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Biological Farming Inputs – A practical guide to on-farm testing

Biological inputs include a wide range of products aimed at supporting soil fertility, biological activity, and plant growth. They include microbial inocula, biostimulants that promote favourable microbial populations and plant growth, composts and compost teas, manures, and biochars. These inputs are often used with the broad aim of reducing the use of traditional chemical fertilisers and agro-chemicals, but they may also form the back-bone of organic farming systems.

Overview

As with many inputs that may be used either in conventional or alternative farming practices, there is a need to examine the extent to which a product of interest may be effective in a particular situation. Results obtained from all farming inputs can differ greatly depending upon soil and climatic constraints at a given site, and this is no different for biological inputs. Indeed, results may be different in different areas of the same property, or even different areas of the same paddock, with variability between seasons also likely.

The first aspect to consider when deciding which products to trial is to understand what factors constrain production in the area in which the product will be trialled, and whether these are similar across the property. Some climate constraints such as drought susceptibility may be relatively widespread, whereas others such as waterlogging and frost may be very localised. Likewise, soil constraints can vary from local constraints such as shallow soil in some areas of a property, to more widespread issues such as excess acidity or alkalinity.

Seasonality and timing in relation to crop rotation may also affect results observed. For example, in an unexpectedly good rainfall year, a product that claims to help improve drought tolerance may have no observed effect, even though in a dry year it might have been effective. It is also known that soils can respond differently at different stages in a rotation e.g. after a legume or a break crop. Consequently, it is important to understand that, while the result observed in one year may either indicate that the product is effective (or not effective), it is quite likely that under different circumstances a different result may be observed.

Finally, even if rotations and climate were to remain constant, many products may have different results in the years after first application, even if the product is not reapplied. Long-lived products such as biochars may have long-term effects on soil physico-chemical properties because slow interactions occur between the biochar and the mineral soil. Other products aimed at manipulating the soil biological community may require several years of re-application before significant results are observed. Thus nature of the biological product being applied is of considerable importance.

Types of biological farming input

Biological inputs include a wide range of products aimed at supporting soil fertility, biological activity, and plant growth. They include inocula (such as rhizobium), biostimulants that promote favourable microbes and plant growth, composts and compost teas, manures, and biochars. These inputs are often used with the broad aim of reducing the use of traditional chemical fertilisers and agro-chemicals, but they may also form the back-bone of organic farming systems. Many inocula, biostimulants, and other less bulky biological inputs such as humates, are available nationally, either supplied direct from manufacturers or importers, or sold through general agricultural suppliers. Other products such as composts, manures and other bulky amendments may only be available locally and may vary considerably in their properties between regions and from batch to batch. Due to their bulk, and the requirements for large addition rates (e.g. tonnes per ha), transport costs can be a major factor in limiting their adoption, especially on properties situated long distances from sources of these amendments.

Many biological inputs are rich in organic carbon and nutrients and/or contain living organisms. Furthermore, the benefits of increasing organic matter to maintain soil function are widely recognised, and often promoted as an important co-benefit of using biological inputs, especially in soils that are low in organic carbon. However, these benefits may be difficult to quantify, especially if they occur over a long period of time.

Which product should I test?

Different types of amendments can affect different aspects of plant growth and yield under different conditions. The particular constraints or stresses that affect plant growth and yield will determine which products could be considered for testing. It is equally important to realise that if plants are not stressed, then these products may have little beneficial effect, especially in the short term. A good analogy is with the use of mineral fertilisers: if the soil is already chemically fertile, addition of excess mineral fertilisers will have limited effect on plant growth or yield. Indeed, too much mineral fertiliser may be detrimental. However, because export of agricultural products through harvests also removes plant nutrients, a lack of mineral fertiliser use will lead to degradation of chemical fertility in the long-term.

Growers should consider if they are looking for short-term or long-term effects. For example, if plant available water is the stress of concern, addition of manures may have little effect in years with good rain (short-term effect) but may alter soil physical properties such as water holding capacity leading to benefits in crop growth and yield in later years if rainfall is low or infrequent (long-term effect).

