SUMMARY OF TWO INDEPENDENT CONSECUTIVE TRIALS OF TwinN BIOFERTILISER IN STRAWBERRIES IN CALIFORNIA, 2020 AND 2021

In 2020 Holden Research and Consulting, an independent reseach company, was contracted to perform a formal, replicated and statistically analysed trial of the capacity of TwinN to produce high yields of strawberries when combined with a 20% reduction in nitrogen fertiliser. The trials were located on a commercial strawberry farm in Ventura County, California.

That initial 2020 trial is summarised in the following document, after a report of the recent 2021 repeat of the trial. The 2020 trial produced two key results:

1. Yields of marketable fruit were increased by 11.7% over the season in the TwinN plots compared to the Grower Standard nutrition program
2. Commercial returns to the grower were increased by 13.3% in the TwinN plots compared to the Grower Standard nutrition program

The trial was repeated in 2021 to confirm this result and to refine the method of TwinN delivery to maximise convenience to commercial growers. The 2021 trial produced these results for the two TwinN treatments:

1. Yields of marketable fruit were increased by 9.1% and 10.5% over the season for the two TwinN delivery methods compared to the Grower Standard nutrition program
2. Commercial returns to the grower were increased by 14% and 13.6% over the season for the two TwinN delivery methods compared to the Grower Standard nutrition program

Results of the recent 2021 trial are presented here, followed by those from 2020. Both trials gave excellent results with the increases in both yield and commercial returns very consistent over two years.

Contacts

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TwinN strawberry trial, Ventura County, California

2021

SUMMARY

The trial was located on a commercial strawberry farm near Oxnard, Ventura County, USA. It was performed by Holden Research and Consulting, an independent company. The trial was a follow-up trial after a successful TwinN trial in 2019-20 in the same strawberry production area. Both trials compared yields and commercial returns from the standard N, P and K program versus those obtained from TwinN combined with a 20% reduction in N, P and K. The percentage increases in yields of marketable strawberries and net commercial returns for 2020 and 2021 are summarised in Table 1.

Both the TwinN programs tested in 2021 produced valuable increases in yields and commercial returns compared to the standard grower program.

Table 1. **Increases in marketable yields and commercial returns to growers in TwinN programs compared to the standard grower program in 2020 and 2021.**

	2021 TwinN 1	2021 TwinN 2	2020 TwinN
Marketable yield in flats per acre	9.1% increase	10.3% increase	11.7% increase
Final grower returns/acre	14% increase (+$2973/acre)	13.6% increase (+$3052/acre)	13.3% increase

TwinN is a microbial biofertiliser supplied from Australia and consists of freeze-dried nitrogen fixing microbes. The microbes fix N, solubilise bound P and produce plant growth regulators. TwinN is used to increase yields and reduce synthetic nitrogen fertiliser requirements in crops. TwinN is also used to improve nitrogen nutrition in organic production systems.

TRIAL PROTOCOL

The variety (Wellpick 3.324) utilized for this trial was grown in a commercial block of strawberries. This trial was set up as a completely randomized strip trial of three treatments with completely randomized data collection of six replicates (6 x 4m plots) maintained during the growing season. All treatments received, at planting, controlled release fertilizer and in season applications of N-P-K, along with seasonally necessitated foliar pest control. The pre-plant controlled release fertilizer application was the same across all treatments, but the in-season N-P-K was reduced to 75% of grower standard for the two TwinN programs to achieve a 80% program of inputs when considered with the pre-plant 100% application of controlled release N-P-K.

Note. A standard commercial recommendation would be to reduce only N, retaining P and K at standard levels (see Recommendations on the last page).

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Table 2. **Grower Standard Strawberry Fertility Program for 2021 Winter Production Trials**

Soil Applied	16 weeks of application	Grower Std.	PrePlant CRF 18-8-13		CAN 17	AN 20	7-21-0	KTS 0-0-25
	Weeks after Planting							
10/12/2020	Planting	W1	750 lb=135 lb N, 69 lb P2O5, 97.5 lb K2O					
10/19/2020	Plant Establishment	W2						
10/26/2020	Plant Establishment	W3	rt injections					
11/2/2020	Plant Establishment	W4						
11/9/2020	Plant Establishment	W5			1 gal		1gal	1 gal
11/16/2020	Plant Establishment	W6						
11/23/2020	Plant Establishment	W7			1.5 gal		1 gal	1 gal
11/30/2020	Plant Establishment	W8						
12/7/2020	Plant Establishment	W9			1.5 gal		3gal	4.37 gal
12/14/2020	Plant Establishment	W10						
12/21/2020	First Flower	W11			6 gal		3.17 gal	4 gal
12/28/2020	Fresh Harvest	W12						
1/4/2021	Fresh Harvest	W13			3 gal		2 gal	4 gal
1/11/2021	Fresh Harvest	W14						
1/18/2021	Fresh Harvest	W15			3 gal		2 gal	3 gal
1/25/2021	Fresh Harvest	W16						
2/1/2021	Fresh Harvest	W17			4 gal		2 gal	4 gal
2/8/2021	Fresh Harvest	W18						
2/15/2021	Fresh Harvest	W19			4.35 gal		2.36 gal	3.5 gal
2/22/2021	Fresh Harvest	W20						
3/1/2021	Fresh Harvest	W21			4.35 gal		0 gal	3.5 gal
3/8/2021	Fresh Harvest	W22						
3/15/2021	Fresh Harvest	W23			5.2 gal		2.16 gal	3.5 gal
3/22/2021	Fresh Harvest	W24						
3/29/2021	Fresh Harvest	W25			5.2 gal			3.5 gal
4/5/2021	Fresh Harvest	W26						
4/12/2021	Fresh Harvest	W27			7.15 gal		0 gal	4.2 gal
4/19/2021	Fresh Harvest	W28						
4/26/2021	Fresh Harvest/Possessor	W29			7.15 gal		0 gal	4.2 gal
5/3/2021	Fresh Harvest/Possessor	W30						
5/10/2021	Fresh Harvest/Possessor	W31			7.15 gal		0 gal	4.2 gal

