Biostimulants: What's behind the name?

Elicitors, phytostimulants, biostimulants, phytoprotectants, biofertilizers, bioactivators, soil enhancers and so on. What is this? What are they? Are they all the same? What do they do? What do they don't do? If you are lost in the jungle of all these products, don't be discouraged: you are not the only one! Still, understanding the differences between these product categories, and more importantly the concepts and the mechanisms that make them work in the field, sometimes with impressive results, is really worth the effort. Some of these products will definitely make their way through in the High Tech Agriculture of the future. And this is probably only the start of something bigger that will happen when molecular biology and agronomy will have become efficient partners in advancing research. As genomes of a number of plants are now completely sequenced or nearing completion (Arabidopsis thaliana, Medicago truncatula, tomato, rice, poplar, etc.), it is possible to look at the effects of e.g. seaweed extracts and components of the seaweeds on the whole genome/transcriptome of plants to better understand the mechanisms of action of seaweed-induced growth response and stress alleviation. In the meantime, seaweed extracts based products, humic acids products and aminoacids based products, the three main categories of substances covered by the "biostimulants" term, are increasingly popular among growers although the mechanisms that drive the way they work on the crop or the soil are not fully documented and known, which may also partly explain why their application on agricultural crops doesn't always end up with outstanding effects.

For some people, from the academic circles in general, biostimulants are defined as materials, other than fertilizers, that promote plant growth when applied in small quantities; they are also referred to as metabolic enhancers.

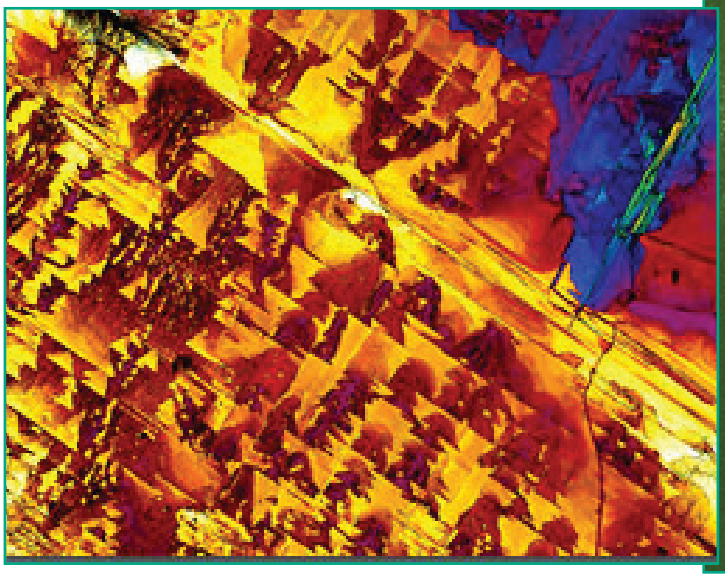
For others, mostly from the industry and in particular the specialty plant nutrition industry, biostimulants should be classified as fertilizers- and therefore benefit from the main advantages attached to this category of products, in particular no need for costly registration, but should be marketed in a different manner with specific claims attached to these products that allow for higher selling prices than fertilizers!

For a third category of people, from the plant protection industry in general but not only, biostimulants such as seaweed extracts in particular, are natural substances for biological control of pests and diseases.

For a fourth category of people that includes growers, most distributors and a sizeable number of regulators around the world, biostimulants are defined by what they do more than by what they are, since the category includes a diversity of substances. And this is perhaps the most sensible approach as it allows putting the same commercial product in several categories, depending how, when and where it is intended to be applied!

As the name suggests, biostimulants stimulate growth-like fertilizers (but water also stimulates growth and is not considered a fertilizer!), but they do much more.