The following sections and table present different stresses and indicate the types of biological amendments that may be worth testing.

Common yield-limiting stresses

Water related stresses

Stresses such as heat, frost, drought, salinity, and low plant available water content (PAWC) all affect the plant’s water relations. Biological stimulants, such as amino acids, humic substances and seaweed extracts may be of some benefit by altering plant physiological responses to water stress making them more resilient to low water availability, or in the case of frost, providing resistance. As these are physiological responses, they are probably only ever short-term and the amendments may need to be applied each season and possibly more than once.

Low plant available water content should be considered differently to drought as it is an enduring soil characteristic whereas drought tends to happen over fixed time periods that are interspersed with periods of sufficient rainfall. Low plant available water content is associated with soil characteristics such as coarse texture, low levels of organic matter, and hydrophobicity; thus any amendments that can alter these characteristics may alter plant available water capacity. Organic amendments such as animal manures, green manures, composted amendments, compost teas and biochars, could be expected to increase soil organic matter and alter soil physical properties, both of which may increase plant available water content. These amendments may have long term effects. Plant available water content may also be increased with the addition of chitosan oligosaccharides, but it is uncertain how long such an effect will last. These compounds can be found in many commercially available biostimulants. Of course, the response will depend on the amount of these amendments added and over what time frame. On a negative side, such a build-up in plant available water content near the soil surface will mean less infiltration to deeper depths, possibly putting that surface moisture at greater risk of loss through evaporation.

Trials with these amendments should be conducted over several seasons for two reasons:

- It is likely to take some time for these amendments to build organic matter or change physical properties (depending on how much is added and how frequently), and
- They may only be effective in a season that is unusually dry or receives infrequent rainfall.

Root diseases

Some root diseases may be suppressed by chitosans and oligosaccharides. It is believed that these compounds elicit a 'disease response'. Chitosans and oligosaccharides are thought to induce disease resistance mechanisms within the plant, or exhibit a direct antimicrobial effect on the growth and development of many pathogens; which would be a plant's normal response to a disease pressure. Compost teas and seaweed extracts have also been claimed to reduce root diseases. Similarly, inoculation of soil with 'beneficial' microorganisms may also suppress disease. For many of these, the response is likely to be short-term unless the soil conditions are conducive to maintaining populations of beneficial microorganisms. It should be noted that soils may already contain many such 'beneficial' microorganisms and some products may encourage their growth.

When testing products for treating root diseases the following should be considered:

- Reapplication over a number of seasons may be required.
- For a continued benefit, it is important to maintain soil organic matter levels.
- Responses may be seasonally dependent and affected by soil type and farming practice.

Soil biological stresses

Low soil biological activity can impede productivity in a number of ways. A lack of a 'healthy' microbiological population may make roots more susceptible to disease; a lack of suitable mycorrhizal fungi may mean that P supply is limiting growth; a lack of rhizobia may mean that legumes cannot effectively fix nitrogen. These issues are commonly underpinned by low soil organic matter and may respond to additions of various manures, composts and biochars in a manner similar to low plant available water content. Increasing soil organic matter helps sustain healthy biological activity by providing a carbon and nutrient source for a microbiological population. Soil biological activity is responsible for mineralising nutrients in soil organic matter and making it available to plant roots. Soils with low mineralisable N fall into this category and may respond to various manures and composts depending on the carbon to nitrogen ratio and the rate of organic matter mineralisation. Another approach to improving soil N content is the use of legumes in combination with rhizobial inoculants. Under healthy soil conditions, for example, legumes may respond to direct addition of appropriate rhizobial inoculants and fix nitrogen.

In contrast to rhizobia, application of inoculant mycorrhizal fungi is likely to be less effective than implementation of management practices, such as optimising use of N and P fertilisers or inclusion of pastures in rotations, for increasing colonisation of roots by these symbiotic fungi. Plants with well developed mycorrhizas may have better uptake of N, Zn and water, where these are limiting factors, than would plants with little colonisation of roots by these fungi. Overall, maintaining a good balance of soil organic matter will aid in maintaining healthy soil microbial populations, including mycorrhizal fungi, free-living N-fixers and organisms that have potential to suppress disease.