Treatment 1 Grower standard nutrient program: Pre-plant CRF 18-8-13 slow release at 500 lbs/ac (560 kg/ha) plus 73 lb/ac (82.5 kg/ha) N (plus standard P, K etc) applied in season via fertigation

Treatment 2 Grower standard nutrient program: Pre-plant CRF 18-8-13 slow release at 500 lbs/ac (560 kg/ha) plus 55 lb/ac (62.5 kg/ha) N (plus standard P, K etc) applied in season via fertigation. Initial **TwinN** application was done by soaking seedlings roots for 50 minutes in TwinN solution before transplant and subsequent applications were made by via fertigation.

Treatment 3 Grower standard nutrient program: Pre-plant CRF 18-8-13 slow release at 500 lbs/ac (560 kg/ha) plus 55 lb/ac (62.5 kg/ha) N (plus standard P, K etc) applied in season via fertigation. The initial **TwinN** dose was applied by applying TwinN solution into planting holes on the day of transplanting and subsequent applications occurred via fertigation.

TwinN application protocols

In the 2020 trial TwinN was applied as a root drench (15 minutes soak) before transplant. In 2021 TwinN Treatment 2 was a root/plant drench (50 minutes) and in Treatment 3 TwinN was applied via fertigation soon after transplant (October 17, 2020). For both Treatments 2 and 3 TwinN was then applied three more

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times through the season on Nov-11-2020, Dec-21-2020, Feb-4-2021. A fifth application was planned for Mar-17-2021 but the picking season was over so this application was not made. Applications were delivered in 200L/ha water via fertigation. All applications used non-chlorinated water.

Yield assessment

Weekly picks of marketable and unmarketable fruit commenced on 5 Jan 2021 and finalised on 27 March 2021. These were recorded, extrapolated to a per acre rate and presented cumulatively (Chart 1). The commercial returns for both treatments were calculated based on USDA Shipping Point Market Prices found at HTTP:\\marketnews.usda.gov/portal for each pick day (Chart 2).