What are biostimulants? Before one can understand the benefits of biostimulants, he must understand the role

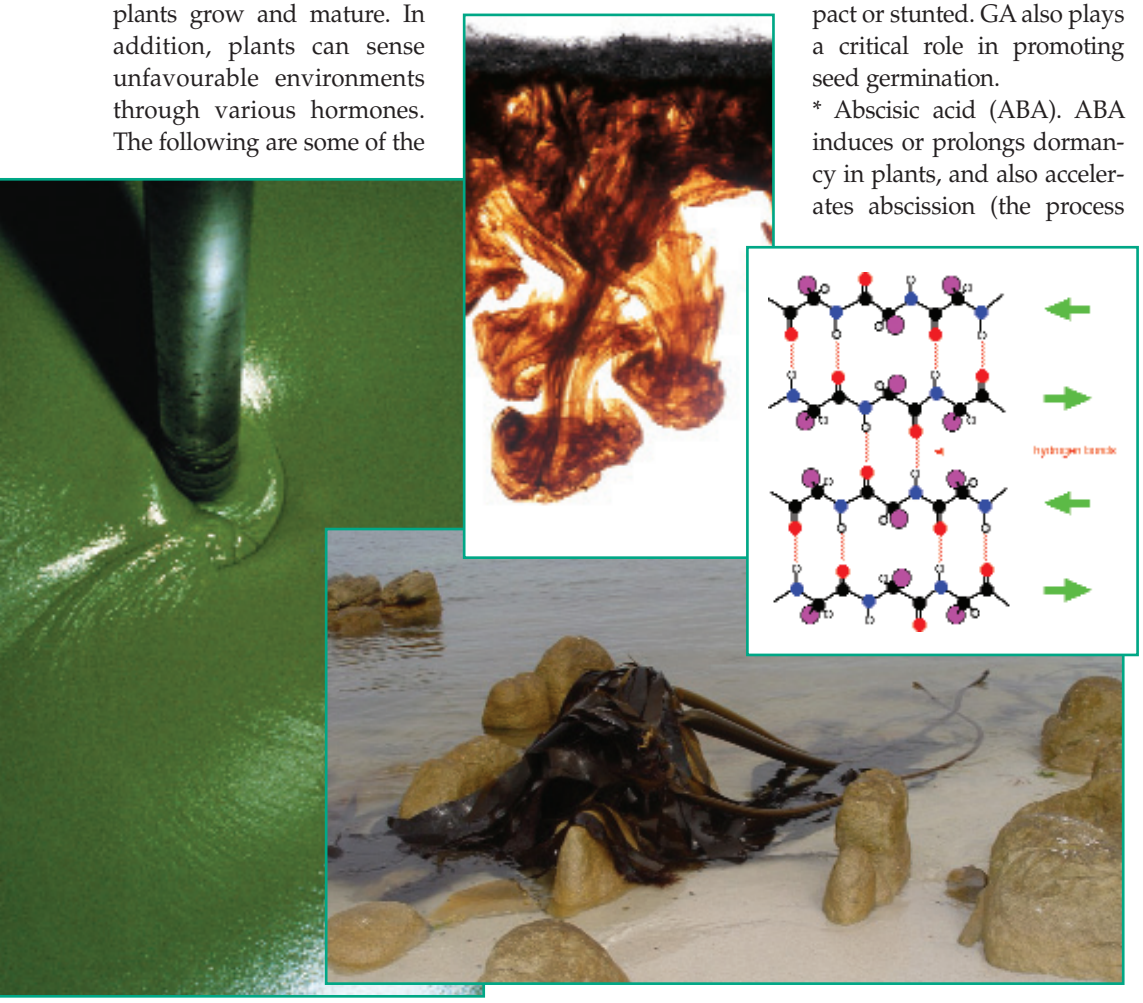


of plant hormones. Many important benefits of biostimulants are rooted in their ability to influence hormonal activity.

THE ABILITY TO INFLUENCE HORMONAL ACTIVITY IN PLANTS

Hormones (often referred as phytohormones in plants) are chemical messengers regulating normal plant development as well as responses to the environment. They regulate tissue growth and differentiation, dictating how plants grow and mature. In addition, plants can sense unfavourable environments through various hormones. The following are some of the major categories of plant hormones.

