# Keeping plants short: Natural gibberellin inhibitors

In this series of posts, we have discussed the different techniques and synthetic chemical substances that can be used to keep plants short. We discussed why keeping plants short is important, how this can be done with synthetic gibberellin inhibitors and how this can also be achieved using day/night temperature differentials. However, there are also a lot of natural substances that can be used to inhibit gibberellins, which can be used to help us achieve this same objective. In this post, we will be talking about the research around natural gibberellin inhibitors, the plant extracts that have shown this activity and what we have discovered these plant extracts contain.



Dried seeds and fruits of the carob plant

Research around plant extracts that could inhibit gibberellins started in the late 1960s. Many different plant extracts were tested for inhibitory activity. The tests were simple, a control plant was not sprayed, a second gibberellin control plant was sprayed with gibberellins and a third plant was

sprayed with a mixture of gibberellins and the tested plant extract. Whenever inhibitory activity was present, the third plant would show very similar characteristics to the control while the gibberellin sprayed plant would usually stretch significantly. You usually see graphs like the one showed below, where the plant sprayed with the pure gibberellins is the control while the extract contains both the gibberellins and the plant extract. When an extract inhibits the gibberellins the plant grows less under the same gibberellin concentration although as the gibberellin concentration is increased the inhibitory effect of the extract is surpassed and the plants reach similar points.

When doing this research, one of the plants that showed the most promise was the carob plant. Cold-pressed extracts of green carob fruits were studied quite extensively and showed this effect repeatedly (1, 2, 3). Different fractions extracted showed the effect and researchers sought to find the specific substances responsible for the inhibition. Eventually, researchers found that the culprit was abscisic acid (4), also known as ABA. Other plant extracts that had gibberellin inhibitory effects, such as lima beans, also proved to contain significant amounts of ABA (5). So why are we not using ABA as a safe and environmentally friendly gibberellin inhibitor?

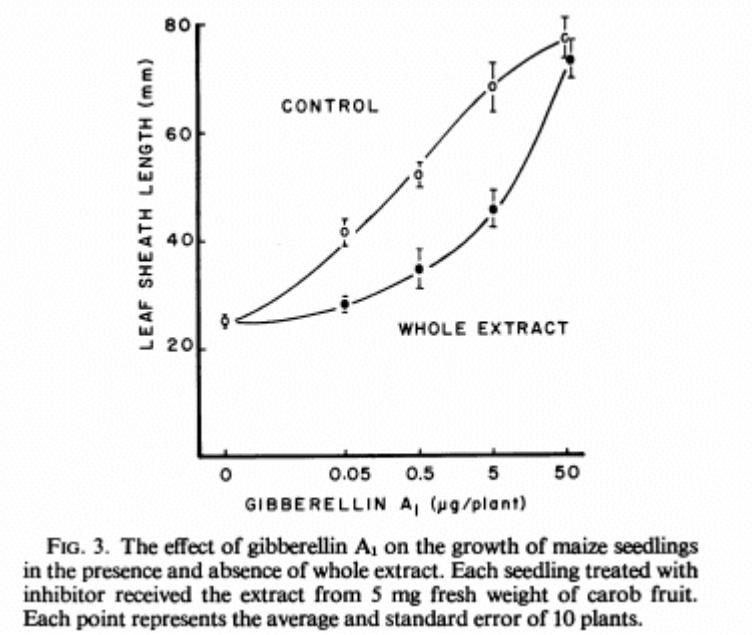



FIG. 3. The effect of gibberellin $A_1$ on the growth of maize seedlings in the presence and absence of whole extract. Each seedling treated with inhibitor received the extract from 5 mg fresh weight of carob fruit. Each point represents the average and standard error of 10 plants.

