The
Oligo Prime® technology

2018-2023 RESULTS

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Agro 100®
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THE CHALLENGE 1

The increase in the world population and the limits to the increase in agricultural productivity have been a concern for experts for many years. The Organisation for Economic Co-operation and Development (OECD) and the Food and Agriculture Organization of the United Nations (FAO), in their report on the Agricultural Outlook 2021-2030, forecast an increase in average food demand per person of 40% in 2030. This increase in average food availability will have to be achieved mainly by increasing agricultural yields (87%), increasing crop intensity (7%) and increasing arable land (6%).[1*]

The environmental sustainability of agricultural practices is a second challenge facing agriculture. Global warming requires a reduction in greenhouse gas emissions; already, greenhouse gases emitted by the agriculture industry represent a net 7% of GHGs emitted in Canada.[2] In addition, the social acceptability of the use of genetically modified organisms, plant protection products and synthetic fertilizers is decreasing. Knowing that converting to "organic" farming will not achieve the objectives of increasing yields[3] farmers must find solutions that will increase productivity, while minimizing the environmental impact.

Increased crop productivity could come from reduced losses to biotic and abiotic stresses. An analysis of the yields observed in several regions of the world indicates that the yields of rice, corn and wheat represent respectively 59%, 35% and 65% of the maximum theoretical yield. The factors that contribute to these yield discrepancies are multiple and include nutrient management, water stress, flooding, soil problems (salinity, heavy metal and chemical toxicities, pH, etc.), diseases, insects, weeds, etc. [4]

Another assessment of the impact of biotic and abiotic stresses, by Buchanan et al. (2000), indicates that crop yields average only 18% of the maximum theoretical yield and that average yield losses associated with biotic stresses range from 5% to 10%, while losses associated with abiotic (environmental) stresses range from 66% to 82%. The most important abiotic stresses remain water stress (water deficit) and soil salinity.[5]

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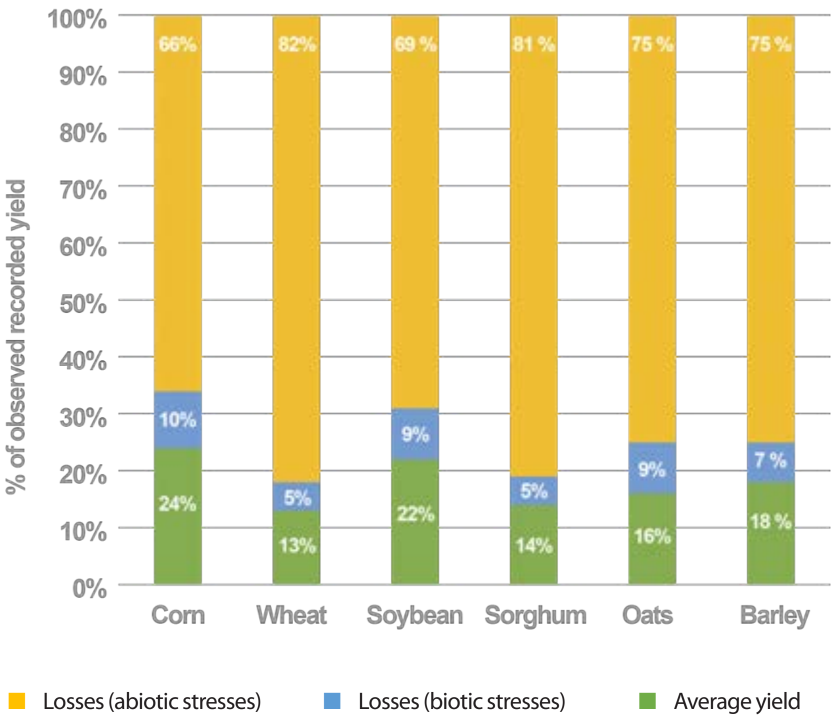
* These numbers refer to the works listed in the bibliography.

Figure 1. Yield losses associated with biotic and abiotic stresses[5]



Farmers cannot control the frequency and severity of environmental stresses. The relatively recent introduction of biostimulants into the range of tools available to farmers makes it possible to envisage a reduction in the negative impacts of abiotic stresses.

A biostimulant is defined as:

> *"[...] a substance or microorganism used on plants to improve their nutrient use efficiency, resistance to abiotic stresses, and thus crop yield and quality, notwithstanding nutrient content. The term biostimulants also refers to products containing mixtures of these substances and/or microorganisms."* [6,7]

The work carried out by Agro-100 Ltd. focused on the use of plant signals that can reduce the impact of abiotic stresses caused by the use of herbicides on corn, soybean and wheat yields. The addition of metabolic signals to a mixture of herbicides and water reduced yield losses associated with herbicide stresses. These results were confirmed by Agro-100 which demonstrated that the use of certain metabolic signals allowed plants to correct the rate of photosynthesis during the period of herbicide-associated stress.[8,9]

The goal is to improve the ability of crops to tolerate abiotic stresses by using biostimulants that will reduce the impact of abiotic stresses on crop yields.

