	176.7	3	20	7.7	2500	2004	24.7
BANDANGA	162	2	15	10.1	1889	1705	10.8
PEMBA	161.1	3	17	8.1	2196	2055	6.9
NAMITETE	160.9	4	21	5.6	2010	1971	2.0
NABOMBA	146.5	2	10	12.2	2101	1833	14.6
MANKHAMBA	133.4	2	12	9.5	1379	1691	-18.5
<b>Total trial means</b>					<b>2052</b>	<b>1886</b>	<b>8.8% ↑</b>

## Year 1 Results

- Carbotech increased leaf N by 25.8% despite a 20% cut in N
- TwinN plus CT increased leaf N by 29% despite a 44% cut in N

### Summary from Report

As expected, there are no appreciable differences in tree health or yield between the trees treated with Carbotech® and/or TwinN® and the control. It is too early in the trial to expect any differences – if any differences are to appear. The concentration of Nitrogen in the trees treated with Carbotech and/or TwinN showed a 25-29% increase in the first season after a 25% N reduction. While these results are promising Westfalia cannot endorse the products without further results.



# Experiencia 2010 - Arándano



**Arándano** is blueberry

Picture on right is 21 days after TwinN application



## 2<sup>nd</sup> Mechanism of Action – larger, more effective roots

TwinN produces **larger root systems** due to auxin synthesis (IAA etc)



Note this picture is from a DPI-measured comparison and root mass rating increased >13% in TwinN plots

- Greatly **increased root hair density** gives better nutrient capture of all nutrients
- **Improved capture** of mineralised and applied N **increases nitrogen use efficiency**
- More vigorous root growth **helps crops fight back from root damage** from pests and diseases

**Improved N nutrition is achieved by a combination of N fixation from TwinN microbes plus better N use efficiency via the effect of TwinN on roots**



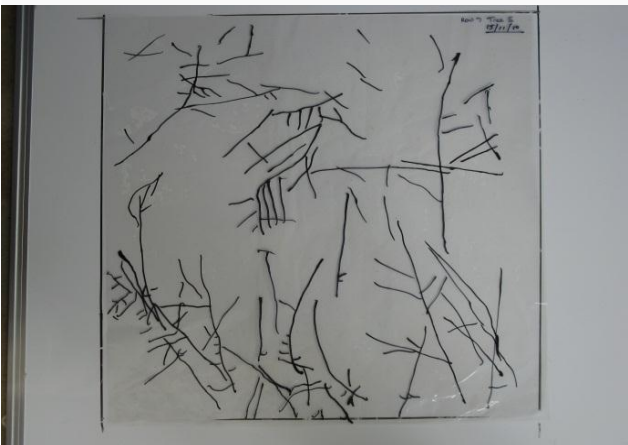




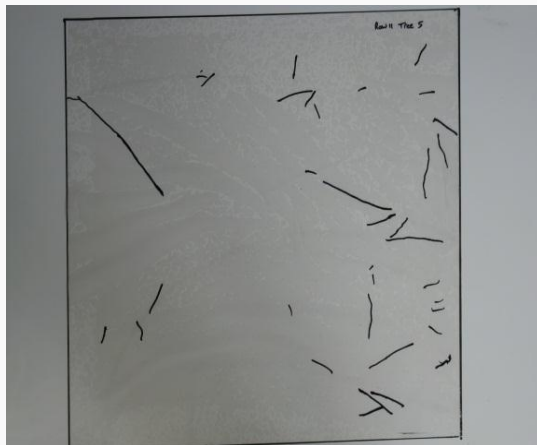
- TwinN soil application 2 months previous
- Perspex windows to monitor root growth
- Avocado Australia trial