RESULTS

Early growth parameters

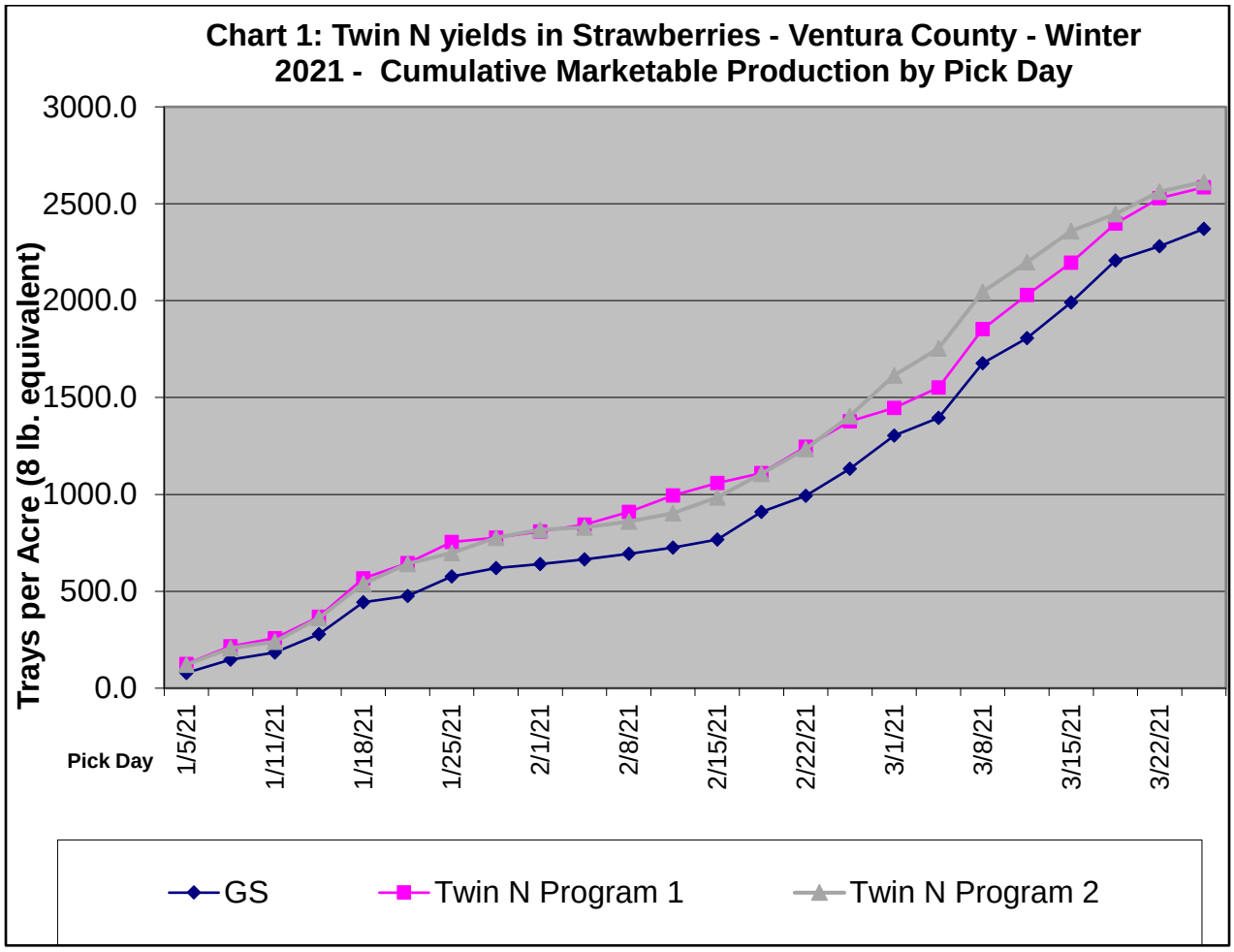
Vigor (rated 1-5) and SPAD measurements (SPAD meters give a relative chlorophyll value) showed no significant differences between the Grower control and the TwinN treatments. Canopy percentages were measured using a Canopeo app from Oklahoma State University. Both TwinN treatments showed a significant increase in canopy development over time compared to the Grower control. This effect was followed by significantly higher early flower development counts in both TwinN treatments compared to the Grower control, with the rating taken in late November, indicating the potential for earlier fruit production. This was confirmed with the first pick analysis on January 5, 2021 with the two TwinN programs showing 56 and 51 percent more fruit by weight over the grower standard on the first pick date (see Chart 1).

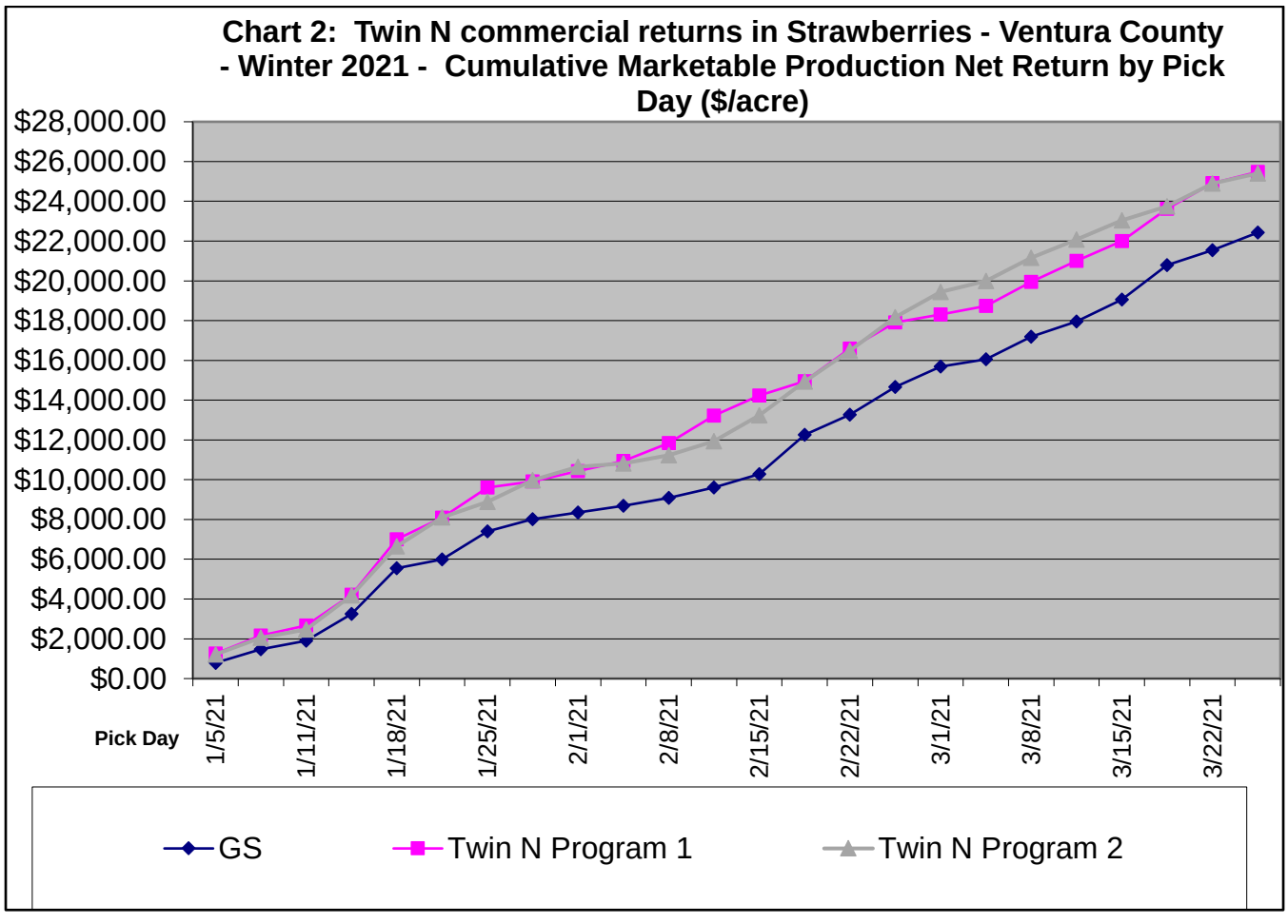
Brix measurements of fruit were taken four times through the season and no significant differences were observed with the exception of one reading date for Treatment 2 which showed increased Brix compared to other treatments.

