TwinN for Sugarcane

Mapleton Agri Biotec Pty Ltd

What is TwinN?

TwinN is a breakthrough product that reduces the amount of N fertiliser needed

- Freeze-dried microbial product - *Diazotrophs*
- Produced in modern, sterile fermentation facility
- Every batch quality control tested by NSW Gov labs
  - > 10^{11} cfu/ha - very high concentration
  - All strains present and free of contaminants
- Reliable shelf life 12 mo – cool (4°C) storage
- Light to transport

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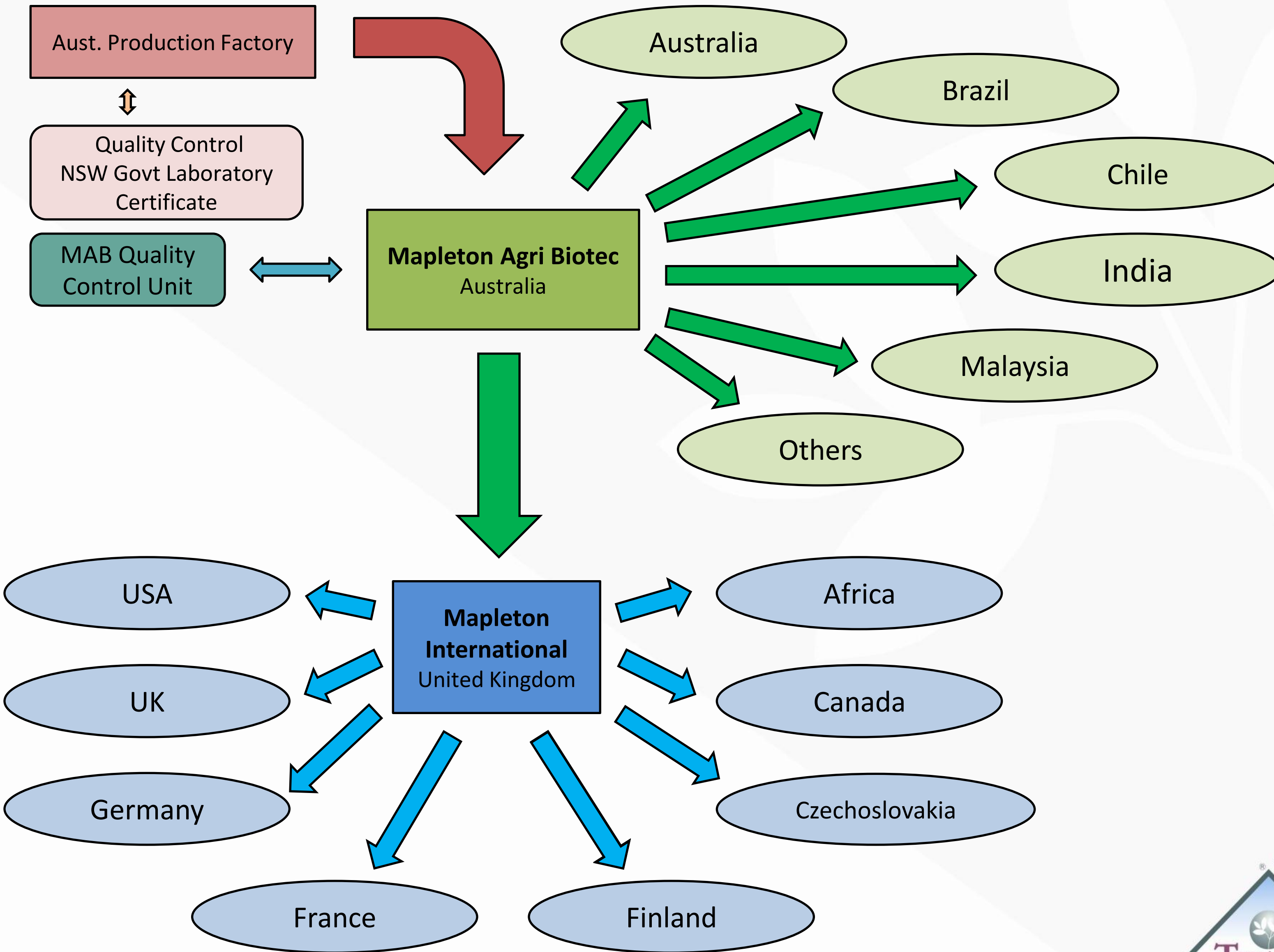
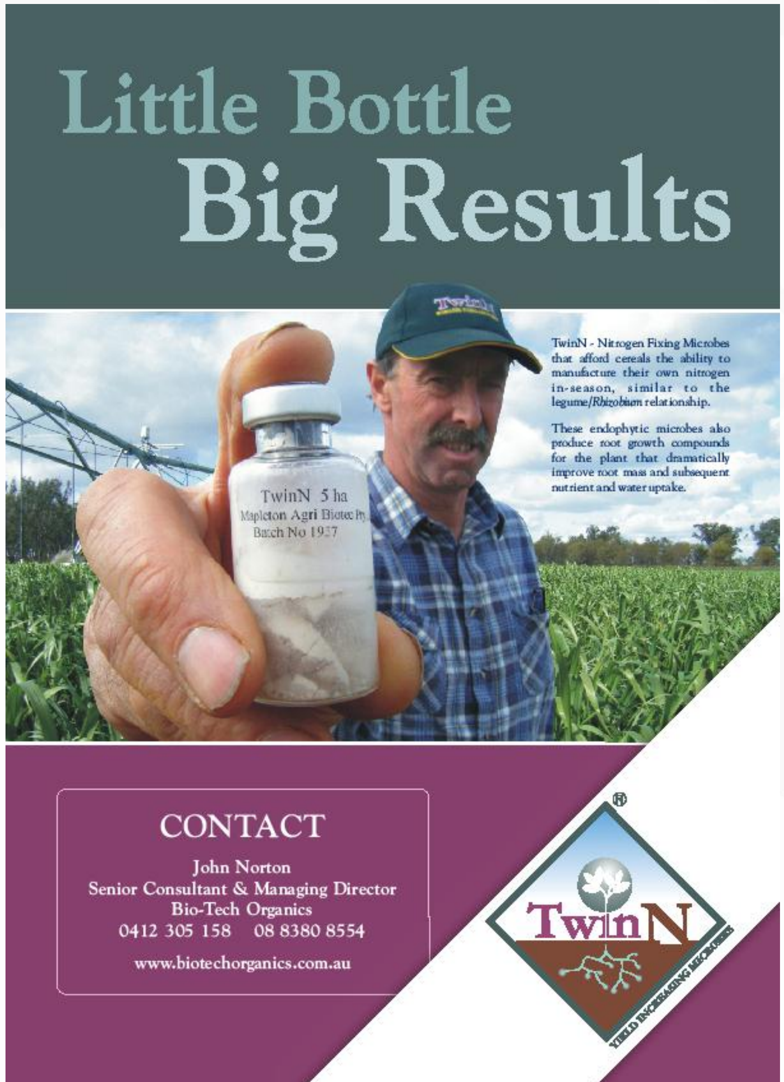