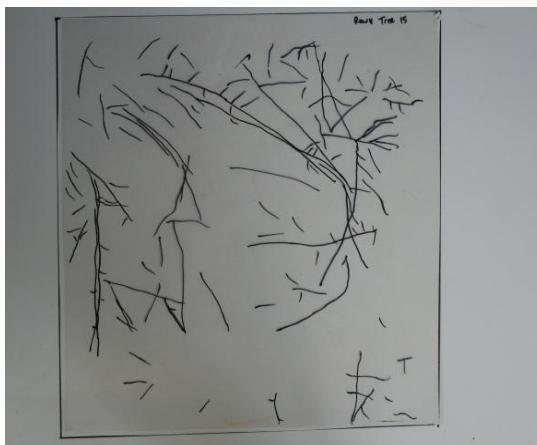
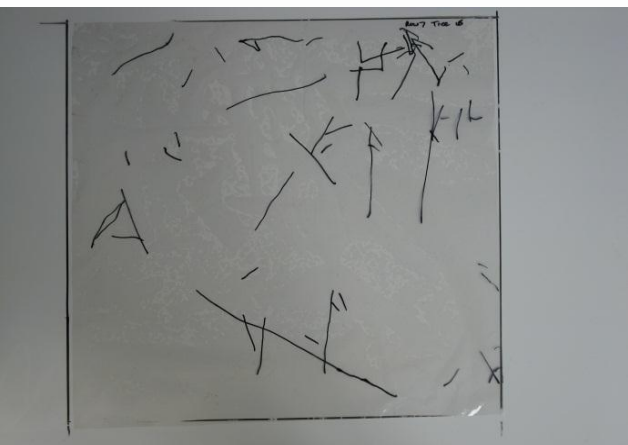
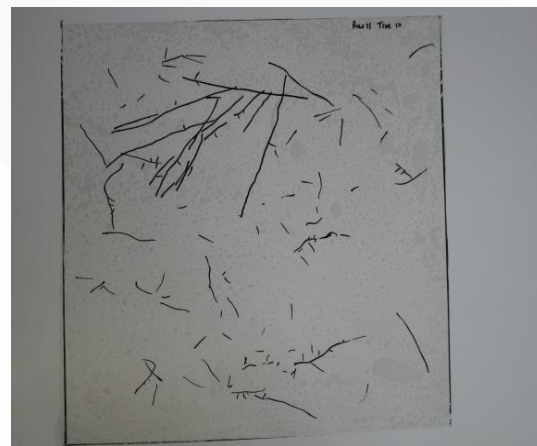
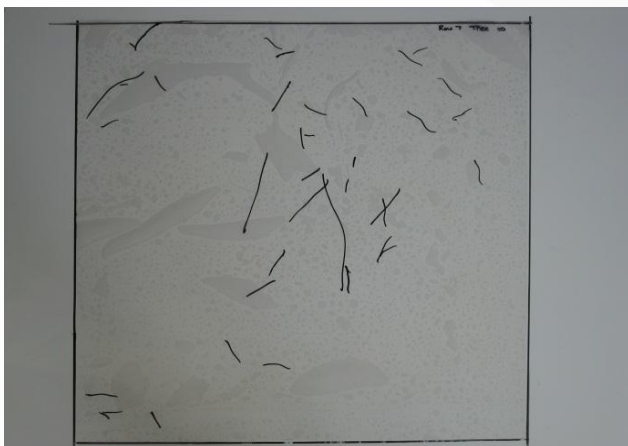
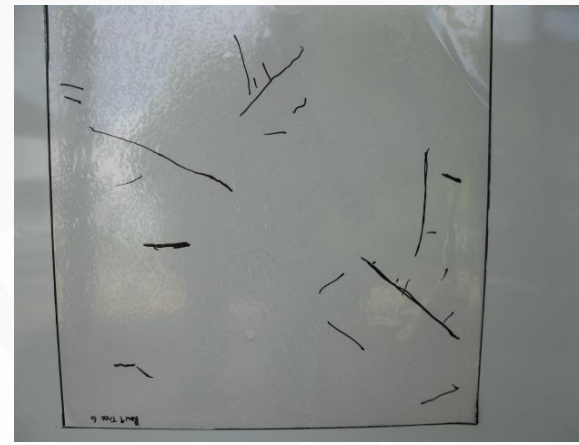
**TwinN**