Biochar, although quite resilient to decomposition, can sometimes provide an improved microclimate and microhabitat for microbiological communities.

When testing products for relieving biological stressors the following should be considered:

- Some trials may respond in the short-term (e.g. improved growth due to rhizobial inoculants)

- Some trials may respond in the longer-term (e.g. build up of a healthy microbial communities)
- Some trials may be seasonally-dependent (e.g. suppression of root diseases)

Soil chemistry stresses

The common soil chemical stresses include low nutrient availability, high nutrient fixation, low or high pH, high salinity, and high sodicity.

Chemical stresses such as low N, K and P as well as micronutrient deficiencies may also be mitigated by organic amendments such as manures and composts as a result of nutrients contained in these products. The degree of response will depend on the amount of amendment added, and over what time frame.

Other chemical stresses such as low pH, low pH buffering capacity (pHBC) and low cation exchange capacity (CEC) also may respond to manures and composts. Increasing pHBC reduces the susceptibility of a soil to pH change and increasing CEC reduces the risk of cation nutrients loss. Addition of composts provide a liming effect and the organic matter provides CEC which also increases pHBC. In addition, these chemical stresses may respond to biochars and humic substances.

Salinity and sodicity may also respond to manures and composts through increased CEC and competing nutrients, especially Ca, Mg, and K. Soils with high P sorption, and thus likely low plant available P, may also respond to increasing the activities of microorganisms which have the capacity to solubilize and facilitate uptake of sorbed P.

Field trials to mitigate soil chemical stresses:

- will usually respond in the short-term (e.g. through addition of nutrients, increase in CEC).
- should be conducted over longer periods of time to judge the benefit in ‘good’ years and ‘poor’ years, and in order to test for the effect of repeated application.

Soil physical stresses

Soil physical characteristics may cause plant stresses in a number of ways. In heavy clay soils, poor physical structure can restrict water and air movement, while in coarse textured soils water retention may be limited due to rapid drainage. Both of these conditions may be ameliorated through addition of organic amendments such as animal manures, green manures, composted amendments, compost teas, and biochars. High soil strength (possibly through compaction) which can cause stress through restriction of root growth and low infiltration rates, may also be ameliorated with manures, composts and biochars. Aggregate stability is another physical characteristic that can affect plant productivity. Where aggregate stability is poor, soil dispersion may cause plant stress and also contribute to erosion. Manures and composts and some microbial inocula may result in a better structured soil. Furthermore, humic substances and chitosan oligosaccharides have reportedly improved aggregate stability.

When testing products for relieving physical stressors the following should be considered:

- In most cases, amelioration of soil physical stresses will require long-term trials.
- To be effective in the long-term, soil organic matter levels will need to be maintained. This will most likely require repeated applications of manures and composts.

Table 1: Summary of major biological input groups and their modes of action

	Mode of action (MoA)	Humic substances	Hydrolysates & AAs	Seaweed derived	Chitosan	Animal manures	Green manures	Composts	Vermicomposts	Teas / brews	Wood-based biochar	Manure-based biochar
	DIRECT NUTRIENT VALUE											
	macro			✓		✓	✓	✓	✓			✓
	micro			✓		✓		✓				✓
	PLANT PHYSIOLOGICAL RESPONSES											
	signal molecules / hormones	✓	✓	✓	✓				✓	✓		
	nutrient uptake / use efficiency / metabolism	✓	✓	✓								
	response to drought, salinity, cold stress: osmoregulation	✓	✓	✓			✓		✓			
	heat / temperature stress: membrane stability											
	plant disease responses			✓	✓	✓	✓	✓	✓	✓		
	SOIL QUALITY											
Physical	structure and stability					✓	✓	✓				
	bulk density and porosity					✓	✓	✓			✓	
	hydraulic properties					✓		✓				
Chemical	pH buffering					✓	✓	✓				✓
	cation exchange capacity					✓	✓	✓			✓	✓
	chelation	✓		✓		✓	✓	✓				✓
Biological	carbon/energy supply		✓			✓	✓	✓	✓			
	nutrient cycling	✓				✓	✓	✓	✓		✓	✓
	disease supression	✓				✓	✓	✓	✓	✓	✓	
	resistance and resilience	✓				✓	✓	✓	✓	✓	✓	
Dominant FORM	*S = solid; G = granule/prill; L = liquid*	*SGL*	*L*	*L*	*L*	*S*	*S*	*S*	*S*	*L*	*S*	*S*
APPLICATION	*L = Land/soil; F = foliar spray; S = seed;*	*LFS*	*FS*	*LFS*	*FS*	*L*	*L*	*L*	*L*	*LFS*	*L*	*L*

