Brief General Description of the "Growth Technique Innovation" ("GTI")

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"GTI", what does it do?

The purpose of Crops Advance's "Growth Technique Innovation" is to significantly increase crop yield

while at the same time decreasing susceptibility to diseases.

How?

By increasing the "carbon use efficiency" (CUE) of a crop by means of preventing the up-regulation of

the Alternative Pathway Respiration (APR, AOX), specifically during the juvenile phase.

Please note: GTI exploits the already present genetic potential for growth.

Background:

More than 90% of the dry matter of plants is produced from CO_2, by means of photosynthesis. In this

process, only a part of the captured carbon is actually sequestered ("fixated") by the plant. The ratio of sequestered carbon versus captured carbon is a way to express the plant´s "carbon use efficiency".

The theoretical maximum of the CUE is approximately 70%. The minimum consumption of approximately 30% is used by the plant´s metabolism, including maintenance processes.

Overview of the Crops Advance developed and built “Micro Climate Station”. The system uses custom built -highly accurate- sensors, a micro-processor and WiFi data transmission.

Average CUE values, both in nature and in agriculture, are often around 35%, meaning there is a 50% yield-gap. In this case, next to the plant using the aforementioned “basic” 30% of the captured carbon for energy for metabolism and maintenance, the crop consumes an extra approx. 35% of the energy initially captured by means of photosynthesis.

At 35% CUE this means that of the 100% CO_2 initially captured, only 35% is actually sequestered (fixated) by the crop and that 65% is being returned to the atmosphere.

CUE of a crop is determined by various factors, as mentioned below. In well managed intensive production processes (as may e.g. be the case in greenhouse production) CUE can be significantly above average, while in adverse conditions it will end up significantly below average.

If a crop’s CUE of 30% can be increased to e.g. 36%, the relative yield (defined as accumulated dry matter) of that crop will be increased by 20% (6 over 30) and 9% less CO_2 will be returned by that crop to the atmosphere (6 over 70).

At low CUE the crop is using a relatively large part of the captured energy for its metabolism and maintenance, including its defense against stress. Thus, little of the initially captured carbon will remain available for dry-matter accumulation, e.g. growth. If the captured energy is insufficient for growth and metabolism, the crop is likely to become susceptible to disease and to subsequently become infected and eventually die.

The part of the theoretical maximum of 70% of the CO_2 that a crop actually sequesters (i.e. of the 100% initially captured) is determined by several variables, such as the availability of CO_2, light, nutrients, temperature and water. A sub-optimal availability of these components likely causes stress.

The plant will spend energy in order to combat that stress. Limited availability of for instance phosphate or nitrogen activates the alternative oxidative pathway, decreasing respirational efficiency, decreasing CUE, reducing dry-matter accumulation to maintain a proper balance between these nutrients and the carbon remaining. These various causes of stress are well known and producers will aim to optimize their combat.

The stressor, however, that is the focus of this description is “ground-level ozone”.

Ground-level ozone is, to a certain extent, also a known stressor and as such the (visual) damage it causes is familiar. However, the effects of ozone on yield have so far not been properly understood and as a consequence have been seriously underestimated.

Present day estimates of annual global yield loss vary from 12 – 20 billion euro, corresponding to approx. 3-5% of global farm output (FAO stat. 2008). However, contrary to the generally accepted supposition, yield loss due to ground-level ozone does not just occur above a threshold value of e.g. 40ppbv.

Moreover, the adverse effect of ozone may be related to ozone flux into plants occurring at ozone peak levels, rather than just to average fluxes.

We show that loss may actually occur at any level of ozone above 0 ppbv. The implication is that almost all yields, worldwide, suffer from a significant hidden percentage of yield loss and disease susceptibility due to the effects of ozone.

By avoiding or reducing this “hidden loss”, yield may be restored to its full potential, or may at least be significantly improved.

Ozone is a unique stressor because the inter-cellular hydrogen peroxide generated acts as a defuse but false feedback signal to the “intra-cellular oxidative processes” of the plant.

As a consequence, within these processes reactions occur in response to the false signals created by ozone. This results in up-regulation of a defense mechanism: the “Alternative Pathway Respiration (APR, also referred to as “Alternative Oxidative Pathway” or “AOX”).

The AOX is in principle able to drain a large part –in extreme cases up to 80%– of the energy captured by means of photosynthesis.

Electrons that bind with oxygen via the alternative pathway in total render 11 ATP per 6 molecules of oxygen respired compared to 29 ATP per 6 molecules of oxygen respired if binding with oxygen through the main Cytochrome Path or COX.

Although the AOX is certainly functional, for the purpose of maximizing dry-matter accumulation it should be kept as low as possible, simply because it costs the plant energy that, as a consequence, cannot be used for its growth/production.

Sugar cane growing in 0 ppbv ozone phytotron (left) and in 60 ppbv ozone phytotron (right). The 60 ppbv plants show no visual damage but it is clear that their growth, i.e. their carbon sequestration is poor.

Without the reference of the 0 ppbv plants, as would be the case in a normal production environment, it would not be obvious that somethingis amiss.

So what’s new?

(1) “Memory effect”: the scientific discovery made by Crops Advance is the existence of an “AOX default setting” along with the fact that this default is determined and fixated during the juvenile phase of the plant. Furthermore it has been established that the level of this default setting is determined by the stress that the plant experiences in its juvenile phase and that the plant retains this “default AOX setting” during its life.