**TwinN**



**No TwinN**



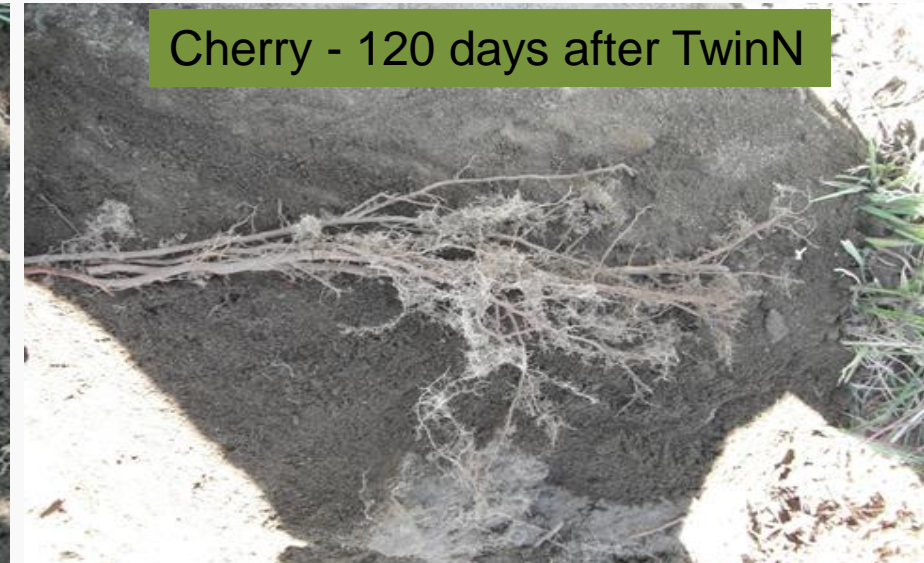


# Root growth effects – Chile 2010

Cherry – No TwinN



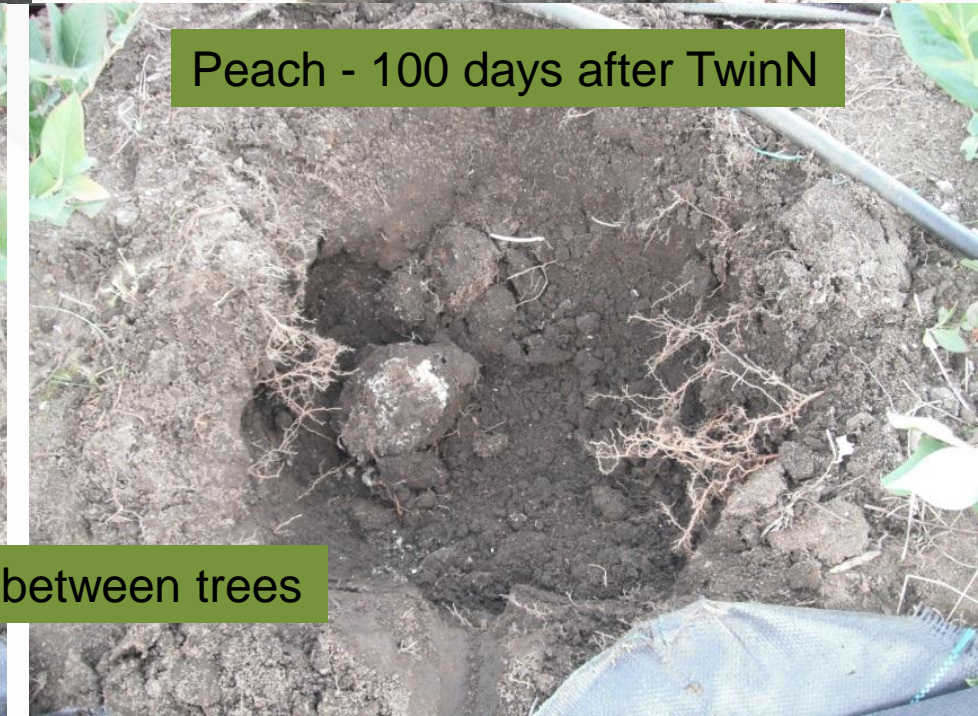
Cherry - 120 days after TwinN



Peach – No TwinN



Peach - 100 days after TwinN



Root growth between trees



# 3<sup>rd</sup> Mechanism of Action – Improved Soil Health

## TwinN can reduce populations of some pathogenic microbes

- TwinN encourages growth of beneficial microbes that help keep soil pathogens in check

## TwinN increases root nodulation in legumes

See USDA soybean trial results next slide and clover pictures this slide

## TwinN enables reduced synthetic N application rates

- Lowers impact on soil organic carbon
- Avoids issues with lowering soil pH
- Lowers impact on soil structure

**Increased nutrient availability** – TwinN microbes release organic acids **improving availability of P (P solubilisation)** and increase the **availability of some micronutrients** in soils.

Most growers understand the importance of soil health and root vigour on annual yield and long term productivity.

TwinN helps achieve these goals



Picture 9: Clover nodulation on 12 September 2009 (four months after application of TwinN and nine months after trace elements)



Picture 8: Original clover nodulation, 11 December 2009

# Soybean – Boone 2007 – US Dept Ag, Illinois

## YIELD EFFECTS

Treatment	Kg/Ha	% increase
No TwinN or Roundup	3,880 (a)	0
+ Roundup	4,270 (b)	10
+ TwinN + Roundup	4,600 (c)	18.6

- 8.6% increase in yield
- Decreased *Fusarium*
- Increase root pseudomonads (beneficial microbes)
- Increased nodule weight
- Trial repeated at second site and at 2 sites in 2008

## SOIL HEALTH EFFECTS

Treatment	Fusarium root colonisation	Root pseudomonads	Nodule weight
No herbicide	67.5 (a)	116.9 (a)	828 (ab)
+ Roundup	106.4 (b)	28.2 (b)	745 (a)
+ TwinN + Roundup	64.0 (a)	80.0 (a)	866 (b) (16%inc)





## Lupin baiting tests for *Phytophthora cinnamomi* (P.c.) under pineapples

Performed by DEEDI QLD

Treatment	% Mortality 10/3/10	Chlamyospore counts 14/7/10
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

“There is an obvious trend towards a reduction in the activity of P.c. with an increase in microbial activity in the soil”

Col Scott – Consultant Agronomist

## *3<sup>rd</sup> Mechanism of Action – Improved Soil Health*

TwinN is not a cure for Phytophthora or other soil pathogens but it has a useful role as part of an integrated management strategy to assist growers cope with long term disease pressures.

MAB is due to release a new product (Research name **BB5**) with additional microbes to enhance this effect.



# Results in vine and tree crops



# Citrus

- TwinN is used to enable 20 – 40% reduction in N up to a maximum cut of 50 U N/ha
- Spread cuts across the season
- Apply in spring and again in autumn if economics allow
- If fruit prices are low then make larger cuts in N to save input costs
- 40U cut (87 kg urea) = \$52/ha @ \$600/T. TwinN ~ \$33/ha inc GST
- If fruit prices are high use smaller N cuts and apply TwinN x 2





# Farm Demonstration

- **Citrus – Turkey – 2009**



- **Full fertiliser regime**

- Noticeable change in tree colour and health
- TwinN applied 4 weeks prior to picture
- Farmer reported clear yield increases

- **60% fertiliser regime - Plus TwinN**

# The TwinN – CFT program in tree crops

- Carbon Fertiliser Technologies (CFT) are distributors of TwinN in South Africa
- Carbotech (CT) is a carbon technology used in intensive irrigated tree crop production systems and enables reductions in P, K etc
- When Carbotech that is used in combination with TwinN nitrogen fertiliser applications are also reduced
- During 2009 a series of citrus and mango farms in South Africa and Australia adapted their systems to CT/TwinN
- The next two slides summarise the leaf N data from the 2009 season (before TwinN) and 2010 season (after a 20% reduction in N fertiliser and TwinN application)
- The data were part of a program to redesign the nutritional programs in commercial farms to enable very high production with reduced inputs of N, P and K