Yield and commercial returns

Chart 1 shows cumulative yield of trays of marketable fruit through the season commencing with the initial pick on 5 January 2021. Both TwinN treatments produced an improved initial harvest and this effect persisted through the season. TwinN treatments 2 and 3 produced more flats of strawberries during the trial period over the Grower's standard with 2585 and 2613 extrapolated flats per acre respectively, compared to 2370 flats per acre for the Grower standard. These were a 9.3% (Treatment 2) and 10.3% (Treatment 3) increase in cumulative seasons yields compared to the Grower control.

A different perspective of how the increased production from the TwinN treatments affected final grower returns is shown in Chart 2 which show the daily marketable returns based on USDA shipping Point Market Prices found at HTTP:\\marketnews.usda.gov/portal for each pick day. This data was used to calculate net dollar returns to growers for the three treatments after costs of approximately $6.00 per tray were removed that would represent picking labour, carton and tray costs, transportation to the cooler, and cooling costs associated with picking the strawberries (Chart 2). Based on this data TwinN treatments 2 and 3 produced seasonal returns of US$25,480/acre (14% increase) and US$25,401/acre (13.65% increase) versus US$22,428/acre for the Grower control.





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Soil analyses

Soil samples were taken on 16 November 2020 and analysed by A & L Western Agricultural Laboratories. These showed an early season base reading of 35 ppm nitrate-nitrogen across treatments. The next soil samples were taken after the 3rd TwinN application (31 December 2020) and showed moderate amounts of nitrate-nitrogen in the Grower standard (15 ppm) and TwinN Treatment 2 (17 ppm) and high amounts of nitrate-nitrogen in TwinN Treatment 3 (39 ppm). End of season soil samples showed a similar pattern with Grower standard (13 ppm) and TwinN Treatment 2 (5 ppm) having reduced nitrate-nitrogen levels and TwinN Treatment 3 having high amounts of nitrate-nitrogen (43 ppm). There was no obvious explanation for the increased nitrate-nitrogen levels in Treatment 3 at the end of the season. In general, nitrogen fixed by the TwinN microbes is produced either within the plant tissues (endophytes) or very close to the root surface and in either case it is taken up very efficiently by the plants, rather than contribution to any residual soil nitrogen bank.

Leaf Analysis

Leaf samples were taken early season (19 Jan 2021) and late season (9 April 2021) and analysed by A & L Western Agriculture Laboratories (Table 3).

Table 3. Leaf analysis data from Grower Standard and TwinN plots at two sampling dates

	Nitrogen (%)	Phosphorous (ppm)	Potassium (ppm)
Grower Standard 19 Jan 2021	3.57	0.46	1.83
TwinN Treat. 2 19 Jan 2021	3.37	0.45	1.84
TwinN Treat. 3 19 Jan 2021	3.40	0.44	1.81
Grower Standard 9 April 2021	3.41	0.26	1.46
TwinN Treat. 2 9 April 2021	3.16	0.27	1.48
TwinN Treat. 3 9 April 2021	3.18	0.29	1.56

Analysis of the 19 Jan 2021 leaf samples showed similar nitrogen levels and elevated phosphorous and potassium levels in all treatments despite reduced fertiliser applications of all three elements in the TwinN plots. The 9 April 2020 samples showed similar levels of P and K in all treatments and slightly lower nitrogen levels in the TwinN plots levels of all three elements in the TwinN plots. However, the TwinN plots continued to yield higher.