LITERATURE REVIEW 2

2.1. Abiotic stresses

Adverse environmental conditions caused by climate change will become more frequent. High temperatures, water deficits, salinity are just examples of conditions that will have significant impacts on plant growths.[10]

Abiotic stress is defined in several ways. It is defined as "an environmental factor that can have harmful effects on plants. These environmental factors can include drought, extreme cold or heat, high winds, ozone, solar radiation, heavy metals, soil salinity, chemicals, mechanical damage, and more.[11] Blumwald's proposed definition simplifies the notion of abiotic stress to "any environmental condition that prevents plants from realizing their full genetic potential."[12] Ben Ari and Lavi add the notion of "specific environment" to the definition. Indeed, plants being sessile, they cannot simply change their environment to avoid abiotic stresses. As a result, they have developed complex systems of physiological and developmental processes to ensure their growth and reproduction.[13] Thus, a water deficit or soil salinity will initially cause a reduction in water potential and cellular dehydration; in a second step, the closure of the stomata and the decrease in the concentration of carbon dioxide will have a negative impact on photosynthesis. The electrons no longer participating in CO_2 fixation will lead to the production of reactive oxygen species[12] (see figure 2).

Abiotic stresses will directly or indirectly result in the production of reactive oxygen species (ROS).[14] These molecules contain at least one free electron in their orbits. The main forms of ROS are superoxide radical ($O_2^{\bullet -}$), perhydroxyl radical ($HO_2^{\bullet}$), hydroxyl radical ($^{\bullet}OH$), peroxyl radical ($RO_2^{\bullet}$) and alkoxyl radical ($RO^{\bullet}$), as well as non-radical forms such as hydrogen peroxide (H_2O_2).[15]

The superoxide radical ($O_2^{\bullet -}$) is usually the first to be formed in chloroplasts. Although its half-life is short (3.1-3.9 µs) and its diffusion distance small (190nm), it can diffuse out of the chloroplast and reach cell membranes to cause damage.[16] However, it is its role as a precursor to the formation of the hydroxyl radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$), which are much more reactive and toxic, that will have a greater impact on cell damage, and more particularly on the peroxidation of cell membrane lipids.[17] Despite a short half-life of 3 µs and a small diffusion distance of 100 nm, singlet oxygen ($^1O_2$) will cause damage to proteins, pigments, nucleic acids and lipids.[18]

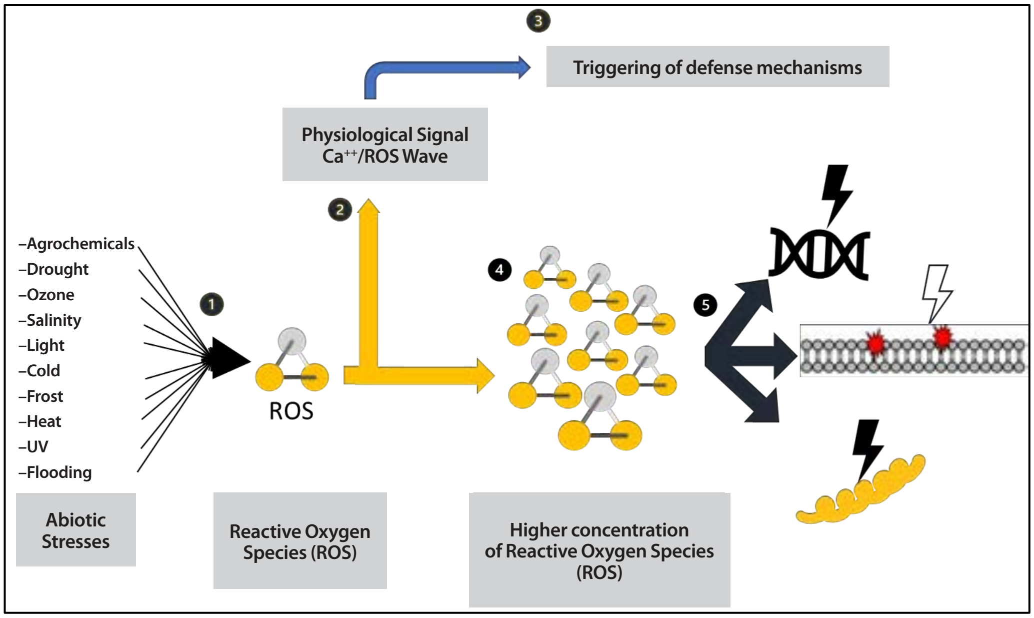


Figure 2. Sequence of events following the appearance of abiotic stresses.

❶ Abiotic stresses cause the production of reactive oxygen species. ❷ ROS are first and foremost signals that with Ca^{++} will propagate through the plant ❸ triggering defense mechanisms. ❹ The concentration of ROS increases and the plant's defense mechanisms are unable to maintain balance in the cells. ❺ ROS will attack cell membranes, DNA, and proteins, causing significant damage to plants.