* Cytokinins. These hormones are responsible for the formation of roots and buds, and promote cell division. Cytokinins tend to counteract the effects of aging and stress in plants.
* Auxins. Hormones in this group produce several growth effects in plants, especially cell enlargement (contrasted with cytokinin-induced growth, which results from cell division). Cell enlargement causes root and shoot elongation in plants and allows them to produce tropic responses, such as shoots bending toward a light source (phototropism) and roots growing downward (geotropism). Indolacetic acid (IAA) is perhaps the best-known auxin.
* Gibberellic acid (GA). GA is another growth promoter known for producing elongation in plants. Conversely, suppressing GA levels can cause plants to become compact or stunted. GA also plays a critical role in promoting seed germination.
* Abscisic acid (ABA). ABA induces or prolongs dormancy in plants, and also accelerates abscission (the process that results in fall leaf drop). Thus, ABA is considered generally as a growth inhibitor, rather than a promoter. ABA also is involved in water regulation within plants. ABA levels rise in plants under drought stress, particularly in leaves, where ABA prompts stomata to close.



The balance of the various hormones within a plant is a complex interaction that controls its overall growth and development. Thus, materials that alter the hormonal status of a plant can exert large influences over its growth and health.

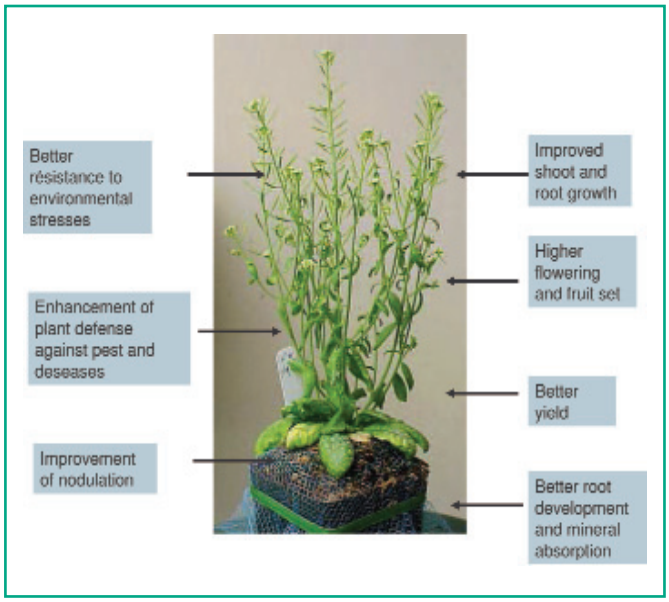
Free radicals and antioxidants

Antioxidants are another group of plant chemicals important to understanding biostimulants. Apparently, some biostimulants promote antioxidant production. Research over the past decade has shown that various environmental stresses, such as drought, heat, ultraviolet light and the use of herbicides, damage plants by causing production of free radicals or reactive oxygen molecules (such as O2-, hydrogen peroxide [H2O2] and hydroxyl radical [OH]). These molecules are strong oxidizing agents and damage lipids, proteins and DNA inside cells.

Antioxidants are metabolites and enzymes that scavenge free radicals and thereby protect plant cells from damage. Antioxidants include lipid-soluble substances such as vitamin E and beta-carotene,

water-soluble materials such as vitamin C and glutathione, and various enzymes. Frequently, several antioxidants work together to suppress free-radical toxicity.

Stress tolerance is perhaps the most important benefit of biostimulants-tolerance of drought, heat, UV light and even diseases. Biostimulants impart stress tolerance partly by stimulating root growth and partly by promoting antioxidant activity. However, we still have much to learn about how biostimulants work, so these and other functions will become better understood with additional research.



MOSTLY NATURAL SUBSTANCES SUCH AS SEAWEED EXTRACTS, HUMIC ACIDS AND AMINOACIDS

During the past 10 years researchers have evaluated many kinds of biostimulants for use in the agricultural industry. Of the various materials examined, the most promising are seaweed extract, humic acid, amino acids, salicylic acid and silicates. All of these products exert beneficial influences either through hormonal effects or by raising antioxidant levels.