Testing biological inputs on-farm

As discussed previously, there is a diverse range of biological farming inputs available for use in Australia. These come in a variety of forms, from concentrated liquids to bulk organics. Each type of product will have its own requirements for handling, storage, and application, and these considerations may also have an implication on costs of use by the end user. Some of these considerations are provided as examples below:

1. Biological inocula: These are generally applied in low quantities, but may require refrigerated storage and brewing prior to use. They may require application at different timings to other inputs, and may not be suitable for tank mixing with other inputs, especially pesticides. They may be applied by liquid injection or by spray, but often require the soil to be moist prior to application.
2. Biostimulants and liquid alternative fertilisers: These are generally applied in relatively low quantities as liquids, and can often be tank-mixed with other inputs, though care needs to be taken to observe incompatibilities that may be listed in the application notes. Application may be by liquid injection or spray, though required timings may differ from other inputs, and repeat applications may be necessary.
3. Pelletised amendments: These can include humates and other pelletised organic amendments such as some products of biochars, manures, compost, etc. As these products are pelletised, they are generally stable so that storage conditions are not as critical as those of biological inocula. Many of these products are designed to be compatible with drill / air seeders to allow application in-furrow. Others may be surface spread prior to seeding.
4. Bulk organic amendments: These can include composts, manures, biosolids and biochars, and are generally applied at high rates to the surface of the soil prior to seeding. Due to their bulky nature and high application rates (generally in the t/ha range), costs of transport and application may be high, especially where large areas need to be covered. For non-stabilised inputs such as manures, odours both during storage or after application may be problematic.

Precision agriculture approaches

Precision agriculture (PA) in this context refers to the use of tools such as global positioning systems (GPS) attached to seeders and headers to allow accurate mapping of input application rates and yield responses. These techniques can be particularly useful to understand within-paddock variability due to changes in topography, soil type, water relations, etc. By combining PA with variable rate technology (VRT), growers can manipulate rates of input based upon previous knowledge of crop performance in a particular area of a paddock. Because VRT allows growers to accurately map areas to which more or less input should be applied and the resultant yield measured, it can also be very useful for designing and interpreting highly replicated on-farm experiments. The GRDC has produced a wealth of information on the use of VRT in both general agricultural practice, and in conducting on-farm experiments, and this can be accessed from: http://www.grdc.com.au/Resources/PALinks.

Whole paddock testing

Though the use of plot-scale seeders and headers allows the testing of potentially a large number of treatments, when conducting experiments using farm equipment at the paddock scale, simplicity in design is paramount. For the purposes of this guide, we will be considering the testing of a single treatment, and the designs below are based upon the use of normal agricultural machinery with only limited location positioning information available.

Experimental designs

There are many experimental approaches that can be taken when conducting on-farm testing of different treatments, varieties, or farming practices. By and large, these are just as applicable for the testing of biological farming inputs as they are for any other treatment (e.g. lime, gypsum, micronutrients, etc.). Here, we focus on strip testing as this is the simplest design by which to obtain valid statistical power to assess whether changes in yield or grain quality are significant. Other experimental designs are possible, especially when combined with the use of VRT technology, but what is presented here is the simplest means of conducting a robust on-farm field experiment with basic apparatus.

Often when testing products on-farm, growers may take a split-paddock approach where the product is applied to one part of a paddock, and no product is applied to the other half. This is not a suitable experimental design unless the paddock is absolutely uniform, and this approach should be discouraged.