Sample graph showing the gibberelin inhibitory effect of a natural extract obtained from carob (taken from here)

It boils down to the chemistry of ABA, which is quite complicated. First of all, ABA contains a chiral center (1′ in the image below), making it the first chiral plant hormone to be discovered. This means that its mirror images are not equivalent – like your right hand is not equivalent to your left hand – which means that these two chemical forms will behave differently in biological systems. This complicates the synthesis of the molecule substantially. Furthermore, ABA contains several double bonds, which, depending on their configuration, can make the molecule completely inactive. Unfortunately, ABA goes through a double bond rearrangement under UV light that causes the molecule to deactivate, making it unstable for everyday use. So while ABA was great on paper, in practice it was never used widely. Several chemical analogs of ABA were developed and a lot of chemistry surrounding ABA and the proteins it binds to have been explored (you can read more in this book).

Phenolic compounds were also of great interest in the 1970s since many of the plant extracts that showed inhibitory activity also contained many of these molecules. These belong to a family of compounds called “tannins” and were then explored in pure form as potential gibberellin inhibitors,

with many of them showing substantial activity (6, 7, 8). This showed that extracts coming from fruits like carob had an inhibitory activity that was independent of the activity they got from ABA, although the phenolic compounds were significantly less active compared to the pure plant hormone.



Labeled diagram of the active form of ABA

In the late 1970s, the research into these natural gibberellin inhibitors stopped as the first successful synthetic gibberellin synthesis inhibitors started to surface. These were much more effective since they did not deal with the gibberellin once produced but mostly attacked the paths that were used to form the chemical within the plants. Substances such as Chloromequat and Paclobutrazol made most of this research into naturally source inhibitors irrelevant, as these were cheap to produce in mass quantities and much more effective.

With the return towards safer and more natural alternatives and advances in chemical synthesis, the direct use of ABA or phenolic substances in order to inhibit gibberellins to prevent shoot elongation starts to become attractive. If you’re interested in this path, looking at past research from the 1970s to come up with test formulations for foliar spray or root drench products would be a good initial approach. If you want to avoid the use of pure substances and all chemical synthesis, using direct extracts from plants like lima beans and carob is also a potential approach, although care needs to be taken to ensure the conditions of the extraction processes and extract storage do not destroy their active properties.

# Using triacontanol to increase yields in hydroponics

Usually additives used in hydroponics need to be added in rather large quantities to obtain palpable results. Molecules like salicylic acid – which we have discussed before – need to be used in concentrations in the order of $10^{-4}$ to $10^{-2}$ M to obtain a significant effect. This means that you need to use quantities in the order of 20-150ppm of most additives in order to see a significant result. However there is a molecule called 1-triacontanol that can generate very significant results with only a fraction of that concentration. Today we will talk about this substance, what it does, how to use it and why it's such a desirable tool in your hydroponic additive arsenal. Many of the things I will talk about in this article are derived from this 2011 review on triacontanol (make sure you read that for a deeper insight into why this molecule works).

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Triacontanol is a very long fatty alcohol. Each molecule has 30 carbon atoms linked in a linear structure which makes this molecule extremely hydrophobic and hence very hard to dissolve in something like water. Using triacontanol therefore involves dissolving this molecule in something other than water – for example Tween 20, chloroform, methanol – before adding water in order to prepare an emulsion for use in either root applications or foliar feeding. Most research using triacontanol has used foliar feeding as this is the easiest

way to control the application of the molecule and also how it seems to have the largest effect.

The effects of this molecule are not short of miraculous. Triacontanol is usually applied in concentrations on the order of $10^{-7}$ to $10^{-9}$ M, which means it is used from around 0.01 to 1 ppm. This means that we use about 1000 times less triacontanol than other additives in order to obtain a meaningful result. The table below shows some of the effects that triacontanol has showed in peer reviewed studies, with plant height, weight and yields increasing across a variety of different species, from tomatoes to japanese mint. Papers on other plants besides those on the chart have also been published, for example triacontanol has showed to significantly increase yields in lettuce crops (here).  Some studies have also found that the effect of triacontanol can also be enhanced through the use of magnesium or in conjunction with other hormones (here).

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With such an impressive array of effects and such a low expected toxicity – due to its very low solubility – it's definitely one of the best additives to use to get production gains in hydroponic crops. This also makes it one of the most commonly used substances in commercially available grow enhancers. Nonetheless since it's used in such a small quantity it's very easy for someone to buy a small amount of triacontanol and use it for years before running out. You can buy small amounts of triacontanol as a powder (there are several reputable sellers on ebay) and you can then prepare your own concentrated triacontanol solution in Tween 20 – not water – that will last you for ages. A liter of 2000ppm solution of triacontanol will last you for 1000-2000 liters of foliar spray. You cannot get more economical than that.