Schematic of supply and distribution of TwinN

TwinN is sold in 1, 5, 10 & 100 ha packs



Why Develop TwinN ?

- Use of nitrogen fertiliser has underpinned large increases in crop yields worldwide for the last 40 years

  But

  - Nitrogen fertiliser prices fluctuate – usually upwards
    - Prices doubled during 2007 – 08, have fallen and will rise again
  - Nitrogen fertiliser is bulky and transport costs are high
  - Nitrogen fertiliser has a very high carbon footprint
  - Nitrogen fertiliser is lost via leaching and volatilisation
  - Nitrogen fertiliser acidifies soil and depletes soil carbon

- Nitrogen fixation in legumes with *Rhizobium* has been very successful
- But other N fixing products are inconsistent and of varying quality
- MAB sought to engage better technology to overcome shortfalls

Mechanisms of Action

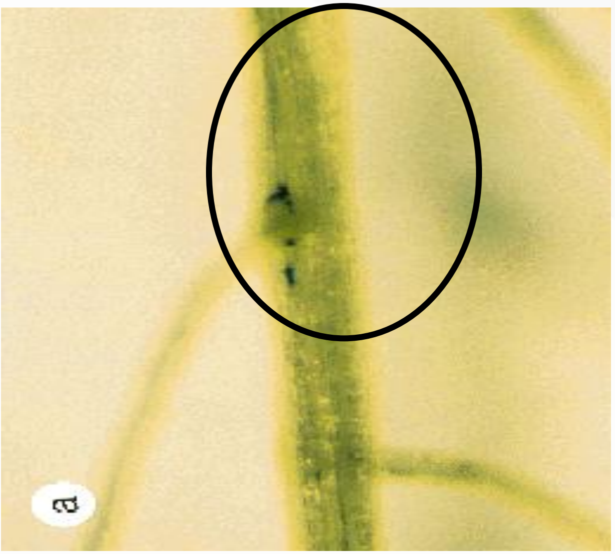
TwinN improves crop performance by four main mechanisms

1. Converts N_2 from the air into a **steady supply of plant available N** through the entire crop season
2. Produces **larger root system** due to production of Plant Growth Factors (PGFs)
3. Microbes release organic acids **improving availability of P** and some micronutrients in some soils
4. **Improved soil health and structure** with longer term use of TwinN lowering soil disease pressure and **builds soil carbon**

1st Mechanism of Action – Biological Nitrogen Fixation

$$N_2 + 8H^+ + 6e^- + 16ATP = 2NH_3 + H_2 + 16\ ADP$$

The microbe species in TwinN all contain the *nif* gene which is used by Rhizobium to fix N_2 from the air into plant available nitrogen