Hydrogen peroxide (H_2O_2) is a moderately active molecule and is obtained by the reduction and protonation of the superoxide radical ($O_2^{\bullet-}$). This molecule can also be produced directly by the process of photorespiration under water-stressed conditions 19 Hydrogen peroxide has a relatively long half-life (1 ms) and can cross cell membranes. At low concentrations, hydrogen peroxide acts as a signal controlling several physiological processes (senescence, photorespiration, photosynthesis, stomata movement).[14] However, at higher concentrations, hydrogen peroxide will oxidize cysteine and methionine, inactivate the enzyme super oxide dismutase (SOD), and reduce the activity of several enzymes by 50%.[17] Reactive oxygen species are therefore able to react rapidly and oxidize a variety of cellular constituents, including proteins, DNA, RNA and lipids.[14,20]

The plant can reduce the impact of reactive oxygen species through natural defense mechanisms divided into two categories. Enzymatic antioxidants, such as SuperOxideDismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), POX, Monodehydroascorbate Reductase (MDHAR), Dehydroascorbate Reductase (DHAR), Glutathione S-Transferase (GST), Glutathione Peroxidase (GPX), AOXs, Peroxiredoxin (PRX), and Thioredoxin (TRX) reduce the concentration of ROS by converting them to H_2O_2 and ultimately H_2O.[21]

Non-enzymatic antioxidants contribute to the reduction of ROS concentration and are the second line of defense. This class of compounds includes carotenoids, tocopherol, ascorbic acid, melatonin, some phenolics, and glutathione.[22] Ascorbic acid and tocopherol recover OH^- and $^1O_2$ ions, thereby protecting chloroplasts.[23] Glutathione is a tripeptide that acts as a reductive and can inactivate ROS. Phenolic compounds, such as flavonoids, tannins and lignin, act by recovering and inactivating ROS in cells.[22]

The amount of ROS produced by the cells will depend on the amount and duration of the stress. If the stress lasts, the accumulation of ROS will exceed the capacity of the plant's natural defense systems.[20] The defense systems of the cells must maintain a balance with the ROS.

Reactive oxygen species act as a metabolic signal that rapidly communicates the perception of a stressful event (mechanical or chemical injury, disease, etc.) and the accumulation of ROS to the whole plant in order to allow acclimation and the establishment of defense mechanisms.[24]

This signal is broadcast thanks to the Ca++/ROS wave. This process, accelerated by the RBOHD protein, automatically spreads from one cell to another; when triggered in a cell, it causes ROS to accumulate in nearby cells and eventually throughout the plant. This signal triggers defense mechanisms in the plant that divert resources usually attributed to supporting growth towards a stress and defense metabolism.[25] It also coordinates the response of stomata to water or heat stress.[26] The signal transmitted by ROS is not specific to a particular stress, but it accompanies other signals that will bring this specificity. These other mechanisms or molecules, such as electrical signals, hormones, calcium waves, are involved in the transmission of signals from the roots to the leaves and vice versa.[27,28]

Increasing the concentration of ROS in cells will lead the cell's organelles to activate signaling cascades that will allow the plant to better resist abiotic stresses. ROS can interact with intermediates such as Ca^{++}, MAPK, jasmonic acid, abscisic acid, and ethylene to trigger other signaling pathways that will lead to changes in gene expression.[22] Increasing the concentration of cellular Ca will activate certain protein kinases that will regulate the expression of certain genes by phosphorylating or dephosphorylating transcription factors.[12] Plant hormones such as abscisic acid, cytokinin, giberrelic acid, auxin, salicylic acid, jasmonic acid and brassinosteroids also play important roles in the acclimatization of plants to abiotic stresses.[29] These plant hormones will have an important role to play in managing interference between defense mechanisms.[12,30]

2.2. The effect of herbicides on the production of ROS in plants

Herbicides are small molecules that act by inhibiting specific molecular target sites within primary plant metabolic pathways resulting in catastrophic and lethal consequences. The stress induced by herbicides generates reactive oxygen species (ROS), but little is known about the nexus between each herbicide mode of action (MoA) and their respective ability to induce ROS formation. Indeed, some herbicides (groups 5, 6, 10, 12, 13, 14, 18, 19, 22, 24 and 27) cause dramatic surges in ROS levels as part of their primary MoA, whereas other herbicides (group 1, 2, 9, 29 and 4) may generate some ROS as a secondary effect of the stress they imposed on plants. (Traxler et al., 2023) (Caverzan et al., 2019).

Herbicide selectivity is defined as the ratio between weed control and crop injury. It is the mechanism by which some plant species are preferentially controlled or killed while others remain unaffected or less affected by the herbicide. The selectivity of herbicides is explained by a combination of the following mechanisms.[31]

- Differences among plants in interception and uptake of the herbicide.
- Different metabolism between crops and weeds; weeds are less able than the crop to metabolize and inactivate the selective herbicide.
- Physical: herbicides may be applied in a different zone or at a different time minimizing the impact on the crop.
- Use of antidotes or safeners that make the herbicide less toxic for the crop.

Differential metabolism of the herbicide is one of the most important factor explaining selectivity of herbicides. Crops have the ability to metabolize more rapidly the herbicides, allowing for a better resistance to the herbicides.[32]

2.3. The Oligo Prime® technology

Crops have the ability to defend themselves from damages caused by ROS created in abiotic stress events or by ROS generated by herbicides. The ROS defense mechanisms include enzymatic and non-enzymatic components which serve to balance the production and the detoxification of ROS.

