

Understanding biostimulants, biofertilisers and on-farm trials

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Table of contents

1. Introduction	4
2. What are biostimulants and biofertilisers?	4
2.1. Biological controls are not biofertilisers or biostimulants	4
2.2. Biostimulants	5
2.3. Biofertilisers	5
3. How to decide if a product works?	5
3.1. Understanding the science	6
3.1.1. Real-world use	6
3.1.2. Appropriate methodology	7
3.2. Measuring the correct parameters	8
3.3. Time frames	8
3.4. How to rig a fertiliser experiment	8
3.5. The experimental dilemma	9
3.6. How to do your own experiments	9
3.7. Return on investment	11
4. How they work	11
5. Putting it all together	12
5.1. Evidence based	12
6. References	13



1. Introduction

There has been a phenomenal growth over the last decade of biostimulants and biological fertiliser (biofertilisers) products. A wide range of claims are made for these products and it can be hard for farmers and growers to tell fact from fiction. There has also been something of a factional spilt on this issue between academics and farmers & growers: many mainstream academics have been sceptical of these products, considering they are not based on 'real' science, while some farmers and growers have been buying and using them successfully in increasing amounts. The opposite is also true, in that some farmers and growers have been sceptical about some of the claims being made and there are academics who have been passionate advocates. The whole topic is therefore something of a mare's nest.

So, diving in where angels fear to tread, this report aims to give you the guidance you need to work out which biostimulant and biofertiliser products have real potential to help you farm better and more profitably and those that should be treated with scepticism. It is going to be a somewhat windy road, as there is no simple answer, there is no simple list of what works and what does not. You need to understand some of the complexities and niceties of scientific experiments, but hopefully the knowledge you gain will be valuable in other areas of your decision making.

2. What are biostimulants and biofertilisers?

There are no formally agreed definitions of biostimulants or biofertilisers, in part because new forms and types have been rapidly appearing and the industries as a whole are evolving quickly.

Broadly, a biostimulant is a substance or microorganism that when applied to plants or the soil, stimulates existing biological & chemical processes in the plant and/or associated microbes (e.g., mycorrhizal fungi) to enhance the plants growth, yield and/or quality through improving nutrient uptake, nutrient use efficiency and/or tolerance to abiotic stress (e.g., heat, saline soils).

Biofertilisers are materials of biological origin, e.g., plants, seaweed, fish, land animals, etc., that contain sufficient levels of plant nutrients (nitrogen, phosphorus, potassium, calcium, magnesium, etc.), in forms that are either directly absorbed by plants, or are sufficiently quickly decomposed to available forms, to cause an increase in plant growth and/or quality.

The main difference therefore is that biostimulants don't contain many nutrients, while biofertilisers do.

The term biofertilisers has both a narrow and broad meaning. Taking the term at face value, it means anything with a fertiliser value that is biological in origin. However, some consider biofertilisers to mean processed, commercial / proprietary products - the narrow meaning. The broader meaning therefore includes traditional, and widely used, materials such as manures and composts. However there is no clear dividing line between the narrow and broad definitions, for example biodigestates (the material produced by biodigesters / anaerobic digestion) come from a controlled process and are heavily modified from the starting material, while some proprietary products are only slightly altered from their raw state. This report therefore uses the wider definition but notes which type of product is being referred to.

2.1. Biological controls are not biofertilisers or biostimulants

It is also important to differentiate between biofertilisers & biostimulants and biological controls (biocontrols). Biocontrols use one or more living organisms, typically microbes and insects, to control pests, such as diseases, insects and weeds. The biocontrol agent therefore has no direct effect on the crop plant, only the benefit of having the pest controlled (although the impact of that can be a profound increase in growth). Biopesticides are a sub-group of microbial biocontrol agents that are



applied / used like chemical pesticides, i.e., sprayed onto crops. These may appear to be similar to biostimulants (many of which are also microbes) but they are different. Confusingly some products, for example *Trichoderma* fungi can act as both a biostimulant and a biocontrol. Adding to the confusion is that a number of biocontrols are marketed as biostimulants to get around the extensive safety and efficacy testing that is required for pest control products which has been designed for agrichemicals, not biological organisms.