Although biostimulants can be synthetic chemicals, naturally occurring organic materials are excellent sources of biostimulants. Humic acids, amino-acids and seaweed extract are three commonly used biostimulants.

* Humic acid has received increasing attention in recent years. Humic substances are naturally occurring organic materials derived from biological sources (i.e. decomposed organic matter). They typically are mixtures of several types of chemical compounds, including humic acids, fulvic acids and humins. Scientists were exploring the benefits of naturally occurring soil humic acid on plants as far back as the 1940s and '50s. In the '70s, researchers found that humic substances exhibited auxin-like activity and chelation properties (chelation of micronutrients, such as iron, aids plant uptake and utilization).

In a more recent research conducted at Virginia Tech in the USA, researchers found that-in addition to increasing root initiation, nutrient uptake, chlorophyll content and photosynthesis-humic acid inhibits indolacetic acid (IAA) oxidase (which destroys IAA). The net result is higher growth-hormone levels, which promote more growth.

* Amino acids are building blocks for proteins and enzymes. Some free amino acids, such as proline, improve osmotic adjustment and water-stress tolerance of plants.

* Seaweed contains various hormones, vitamins, amino acids, mineral nutrients and other components. Thus, it may affect plants in several ways. However, its stimulating influence-particularly for crops growing under environmental stresses-has been attributed to its hormonal activity, especially that of cytokinins and auxins.

* Salicylic acid, the active ingredient in aspirin, seems to improve plant resistance to disease and environmental stresses.

Silicate is not usually listed among the generally essential elements or nutrients for a number of crops, but it has been shown to enhance plant growth and metabolism, regulate nutrient balance, enhance antioxidant activity and improve plant resistance to various stresses.

All the biostimulants mentioned above, including amino acid products and silicates, promote antioxidant activity and enhance stress tolerance. Most biostimulants are formulated as liquid products (except salicylic acid, which is a powder, and some humic extracts).

BIOSTIMULANTS PERFORM MULTIPLE FUNCTIONS: BIOSTIMULANTS HELP PLANTS HELP THEMSELVES!

Because biostimulants contain a diversity of chemicals, it's not surprising that the benefits associated with their use vary as well.

To some extent, it's useful to evaluate biostimulants visually and with growth parameters (such as root mass, clip-

Table 2: legislation on Seaweed Extracts around the world

Country	Fertilizer(1)	Plant Protection Product	Specific category	registration (2)	no registration (3)
Australia					
Belgium		yes- 91/414			
Brazil	organic				
France		yes-91/414			
Germany		yes 91/414			
Greece		yes 91/414			
Italy	organic	yes-91/414		yes-fertilizer	
New Zealand					
Spain		yes-91/414			

(1) mineral or organic fertilizer. (2) means each product must be separately registered through a specific procedure.
(3) means products can be freely marketed without specific registration.

Table 3: legislation on Humic acids around the world

Country	Fertilizer(1)	Plant Protection Product	Specific category	registration (2)	no registration (3)
Australia					
Brazil			category of fertilizer		
Greece			amendment		
France					
Italy			amendment		
Spain					
USA (California)					
USA (Florida)					
USA (others)					

(1) mineral or organic fertilizer. (2) means each product must be separately registered through a specific procedure.
(3) means products can be freely marketed without specific registration.

ping weight and root strength). However, physiological parameters (photosynthetic effects, antioxidant analysis and hormone activity, for example) provide a more in-depth understanding of the influence of biostimulants. As appears in table 1 presented by Dr J F Morot-Gaudry from INRA in France and a member of the French Academy of Agriculture, biostimulants perform multiple functions. Although the illustration of Morot-Gaudry was focusing on seaweed, it may apply to the whole Group of biostimulants, despite the specifics of one or the other product category. (1) Enhanced root and shoot development; (2) Drought tolerance; (3)Salt tolerance; (4)Disease resistance; (5) UV light tolerance; (6)Heat tolerance; All these translating in better yield, higher flowering and fruit set, etc.