If stress reaches a value whereby the “default AOX level” should prove to be too low for an effective defense, the plant can and will as yet up-regulate its AOX. However, when the level of stress again decreases, the plant will reduce its AOX level correspondingly. Unfortunately the plant is unable to decrease its AOX to a level below the (permanently fixated) default level.

In this way the “default AOX setting” determines in a static manner the level of what in fact is a very significant energy drain. If this setting can be kept low, the result is that the plant will have a lower level static (permanent) energy drain and therefore a larger dynamic control range at its disposal. As a consequence the plant will be able to better match the energy it spends on maintenance/defense to the level of stress that, at any time, is experienced.

(2) Ozone creates a false signal of oxidative stress at the cellular level: ozone plays an especially important role as “stressor”, because of the way it leverages its effect through interference in the internal processes at cellular level of the plant. Because of this, the real total damage of ozone far exceeds the known direct damage that it causes and for which it is known.

Note: a number of plants possess, to a varying degree, a natural defense against ozone damage.

This is the case e.g. with oak and eucalyptus.

Until now ozone damage, both for production and climate models has been assumed to occur above the level of roughly 40ppbv. This level has been chosen because only at, or above, this level visual ozone damage occurs. However, this visual ozone damage only represents the "top of the iceberg": ozone damage does not just occur from 40ppbv but from 0 ppbv and is not just limited to what is visible!

Additional clarification:

For the proper understanding of this description it is important to note that the technique outlined here ("GTI") does not provide "super plants" in the sense that it does not alter crops' genetic potential for growth. GTI merely enables the crop to better develop its inherent potential for growth. It achieves this by avoiding a strategy, adopted by the crop in its juvenile phase that is likely to be wasteful from a production point of view.

This wasteful strategy results in undesirable and unnecessary loss of energy over the whole of the productive life span of the crop.

Of course, adequate growth conditions and management of a crop remain important; although the application of GTI will improve the yield of both poorly and well managed crops, a crop grown in inadequate conditions and/or poorly managed will, as is to be expected, provide a sub-optimal yield.

However, if any two identical crops are grown under identical conditions, the crop with the lower AOX default will provide a correspondingly better yield.

Patents & Implementation:

Patent applications for "GTI" have been submitted in 2008 and 2009. The essential criteria for successful patent application are: (1) inventive step, (2) novelty and (3) industrially applicability. The inventive step of GTI is specifically: to minimize a crop's AOX default level by avoiding or minimizing ozone stress during the juvenile period of that crop for the purpose of increasing its carbon use efficiency. This can be achieved by means of:

1. adequately equipped greenhouses

2. application of a microbiological/agro-biological compound

3. selective expression of a protective substance achieved through genetic manipulation.

In the course of 2011 the PCT Examiner has confirmed that Crops Advance's "GTI" is novel, inventive and industrially applicable and as such meets the essential PCT criteria.

“Proof” to date:

The effects of ozone on yield and disease susceptibility as briefly outlined above are supported by extensive and well documented field observations over a period of approx. five years among several large production areas in São Paulo and Minas Gerais.

Subsequently laboratory tests have been conducted to verify the effects of ozone as found in practice.

These tests, using high-tech climate chambers (“Phytotrons”) and measuring techniques provided “Proof of Concept” (POC), with respect to Crops Advance’s discovery as outlined above. This was an essential requirement within the patent application process.

The novelty, inventiveness and industrial applicability of “GTI” have been initially verified, with positive outcome, by “Vereenigde Octrooi Bureaux”. Patent Attorneys in The Hague, The Netherlands.

In 2011 this outcome was confirmed by PCT authorities.

In view of the importance for the large horticultural sector in The Netherlands and also because its relevancy to climate modeling, the Ministry of Economic Affairs, Agriculture and Innovation of The Netherlands ordered an evaluation of Crops Advance’s discovery and claims by Wageningen University and Research Centre (WUR). The WUR evaluation report, published May 2011, confirms Crops Advance’s findings, including the potential for up to 40% yield increase.

Control hardware for light, CO2, humidity, nutrition, irrigation, temperature, ventilation and ozone levels in the phytotrons.

Business Case

Crops Advance has, over a period of five years, conducted and financed the research leading to the discovery outlined above and has subsequently provided the POC for that discovery.

The application of the discovery in practice requires the development of specific products in the three fields mentioned in the above paragraph “Patents & Implementation”. The development of such products (1) falls outside the scope of Crops Advance, (2) in as far as g.m. is concerned falls outside the competence of Crops Advance and (3) requires specific capital outlay.

Business cases will therefore need to be made for each specific application and product whereby it stands to reason that this is to be done by the entities that choose to develop commercial products on the basis of GTI.

Phytotron controller designed & built by Crops Advance, showing the processor board

Conclusion:

Implementation of GTI will positively affect the following aspects of production:

1. yield
2. resistance to diseases
3. the cost of the crops
4. the reliability/availability of the crops
5. the quality of crops
6. optimization of the use of available acreage
7. reduction of de-forestation and violation of natural lands
8. reduction of competition between food and biomass/energy crops
9. reduction of chemical pollution of soils and groundwater
10. reduction of pollution/alteration of the atmosphere