2.2. Biostimulants

Biostimulants are in-turn divided into four major sub-types, some with sub-types of their own [1]

1. Microbial inoculants
 - Free-living fungi
 - Arbuscular mycorrhizal fungi (AMF)
 - Free-living bacteria
2. Protein hydrolysates and amino acids
3. Humic Substances
 - Humic acids
 - Fulvic acids
4. Seaweed extracts

This is not an exhaustive or exclusive list, for example, compost teas contain microbes, so they could be included in '1. Microbial Inoculants', even though they contain more microbe species than are listed, they may also contain proteins, amino acids and humic substances. Seaweeds, while the most common, are not the only plants that extracts are made from - many terrestrial plants are also used in both commercial and farm-made extracts. Despite these limitations the framework highlights the main types and shows the broad range of organisms and substances that are classed as biostimulants.

2.3. Biofertilisers

As biofertilisers can be made from any previously living entity, either animal or plant, there is no equivalent categorisation as for biostimulants. Generally they can be grouped by how processed or decomposed they are. For the broad definition of biofertiliser, materials that are in a raw, or close to raw state, include slurry and farm yard manure (FYM) while those that are well decomposed include compost and biodigestate. For the narrower definition raw (undecomposed) seaweed can be used as a fertiliser, while proprietary biological seaweed fertilisers and biostimulants process the raw seaweed using a range of techniques to concentrate the desired components and/or enhance certain components to create the final product.

3. How to decide if a product works?

Determining whether any particular product works is where things get complex and a bit tricky. Perhaps a good way to start is with an anecdote...

Back in the 1980s the first wave of seaweed biostimulants came to market. A vining pea grower in the UK was interested in the claims being made, but though he would test the products before using them on the whole farm. Sensibly, he set up a simple experiment in his field by spraying several strips of the seaweed product up his field of peas with unsprayed strips in-between. It was soon pretty obvious where the peas had been sprayed, as the plants were both bigger and greener, a property that lasted until harvest time. To test the effect on yield he drove the harvester across the strips: every time he hit one of the sprayed strips the harvester groaned and he had to put it down a gear and hit the throttle, so he assumed that it was from all the weight of extra peas that he had grown.



Fortunately his farm adviser was there and suggested that he get off the harvester and actually have a close look at the crop itself. It did not take long for the grower to realise that the extra work the harvester was doing on the sprayed strips was nothing to do with peas, because there were hardly any pods on the sprayed vines, let alone peas. The seaweed biostimulant was one that contained plant hormones (phytohormones) which are chemicals that regulate plant growth. In this case the phytohormone was one that encouraged vegetative growth in peas - i.e., making vine, and suppressed reproductive growth - i.e., making pods and peas. The strain on the harvester was not lots of peas, it was lots of vine!

There are a couple of key lessons to take from this true story.

- The plant-soil-climate system is one of the most complex things in the universe. It is simply impossible to predict the effect of complex products (i.e., those containing several ingredients, as opposed to one or two) on plants and the rest of the system. The only way to determine the effects is by empirical scientific experiment (spraying strips up the field). However, you must make a direct measurement of the results (counting the number of pods and peas on the vines) not rely on indirect measurements (the response of the harvester).
- Where products have real and consistent effect, it may not be the one you want (vine instead of peas). It is essential that the product has been tested on your specific crop (even specific cultivars), for the effect you want to achieve, at the point in the crops lifecycle you plan to use it (e.g., early growth, post flowering). Just as there are selective herbicides that will kill weeds and not the crop, any one biofertilisers or biostimulant may have quite different results on one crop species than another as well as different effects at different growth stages.
- Farm advisers can sometimes be worth their weight in gold (well nearly)!

3.1. Understanding the science

Having said that empirical scientific experiments are the only way to determine the effectiveness of a product, designing the experiments to truly measure outcomes and responses can be a quagmire.

The key overarching questions are:

- Does the research replicate real-world use?
- Is the experimental methodology appropriate?
- Does the experimental design measure the correct parameters?
- Do we know what to measure?
- Is the experiment run over a long enough time frame?