CONCLUSIONS

This independent trial in 2021 reproduced the excellent results recorded in the 2020 trial. The results of both trials showed clearly that four applications of TwinN increased yields of marketable strawberries and produced a very substantial increase in net commercial returns compared to the non-TwinN plots. This was achieved in combination with a 20% reduction in fertiliser inputs. While commercial applications of TwinN in strawberries will not typically be used to reduce P and K rates, the reduced nitrogen applications will reduce the carbon footprint of production and reduce leaching of nitrates into streams, lakes, aquifers and oceans. TwinN is a useful tool for strawberry growers wanting to increase yields and commercial returns and improve sustainability of production.

COMMERCIAL GROWER RECOMMENDATIONS

These are some guidelines for use of TwinN in strawberry production.

- Apply the pre-plant fertiliser at standard rates
- Reduce in-crop nitrogen fertiliser to 75 - 80% of the standard rate
- Make reductions to N as evenly across the season as possible
- Do not cut other nutrients such as P and K
- If TwinN is being applied in organic strawberry crops (TwinN is OMRI certified) do not reduce any organic nutrients. Apply TwinN on top of the organic nutrition program.
- Apply TwinN to the crop via fertigation immediately after transplant to speed early root growth and establishment
- Apply TwinN another four times spreading the five applications evenly across the season
- Apply via fertigation. Be sure to avoid doing applications during hot sunny periods if there is a risk that black plastic irrigation lines can become hot in the direct sun.
- Do not co-apply TwinN microbes with agrochemicals including fertilisers. Avoid applying these for 24hrs before and after the TwinN application.
- Ensure the irrigation lines are free of agrochemicals before TwinN application
- Do not expose the TwinN microbes to chlorinated water during application. Chlorinated water can be used for the crop after TwinN application is complete.
- If only chlorinated waster is available for application of TwinN de-chlorinate with sodium thiosulfate (STS). See www.mabiotec.com for instructions or contact your distributor. For organic methods to de-chlorinate see www.mabiotec.com.

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TwinN strawberry trial, Ventura County, California 2019-20

SUMMARY

The trial was located on a commercial strawberry farm near Oxnard, Ventura County, USA. It was performed by Holden Research and Consulting, an independent company. The trial compared yields and commercial returns from the standard N, P and K program versus those obtained from TwinN combined with a 20% reduction in N, P and K. The TwinN program increased yields of marketable strawberries by 11.7% and increased net commercial returns by 14% compared to the standard nutrition program. Average Brix from four readings through the picking season was statistically significantly improved in ripe fruit from the TwinN plots compared to the non-TwinN plots.

TwinN is a microbial biofertiliser supplied from Australia and consists of freeze-dried nitrogen fixing microbes. The microbes fix N, solubilise bound P and produce plant growth regulators. Twinn is used to increase yields and reduce synthetic nitrogen fertiliser requirements in crops. TwinN is also certified organic with OMRI.

TRIAL PROTOCOL

Trial design: The trial was a Randomised Complete Block design with 6 replicates and two treatments. Replicates consisted of strawberry (Fragaria vesca var. americana, variety Wellpick 3.324) in strip plots 4m x 6m ($24m^2$). Statistical analysis used New Duncan's Multiple Test Range.

Nutrition treatments: The trial compared yields and commercial returns from a standard strawberry nutrition program (Treatment 1, Grower Standard) with those obtained from use of TwinN combined with a reduced fertiliser rate (Treatment 2, TwinN). Both treatments received the standard pre-plant fertiliser application. Grower Standard then received the standard in-crop nutrition program listed below (Nutrition Program)

TwinN plots received the same pre-plant rate as the Grower Standard and 75% of in crop N-P-K nutrition to deliver an overall N-P-K rate of 80% of the Grower Standard (20% reduction of N-P-K). A standard commercial recommendation would be to reduce only N, retaining P and K at standard levels (see Recommendations on the last page).

TwinN was applied to Treatment 2 plots four times during the season as per the Nutrition Program below.

The initial TwinN application was a root drench for 15 minutes prior to transplant. The subsequent applications were delivered via field drip irrigation system in 200 L/ha rate of non-chlorinated water.