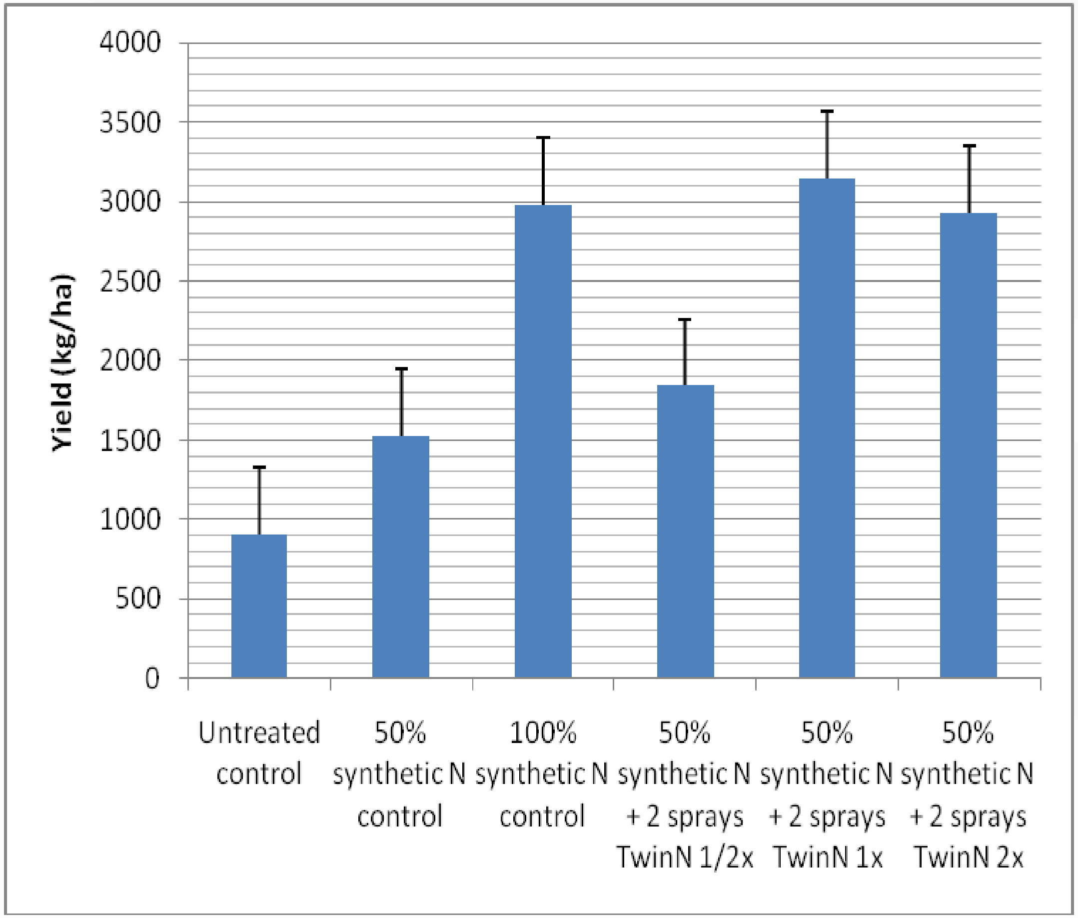


Nitrogen Fixing Bacteria – Diazotrophs

Symbiotic Nitrogen Fixing Bacteria – Rhizobium

1st Mechanism of Action

Wheat – Western Cape South Africa - 2010



- 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).
- Half rate TwinN did not perform and is not recommended at all.
- 2X rate TwinN performed no better than 1X