3.1.1. Real-world use

A recent review paper on biostimulants [1] listed a wide range and number of experiments that had been undertaken. However, a majority of these were not undertaken in real-world conditions - an important caveat. Typically when scientists start working on a biostimulant, or undertaking other research using living thing, such as biocontrol agents or allelopathic chemicals, they start work in the laboratory (*in vitro* (meaning in glass) in the jargon). Mostly this is because it is quick and cheap and they can get a research publication out of the work. If the lab work looks promising, and just as often when it does not, they then proceed to pot trials, i.e., growing plants in pots in a glasshouse. This is more expensive than the lab, but more realistic, and it produces another paper. However, those scientists that have been in the game for a long time know that the results of lab trials, and often pot trials, bear no relation to performance in the real world. Therefore, experienced scientists, as a starting, not an end point, often undertake trials in situations as close to real world use as possible, and skip the whole lab and pot trial, publication gravy train.



What that means is research, even high quality research, that is not conducted under real-world conditions that match your crop and farm, i.e., the exact crop species, even the same cultivars for some species (e.g., grapes), on similar soils and similar climates may not be relevant to your operation. Due to the huge number of soils and climates around the world you can't be too picky about the conditions being similar, e.g., in New Zealand, Canterbury and Hawkes Bay results should be considered comparable, but anywhere in NZ with Australian dryland would not be comparable. So, as farmers and growers you should pretty much just ignore lab and pot based experiments - their results are signposts but almost meaningless as far as your crops and pasture are concerned. Results from experiments that sound like they could have been done on your farm, orchard, vineyard etc., are the ones you should pay the closest attention to.

3.1.2. Appropriate methodology

Experimental methodology is science jargon for how an experiment was done. It covers things such as the treatments, e.g., amount and type of fertiliser, the untreated 'null' controls, the statistical analysis, the general setup, e.g., in-vitro lab experiment, pot experiment, field experiment, and all the details, e.g., soil type, soil tests, soil moisture, weather for the whole experiment, plant species & cultivar, age, when planted, and uncle Tom Cobley and all.

Determining if the experimental methodology is appropriate is unfortunately where the quagmire gives way to the snake pit. It is surprisingly easy for scientists to set experiments up to get the result they want, and it is even easier for scientists that don't have the right expertise, to set an experiment up that fools them into thinking they have an accurate result. Then there is the interpretation: scientists can disagree over what the results mean - one of the most famous was the UK's multi-million dollar experiments culling badgers for TB (tuberculosis) control in cattle - where opposing camps of scientists, very politely, tore strips off each other in public over their contrary interpretation of the results.

Also just because a paper has been "published in a peer reviewed journal" does not mean that the information is inviolable.. Often when scientists undertake a 'meta-analysis' (taking all the experiments in journal papers that have researched a particular topic (e.g., culling badgers to reduce TB in cattle) and combining the results into one giant statistical analysis, they often throw out 10 to 40% of the papers due to invalid methodology, i.e., they consider the results of those trials to be unreliable. It is also pretty common for different experiments to give contrary results, due to the vagaries of nature, and agricultural science. As an example, in the European Union, cultivar comparison experiments have to comply with the 5 × 5 rule: the comparisons have to be done in at least five locations for a minimum of five years for the data to be considered reliable - 25 repeats of the same field experiment!

It takes a lot of scientific training and even more experience to make a good call on the methods for an individual experiment, and in the end it is a subjective decision. Therefore there is little chance that the layperson can make that judgement, so if you want a view on a particular experiment, then you need to find an independent scientist who is trained and experienced in the same specialism as the experiment, to give you advice, and then, it is only an opinion.

At the end of the day, individual papers count for little, it is the amassed results from across a large number of experiments, across many years, plus the experiences from farmers and growers using products and techniques for real, that eventually determines if an effect is real or not. Until such broad consensus it built up, caveat emptor applies.



3.2. Measuring the correct parameters

From a farmer and growers perspective, it may seem pretty obvious what parameters to measure - the ones that get turned into profit, i.e., the stuff you harvest: lambs, grapes, apples, lettuces, wheat grains, etc. Disappointingly this is too frequently the measurement that gets missed by scientists. However, it is also important to measure 'intermediate' parameters, such as growth during the whole season, plant nutrient levels, etc., as these are important for helping understand what is going on, i.e., the cause and effect relationships. There is a mantra in science that 'correlation does not imply causation'¹. What this means is if you only measure yield, you don't know why the yield increased, so you only have a correlation, which is weak science, while if you measure other parameters these can point to how the increase was caused, then you have stronger science.