The **OligoPrime® technology** is designed to increase the effectiveness of the natural defense mechanisms present in all plants. It is based on four components:

① **Metabolic signals**;
② **C-plex® technology**;
③ **Fulvic acid**;
④ **Chitosan**.

The synergy between these four technologies allows products containing Oligo Prime® technology to perform better and deliver economic returns in excess of 3 to 1.

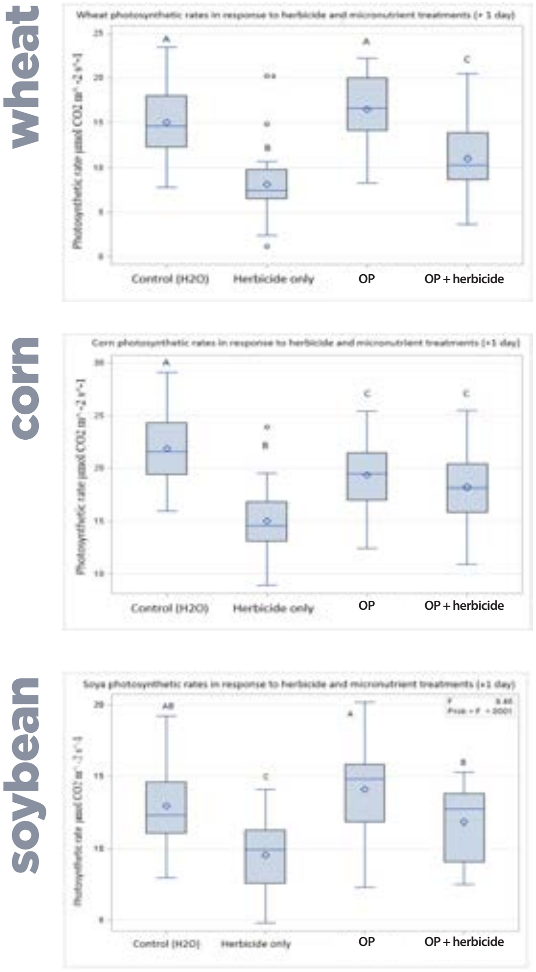
2.3.1. The role of metabolic signals

Until recently, many studies on the role of phytohormones in plant secondary metabolism focused on jasmonic acid (JA), salicylic acid (SA), gibberellins (GA), and abscisic acid (ABA). It is now clear that phytohormone-induced regulation of signaling occurs via regulation of the genome, or an increase in secondary metabolite production.

Secondary metabolites are synthesized in different cellular compartments and include phenolics, terpenoids, and alkaloids whose main functions include protection against biotic and abiotic stresses.

The addition of secondary metabolites to the Oligo Prime® technology reduces the negative impact of herbicide-associated stresses on photosynthesis. Thus, the rate of photosynthesis decreases by 50% when the herbicide (wheat: bromoxynil/MCPA, corn: glyphosate, soybean: glyphosate) is used alone, the rate of photosynthesis decreases by 44% for wheat, 30% for corn and 20% for soybeans when measured one day after application. The addition of secondary metabolites to herbicides reduces the decline in photosynthesis rates to 22% for wheat, 16% for corn, and 10% for soybeans[8] (see figure 3).

Figure 3. Effect des secondary metabolites on the photosynthetic rate



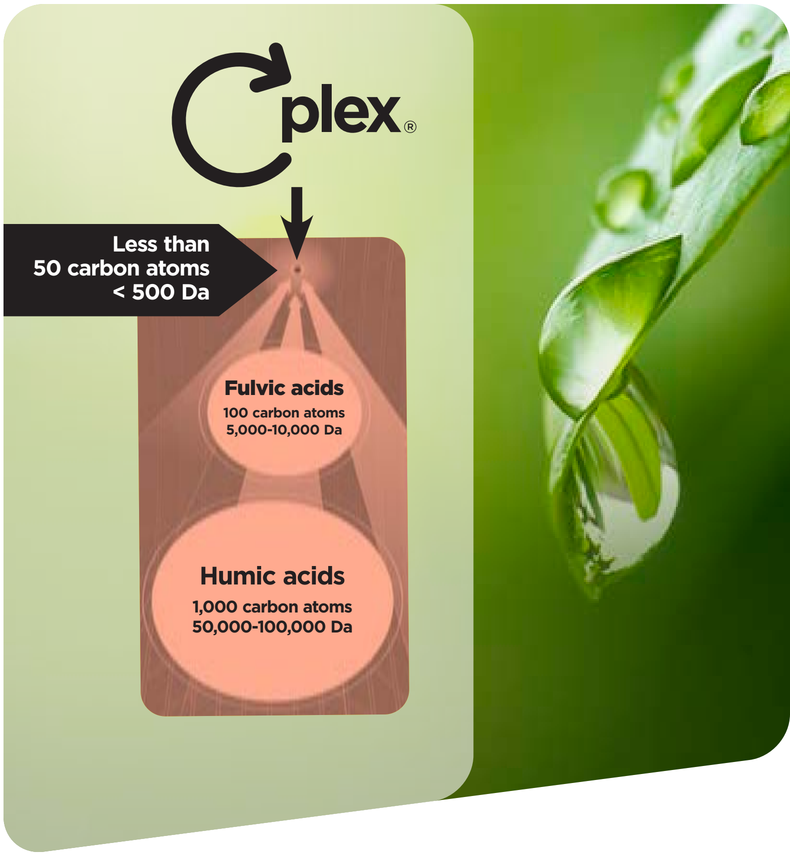
The secondary metabolites included in the Oligo Prime® technology also impact the plant genome and activate the production of 71 defensive proteins and enzymes that play a role in reducing the impact of ROS.[33]