# Leaf N levels in citrus - Australia

Client	Ha	Block Name:		No TwinN 2009	Plus TwinN 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	
			Late	3.53	
			2.3 A Murcott	2.95	2.32
			2.3 B Murcott	2.78	2.4
	8.1	Kay Farm 3	Clementine	2.36	
			Clementine	2.42	
			3.1A Navel		2.87
			3.1B Navel		2.81
Twynam	8.34 47.9 53.2	Stage 1	Stage 1 South	2.97	2.87
		Stage 2	Stage 2	2.79	2.67
		Stage 3	Stage 3	2.9	2.61
John Davidson	55	Farm 484	Salistiana	2.6	2.9
			Hamlin	2.5	2.9
<b>Total Average</b>				<b>2.87</b>	<b>2.65</b>

Crop / Cultivar	N	
	Min	Max
Navel (Aus)	2.50	3.00
Valencia (fresh) (Aus)	2.20	2.50
Valencia (juice) (Aus)	2.50	3.00
Grapefruit (AUS)	2.50	3.00
Mandarin (AUS)	2.50	3.00
Lemon (AUS)	2.50	3.00
Midnight (AUS)	2.50	3.00

- 2009 data is pre TwinN – CT
- 2010 data is after >20% N reduction + TwinN
- In blocks with excess leaf N the N reductions were larger

CT program also reduced P, K etc applications



# Leaf N levels in citrus & mango - South Africa

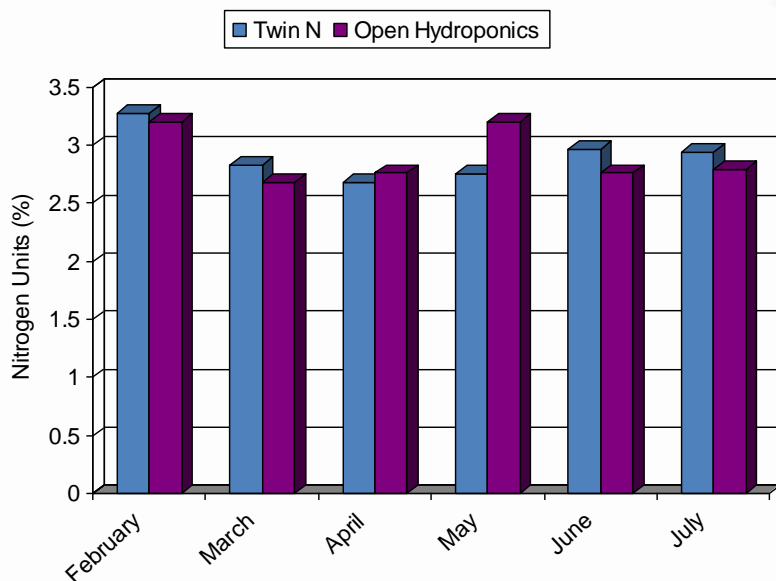
Citrus in RSA:			N leaf analyses:	
Client	Ha	Block Name:	No TwinN 2009	Plus TwinN 2010
Piet Engelbrecht Drip	49	24 Nawels	2.4	2.57
PLM	9	PLM 36 Midknigh Drup	1.92	2.5
Schoonbee	13	Schoonbee SL1 Eureka	1.82	1.97
Petrus Berg Groep 1	20	4Jong Nova	2.34	2.55
		Mid 1 & 2	1.87	1.73
Bosveld Midknights	27	2C 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
<b>Total Average</b>			<b>2.2</b>	<b>2.22</b>
<b>Mangoes in RSA:</b>				
Bavaria Mango Grovedale	6	Grovedale Groep 5 H3	0.87	0.97

Leaf analyses norms (RSA):	N
Delta (small fruit)	2.1-2.3
Lemon (oil)	2.2-2.6
Midnight 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

- 2009 data is pre TwinN – CT  
 - 2010 data is after >20 – 25% N reduction + TwinN and CT  
 - In blocks with **excess leaf N** the N reductions were larger  
 We recommend a 20 – 40% reduction in nitrogen fertiliser plus TwinN in Spring and Autumn. Use a larger reduction in N when N prices are high and/or when returns are low. TwinN will also assist in maintaining a strong healthy root system and coping with short dry spells

# Nitrogen Levels in Citrus Trees

2009	February	March	April	May	June	July
Nitrogen Levels (%) after Twin N	3.28	2.83	2.68	2.75	2.97	2.94
Nitrogen Levels (%) after Open Hydroponics	3.2	2.68	2.76	3.2	2.76	2.79



**Location:** Forbes NSW Australia (Jemmalong)

**Crop:** Citrus Trees

**Treatments:**

- 1) Twin N nitrogen fixing bacteria
- 2) Open Hydroponics – regular application of citrus specific hydroponic formulation including calcium nitrate and potassium nitrate

**Results:**

Growth parameters (trunk girth and height) – no significant difference.

Leaf analysis shows Twin N is maintaining leaf nitrogen in ideal ranges, often at a higher level than the open hydroponic trees.