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Nutrition Program

Date	Grower Standard (Treatment 1)	TwinN (Treatment 2)
1 Sept 2019	Pre-plant CRF 18-8-13 slow release 500 lbs/ac (560 kg/ha)	Pre-plant CRF 18-8-13 slow release 500 lbs/ac (560 kg/ha)
16 Oct 2019		**TwinN drench prior to transplant**
22 Nov 2019	N as CAN 17 (100% rate)	N as CAN 17 (75% rate)
3 Dec 2019	N as CAN 17 (100% rate)	N as CAN 17 (75% rate). **TwinN applied via fertigation.**
17 Dec 2019	NPK as CAN and DiKap (100% rate)	NPK as CAN and DiKap (75% rate)
31 Dec 2019	NPK as CAN and DiKap 0-31-52 (100% rate)	NPK as CAN and DiKap (75% rate)
10 Jan 2020	N as CAN 17 (100% rate)	N as CAN 17 (75% rate) **TwinN applied via fertigation.**
22 Jan 2020	NPK as CAN and DiKap (100% rate)	NPK as CAN and DiKap (75% rate)
6 Feb 2020	N as CAN 17 (100% rate)	N as CAN 17 (75% rate) **TwinN applied via fertigation.**
19 Feb 2020	N as CAN 17 and KTS 0-0-25 (100% rate)	N as CAN 17 and KTS 0-0-25 (75% rate)
4 March 2020	N as CAN 17 and KTS 0-0-25 (100% rate)	N as CAN 17 and KTS 0-0-25 (75% rate)
20 March 2020	N as CAN 17 and KTS 0-0-25 (100% rate)	N as CAN 17 and KTS 0-0-25 (75% rate)
30 March 2020	N as CAN 17 and KTS 0-0-25 (100% rate)	N as CAN 17 and KTS 0-0-25 (75% rate)
16 April 2020	N as CAN 17 and KTS 0-0-25 (100% rate)	N as CAN 17 and KTS 0-0-25 (75% rate)
30 April 2020	N as CAN 17 and KTS 0-0-25 (100% rate)	N as CAN 17 and KTS 0-0-25 (75% rate)

Yield assessment

Weekly picks of marketable and unmarketable fruit commenced on 8 Jan 2019 and finalised on 16 April 2020. These were recorded, extrapolated to a per acre rate and presented cumulatively (Chart 1). The commercial returns for both treatments were calculated based on USDA Shipping Point Market Prices found at HTTP:\\marketnews.usda.gov/portal for each pick day (Chart 2).

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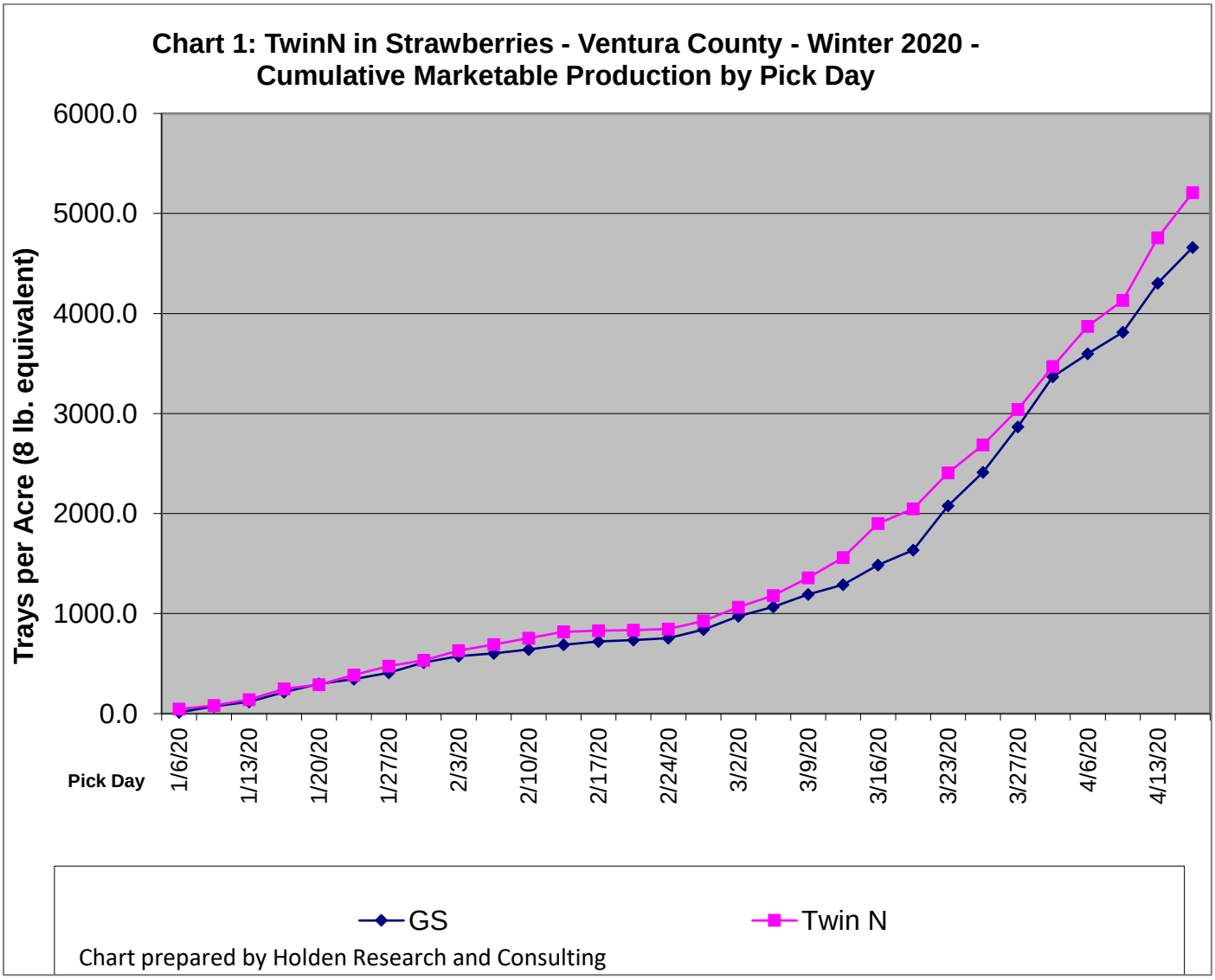
RESULTS