In-field variation

Because soils, and ultimately therefore crop performance, can be highly variable across even a seemingly flat paddock, experimental designs must take into account this variation in order to avoid missing a real effect. A poorly designed experiment may indicate that a treatment has had an effect when in fact the result is just due to underlying variation, which might lead to investment in product that in actual fact had little effect on yield.

In Figure 1, three example paddocks are presented with increasing yield gradients across them. While a north-south gradient such as this may be simplistic in the real world, it allows a clear demonstration of why strong experimental design is needed to avoid drawing false conclusions from your experiment. More complex variability such as that commonly seen on the ground might further reduce reliability.

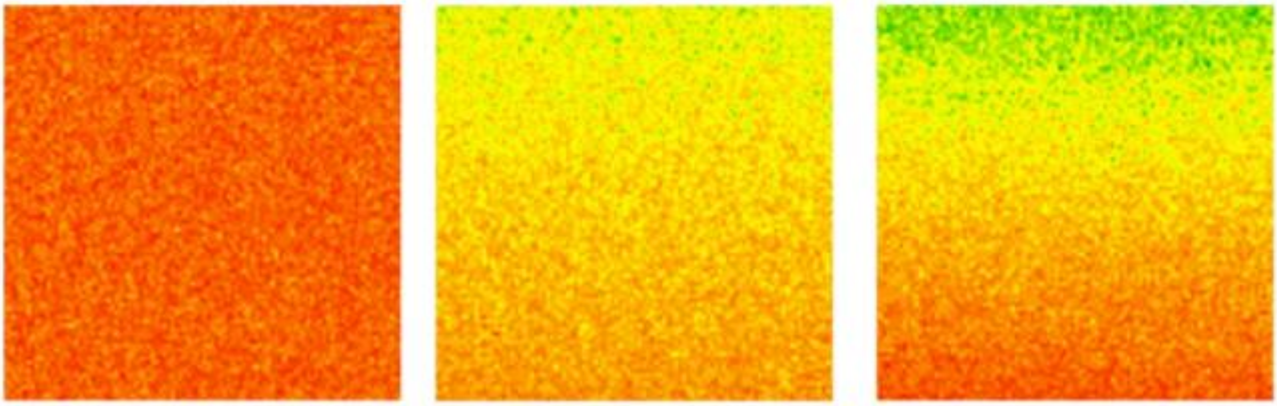

Figure 1: Three example paddocks, L-R: one with no gradient, one with a 1 t/ha gradient from the bottom row to the top row, and one with a 2 t/ha gradient from the bottom row to the top row. Reds indicate low yield, ranging through to greens indicating higher yield.

Focussing on the paddock with the strong gradient, Figure 2 shows the contrasting conclusions that could be drawn if a product is only applied to half a paddock in a test. First, in the left hand panel, if the product actually had no effect, but was applied to the top half of the paddock, an increase in yield of 1 t/ha would be incorrectly attributed to the product (the bottom half of the paddock has an average yield of 3 t/ha, the top half of the paddock has an average yield of 4 t/ha).