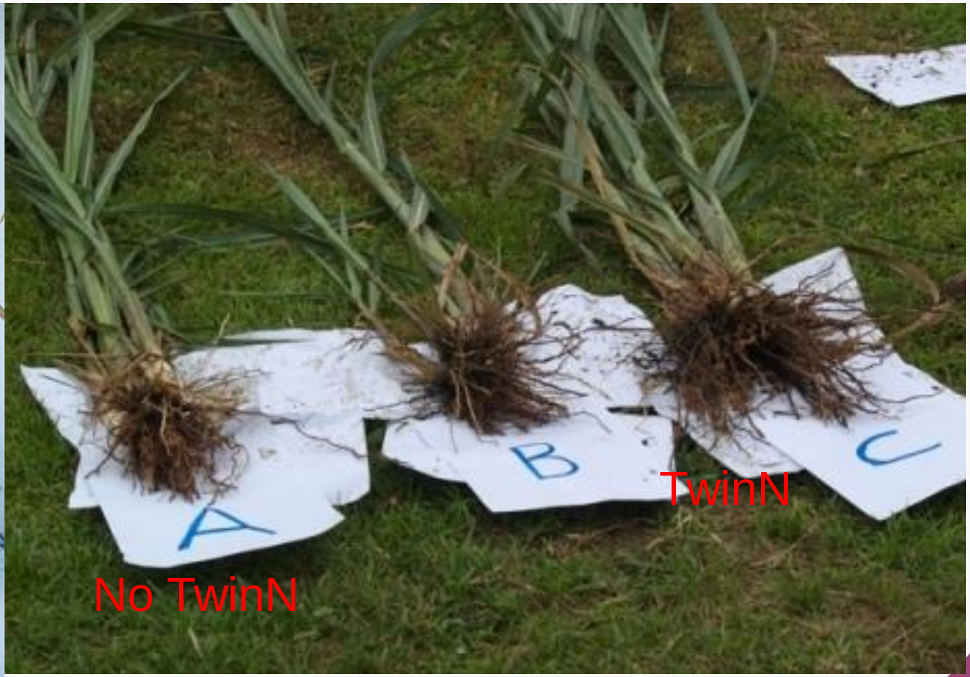
100%N = 112kgN

2nd Mechanism of Action – larger, more effective roots

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

- Greatly **increased root hair density** gives better nutrient capture of all nutrients
- **Improved capture** of mineralised and applied N **increases nitrogen use efficiency**
- Larger roots harbor more TwinN microbes to fix **nitrogen** in the rhizosphere
- Larger root mass leaves more organic carbon residues
- More vigorous root growth **helps crops fight back from root damage** from pests and diseases

2nd Mechanism of Action – larger roots



Wheat

Sugarcane
Ilovo trial Zambia

2nd Mechanism of Action – larger roots

Sweet corn roots

3rd Mechanism of Action – Phosphorous Availability

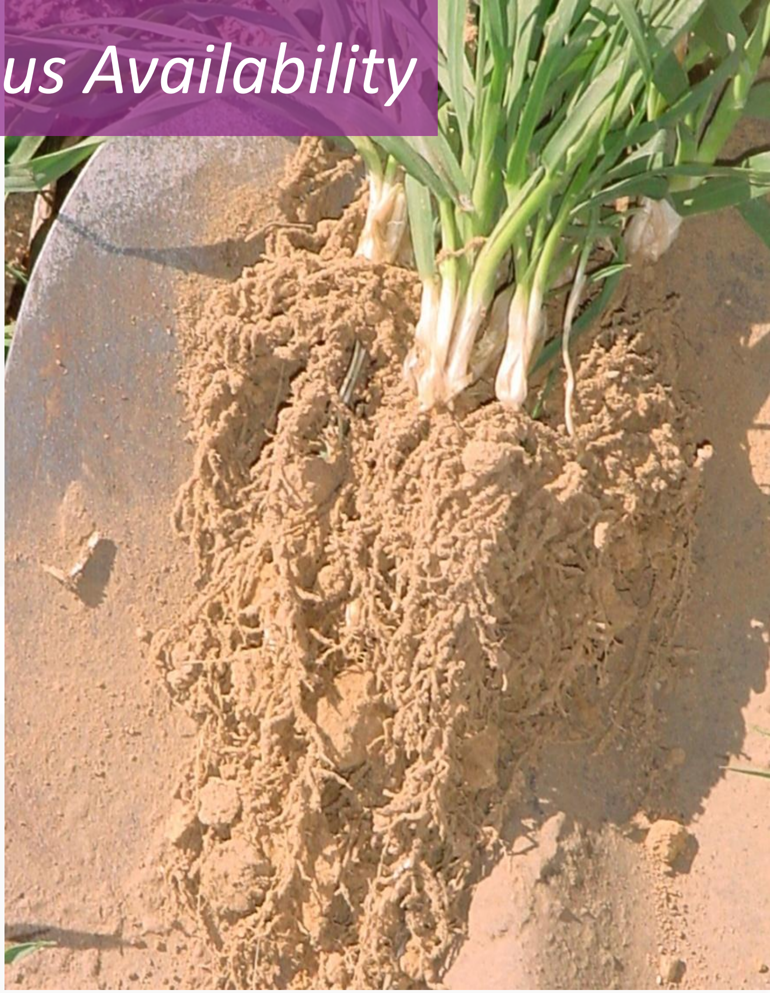
- Microbes release organic acids such as gluconic, oxalic, citric acid etc.
- Releases bound nutrients in the soil

