

TwinN – Frequently asked questions:

1. What is TwinN and how does TwinN work?

TwinN is a mix of free-living, nitrogen-fixing endophytes that are capable of fixing atmospheric nitrogen both within the plant and around the root zone. Nitrogen-fixing bacteria are single celled organisms that essentially turn nitrogen gas from the atmosphere into plant available amines and ammonium, via a specific and unique enzyme they possess called nitrogenase. Sprayed onto the leaf surface or root zone, **TwinN** microbes are effectively inoculated into the plant via stomata, leaf abrasions and lateral root cracks, diluting and multiplying through the plant via the vascular system.

2. Nitrogen fixation has been known about and studied for many years but in the end, inconsistent results have prevented farmers from risking money and time with such products. What is different with TwinN today?

Nitrogen fixation is a numbers game with each microbe colony acting like a tiny urea factory. The secret is to get sufficient colony forming units (CFU) onto and into the plant to generate a commercial amount of nitrogen. With recent advances and decreased costs in freeze drying and related technologies, we are able to have a synergistic mix of microbes in a single vial and consistent CFU dilution rates through a short rehydration step. A second most distinguishing feature of **TwinN** is the foliage application and inoculation of the plant via the stomata, reducing the impact of variable soils and soil conditions.

3. How many units of nitrogen can I expect from a TwinN application?

Though we have observed crops treated with **TwinN** yielding equivalent to commercial chemical nitrogen applications of 30 to 150 units, **TwinN** applications work more on a percentage of nitrogen replaced rather than the standard unit. This is because of the prolonged uptake and working period of fixed nitrogen,

and the fact that the plant controls the amount of nitrogen supplied and utilized as demand varies within the growth cycle. As a general start rule, aim to replace 50-60% of your current chemical nitrogen application with **TwinN**. Application of this chemical nitrogen in the seed bed allows a foliar application of **TwinN** time to establish within the plant and no early nitrogen stress.

4. Is TwinN organic? What crops are suitable for TwinN application?

Yes, **TwinN** is certified organic by a number of regulators such as NASSA, IFOAM and the Soil Association. It is an invaluable nitrogen source in organic farming. It can be sprayed on all crops, in both organic and conventional agriculture. This includes legumes where **TwinN** stimulates nodulation, monocots (grasses), dicots (broad leaved crops), vegetables, flowers, vines, fruiting trees, tea, coffee and forestry.

5. How will the TwinN microbes affect my soils and can there be detrimental effects to native microbe populations?

TwinN is expected to have a positive impact on soils. The microbes in **TwinN** are found in all five continents of the world, and though applied in a concentrated form to enable commercial nitrogen fixation, do not out-compete native microbes. They are mainly endophytes and live within the plant vascular system or close to the root zone, populations dying back once the host plant is harvested or removed. Synergistic effects have been observed with applications of other beneficial microbes such as Trichoderma and AMF and plants grown in healthy soils, rich in organic matter (being an advantage but not a necessity), with the correct mineral and trace element balance and microbe density thrive on **TwinN** applications.

6. Can I expect any residual carryover to my next crop, especially where I have a rapid rotation?

Maybe. In general we recommend a new application of **TwinN** per crop to ensure commercial nitrogen availability but in certain soils **TwinN** can improve carryover soil nitrogen levels.

7. How should I store TwinN and what is its shelf life?

In its original container, **TwinN** can be stored at room temperature, out of the sun for up to 18 months. This stability is achieved by the total removal of moisture from the vial by the patented freeze-drying process.

8. Please carefully explain the re-hydration process, why I should leave it to stand for three hours before spraying and how long can I store the product after re-hydration?

Add 100mls of (rain) clean water to the rehydration vial and dissolve the food source. Open the sealed **TwinN** vial and use this rehydrate water to dissolve the freeze dried material, emptying all the vial contents into the rehydration container. Replace lid onto container. Leave to stand at room temperature for 3 to 4 hours, swirling occasionally to help disperse the clumps. If you are spraying early in the morning, rehydrate the freeze-dried **TwinN** the night before. If you are spraying in the evening rehydrate at 1pm that day.

Remember, the application and efficacy of **TwinN** is based on the known number of microbes per dilution. The 3-4 hours is roughly the time needed to disperse and break the freeze dried clumps but is short enough to prevent any external microbe contamination competing with, and altering the formulation composition. Knowing the number of viable CFU's per ml of rehydrate, enables accurate dilution rates in the spray tank and thus consistent inoculation of the plant.

Once re-hydrated and activated TwinN should be used within 12 hours. The unused portion of the rehydrate can be stored in a fridge at +/- 4 deg. C for a further 30 days.

9. Should I use chlorinated or distilled water for re-hydration?

NO. Use non-chlorinated clean water, such as rain water. Distilled water can rupture microbe cell walls and should also be avoided.

10. How should I apply TwinN?

In water, by whatever conventional sprayer, spray system or irrigation system at hand. Apply as a foliar sprayed top dressing to the leaves of the crop or via drip irrigation to the root zone.

11. Does it matter what equipment I use, what nozzle or what pressure I should apply at?

Do not use hydrostatic sprayers. Avoid 'T' nozzles where a jet of water is sprayed onto a plate that disperses and breaks the jet into fine droplets. Other nozzles tested, up to a pressure of 45 psi, have no detrimental effect on the predicted CFU test count before and after the nozzle.

However, as plant inoculation requires water on the leaf surface we recommend larger droplets that can be achieved by using coarse to medium nozzles or air-induction nozzles. Remove any fine filters, especially those inside the nozzle barrel.