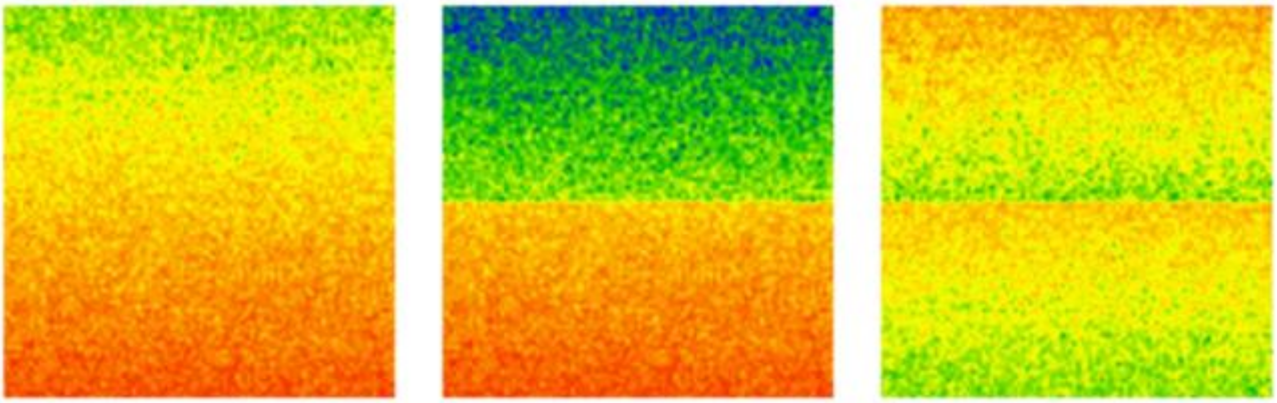

Figure 2: Left - Paddock with high gradient and no treatment; Centre – Paddock with high gradient, and a 20% increase in yield as a result of a treatment on the higher-yielding side of the paddock; Right – Paddock with high gradient, and a 20% increase in yield as a result of a treatment on the lower-yielding side of the paddock. Reds indicate low yield, ranging through to greens and then blues indicating higher yield.

In the centre panel of Figure 2, a treatment producing a 20% increase in yield has been applied. However, the increase in yield as a result of the treatment appears to be more than 0.5 t/ha higher than would be expected of a 20% yield increase on 3.5 t/ha, and this is purely down to the underlying gradient. In the right-hand panel, the same treatment resulting in a 20% increase in yield has been applied to the bottom half of the paddock. Here, the resulting yield increase is masked by the natural variation in the paddock, with the top half of the paddock giving a similar yield. Consequently, such a simple experimental design where half a paddock is treated and half a paddock is not can result in the wrong conclusions being drawn from the experiment. The result of this may be either that a true opportunity to increase productivity is missed, or alternatively, that resources are wasted on a product that actually has limited effect.

Understanding variation, replication of treatments and statistical testing

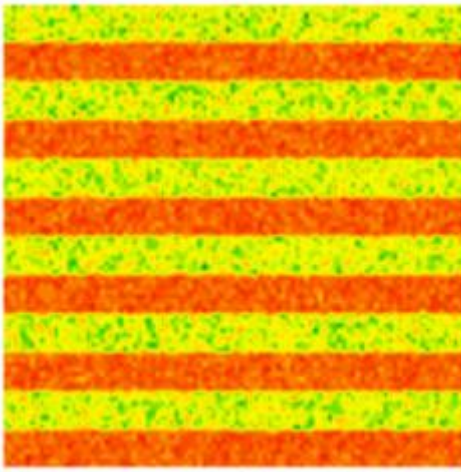

Figure 3: Example of a treatment replicated in strips across a paddock

Given the pitfalls of simple split-paddock experimental designs due to underlying variation as outlined above, it is advisable to use replicates when assessing the effects of any treatment in an on-farm experiment. Where PA approaches such as utilising VRT are not available, a parallel strip design should be implemented, using sprayers, spreaders or seeders as appropriate to apply the treatment in parallel strips across the paddock. Given that variations in harvesting efficiency may occur whether the header is running up or down a paddock (often due to the direction the crop may be leaning relative to prevailing winds throughout its growth), these strips should be an even number of up/down header widths. An example of such a design is provided in Figure 3, where a treatment resulting in a 20% increase in yield has been replicated in six strips across the paddock. As this hypothetical paddock has no underlying gradient, it is clear that there is a difference between the control and the treatment for each replicate.

It is good practice to have several replicates whatever the experimental question. This ensures that the result you observe isn't a 'fluke', or a product of underlying variation as outlined above. By using a greater number of replicates, you are able observe increasingly small changes in yield (or grain quality). Where you have more than one replicate per treatment (and in the case of Figure 3 there are six replicates), it is usual to take the average (or mean) of those values. In this case, the control (no treatment) yielded an average of 3.5 t/ha, while the treatment yielded 4.2 t/ha. In the case of a perfectly uniform paddock such as this, very few replicates would be required to discover whether or not a treatment had a significant effect over background variation. However, where underlying variation (known or otherwise) may be high, an increased number of replicates will allow greater power when analysing the data to verify whether a treatment has had a significant (and therefore hopefully repeatable) effect.