Yield and commercial returns

TwinN (Treatment 2) produced statistically significantly more flats of strawberries during the trial period compared to Grower Standard with 5208 extrapolated flats per acre, compared to 4661 flats per acre for the Grower Standard (Chart 1). That represents an 11.7% increase in yield. This data was used to calculate net dollar returns to growers for the two treatments after costs of approximately $6.00 per tray were removed that would represent picking labour, carton and tray costs, transportation to the cooler, and cooling costs associated with picking the strawberries (Chart 2). Treatment 2 (TwinN) produced an extrapolated cumulative total return of US$36,357/ac (AU$52,163/ha) compared to Treatment 1 (Grower Standard) of US$31,892/ac (AU$45,757/ha). This represents a substantial 14% (US$4,483, AU$6,431) increase in net returns per acre.



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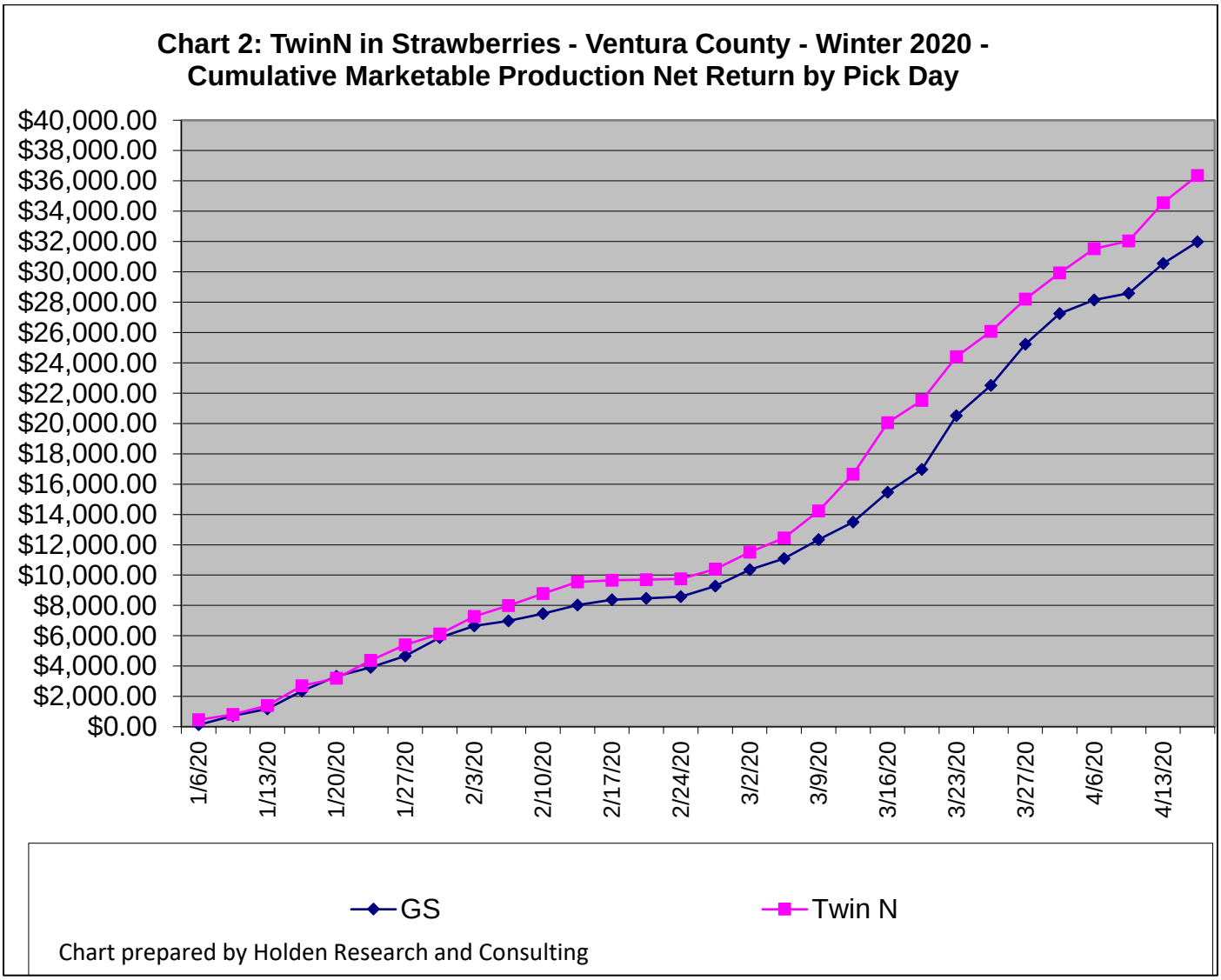

Brix

Brix readings were taken from ripe fruit samples at four dates through the season. Brix readings were consistently improved in fruit from the TwinN plots. Average Brix from the four readings was statistically significantly improved in fruit from the TwinN plots.