2.3.2. The role of fulvic acid and C-plex®

Humic substances are divided into different categories that include humic acids, fuvic acids, and humins. Historically, humic acids were considered to be larger molecules with molecular weights ranging to a hundred thousand daltons, while fulvic acids are typically only a few thousand daltons. As stated above, fulvic acid is considered to be the soil organic fraction that is soluble in both alkali and acid. Fulvic acids have greater total acidity, greater numbers of carboxyl groups, and higher adsorption and cation exchange capacities than humic acid. Fulvic acids are responsible for chelation and mobilization of metal ions, including Fe and Al. Given their small molecular size, fulvic acids can pass through micropores of biological or artificial membrane systems, while humic acids cannot.[6]

The capacity of fulvic acid to enhance uptake of nutrients has been reported in diverse systems. Early work recorded enhanced uptake of N, P, K, Ca, Mg, Cu, Fe, and Zn in cucumber plants grown in Hoagland solution. Fulvic acids were reported to enhance uptake of ^{32}P phosphate in wheat and corn. Several studies focused on the interaction of fulvic acids with Fe. In a model soil system without plants, fulvic acid was reported to complex Fe^{3+} in soil in a soluble form that could be taken up by plants.[6]

The capacity of humic materials to complex cations was demonstrated with fulvic acid. C-plex® is a molecule derived from fulvic acid that has a smaller size and a higher cation exchange capacity. This smaller size is obtained through an exclusive digestive process that yields molecules of a few hundred daltons. This small size allows for quick and easy uptake by the plant and a very high complexing capacity.



2.3.3. The role of chitosan

Chitosan is a natural biopolymer modified from chitins that acts as a biostimulant and elicitor in agriculture. It is non-toxic, biodegradable and biocompatible which promotes a very wide application. It improves the physiological response and mitigates the harmful effect of abiotic stresses. Chitosan treatment stimulates the rate of photosynthesis and closure of stomata, increases the concentration of antioxidant enzymes, and induces the production of organic acids, sugars, amino acids, and other metabolites that are required for stomata control, production of metabolic stress signals, and energy management under stress conditions.[34]

Chitosan induces several responsive genes, proteins, and secondary metabolites in plants. Chitosan elicits a signal transduction pathway and transduces secondary molecules such as hydrogen peroxide and nitric oxide. Under biotic stress, chitosan can stimulate phytoalexins, pathogenesis-related proteins, and proteinase inhibitors. Pretreatment of chitosan before exposure to abiotic stresses induces plant growth, production of antioxidant enzymes, secondary metabolites that elicit the production of defensive enzymes, and abscisic acid (ABA). However, plant responses depend on the type of chitosan-based structures, concentrations, species, and crop developmental stages.[35]

The addition of chitosan to the Oligo Prime® technology increases the ability of cells to produce the defensive enzymes necessary for the inactivation of ROS caused by herbicides.

HYPOTHESES AND OBJECTIVES 3

3.1. Research Hypotheses

The use of herbicides causes an increase in the production of ROS. The Oligo Prime® technology promotes an increase in the concentration of enzymes specific to the inactivation of ROS. The specific hypotheses tested in this research proposal are:

- The use of Oligo Prime® in a mixture with the herbicide glyphosate will reduce the impact of this herbicide on the production of ROS and increase the yield of the crop;
- The use of Oligo Prime® in a mixture with herbicides that generate high concentrations of ROS will reduce the impact of these herbicides on ROS production and increase crop yield.

Figure 4. Research sites in Eastern Canada

METHODOLOGY 4

4.1. Sites

The trials were conducted on research farms located in Quebec and Ontario between 2018 and 2023.

4.1.1. CEROM

The CEROM research farm is located at 740, chemin Trudeau, Saint-Mathieu-de-Beloeil (Québec) J3G 0E2. For the period from April 1 to October 31, the average accumulation (between 1979 and 2008) of degree days (base 0) is between 3002 and 3189. The number of corn heat units is between 2900 and 3100 (8 years out of 10).

4.1.2. University of Guelph

The University of Guelph research farm is located at 12088 Baker Road, Winchester, ON, K0C 2K0.

4.1.3. Université Laval

The Université Laval Research Farm is located at 521,QC-138, Saint-Augustin-de-Desmaures (Québec)G3A 1W7. For the period from April 1 to October 31, the average accumulation (between 1979 and 2008) of degree days (base 0) is between 2627 and 2814. The number of corn heat units is between 2300 and 2500 (8 years out of 10).

4.1.4. BlackCreek Research

BlackCreek Research's research farm is located at 886613 Oxford Rd 8, Bright, ON N0J 1B0.