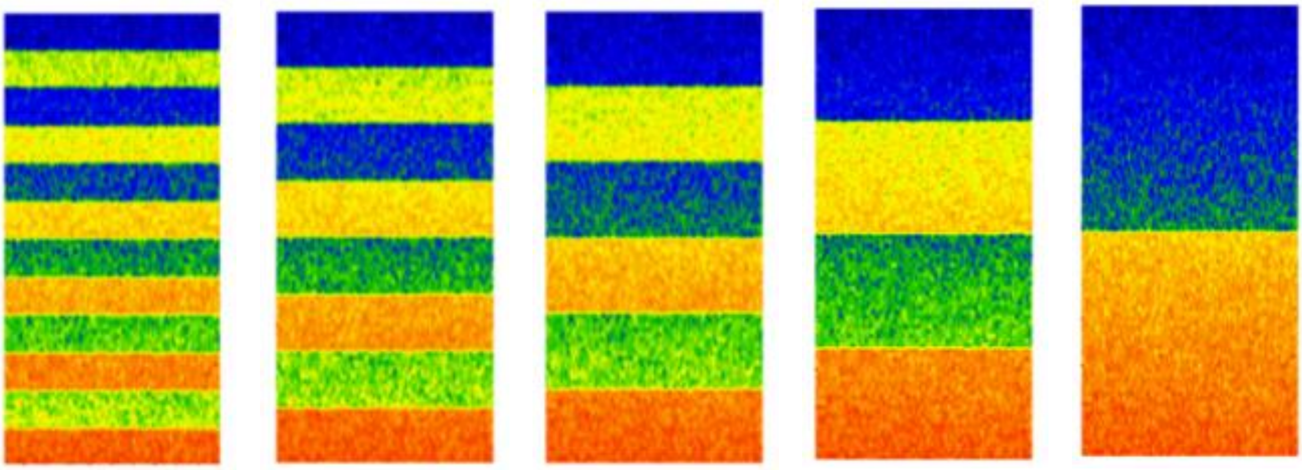

Figure 4: The same paddock as Figure 2, with a treatment resulting in a 40% increase in yield. Here, the number of replicates decreases from six (L) to one (R)

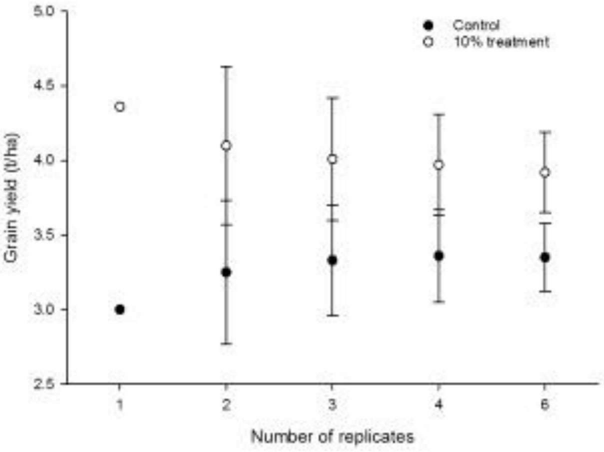

Figure 5: The average yields of a control and treatment applied across a paddock with a high gradient with an increasing number of replicates as depicted in Figure 4. The dots represent the average, and the lines extending below and above are error bars, showing the variance or uncertainty

When you have more than one replicate, at the same time as calculating the average yield from the control and the treatment, variance should also be calculated. This allows you to see how variable the results for each individual replicate in the treatment and control are. Understanding variance is important as it provides information on how much difference there might have been between strips (replicates) even if no treatment had been applied. Generally in experiments, variance is calculated as the "Standard Error of the Mean" (SEM). This is a metric that takes into account the "Standard Deviation" (SD) and the number of replicates to produce a value that provides some insight into how certain we are that the measured average from the replicate strips reflects the "true" average value. Standard errors are usually shown on graphs as error bars above, or above and below the mean, as shown in Figure 5. They might also be presented in tables in parentheses or after a "±" symbol. Where only one replicate was used, we cannot estimate variance, but where an increasing number of replicates have been used, the error bars decrease in size, showing an increasing certainty in the average value.