**NOTE** Total replacement of N fertiliser with TwinN is not recommended

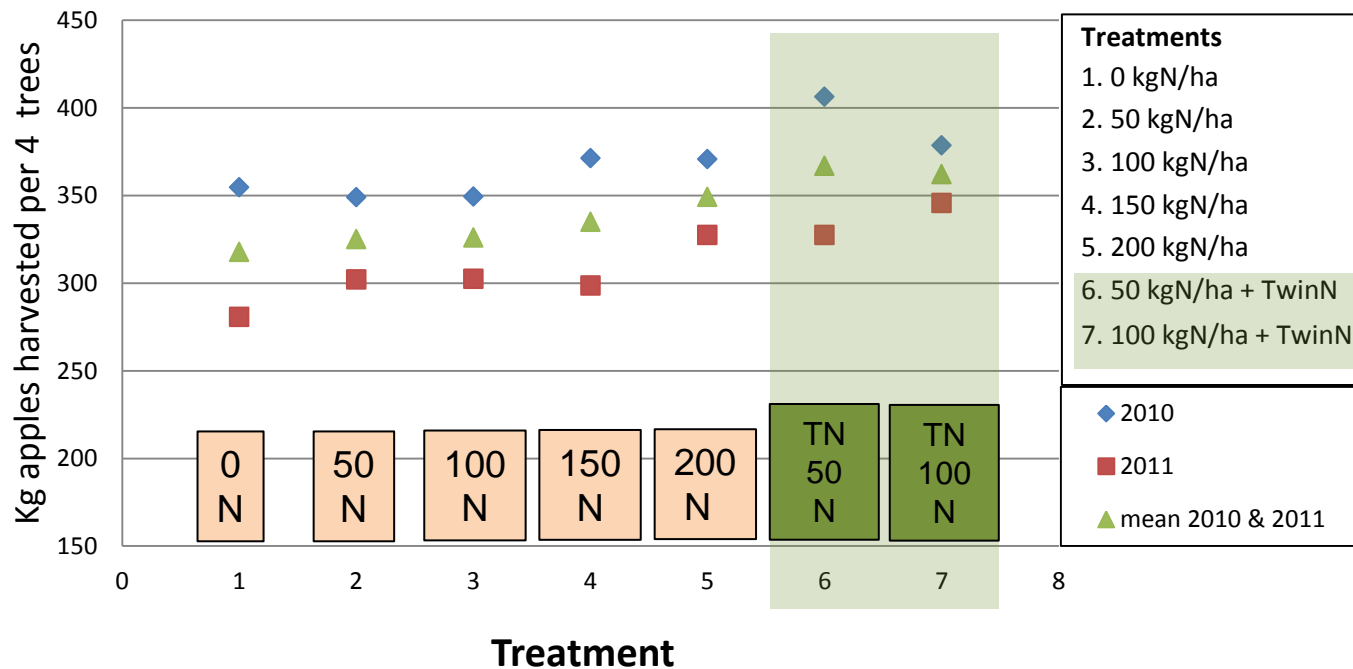
# Apple, Nectarine & Peach

- TwinN is used to enable 20 – 40% reduction in N up to a maximum cut of 50 U N/ha
- Spread cuts across the season
- Apply in spring and again before trees cease growth at the end of the season if economics allow
- If fruit prices are low then make larger cuts in N to save input costs
- 40U cut (87 kg urea) = \$52/ha @ \$600/T. TwinN ~ \$33/ha inc GST
- If fruit prices are high use smaller N cuts and apply TwinN x 2



# Bulmer Cider, Independent replicated trial, Apples, UK, 2010 & 2011

Yield of apples in 2010 and 2011 with and without TwinN



TwinN was applied to soil via two applications per year in spring and late summer

This trial was conducted to test TwinN's capacity to increase profits via improved yields and reduced fertiliser costs.

Improved sustainability of production was also noted.

## Conclusions

- TwinN plus 50 or 100 kgN produced the highest yield in 2010 & 2011
- TwinN plus 50 kgN/ gave a 12.6% yield increase over 100 kgN with no TwinN
- This translates to reduced N costs, increased returns, decreased C footprint (see next slide) and improved long term soil health due to reduced N fertiliser applications

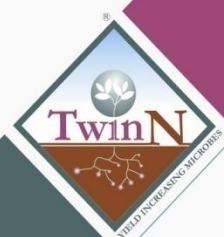


# Peach, nectarine & apple farm use - Oregon USA 2011

		3/6/2011	7/11/2011
Block	lbs N Soil	% Leaf N	% Leaf N
14 A Gala Apple Grower Std.	27	2.31	2.00
14 A Gala Apple Twin N	28	2.66	2.23
14 B Nectarines Grower Std.	55	4.92	3.84
14 B Nectarines Twin N	55	4.50	4.04
Block 21 Peaches Grower Std.	63	4.81	3.96
Block 21 Peaches Twin N	53	4.98	3.87
Block 15 A Jazz Apple Grower Std.	49	3.43	2.50
Block 15 A Jazz Apple Twin N	64	3.24	2.61

TwinN in commercial farm use in Oregon State, USA, Two drip irrigation applications of TwinN – spring and autumn  
Approx. 40% N reduction

Data provided by GS Long Pty Ltd





# Peach, nectarine & apple farm use - Oregon USA 2012

		N	P	K	S	Ca	Mg	B	Mn	Zn
Apple Gala	Standard	2.1	0.17	1.74	0.13	2.78	0.6	25.9	105.4	15.9
	TwinN	2.38	0.2	1.57	0.15	2.39	0.54	24.2	83.9	15.8
Nectarines	Standard	3.88	0.35	2.6	0.37	1.84	0.59	28.5	45.4	47.3
	TwinN	3.09	0.22	2.07	0.19	2.24	0.66	27.3	46.9	26.1
Peaches	Standard	4.3	0.35	2.5	0.27	2.4	0.53	32.7	37.9	38.7
	TwinN	4.38	0.45	2.67	0.35	1.89	0.53	38.3	55.2	42.9
Apple Jazz	Standard	2.86	0.26	1.99	0.2	2.34	0.59	32.1	82.5	27.4
	TwinN	2.62	0.2	1.52	0.16	1.88	0.56	29.1	92.1	20.2