Dicalcium phosphate

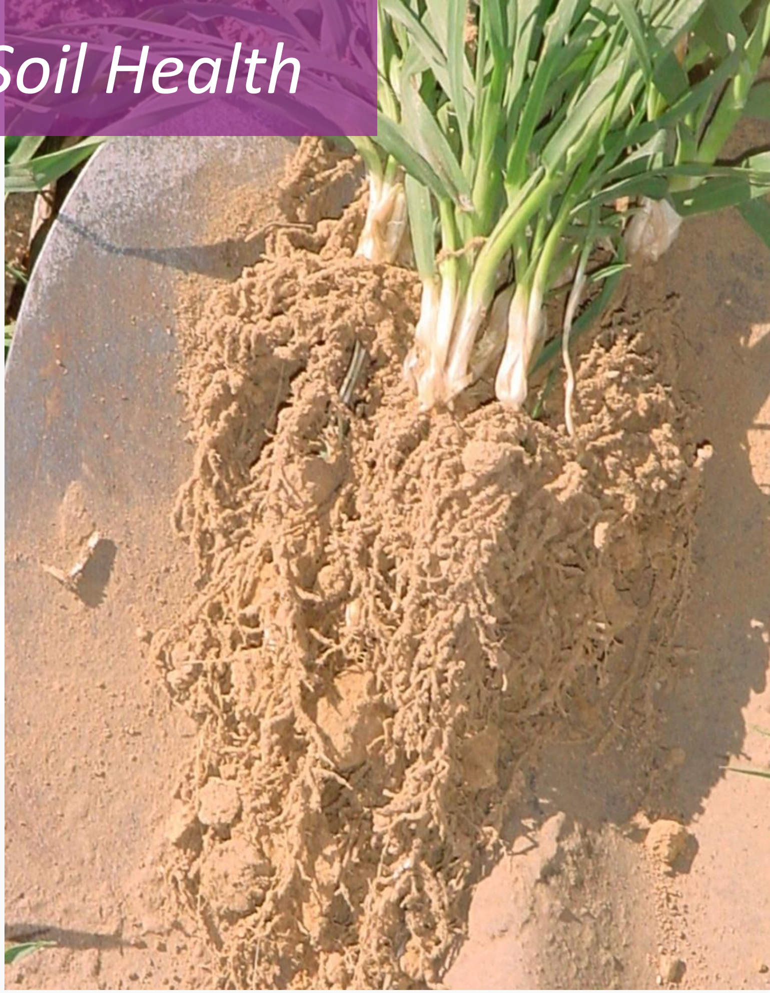
$$CaHPO_4 + H^+ \longrightarrow H_2PO_4 + Ca^{2+}$$

(Not available) → **(Available)**

Mechanisms of P binding varies in different soils so do NOT reduce P applications to TwinN crops



4^{th} Mechanism of Action – Improved Soil Health



Increased root exudates

- Encourage higher populations of beneficial microbes
- Are carbon based building active soil carbon

Can reduce populations of pathogenic microbes

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

Increases root nodulation in legumes

Reduced N application rates

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

4^{th} Mechanism of Action – Improved Soil Health

•Soybean – Boone – US Dept Ag

Treatment	Fusarium root colonisation	Root pseudomonads	Mn-reducing bacteria	Mn-oxidising bacteria	Nodule weight
No herbicide	67.5 a	116.9 a	73.25 a	104.75 a	828 ab
+ Roundup	106.4 b	28.2 b	35.12 a	169.5 b	745 a
+ TwinN + Roundup	64.0 a	80.0 a	56.25 a	101.5 a	866 b
LSD (0.05)	19.2	62.9	41.8	50.0	99

TwinN:

- Decreased *Fusarium* (pathogens) numbers by increasing beneficial soil microflora (root pseudomonads)
- Improved the ratio of Mn-reducing to Mn-oxidising bacteria which improves micronutrient availability
- Increased nodulation
- Reversed the negative effects of Roundup on each of these factors

Carbon Footprint

TwinN has a super low Carbon Footprint

MAB has had the full process of production and transport of TwinN audited by Carbon Associates, Australia.

- TwinN carbon footprint is *1.44 Kg CO_2-e per 1 hectare*
  - Delivered to farm gate
  - Applied longest international transport route during audit
  - Allow 2 kg CO_2-e/ha for application of TwinN

TwinN Total Carbon Footprint applied to crop

3.44kg CO_2-e /ha

By Comparison to Urea

- Urea manufacture accounts for 4.0 kg CO_2-e per kg
- Post-application release of nitrous oxide and other effects brings this to

10 kg CO_2-e per kg urea (Fertiliser Industry Figures)

- Application of **70 kg/ha urea = 700 kg CO_2-e/ha** for full CO_2-e accounting

Carbon Footprint

Enough Urea to Treat 1000 ha applied @ ~70kg/ha

70 tons sent by Road Train

700kg CO_2-e / ha

Enough Twin N™ to Treat 1000 ha applied at 1 vial / 5 ha

200 vials sent by Post

3.44kg CO_2-e / ha

MAB has purchased official carbon offsets for all TwinN sold annually
See website for certificate

TwinN is convenient to apply

Foliar application

- Boom spray – at 100 – 150 L/ha or more
- Aerial – at 30L/ha for larger areas – onto wet foliage
- Backpack – for small producers
  - Must be applied to moist/wet foliage
  - Tank mixing with herbicides, pesticides etc not OK

Soil application

- Centre pivot
- Fertigation or drip irrigation
- Liquid Inject into furrow at sowing

TwinN by aerial spray onto wheat – Lake Grace, Western Australia 2008

Western Minerals Fertiliser (80 kg/ha: 9 units N) + TwinN

Yield av: 3.5 t/ha

Application by air onto a heavy dew

Where do the TwinN microbes act after application?