Table 1. Brix (°Bx) of fruit sampled at four time points through the season

Sample date (days after transplant)	113	131	145	160	Average Brix
Grower Standard	6.2 b	6.8 a	6.6 a	6.6 b	6.5 b
TwinN	6.5 a	7.0 a	6.8 a	6.8 a	6.8 a

Means followed by different letters (a or b) are statistically different.

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Soil analyses

Soil samples were taken on 14 January 2020 and 28 April 2020 and analysed by A & L Western Agricultural Laboratories.

Table 2. Soil analysis data

Sampled 14 Jan 2020	Nitrogen (NO_2 ppm)	Phosphorous (ppm)	Potassium (ppm)	pH
Grower Standard	6	79	158	8.0
TwinN	13	85	147	8.0
Sampled 28 Apr 2020	Nitrogen (NO_2 ppm)	Phosphorous (ppm)	Potassium (ppm)	pH
Grower Standard	1	19	141	7.2
TwinN	9	25	125	7.3

Nitrate nitrogen was elevated in the TwinN plots compared to the Grower Standard plots, particularly in the second sample date (28 April 2020). This was presumably due to nitrogen supplied by TwinN microbes. Phosphorous levels were higher in the TwinN plots. This was presumed to be due to phosphorous solubilising activity of the TwinN microbes. Potassium levels were lower in the TwinN plots presumably due to the reduced K rates applied.

Leaf Analysis

Leaf samples were taken early season (31 Jan 2020) and late season (28 April 2020) and analysed by A & L Western Agriculture Laboratories (Table 3).

Table 3. Leaf analysis data from Grower Standard and TwinN plots at two sampling dates

	Nitrogen (%)	Phosphorous (ppm)	Potassium (ppm)
Grower Standard 31 Jan 2020	3.02	0.19	1.62
TwinN 31 Jan 2020	2.97	0.43	1.75
Grower Standard 28 April 2020	2.95	0.26	1.46
TwinN 28 April 2020	2.52	0.20	1.18

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Analysis of the 31 Jan 2020 leaf samples showed similar nitrogen levels and elevated phosphorous and potassium levels in both treatments despite reduced fertiliser applications of all three elements in the TwinN plots. The 28 April 2020 samples showed reduced levels of all three elements in the TwinN plots. However, the TwinN plots continued to yield higher. These samples were taken 75 days after the last TwinN application and a commercial recommendation would use another TwinN application to finish the season (see Recommendations on the last page).

Botrytis observation

Anecdotal observation: Rain over 4 days of 2 plus inches from 3/11-3/15. Noted on Pick day 3/16/20 that unmarketable fruit in the grower standard was approximately 90% due to Botrytis infection and only about 10% in the TwinN treated section(s).

CONCLUSIONS

This independent trial showed clearly that four applications of TwinN increased yields of marketable strawberries and produced a very substantial increase in net commercial returns compared to the non-TwinN plots. This was achieved in combination with a 20% reduction in fertiliser inputs. While commercial applications of TwinN in strawberries will not typically be used to reduce P and K rates, the reduced nitrogen applications will reduce the carbon footprint of production, reduce leaching of nitrates into streams, lakes, aquifers and oceans.

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COMMERCIAL GROWER RECOMMENDATIONS

These are some guidelines for use of TwinN in strawberry production.

- Apply the pre-plant fertiliser at standard rates
- Reduce in-crop nitrogen fertiliser to 75 - 80% of the standard rate
- Make reductions to N as evenly across the season as possible
- Do not cut other nutrients such as P and K
- If TwinN is being applied in organic strawberry crops (TwinN is OMRI certified) do not reduce any organic nutrients. Apply TwinN on top of the organic nutrition program.
- Apply TwinN to the crop via fertigation immediately after transplant to speed early root growth and establishment
- Apply TwinN another four times spreading the five applications evenly across the season
- Apply via fertigation. Be sure to avoid doing applications during hot sunny periods if there is a risk that black plastic irrigation lines can become hot in the direct sun.
- Do not co-apply TwinN microbes with agrochemicals including fertilisers. Avoid applying these for at least 24hrs before and after the TwinN application.
- Ensure the irrigation lines are free of agrochemicals before TwinN application
- Do not expose the TwinN microbes to chlorinated water during application. Chlorinated water can be used for the crop after application is complete.
- If only chlorinated waster is available for application of TwinN de-chlorinate with sodium thiosulfate (STS). See www.mabiotec.com for instructions or contact your distributor. For organic methods to de-chlorinate see www.mabiotec.com.

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