4.1.5. Eastern Crop Doctor

The Eastern Crop Doctor research farm is located at 11343 Vancamp Road, Winchester, Ontario, K0C 2K0.

4.2. Protocols

The trials were conducted on corn and soybean fields. The cultivars and hybrids used were chosen according to the area; in all cases, these were glyphosate-resistant cultivars and hybrids. The seeding rates used ranged from 75,000 to 84,000 plants/hectare for corn and 250,000 to 290,000 plants/hectare for soybeans. Sowing was carried out with precision seeders at depths of 3.5 to 5 cm.

4.2.1. Corn glyphosate (group 9) trial

CropBooster OP, a foliar biostimulant (15% N – 3% P_2O_5 – 6% K_2O with 2% S, 0.02% B, 0.05% Mn, 0.05% Mo, 0.05% Zn) enriched with the Oligo Prime® technology (0.34% C-plex®, 0.12% fulvic acid, 0.25% metabolic signals, 0.1% chitosan, 0.5% EDTA) was added to a herbicide tank mix containing glyphosate (RoundUp Weathermax, 900 g a.i./hectare). Group 9 herbicides inhibit the production of the EPSPS enzyme and the formation of essential amino acids. They contribute to the production of significant amounts of ROS.

The treatments are presented in Table 1.

The control treatment was glyphosate (RoundUp Weathermax, 900 g a.i./hectare) applied alone. The resulting spray was applied at a rate of 200 litres/hectare. Treatments were performed between stages V3 and V8. The treatments were replicated 4 or 6 times, and arranged in completely randomized blocks. Statistical analyses were performed with R 4.2.2.[36] An analysis of variance and a comparison of the means obtained was made, using the TukeyHSD function of the statistical program.

Table 1. List of treatments in the **corn** group 9 trial

TRT No.	Treatment	HRAC (group) classification	Application rate	
1	Weedy Control	—	—	—
2	Glyphosate	9	900	g a.i./ha
	Agral 90	adjuvant	0,2	% v/v
3	Glyphosate	9	900	g a.i./ha
	Agral 90	adjuvant	0.2	% v/v
	CropBooster OP	biostimulant	2	L/ha

4.2.2. Soybean glyphosate (group 9) trial

CropBooster OP, a foliar biostimulant (15% N – 3% P_2O_5 – 6% K_2O with 2% S, 0.02% B, 0.05% Mn, 0.05% Mo, 0.05% Zn) enriched with the Oligo Prime® technology (0.34% C-plex®, 0.12% fulvic acid, 0.25% metabolic signals, 0.1% chitosan, 0.5% EDTA) was added to a herbicide tank mix containing glyphosate (RoundUp Weathermax, 900 g a.i./hectare). Group 9 herbicides inhibit the production of the EPSPS enzyme and the formation of essential amino acids. They contribute to the production of significant amounts of ROS.

The treatments are presented in Table 2.

The control treatment was glyphosate (RoundUp Weathermax, 900 g a.i./hectare) applied alone. The resulting spray was applied at a rate of 200 litres/hectare. Treatments were carried out between the V3 and V4 stages of soybeans. The treatments were replicated 4 or 6 times and arranged in completely randomized blocks. Statistical analyses were performed with R 4.2.2.[36] An analysis of variance and a comparison of the means obtained was made, using the TukeyHSD function of the statistical program.

Table 2. List of treatments in the **soybean** group 9 trial

TRT No.	Treatment	HRAC (group) classification	Application rate	
1	Weedy Control	—	—	—
2	Glyphosate	9	900	g a.i./ha
	Agral 90	adjuvant	0,2	% v/v
3	Glyphosate	9	900	g a.i./ha
	Agral 90	adjuvant	0.2	% v/v
	CropBooster OP	biostimulant	2	L/ha

4.2.3. Herbicides + biostimulant in corn trial

CropBooster OP, a foliar biostimulant (15% N – 3% P_2O_5 – 6% K_2O with 2% S, 0.02% B, 0.05% Mn, 0.05% Mo, 0.05% Zn) enriched with the Oligo Prime® technology (0.34% C-plex®, 0.12% fulvic acid, 0.25% metabolic signals, 0.1% chitosan, 0.5% EDTA) was added to herbicides identified as parts of groups 5, 15 and 27.

Group 5 herbicides inhibit the D1 protein of the photosynthetic II system, and promote the production of a significant amount of ROS. Group 14 herbicides inhibit an enzyme (PPO) involved in photosynthesis. Group 15 herbicides inhibit the formation of fatty acids; these herbicides generate little ROS. Group 27 herbicides inhibit the production of the enzyme 4-hydroxyphenylpyruvate dioxygenase (4-HPPD). This enzyme is necessary for the production of carotenoids by plants. Herbicides in these groups generate significant amounts of ROS.[37]

Table 3 shows the treatments performed in these trials.

The control treatments were the herbicides applied alone (treatments 2, 4 and 6). The resulting spray was applied at a rate of 200 litres/hectare. Treatments were carried out between the V3 and V8 stages of corn. The treatments were replicated 4 or 6 times and arranged in completely randomized blocks. Statistical analyses were performed with R 4.2.2.[36] An analysis of variance and a comparison of the means obtained was made, using the TukeyHSD function of the statistical program.