- **If applied to the foliage** under moist conditions TwinN microbes enter leaves and colonise the leaves, stems and roots as endophytes

- **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**

- Microbes need a vigorous plant to work with. **Don’t starve the crop of N and then apply TwinN too late.**

Fitting TwinN into crop systems

Distributors and agronomists need to consider a number of factors when advising how to fit TwinN into a clients cropping system.

Key point. Most good farmers have a well optimised system and its important not to lower the efficiency of the system while adding TwinN into it.

- Don't cut N at planting and then do a late application of TwinN – the crop falls behind in early establishment and never catches up. If N is applied in splits **keep the at plant application standard and reduce later N rates**
- **Don't make cuts to other nutrients** while cutting N eg MAP, DAP etc
- **Don't modify timing of 2nd and 3rd N applications** to the crop. They are usually optimised for the crop.

TwinN has been tested in many crops in many countries

- On-farm demonstrations
- Independent contracted trials
- University trials
- World bank trials

Go to www.mabiotec.com and download trial results

Trial Results

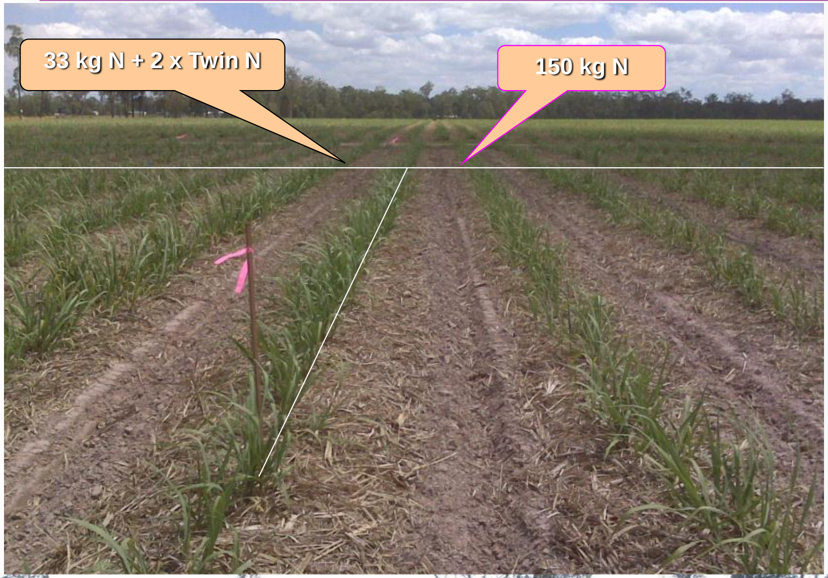
- **Sugar – Maryborough – QLD - Australia - BSES trial – 2008-09**

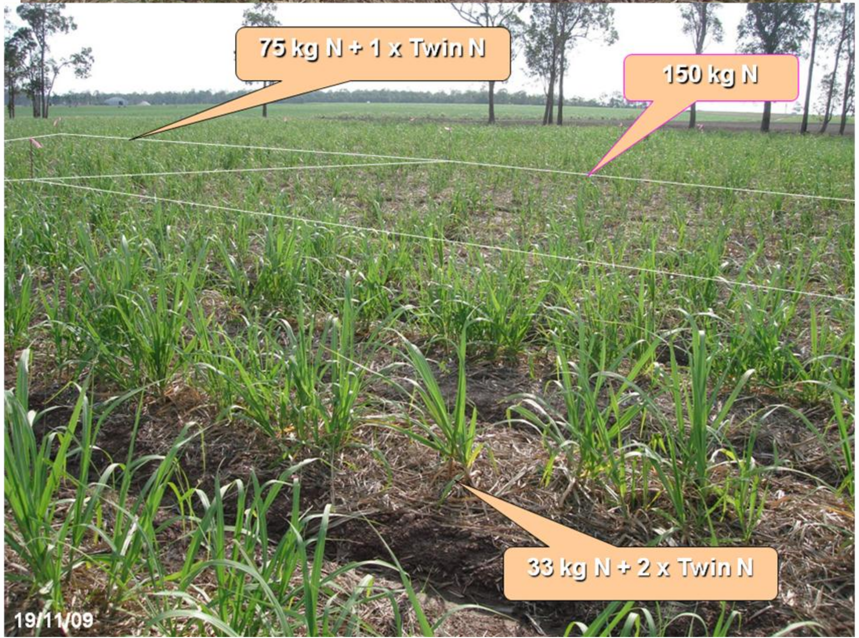
Cane yield TC/ha	
Treatment	TC/ha
75N+1TwN	82.0 A
150N	81.9 A
75N+2TwN	73.8 A
33N+2TwN	58.9 B
LSD p0.01=12.8	