Standard deviation and SEM are useful methods to understand variance around the average value, and give some confidence that trends you are observing are real effects (and not simply a factor of underlying variance as per Figure 2). The next stage in data analysis is to use a relatively basic statistical test known as the "t-test". There are several variants of this method, and as with all statistical analysis, certain criteria or assumptions must be met. Given that the aim of this guide is to facilitate on-farm testing of amendments, we are focussing on the test that requires the fewest assumptions to be met, i.e. the two-tailed independent samples t-test. Compared to other variants that you may be aware of, this test will tend to be conservative in its results, so it is very unlikely to suggest a significant difference when there isn't one (a false positive).

Put simply, the t-test compares the values of all replicates in each treatment, and uses the variance around the mean and the number of replicates to assess whether or not the treatment differs significantly from the control (i.e. whether the crop yield in strips treated with an amendment are different from the yield in strips that were not treated). The result of the test is expressed as a "P" value, and if this value is less than or equal to 0.05, then it is considered a significant result. This means that there is a 95% chance that the 'significant' result being observed was due to the treatment and not to some other random effect.

The number of replicates you use greatly increases the sensitivity of the analysis, especially where there is underlying variability. In the case of the paddocks investigated in Figure 5 where a product has been applied that imparts a 10% yield increase on a paddock which itself has a 2 t/ha gradient across it (Figure 1), a significant effect is only observed when there are six or more replicates. A 10% yield increase is a strong result for a new practice, yet it this effect would have been undetected with fewer than six replicates. Across a paddock with a shallower underlying gradient of 1 t/ha, sensitivity increases so that a treatment difference of 10% can be resolved with four replicates, but a 5% yield increase is still undetectable with six replicates.

One question that might be asked of the relevance of statistical testing to on-farm experiments conducted by growers is:

> "*why should I care?*",

shortly followed by:

> *"well, I've grown more crop anyway, haven't I? Who cares if statistics tell me I have or not?"*

These questions raise interesting points. It is true that if you have a 5% increase in yield then that is indeed more grain in the bin, and money in the bank. However, the objective of the statistical analysis is to discern effects attributed to treatments from those that occur due to chance. Results from a well replicated experiment may vary from year to year, or location to location. This is especially true of experiments testing biological inputs that may be more sensitive to changes in rainfall / temperature / soil properties. The purpose of the statistical analysis is to give an increased level of certainty that the observed effect is due to the treatment and not due to chance so the results should be repeatable, and to help guide investment into possible changes in practice.

Economic considerations

As with any other agricultural input, the application of biological inputs has costs which must be offset by increases in yield / quality (and subsequent income), or decreases in costs associated with reduced application of traditional inputs. The "Biological Product Calculator" that accompanies this guide allows for the exploration of various scenarios out to a 10-year timeframe after initial application. It accounts for the cost of the product, its application, and transport. It allows the user to vary expected increases in yield and gross margin, and then provides options for altering conventional inputs such as fertiliser and yield. Finally, it allows for you to estimate the amount of the initial improvement you expect to see in subsequent years, with or without reapplication, or the changes to conventional inputs. There is also the option to alter the cost of funds loaned to your business.

Taking all these factors into consideration, the calculator then presents the cost of the initial product outlay relative to the income per hectare as a result of application. This calculation takes into account all the factors listed above, and results in a recommendation as to whether or not it is advisable to proceed with product application.

The output generated by the calculator should be taken as a guide. The calculator is designed as an exploratory tool that will enable growers to test scenarios where break-even point is reached. By allowing users to vary many different aspects including cost of biological input, reduced cost of conventional inputs, expected initial yield difference, and scenarios in subsequent years, we hope that the calculator is a useful practical tool to explore what factors drive profitability when biological inputs are being considered. This then allows growers to weigh up likelihood of achieving the required yield increase or conventional input reduction, and thus whether application of the biological input will be profitable.

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