Table 3. List of treatments for the **corn** herbicides + biostimulant trial

TRT No.	Treatment	HRAC (group) classification	Application rate	
1	Weedy Control	—	—	—
2	Glyphosate/s-metolachlor/mesotrione	9/15/27	4.2	L/ha
	Atrazine	5	0.6	L/ha
	Agral 90	adjuvant	0.2	% v/v
3	Glyphosate/s-metolachlor/mesotrione	9/15/27	4.2	L/ha
	Atrazine	5	0.6	L/ha
	Agral 90	adjuvant	0.2	% v/v
	CropBooster OP	biostimulant	2	L/ha
4	Glyphosate	9	900	g a.i./ha
	Tembotrione (Laudis)	27	65	g a.i./ha
	Atrazine	5	576	g a.i./ha
	Hasten	adjuvant	1,75	L/ha
5	Glyphosate	9	900	g a.i./ha
	Tembotrione (Laudis)	27	65	g a.i./ha
	Atrazine	5	576	g a.i./ha
	Hasten	adjuvant	1.75	L/ha
	CropBooster OP	biostimulant	2	L/ha
6	S-metolachlor/mesotrione/bicyclopyrone	15/27/27	3.952	L/ha
	Glyphosate	9	900	g a.i./ha
7	S-metolachlor/mesotrione/bicyclopyrone	15/27/27	3.952	L/ha
	Glyphosate	9	900	g a.i./ha
	CropBooster OP	biostimulant	2	L/ha
8	S-metolachlor/atrazine	15/5	3.5	L/ha
	Glyphosate	9	900	g a.i./ha
	Saflufenacil	14	75	g a.i./ha
9	S-metolachlor/atrazine	15/5	3.5	L/ha
	Glyphosate	9	900	g a.i./ha
	Saflufenacil	14	75	g a.i./ha
	CropBooster OP	biostimulant	2	L/ha

4.2.4. Herbicides + biostimulant in soybean trial

CropBooster OP, a foliar biostimulant (15% N – 3% P_2O_5 – 6% K_2O with 2% S, 0.02% B, 0.05% Mn, 0.05% Mo, 0.05% Zn) enriched with the Oligo Prime® technology (0.34% C-plex®, 0.12% fulvic acid, 0.25% metabolic signals, 0.1% chitosan, 0.5% EDTA) was added to identified herbicides commonly used in soybean production. Glyphosate is a Group 9 herbicide. Chlorimuron-ethyl is a Group 2 herbicide; this group of herbicides inhibits the production of ALS enzymes, stopping the production of amino acids. Group 4 herbicides regulate certain genes responsible for auxin production. Group 14 herbicides inhibit an enzyme (PPO) involved in photosynthesis. Group 2, 4, 9 and 14 herbicides generate significant amounts of ROS.

Table 4 shows the treatments carried out.

The control treatments were the herbicides applied alone (treatments 2, 4 and 6). The resulting spray was applied at a rate of 200 litres/hectare. Treatments were carried out between the V3 and V4 stages of soybeans. The treatments were replicated 4 or 6 times and arranged in completely randomized blocks. Statistical analyses were performed with R 4.2.2.[36] An analysis of variance and a comparison of the means obtained was made, using the TukeyHSD function of the statistical program.

Table 4. List of treatments for the **soybean** herbicides + biostimulant trial

TRT No.	Treatment	HRAC (group) classification	Application rate	
1	Weedy Control	—	—	—
2	Chlorimuron-ethyl	2	9	g a.i./ha
	Glyphosate	9	900	g a.i./ha
	Agral 90	adjuvant	0.2	% v/v
3	Chlorimuron-ethyl	2	9	g a.i./ha
	Glyphosate	9	900	g a.i./ha
	Agral 90	adjuvant	0.2	% v/v
	CropBooster OP	biostimulant	2	L/ha
4	Glyphosate	9	900	g a.i./ha
	2,4-D choline	4	817	g a.i./ha
	Agral 90	adjuvant	0.2	% v/v
5	Glyphosate	9	900	g a.i./ha
	2,4-D choline	4	817	g a.i./ha
	Agral 90	adjuvant	0.2	% v/v
	CropBooster OP	biostimulant	2	L/ha
6	Glyphosate	9	900	g a.i./ha
	Fomesafen	14	240	g a.i./ha
	Turbocharge	adjuvant	0.25	% v/v
7	Glyphosate	9	900	g a.i./ha
	Fomesafen	14	240	g a.i./ha
	Turbocharge	adjuvant	0.25	% v/v
	CropBooster OP	biostimulant	2	L/ha

RESULTS

5

5.1. Glyphosate (Group 9) trial in corn

The objective of this trial was to verify the effect of the Oligo Prime® technology on corn yield, when used in mixture with glyphosate. The yield obtained when glyphosate was applied with the CropBooster OP was significantly higher than when glyphosate was applied alone. The difference observed was 752 kg/ha (12 bu/acre).

The positive impact of Oligo Prime ® technology has been confirmed at all locations and in all years.