Sugar yield TS/ha	
Treatment	TS/ha
75N+1TwN	13.6 A
150N	13.4 A
75N+2TwN	12.5 A
33N+2TwN	9.9 B
LSD p0.01=2.3	

TwinN enabled the same yield from 50% N plus TwinN as from the usual 100% N fertiliser program

Effects of TwinN on next seasons ratoon growth





Ratoon effects

Treatment	Shoots /m^2
75 kgN + 1 TwN	12.42 A
75 kgN + 2 TwN	11.51 A
33 kgN + 2 TwN	11.14 AB
150 kgN + 0 TwN	8.86 B

Nakambala Sugar Estate , Ilovo Sugar Co, Zambia, 2009

Treatment	TCH (T/ha)	Pol (%)	Total sucrose (T/ha)
1. Standard fertiliser (140 kgN) No TwinN	155	15.4	23.9
2. 50% N (70 kgN/ha) 2 TwinN applications	148	16.3	24.2
3. 65% N (90 kgN/ha) 2 TwinN applications	151	16.3	24.7

- 3 Treatments x 3 reps, RCB design
- Variety N41
- 2009 - 10

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Nakambala Sugar Estate , Ilovo Sugar Co, Zambia, 2010

Treatment		Plant basal dressing (kg/ha)	Urea top dress (kg N/ha)	Total inorganic N (kg/ha)
1. 100% inorganic N Zero TwinN	N	60	80	140
	P	70	0	
	K	100	0	
2. 50% inorganic N + 2 TwinN	N	60	10	70
	P	70	0	
	K	100	0	
3. 65% inorganic N + 2 TwinN	N	60	31	91
	P	70	0	
	K	100	0	

- 3 sites – light, med, heavy soils
- 2 cvs N25, N41
- 3 reps

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Nakambala Sugar Estate , Ilovo Sugar Co, Zambia, 2010

Treatment	Total yield (Tons/ha)	Pol %	Total Pol (Tons/ha)
1. 100% inorganic N - Zero TwinN	119	17.1	20.4
2. 50% inorganic N + 2 TwinN	107	17.2	18.5
3. 65% inorganic N + 2 TwinN	121	16.4	19.8

Treatment	Total yield (Tons/ha)	Pol %	Total Pol (Tons/ha)
1. 100% inorganic N - Zero TwinN	142	13.3	18.8
2. 50% inorganic N + 2 TwinN	137	16.2	22.2
3. 65% inorganic N + 2 TwinN	125	15.2	18.9

Treatment	Total yield (Tons/ha)	Pol %	Total Pol (Tons/ha)
1. 100% inorganic N - Zero TwinN	155	15.4	23.9
2. 50% inorganic N + 2 TwinN	148	16.3	24.2
3. 65% inorganic N + 2 TwinN	151	16.3	24.7

Tambankulu Estate Swaziland, 2010

	T1	T2	T3	T4	T5	T6
TCH	96.28	81.23	87.69	81.73	77.92	78.07
TSH	11.35	12.5	12.64	12.4	12.6	12.54
Suc %	11.78	15.38	14.41	15.17	16.17	16.06

- 4 reps
- 4 rows x 150m
- Grown 10 months

Treat.	1st dress N (kg/ha)	2nd dress N (kg/ha)	% N applied	Treatment combinations
T1	70	70	100%	140N
T2	70	0	50%	70N+TwinN
T3	70	35	75%	105N+TwinN
T4	70	0	50%	70N+CyFlo
T5	70	35	75%	105N+CyFlo
T6	70	0	50%	70N+TwinN+CyFlo

Tambankulu Estate Cost - Benefit

Rate of N kgN/ha	Price of N $/ha	Cost of N + TwinN $/ha	Cost advantage over 100% $/ha	Sugar yield T/ha	Gross revenue $/ha	Revenue advantage over 100% $/ha	Cost advantage + Revenue advantage $/ha
70 (50%)	99	144	53.70	12.5	3700	340	394
105 (75%)	148	193	4.35	12.64	3741	382	386
140 (100%)	197	197	0	11.35	3360	0	0

- Price of N = $1.41/kgN ($650/T urea)
- Sugar price = $296/T
- Price of Twin = $45/ha

Sugarcane, Mandya, Karnataka, India, 2010

- Independent trial conducted at Zonal Agricultural Research Station

Trial design	Randomised complete block, 12 treatments, 3 replicates
Plot size	8 rows x 7m
Cultivar	Co 62175
TwinN applications	1) At 5 leaf stage 2) And 2 months after the first application.