12. Are factors such as pH, water volume, water quality important?

Yes. The PH of spray water should be in the range of pH 4.5 to 8.5. **TwinN** has been successfully sprayed by air in 30 litres of water per hectare and by boom sprayer in 600 litres of water per hectare, but the ideal is between 50 to 150 litres

water per hectare. In drier conditions it is advisable to use higher water volumes to try and keep the leaf wet for as long as possible.

Avoid high microbe contaminated water as microbial competition in the spray tank will affect the predicted CFU application rate of the **TwinN** microbes.

13. What should I do if I only have access to chlorinated water?

1Kg of sugar per 1,000 litres of water, dissolved in the spray tank will help neutralize the chlorine.

14. What should I do if my spray water is suspected of being contaminated with a high load of organic matter, soil or microbes?

If possible filter or allow to settle before adding to the spray tank. Where microbe contamination is suspected decrease the spray water volume to reduce the overall competing population.

15. Can I mix TwinN with other products, chemicals, fertilizers or micronutrient blends?

Compatibility trials are ongoing and will be communicated shortly. Current indications are that **TwinN** is compatible with most organic products such as Kelp, Humic and Fulvic acids, liquid fish etc., and with micro-nutrient top dressings, including Boron, when applied at low concentrations. **TwinN** is not compatible with high rates of copper.

In any tank mix always add **TwinN** last and spray immediately.

Until compatibilities are established it is recommended that **TwinN** be applied alone and not tank mixed with herbicides, insecticides or fungicides. To avoid detrimental residues on the leaf it is also advisable to spray **TwinN** a few days before, rather than a few days after a pesticide treatment. Stick to the motto; "Make **TwinN** your first spray."

16. Why is the time of day important to the TwinN application?

Early morning is the best time to spray **TwinN**.

Open stomata, dew on the leaves and sugars carried upwards through transpiration and on the leaf surface all help inoculation. Low damaging UV light and evaporation levels give the microbes time to gain a foothold. Like any disease, **TwinN** microbes thrive in warm, humid conditions that aid multiplication and movement. Hot, dry, midday sun and wind (drift and evaporation) dry the leaf surface quickly, close stomata and hinder inoculation.

The next best time to spray is late evening targeting the night dew for CFU survival and inoculation at stomata opening the next morning. (For CAM plants like pineapples, that open their stomata at night, spray in the evening.)

17. If I do not get optimum spray conditions and growing weather should I apply TwinN?

If your crop is distressed from drought, disease or cold (frost) do not apply **TwinN**. **TwinN** struggles in a diseased crop. Inoculation levels will be low and yields disappointing.

18. If I successfully apply TwinN and then get a dry stress period, can I expect TwinN to still help yield generation?

Yes. Once established in and on the plant, **TwinN** microbes are hardy. Nitrogen-fixing bacteria use plant carbon as a high calorie energy source to fuel the biological reaction that converts N₂ gas into plant available nitrogen compounds. Whether it be the soil dwelling species or the endophyte nitrogen-fixing species, the plant is controlling the amount of energy (plant carbon) the nitrogen-fixing bacteria receive to perform their nitrogen fixation function. As such the quantity of

nitrogen being fixed for plant use is controlled by the plant itself.

For example, when there is limited soil moisture nitrogen fixation slows down, dictated by the plants diminishing nitrogen requirements and subsequent frugal supply of carbon to the bacteria.

When soil conditions are optimum, nitrogen fixation is maximized by the plant's increasing supply of carbon to the bacterial colony, in turn increasing nitrogen fixation to meet the increasing nitrogen requirements. It works like a feedback system and assures the plant receives just the right amount of nitrogen it requires based on the growing conditions at the time.

This is in stark contrast to physically applying nitrogen products where the grower must guess how much nitrogen to apply at the start of the season. If we get this wrong we can oversupply nitrogen in a dry year, dramatically reducing water use efficiency and plant health, 'cooking' the crop. In a wet year we may not be applying enough to capitalize on the good moisture conditions and subsequent nitrogen requirement. Nitrogen fixation solves this problem via this natural feedback system

19. At what growth stage should I apply TwinN on my crop?

This depends on the crop but in general apply early in the crop cycle.

Actively growing, young, healthy plants respond well to **TwinN**. Inoculation and multiplication of the microbes to commercial levels of nitrogen can take about two weeks, so apply earlier than you would normally apply a chemical nitrogen top dressing. Microbe colonies are established at the point of entry and then dilute down and up through the plant, highest concentrations developing at the point of entry and in the growing tips where most energy and sugars are formed. The

colonies thus grow with the plant in a win/win situation.

Make **TwinN** your first spray in annual crops, applying when there is enough leaf surface. On potatoes apply at tuber formation.

20. How often do I need to apply TwinN?

Again this varies per crop and potential yield, but as a guideline apply as many times as you would a conventional nitrogen top dressing. A second application maybe required in advance of the normal chemical nitrogen treatment when crop growth outstrips microbe multiplication within the vascular system, such as rapidly growing sugar cane.

Examples of applications for different crops:

- Small grain cereals - apply once, early tillering.
- Nitrogen hungry crops like maize and cotton - apply twice, at 15cms and at flowering.
- Rapidly growing, nitrogen hungry crops like sugar cane - look at partially substituting chemical nitrogen. Apply nitrogen early and **TwinN** 30-60 days later.
- Tree crops, fruiting or leaf (tea) - apply two to three times per year. At bud set, two months later to improve fruit size and quality, and post harvest to set up the tree for the following season's crop.