3.3. Time frames

For products such as biostimulants that have an immediate and relatively short term effect, trial duration is typically one crop cycle, based on the assumption that there is little or no residual effect, i.e., if you stop using the product, then the effect stops after a week to a few months. However, it is rare for effects to be truly short term, e.g., annual plants may be bigger, so if resources allow, the experiment should be run for three to five years to see what the long-term effects are - which is how they will be used for real.

For products, such as biofertilisers, or anything that impacts on soil processes, duration should be as long as possible. This is because soil processes and performance change very slowly. It really can take decades for the long-term effects to be fully shown. The truly long-term soil experiments around the world have now been running for over a century. Data from these shows that it takes up to 50 years (half a century) for soil to truly reach a new equilibrium. When the first ten to 30 years data from these experiments are analysed they often give quite different results compared with analysing 50 years. If scientists are being really hard core about such trials, they will throw out the data from the first five years, have a look to see if there are any trends in the next five years, and then consider data after the first decade as starting to become reliable. So, if you are running experiments that affect the soil, a trial should really be kept running for five years at a very minimum, and ideally a decade.

3.4. How to rig a fertiliser experiment

The results from fertiliser experiments are particularly noteworthy for their ability to get the results required, it is worth explaining how and why. For the full details underpinning this see the FFC Bulletin article "The Fundamentals of Soil Nutrient Management, Soil Testing and Fertiliser Recommendations" [3], and also "Understanding biological / organic fertilisers using kelp (*Macrocystis pyrifera*) as an example [2].

A plant's response to fertiliser does not just depend on the fertiliser, its growth response is to the total amounts of nutrients supplied from both the soil (or potting mix) and the fertiliser. There is an optimum amount of each nutrient that any given plant needs to maximise yield (or any other measurement). If there are excessive amounts of nutrients it will harm the plant and suppress yield, and if there are too few nutrients yield will also be suppressed. So, if a plant is grown in nutrient deficient soil and nutrients are supplied via fertiliser, the plant will grow more, potentially a lot more if the soil is really deficient. If the soil is around optimum, applying the same amount of the same fertiliser will have no effect on yield. If the soil is already in excess, applying the same level of fertiliser will cause a decrease in yield. So, the same application of fertiliser will result in increased yield, no yield change, and a decrease in yield depending on the nutrient level in the soil used.

¹ https://en.wikipedia.org/wiki/Correlation_does_not_imply_causation



Clearly to sell more fertiliser, the companies selling it will want to do tests in nutrient deficient soil not soil with excessive nutrients. To pick this kind of trick up, the person looking at the experiment's results needs to know what the nutrient level of the soil that was used in the tests and they then need to know if that is deficient, optimum or excessive, and then interpret the results on that basis. In short, always take the results of fertiliser trials with a pinch of salt.

3.5. The experimental dilemma

One of the 'advantages' of chemical agricultural technologies, such as herbicides and insecticides, is that they work pretty much the same pretty much anywhere in the world. A herbicide or insecticide that kills a weed or insect in the UK will do the same in Africa. Conversely one of the 'downsides' with biostimulants and biofertilisers is that their effects can vary widely depending on things such as the crop species, soil type, soil nutrient levels, weather/climate etc. Therefore while a pot experiment that shows a particular weedkiller kills a given weed is transferable pretty much to any farm anywhere in the world, the same is not true for biofertilisers and especially biostimulants. Experiments on biofertilisers and biostimulants need to be done under local conditions, which means that lots and lots of experiments are required to demonstrate efficacy for all the different crops, soils, climates, countries, etc., around the world.

This is the 'experimental dilemma' - experiments are expensive, so to conduct all the experiments needed to demonstrate widespread efficacy of biostimulants and biofertilisers, would be prohibitively expensive. In addition there is very little reason for scientists that don't work for the companies producing biostimulants to test these products. Such experiments are rote work and so are the least rewarding to undertake as they don't create valuable new knowledge or understanding. And, they are almost worthless to scientists trying to travel their career path. And they still cost significant amounts of money, that could be spent on ground-breaking, career boosting experiments, so the opportunity cost is pretty big for a scientist to test such products. So they don't.

However, with the wide range of products out there, there is likely to be one that will improve your production and profit, the question is how to identify them. The answer, DIY experiments.