T1 100%N (250 kgN/ha)	T7 25%N + 1 TwinN
T2 100%N +2 TwinN	T8 25%N + 2 TwinN
T3 75%N + 1 TwinN	T9 Zero N + 1 TwinN
T4 75%N + 2 TwinN	T10 Zero N + 2 TwinN
T5 50%N + 1 TwinN	T11 N equivalents through organics + 2 TwinN
T6 50%.N + 2 TwinN	T12 Absolute control (Zero N + standard P & K)

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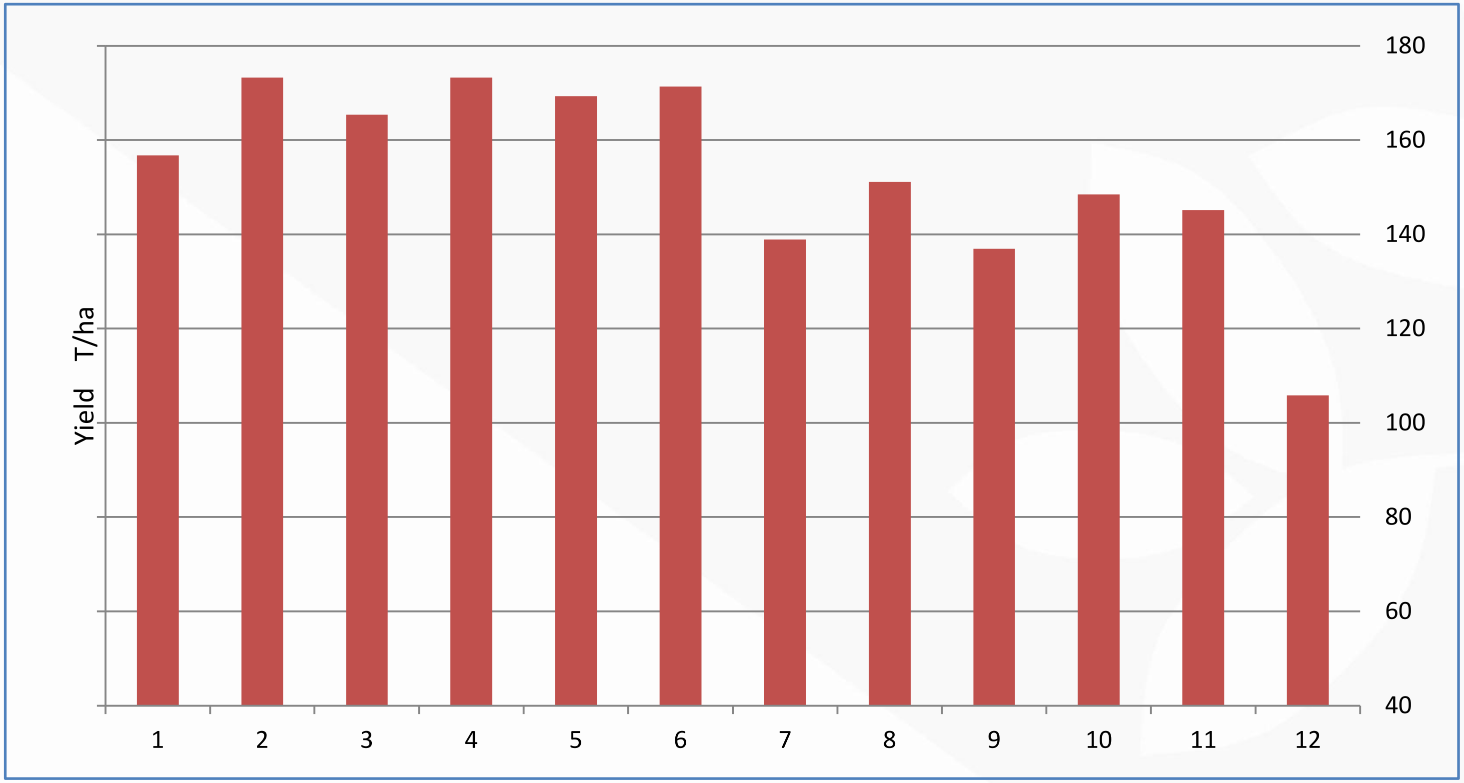
Sugarcane, Mandya, Karnataka, India, 2010



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T5 50%N + 1 TwinN	T11 N equivalents through organics + 2 TwinN
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Conclusions from trials and commercial use

- One application of TwinN enables high yield with reduced N rates

- 50% N gives the same yield as 100% in many situations but is probably too close to the edge of TwinN's performance capacity

- 65% N is a more reliable rate to use to allow for varied farming conditions

- Farmers should use a single application of TwinN plus 75% of standard N for their initial use of TwinN to try the technology
- Apply at 10 – 50 cm plant height
- Keep first fertiliser application standard and reduce N later in the crop cycle

- TwinN will improve soil health and reduced nitrogen applications also improve soil pH etc

- Ensure that the application system delivers TwinN microbes into the moist root zone

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TwinN

YIELD INCREASING MICROBES

Mapleton Agri Biotec Pty Ltd