Actually biostimulants help plants help themselves! One of the roles of plant hormones is that of chemical messengers that tell plants when stressful environmental conditions exists. In response, plants may initiate or increase physiological processes that increase their tolerance to stress. For example, small, rapid changes in abscisic acid levels (which occur when plants are under moisture stress) cause stomata to close. Also, water stress reduces cytokinin activity. This change signals the plant to initiate a defence system to drought. Thus, another function biostimulants may perform is that they act as "switches" that turn on plants' preparations for adverse conditions by altering hormonal balances.

Because humic acid and seaweed extract exhibit cytokinin-like auxin-like activity, these and other biostimulants that contain hormonal materials may signal plants to switch on their defence system to harsh environments.

In a similar manner, salicylic acid is a factor in disease resistance. An application of salicylic acid induces a typical disease-resistance response called systemic acquired resistance, or SAR. Other materials, such as jasmonic acid, peptides, amino-acid analogs, phenolic compounds and silicates also induce systemic resistance to pathogen attack.

Responses from biostimulant treatments generally increase with the level of stress. To put it another way, plants growing under minimal stress may perform similarly regardless of biostimulants.

NOT ALL SEAWEEDS ARE THE SAME!

Seaweeds are simple organisms that are hard to understand. They are not quite plants and they are definitely not animals!

In recent days the use of seaweed itself (e.g. focus serratus) has been replaced by the use of powder (and now also microgranular) and liquid extracts from dry or fresh seaweed. More than 25,000 species of seaweed have been identified in the world. However mostly brown seaweed species that are growing in cold waters have been used in agriculture, such as the most widely used Ascophyllum nodosum in the Northern hemisphere, while Ecklonia maxima and Durvillea potatorum species are used in the Southern hemisphere. Other species such as Laminaria and Sargassum (the tropical equivalent of Ascophyllum in terms of use) are also used by some producers of seaweed based products for agricultural use. Recently benefits from the use of green and red seaweed extracts have been reported.

The question whether all seaweed products are similar is often asked. There are a number of ways to address it. However the logic today is to address it from the perspective that the main contribution to crop improvement from seaweed products is caused by the presence of plant growth regulators and other components acting in a similar way. Seaweed products harvested from different species therefore can differ appreciably, not only in levels of auxins and cytokinins, but also in composition of types within each group.

As seaweed products contain both auxins and cytokinins, it also appears increasingly important to look at the ratios of auxins to cytokinins rather than focusing on only the auxin or cytokinin content alone. A product where the auxins in the ratio are dominating would be expected to give a more auxin-like response from a treated plant. Similarly, a product is expected to give a more cytokinin-like response when the cytokinins are dominating in the ratio.

Seaweed products can be extracted from the raw material in various ways. Processes such as heat, alkaline hydrolysis, fermentation, freezing, pressure differential cell burst, or a combination of some of the above, have been used to formulate seaweed products. The key is the impact of the various processes on the hydrolysis of organic compounds. In other words, it is not because a particular seaweed is supposed to have "superior" qualities when fresh that the same claim can be made for its extracts. The extraction method used obviously will also have an effect on the growth regulator composition

of the end product. Cytokinins are fairly stable molecules and will not be affected much by extraction procedure. However auxins, and especially naturally occurring auxins, are less stable molecules.

A seaweed product favouring auxin content to that of cytokinin therefore will have different biological responses occurring at different growth stages to that of a seaweed product favouring cytokinins. Not only different responses are expected, but optimal biological response will also necessitate different plant growth stages for application timings. In other words, seaweed extract products are in general crop specific and can result in a variety of crop responses.

Last but not least, and this is not to simplify matters, there may be some differences in composition of a given seaweed with a given production process, depending on…the harvesting time!

HUMIC ACIDS SOIL OR FOLIAR APPLIED: A WORLD OF DIFFERENCE!