3.6. How to do your own experiments

Scientific experiments can seem like they are shrouded in an aura of mystery. To be fair, there are some really insanely complicated experiments out there. However, we are blessed in agriculture in that many of our experiments are the simplest there are. The value of DIY experiments is that they are done on your crop or pasture so the results are 100% meaningful for your operation. It is therefore entirely possible for farmers and growers to conduct their own if you follow a few simple rules.

Treatments

Treatments are the different products you want to test. More is not always merrier as the amount of work increases considerably the more treatments.

It is important to decide the application regime from the start: is the product to be applied once at the start of the trial, or sprayed on weekly? The application regime should match what would be done in the real crop.

A control

You need to have something to compare the treatment with which is called, a 'null' control i.e., nothing is applied to the crop, and/or, current practice, e.g., current fertilisers. The control needs to be replicated and randomised just the same as the treatments.



Duration

As noted in section 3.3 getting the experimental duration right is really important. For biostimulants that is typically one crop cycle but ideally three or more, while for biofertilisers, that impact on soil processes, duration should be as long as possible, ideally a trial should last five years but a decade is much better. For field crops, 10 × 10 meters is a good starting point, and for perennials at least ten plants down a row / 20 meters.

Replication

Like the farmer spraying several strips of seaweed fertiliser on his peas, you need to have replication, i.e., several applications of the treatments. Traditionally four replicates are used (really the minimum) but in a perfect world six to eight are best.

Randomisation

It is impossible to emphasise how important proper randomisation is. It would not have been good enough for the farmer spaying strips up his field to just spray every other spray bout. To do the job properly, he should of stood at the bottom of each bout, flipped a coin, head for spray, tails for a control, and kept going until he had enough replicates (spray strips) of the seaweed and unsprayed (control). Randomisation helps take chance out of the experiment, so you didn't accidentally add all the treatment you are testing on an area that by chance had higher or lower fertility anyway.

Layout

The standard layout for field trials is the randomised complete block (RCB). Figure 1 shows a RCB experiment layout with four treatments (a,b,c,d) and four replicates. The key to blocking is that each of the four treatments (or however many there are) are found in each and every one of the blocks (hence complete block). Each one of the squares is referred to as a plot, i.e., it is a group of plants or area of pasture that has one treatment applied to it and is one replicate.

Block 1	d	a	c	b
Block 2	d	c	a	b
Block 3	a	d	b	c
Block 4	a	c	b	d

Figure 1. Randomised complete block experimental layout.

Plot size

Plots need to be big enough so that the natural variation found in agriculture is minimised. Therefore the bigger the plot the better.

Measure what matters

Don't make the mistake the pea grower did of taking his harvesters performance as a measure of pea volumes. It is essential to measure the final product, i.e., the thing you sell to make money. For most of horticulture this is easy, you harvest the crop for each plot and count or weigh it. For livestock it is very hard to measure the effect on the stock (very large plots and lots of stock are required), so for livestock the surrogate measure of pasture growth and laboratory analysis is mostly used.

Statistical analysis

The statistics is typically the most confusing part. Fortunately the ANOVA test, which can be found in most spreadsheets is typically used. However, if you are not comfortable with statistics, as many aren't there are a number of people who can help.



Getting some advice

While the basics of an experiment, as outlined above, are really pretty straight forward, there are niceties in the details that take experience to get right. Getting advice from a real scientist is therefore important. However, what the above illustrates is that it is perfectly possible for a farmer or grower to carry out the experiment themselves, with some expert advice, and create their own empirical evidence about the effectiveness of any given biostimulant or biofertiliser.

3.7. Return on investment

Finally, the fundamental reason for applying any type of fertiliser, pesticides, biostimulants and all the other agricultural inputs available is to increase yield and therefore profit. If the product you are applying costs \$200/ha to use and increases income by \$100 you are \$100 out of pocket (profit has reduced \$100). Unless there is some other benefit, e.g., increasing soil organic matter over the longer term, which results in bigger yields in future, using products that lose you money is not a great idea. So, the ultimate measurement of an experiment, is not yield, it is profit, so it is critical that gross margins for all the treatments are calculated to test for the level of profit or loss.

4. How they work

Explaining how biofertilisers and biostimulants work as a whole is impossible as just about every single one has a different mode of action. This is why it is so hard to generalise about the products - they all work in different ways. A few examples, to give a flavour of the mechanisms involved, are given below.