The optimum application rate and frequency for triacontanol varies across different species but if you want to take an initial guess use a foliar application of a 0.5 ppm solution every week. There is usually a sweet spot for concentration – after that you start to see a decrease in results compared to the highest point – so you want to start below a 1 ppm application rate. For some crops repeated applications might be unnecessary – with just one or two applications giving most of the effect through the entire crop cycle – while for others you do want to apply every week. How you initially dissolve the triacontanol to make your concentrated solution is also important with Tween 20 being the most ecologically friendly – although not the easiest – option.

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# Salicylic acid and its positive effect in hydroponics

When looking for ways to increase crop yields we usually want something that is safe for the environment, safe for us and able to give us a substantial bang for our buck. From the multitude of additives that have been researched during the past 30 years one simple organic molecule seems to fit all the requirements very well: salicylic acid. Today we are going to talk about why this additive is so interesting for use in hydroponic culture, the results it has shown in peer reviewed publications and how we can use it to increase our crop yields. For those of you interested in this molecule I would also recommend reading this 2010 review which contains a much more detailed look into the scientific literature surrounding salicylic acid research in higher plants.

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Salicylic acid is a simple organic molecule with the structure showed above. We have known for a long time that plants produce it and we knew almost right from the start that it played a key role in plants' response to diseases and stress (see here for some early insights from Tobacco cultivation). Salicylic acid is used as a signaling molecule in plants (a.k.a hormone), moving from stressed organs to non-stressed ones as the plant is attacked. However its role is much more complex, having functions related with chloroplast creation, inhibition of fruit ripening and many other important processes.

After learning that this was an interesting molecule it wasn't long before people started studying whether exogenous applications provided any benefit. We have learned that it enhances dry mass and leaf area in corn and soybean (here), that it can enhance germination in wheat (here), the oil content in basil (here), the carbohydrate content in maize, etc. There are also several studies pointing to improvement in root development – even from foliar applications (here) – suggesting that this hormone is able to increase plant productivity through several different mechanisms. The incidence of diseases can also be reduced dramatically by salicylic acid applications (here).

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We also know this molecule has important effects on the flowering process. It can induce earlier flowering in plants

and can often cause larger fruit settings in some plants (like papaya (here)). Most importantly foliar spraying of tomato and cucumber plants with salicylic acid has showed important increases in yields (here). It is therefore clear that exogenous applications of salicylic acid can have many important benefits in crop production and this is therefore an important candidate to consider for enhancing crop production.

But how do we apply it? Most commonly this molecule is applied in foliar feeding regimes, although in some cases it is also applied directly in hydroponic solutions. Most commonly concentrations in the order of $10^{-5}$ -$10^{-4}$ M are used since it has been showed across a few studies that negative effects start to show up when the concentration level reaches 1mM. This means that regular doses will be around 1-100 ppm with the lowest spectrum of dosage being preferred if the effect on the particular plant is unknown. The solubility of salicylic acid is 2.48g/L at 25°C so concentrated solutions of up to around 20-30x can be prepared without issues to make it easier to apply on plants. The preparation of more concentrated solutions requires some tricks but it certainly can be done.

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Salicylic acid also has the advantage of being a very safe molecule so it can be applied without a lot of worry in order to experiment with its effects. For testing on new plants foliar applications of 20-30ppm would be most common, with applications usually carried out once every 5-10 days. The frequency of application as well as the best concentration to use will of course depend on the particular plant you're growing. There are also several other molecules that can be used with salicylic acid to enhance its effect on some plants, but this will be the focus of a future post.

Finally it is also worth noting that ***salicylic acid is not***

***aspirin*** (aspirin is acetylsalicylic acid, a related yet different molecule) so if you want to experiment with this additive you should buy salicylic acid instead of just “dumping some aspirin” into your foliar or hydroponic nutrient solution.