TwinN in commercial farm use in Oregon State, USA, Two drip irrigation applications of TwinN – spring and autumn  
Approx. 40% N reduction

Data provided by GS Long Pty Ltd

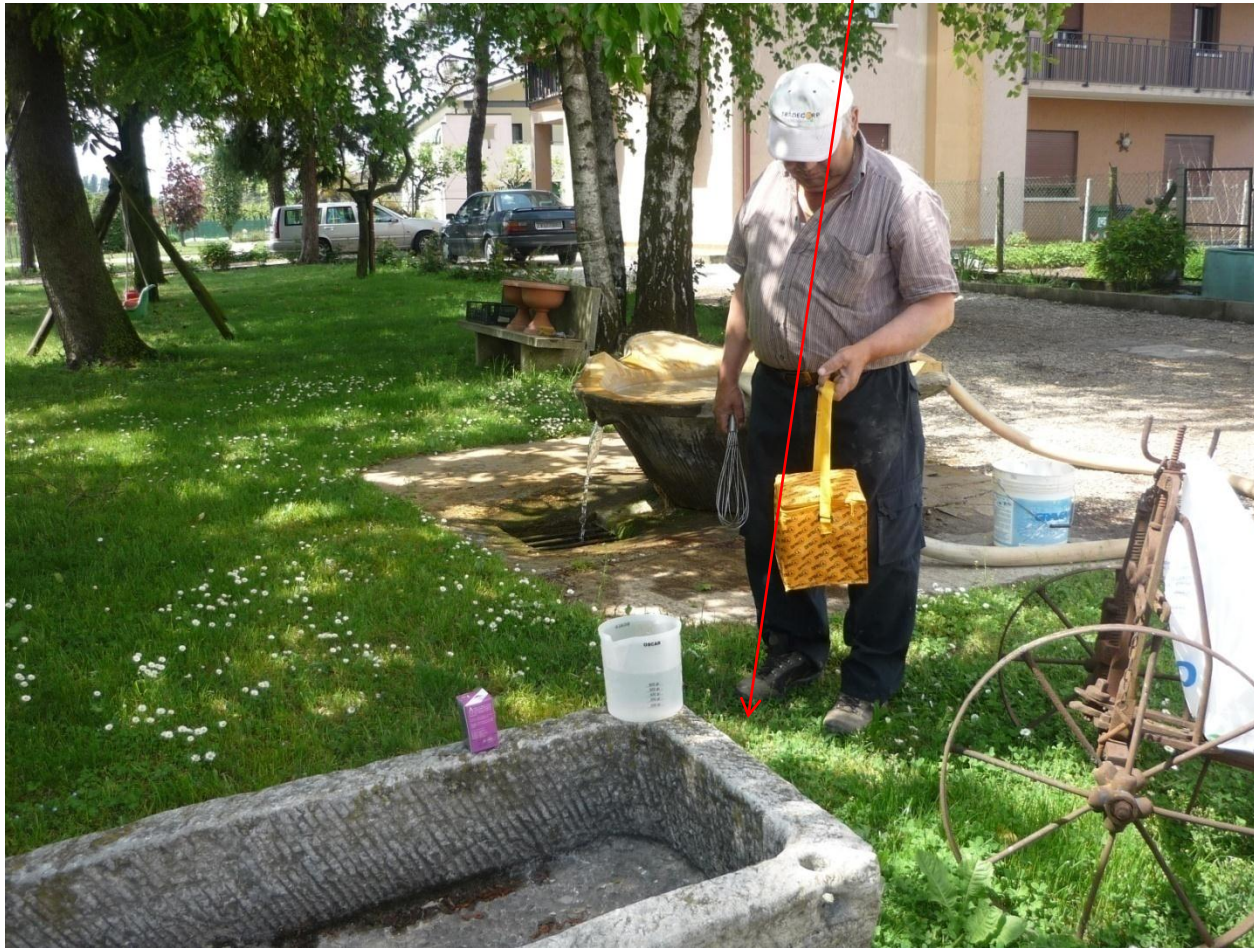


# Grapes

- TwinN is used to enable 20 – 50% reduction in N up to a maximum cut of 50 U N/ha
- Spread cuts across the season
- Apply in spring and again before vines cease growth at the end of the season if economics allow
- If grape prices are low then make larger cuts in N to save input costs
- 40U cut (87 kg urea) = \$52/ha @ \$600/T. TwinN ~ \$33/ha inc GST
- If fruit prices are high use smaller N cuts and apply TwinN x 2