Humic acids have physical benefits (physically modifying the structure of the soil), chemical benefits (chemically change the fixation properties of the soil) and biological benefits (biologically stimulate the plant and the activities of soil micro-organisms). You may think you're ready to go to the market and select the best products! Wait a second: you may need to know a few other things! A brief glance at the literature reveals some contradictory results; and for every reference to the success of humus could be given a reference to its failure! It is obvious that out of that broad group of soil substances that may be separated by alkaline extraction, the humic acids, there are some that promote soil fertility and some that don't! As simple as that. Not to be forgotten is also the fact that a claim may be correct when associated to a certain application method and/or rate, however it becomes invalid when the product is not correctly used.

A number of natural sources may be considered as raw material for the manufacture of humic acid/fulvic acid based commercial specialities. However, it clearly transpires that from the "traditional" raw materials, Leonardite is the best source material. What is Leonardite? Indeed a soft, earthy, medium-brown coal-like substance associated with lignite. Leonardite is organic matter, which has not reached the state of coal and differs from soft brown coal by its high oxidation degree, a result of the process of coal formation (bog>peat>coal).

However it was already in 1980-ies that specialists of Leningrad Technology Institute suggested and then VNII Gidroliz Institute and "NPO RET" LLC (Saint Petersburg) further developed new methods of producing environmentally friendly concentrated humic salts by way of oxidizing hydrolytic breakdown of hydrolytic or pulp&paper lignin-containing by-products (industrial lignin and lignin sulfonates). Such materials contain up to 85% of organic matter of which 60-65% is lignin, which determines their humus-forming properties, because it is lignin that is the raw material for natural soil humification.

Whatever the source, determining the soluble humate and fulvate content of materials is relatively straightforward by extraction at high pH. The determination of humic acids is not so simple and the methods used can significantly influence the result obtained. Therefore it is difficult to compare claims from different companies regarding the humic acid content of their products.

As important as the type of product in the effectiveness of the treatment is the type of application. Applications go from dry soil application of large quantities (like a normal fertilizer) or blending with fertilizers or potting soil, all the way to foliar spraying of concentrated liquids and fertigation. And this is where suppliers have to be consistent in their approach to the buyers/end users in order not to confuse them! Each application has its own purpose, meaning that a claim that is documented for soil application may be totally wrong when foliar spraying.

AMINOACIDS: WHICH ONES?

Plants make 300 kinds of amino acids. Only 20 are used to make proteins. What are the rest used for? The rest are indeed used for defence (non-protein amino acids) against

Table 4: legislation on amino acids around the world (source NAI database from suppliers interviews)

Country	Fertilizer(1)	Plant Protection Product	Specific category	registration (2)	no registration (3)
Australia					
Belgium					
Brazil					
Chile					
China	(b)				
Egypt				only plant origin	
Germany					(a)
Greece					
Italy			specific fertilizer		
Mexico					
Morocco					
New Zealand					
South Africa					
Spain					
USA (California)	©			(d)	
USA (Florida)	©			(d)	
USA (others)	©			(d)	

(1) mineral or organic fertilizer. (2) means each product must be separately registered through a specific procedure.
(3) means products can be freely marketed without specific registration.
(a) product to be included previously in a list. (b) micronutrient to be added © no mention of biostimulants allowed.
(d) customs' permit required for each consignment entering the country.

stress. For any living organism, the synthesis and/or the collection of amino acids is critical for cell survival. They not only serve as the building blocks for proteins but also as starting points for the synthesis of many important cellular molecules including vitamins and nucleotides. So, if plants synthesize amino-acids by themselves, why bringing them amino-acids? It is very different from feeding them with nutrients (N, P,K and all other micronutrients) that they do not synthesize! The truth is that in most cases plants or bacteria are lazy: they would rather use amino acids in their environment than make them from scratch. It takes a considerable amount of energy to make the enzymes for the pathway as well as the energy required to drive some of the reactions of amino acid biosynthesis! The genes that code for amino acid synthesis enzymes and the enzymes themselves are under tight control and are only turned on when they are needed. So bringing amino acids to plants makes their life easier, not different: in other words, when a crop wouldn't survive without the addition of water or nutrients, it would without the addition of amino-acids!