Starting with biofertilisers. Foliar applied biofertilisers (and mineral fertilisers) can boost nutrient uptake even when soil nutrient levels are at an optimum - i.e., adding more nutrients to the soil does not increase yield. This is because they bypass the roots' limitations on nutrient uptake. In some cases this can increase yield and quality but in other cases it can also lead to luxury uptake which can have negative effects, such as lodging, sappy growth, increased pest attack etc. So, more is not always better.

Soil applied biofertilisers cannot be taken up by plant roots because the molecules are mostly too big to get across the root epidermis. They have to be decomposed (mineralised) into inorganic salts / minerals to be absorbed. Nutrients supplied by biofertilisers therefore sit in the same queue for plant uptake as the existing soil nutrients, so the potential for a biological form of a nutrient to have a markedly different effect to a mineral form on immediate plant uptake is small.

There are however important system level effects to take into account. Mineral fertilisers don't contain biological forms of carbon so they don't supply energy to soil biology so they can cause a reduction in soil organic matter as microbes use up the soil organic matter to make use of the extra mineral nutrients. By definition, biofertilisers do contain biological carbon so there is a much reduced likelihood that they will cause microbes to consume soil organic matter. Whether they cause a significant increase in soil organic matter depends on how much is applied. For example, compost, manure, biodigestate, wood chips and similar bulky materials, applied at tens of tonnes per hectare on a regular basis, are just about guaranteed to noticeably increase soil organic matter. Highly processed products applied at kilos per hectare are unlikely to result in comparable increases in soil organic matter.



Biostimulants are where things get really complex. The range of mechanisms by which these products can impact plant growth and quality are almost limitless. They include:

- Enhancing nutrient availability in soil, for example, through increased mineralisation of soil organic matter by microbes.
- Increasing root biomass or root surface area, e.g., bacteria that release plant growth promoting chemicals.
- Increasing the plant's nutrient uptake capacity, e.g., mycorrhizal fungal association and, bacterial inoculants for legumes increase nitrogen uptake, and can therefore be considered a biostimulant.
- Resistance to drought and salinity stress, through microbes that produce protective compounds or induce the plants to produce more of their own protectants.

5. Putting it all together

So, what conclusions can we draw about biostimulants and biofertilisers? Going back to the start, there are some good reasons for mainstream academics to have been sceptical of these products: their modes of action were outside of accepted wisdom at the time, and therefore, the required standard of proof that the effects were real, was, justifiably higher, or as Carl Sagan, one of the world's great scientists and science popularisers, said "Extraordinary claims require extraordinary evidence." However, many academics dismissed biostimulants and biofertilisers out of hand, regardless of the evidence, or without any evidence at all, which in-turn is also clearly unscientific.

On the other side of the ledger Carl also has a pearl of wisdom for academics and producers who have been insufficiently sceptical and promoted and used biostimulants & biofertilisers on the basis that they all work. "Keep an open mind, but not so open that your brains fall out."

Dismissing ideas out of hand is not scientific, but uncritically accepting them, is also unscientific: Somewhere in the murky middle ground between these two extremes, the truth lives. The most effective way for navigating through the murk is empirical evidence, i.e., an experiment.

5.1. Evidence based

The only way to reliably determine if a biostimulant or biofertiliser does what it claims on the tin is to check if it has a body of experimental evidence to back it up. This means not just one peer reviewed paper, but a suite of them that are relevant to your production system. Due to the experimental dilemma (see section 4.3) there is a dearth of good experiments on biostimulants and biofertilisers. There are many biostimulants and biofertilisers that are backed up by large amounts of good quality science. There are many, many more that don't have experimental backing. Some of these will be effective and profitable, some won't be. The answer to the experimental dilemma is therefore to do your own experiments, or get a group of your mates together to do them, or as a whole industry, with some good advice from independent scientists.

In a nutshell, the only way only way to sort the wheat from the chaff is to do the experiments yourself on your land. That way you will really know for sure.



6. Futher information

The very informative "On-Farm Trial Guide" was produced a while back by MAF (!) FAR and LandWISE and hardcopy can still be purchased via their website <http://www.landwise.org.nz/publications/on-farm-trial-guide/>

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