## Twin N box with N-bacteria



These slides are from an Italian company that sells TwinN into grape farms

Growers reduce N by 40 – 50% and use 2 TwinN per season





Cecchetto farm - San Polo di Piave TV

Crop stage at the application 1 of Twin N

Grapes cv. Raboso del Piave

Grapes cv. Prosecco





Twin N :application 2 stage on the clusters cv.Raboso del Piave





Cecchetto farm - San Polo di Piave TV

Twin N :application 2 stage on cv. Prosecco







Cecchetto farm - San Polo di Piave TV

Twin N : 40 d.a.t. on cv.Prosecco





Cecchetto farm - San Polo di Piave TV

Twin N on cv. Prosecco







Cecchetto farm - San Polo di Piave TV

Check plot on cv.Prosecco





Cecchetto farm - San Polo di Piave TV

Twin N on cv.Prosecco







Cecchetto farm - San Polo di Piave TV

Check plot on cv.Prosecco





## Mg deficiency symptoms on the check plot





**NPK CHEM Srl**

Cecchetto farm - San Polo di Piave TV

**Twin N treatment with less Mg deficiency symptoms**

**Twin N on cv. Prosecco**







Cecchetto farm - San Polo di Piave TV

Check plot on cv.Prosecco



Azi. Cecchetto - San Polo di Piave TV

STANDARD

15/03/2010

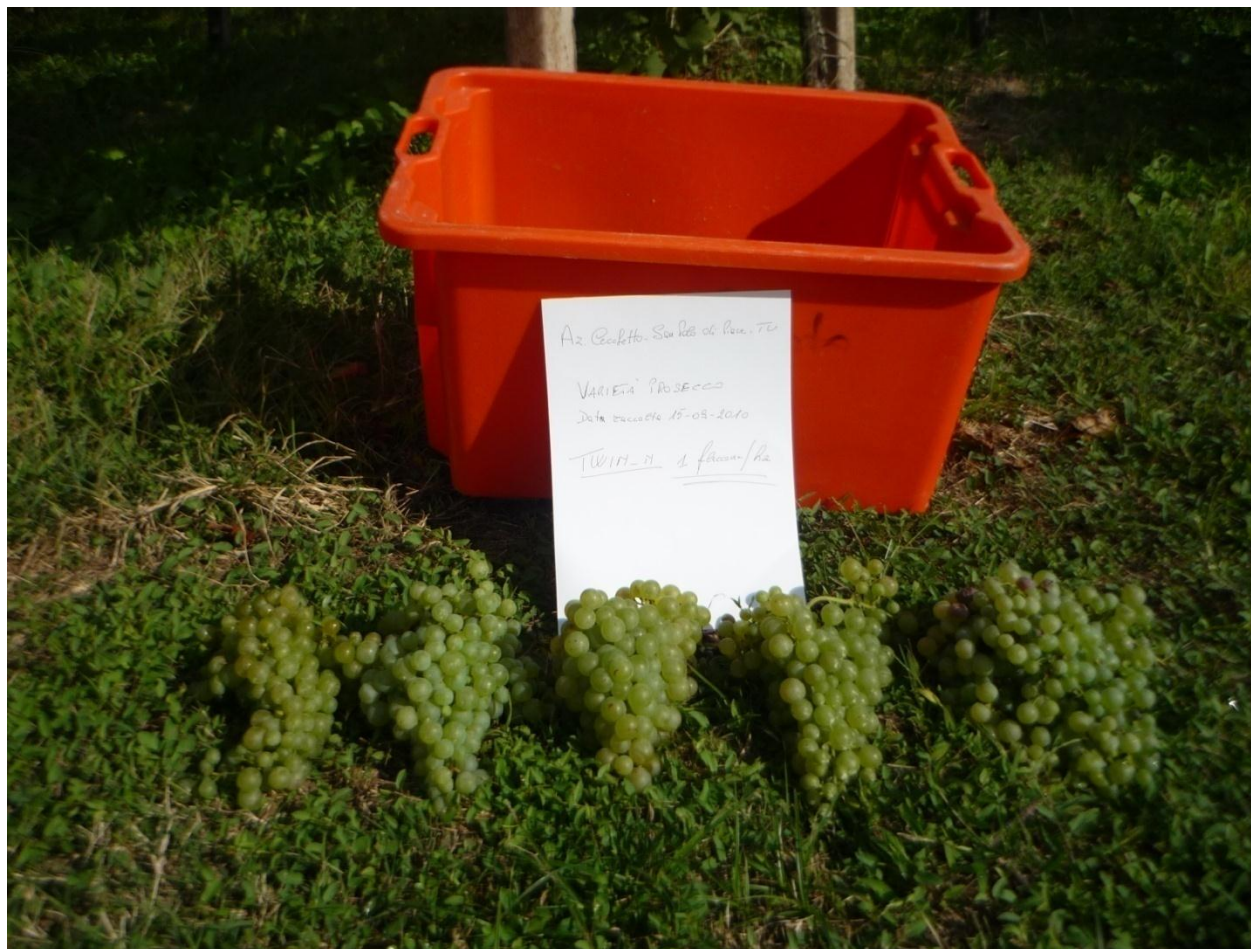




**NPK CHEM Srl**

Cecchetto farm - San Polo di Piave TV

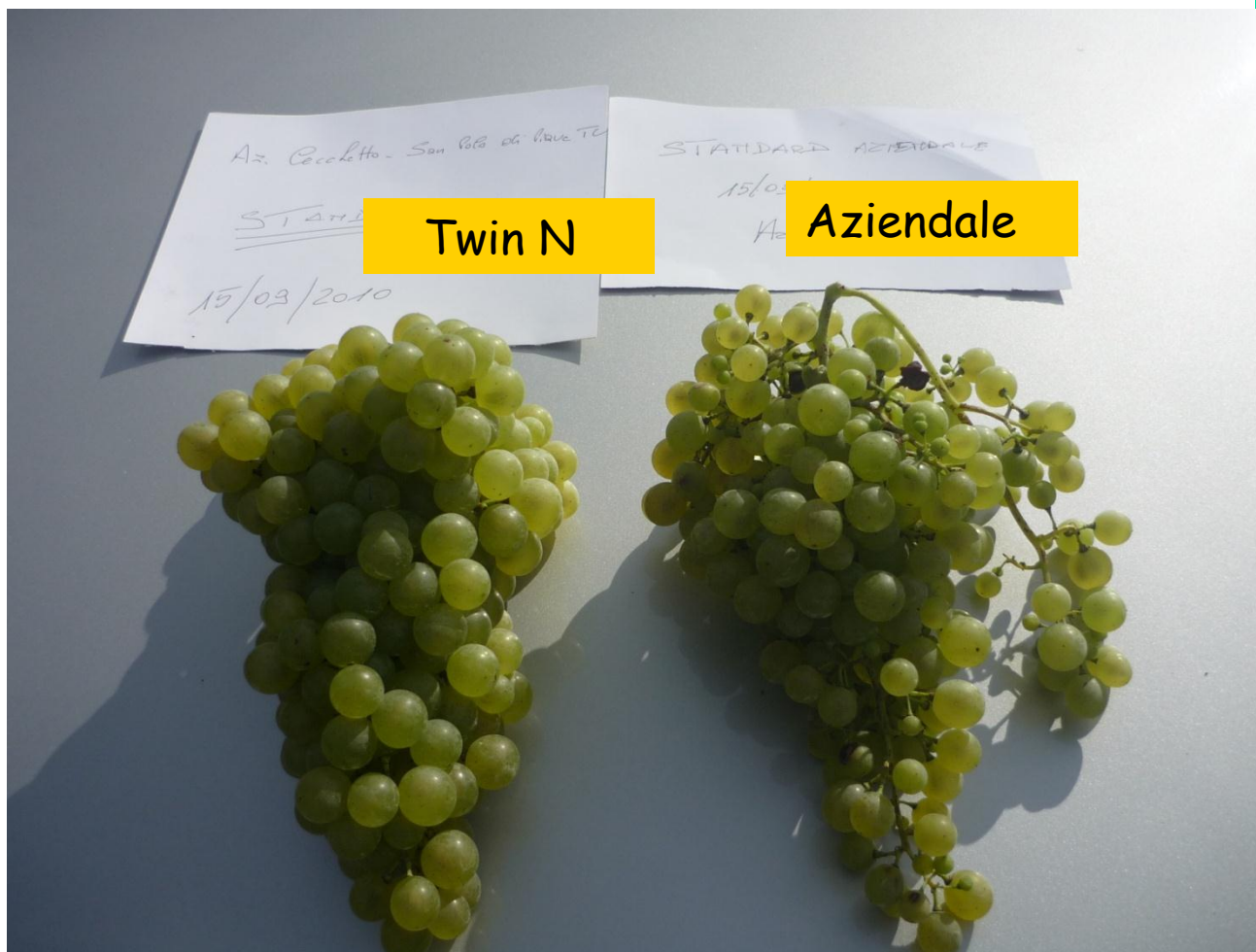
**Twin N on cv.Prosecco**



**NPK CHEM S.r.l.**

Cecchetto farm - San Polo di Piave TV

**Varietà Prosecco**



Note uniformity  
of bunch  
development

Aziendale is  
standard  
treatment





**Results on cv. Prosecco**

**Trial results**



	Avg. weight of 70 clusters	Yielding % incr.
Check	Kg. 20,8	0
Twin N	Kg. 24,5	+ 18%