In amino acid based products offered in the AG market, the list of active ingredients (amino acids and peptides) is roughly the same; However many mechanisms of action are dependent on the kind, the concentration and the ratios of some specific amino acids and peptides. In other words, and in simpler terms, the right combination of a number of fundamental amino acids will impact on a number of biochemical and physiological functions and processes in the plant that may then translate into yield, quality, earlyness and stress resistance improvements when the crop is foliar sprayed with such mixture. Similarly, the right combination of amino acids brought to the soil may positively impact on the microbial flora in the soil and on the micorrhyzae, which will then translate in an improvement of the soil structure.

Amino-acids used in the Agriculture market come from several origins: however most of them come from the Hydrolysis of proteins. It consists in breaking down the original protein from vegetal or animal source (collagen) into its elemental units: the amino acids. There are several methods for protein hydrolysis: chemical process (acidic attack, alkaline attack), enzymatic hydrolysis, anaerobic saline conditions with acetic acid, microwave hydrolysis, etc. Protein hydrolysates of animal origin obtained through the chemical process have been the first to be used in agriculture, and represent today the great majority of the products in the global market.

BIOSTIMULANTS AS PREVENTIVE MEASURES?

Most crops typically grow well without biostimulants when the environment is favourable. In these situations, the beneficial effects of biostimulants may not be easy to identify. When the plants become stressed, however, biostimulants-treated crops perform better because they have developed a better defence system, apparently due to higher levels of antioxidants.

As with mineral fertilizers, biostimulants take time to exhibit their influence on crops physiology. Under the conditions of some studies, for example, growth stimulation was not large enough to measure until 4 weeks after treatment. After 6 weeks, by contrast, significant differences were found in leaf and shoot number, as well as shoot and root weight of treated vs. non-treated plants. These findings suggest that biostimulants yield the best results when one applies them several weeks before a stress occurs-that is, as pre-stress conditioners.

In other cases that support this concept, research showed that application of biostimulants in the spring significantly improved photosynthetic efficiency and reduced the incidence of some diseases in summer.

THE NEED FOR A HARMONIZED AND SENSIBLE APPROACH

At every corner of the world, urea is urea, mancozebe is mancozebe, copper sulphate is copper sulphate, atrazine is atrazine, glyphosate is glyphosate, etc. In other words, there is a common language and a common word to describe a given product or molecule! What about biostimulants? The answer is NO! A rapid look at the legislation in various countries (see table 2-4) shows that the approach is different. Humic acids: very often considered as fertilizers or amendments but needing registration in some places and no registration in others. Amino-acids: same story but sometimes needing the addition of microelements to be authorized and some places where the mention "biostimulant" is not allowed! Seaweed: this is where there is the most distortion between approaches: sometimes fertilizer, sometimes plant protection, most of the time however with a need for registration.

And there are also some places where all products are treated as "foliar fertilizers" but where people have to live up to what is on the label!

Which approach is best? Difficult to say but it is time to work on the shaping of a legislation that will be harmonized and fair.

The recent challenges to food production due to the increasing occurrence of biotic and abiotic stresses is likely due to climate change and will further reduce yields and/or will have an impact on crops in the 21st century (IPCC 2007). Therefore, research into developing sustainable methods to alleviate these stresses should be a priority.

Recent studies have shown that biostimulants protect plants against a number of biotic and abiotic stresses and offer great potential for field application. Still a number of suppliers formulate biostimulants with little or no scientific input. Many biostimulants are on the market. With proper use, some may enhance crop stress tolerance, yield and/or quality. Others do not. The question that arises is, which ones do and which ones don't? And why: Today, biostimulants are defined in the market by what they do more than by what they are. The time has come to link the "do" and the "are"! ■