# TwinN at Yalumba's Oxford Landing Estate

## Comments from OLE Viticulturist

TwinN was applied to 40ha of vines in late October 2011, pre flowering between **growth stage 15 and 17**. It was applied **via fertigation** through our dripper system at the recommended hectare rate. Previous Nitrogen applications on these blocks were between **40 to 60 units of N per season**. During the 2011-12 season we applied **20 Units of N via SOA plus TwinN** and various other products like fish, kelp, humus etc. The **yield comparison between the blocks with TwinN applied and not applied were virtually identical**, even where the nitrogen levels applied were the same. There will have to be more trial work conducted at OLE, comparing yields and vine vigour to make any further comments on Twin N's efficacy.

**Positive comments would be its low carbon footprint, costs and the ease of use.**



# TwinN at Angoves' Nanya Vineyard

## Comments from Angoves viticulturist (after 3 seasons with TwinN):

- As a rule we wet the soil profile down to the root zone before the fertigation of TwinN begins then we apply the TwinN at the recommended rate and keep the irrigation going after the fertigation of the TwinN until we are satisfied that the TwinN would have reached the root zone.
- Using TwinN is like having nitrogen on demand, when the vine needs it, it uses it...
- Single, easy application done by fertigation once per season makes it time and cost effective, much cheaper and more beneficial than using/applying more synthetic products

TwinN is applied via drip irrigation in the 2<sup>nd</sup> or 3<sup>rd</sup> week in October after vine growth has commenced.

	08-09	09-10	10-11	11-12	06-09 average	09-12 average
TwinN	No	Yes	Yes	Yes	No	Yes
Kg synthetic N/L applied	2218	498	1580	921	1820	1000





# Conclusion

## **TwinN will:**

- **Improve profitability**
- **Increase sustainability**
- **Increase health and vigour of vines and trees**
- **Provide flexible options to control input costs as input costs and grower returns vary over the seasons**

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