Corn

	2018 (kg/ha)	2019 (kg/ha)	2021 (kg/ha)	2022 (kg/ha)	2023 (kg/ha)	Average 2018-2023 (kg/ha)	
Glyphosate alone	12,247	6,091	11,359	17,869	15,723	12,890	
Glyphosate with CropBooster OP	**13,585**	**6,975**	**11,404**	**18,437**	**16,069**	**13,641**	
Effect of the CropBooster OP technology	**1,338**	**884**	**46**	**568**	**346**	**752**	***

Corn

	2018 (kg/ha)	2019 (kg/ha)	2021 (kg/ha)	2022 (kg/ha)	2023 (kg/ha)	Average 2018-2023 (kg/ha)
Glyphosate with CropBooster OP	**13,585**	**6,975**	**11,404**	**18,437**	**16,069**	**13,641**
Beloeil	11,974	6,975	11,404	—	—	10,118
Bright	—	—	—	18,437	14,698	16,567
Winchester ECD	14,659	—	—	—	—	14,659
Winchester UOG	—	—	—	—	17,898	17,898
Glyphosate alone	12,247	6,091	11,359	17,869	15,723	12,890
Beloeil	11,740	6,091	11,359	—	—	9,730
Bright	—	—	—	17,869	14,497	16,183
Winchester ECD	12,584	—	—	—	—	12,584
Winchester UOG	—	—	—	—	17,358	17,358

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Traitement	1	8189803.066	8189803.066	7.450628475	0.0091	**
Annee_Site	6	727832191.1	121305365.2	110.3568915	7.37699E-25	***
Traitement x Annee_Site	6	7557709.135	1259618.189	1.145930747	0.3523	ns
Residuals	44	48365226.65	1099209.697	NA	NA	

5.2. Glyphosate (Group 9) trial in soybean

The objective of this trial was to verify the effect of the Oligo Prime® technology on soybean yield, when used in mixture with glyphosate. The yield obtained when glyphosate was applied with CropBooster OP was higher than when glyphosate was applied alone. The difference observed was 107 kg/ha (1.6 bu/acre). This difference is not significant. The difficulties encountered by soybean production in 2022 and 2023 explain these results.

Soybean

	2018 (kg/ha)	2019 (kg/ha)	2021 (kg/ha)	2022 (kg/ha)	2023 (kg/ha)	Average 2018-2023 (kg/ha)	
Glyphosate without CropBooster OP	**3,530**	**3,883**	**6,603**	**3,510**	**4,642**	**4,434**	
Glyphosate with CropBooster OP	**3,665**	**4,038**	**6,786**	**3,566**	**4,653**	**4,541**	
Effect of the CropBooster OP technology	**135**	**155**	**183**	**55**	**11**	**107**	ns

Soybean

	2018 (kg/ha)	2019 (kg/ha)	2021 (kg/ha)	2022 (kg/ha)	2023 (kg/ha)	Average 2018-2023 (kg/ha)
Glyphosate with CropBooster OP	**3,665**	**4,038**	**6,786**	**3,566**	**4,653**	**4,541**
Beloeil	3,261	4,038	6,786	—	—	4,695
Bright	—	—	—	3,566	4,193	3,879
St-Augustin	2,546	—	—	—	—	2,546
Winchester ECD	4,680	—	—	—	—	4,680
Winchester UOG	—	—	—	—	5,113	5,113
Glyphosate without CropBooster OP	**3,530**	**3,883**	**6,603**	**3,510**	**4,642**	**4,434**
Beloeil	3,146	3,883	6,603	—	—	4,604
Bright	—	—	—	3,510	4,062	3,786
St-Augustin	2,459	—	—	—	—	2,459
Winchester ECD	4,718	—	—	—	—	4,718
Winchester UOG	—	—	—	—	5,222	5,222

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Traitement	1	12304.98	12304.98	0.241625	0.625275	ns
Annee_Site	7	91218552	13031222	255.8862	9.47E-36	***
Traitement:Annee_Site	7	142277.8	20325.4	0.399118	0.898154	ns
Residuals	48	2444441	50925.85	NA	NA	

5.3. Herbicides + biostimulant trial in corn

The objective of this trial was to test the effect of the Oligo Prime® technology on corn yield, when used in a mixture with herbicides generating high levels of ROS. The yield obtained when these herbicides were applied with the CropBooster OP was higher than when these herbicides were applied alone. The difference observed was 658 kg/ha (10.5 bu/acre). This difference is significant.

These positive differences have been repeated every year and at all sites.

The comparison of yields obtained when subjected to different herbicide programs is not significant. There is no interaction between herbicides and biostimulant treatments.

Corn

	2021 (kg/ha)	**2022** (kg/ha)	**2023** (kg/ha)	**Average** 2021-2023 (kg/ha)	
Herbicide without CropBooster OP	**12,390**	**7,487**	**16,094**	**12,039**	
Herbicide with CropBooster OP	**15,785**	**8,101**	**16,505**	**12,698**	
Effect of the CropBooster OP technology	**3,395**	**613**	**411**	**658**	***

Corn

	2021 (kg/ha)	**2022** (kg/ha)	**2023** (kg/ha)	**Average** 2021-2023 (kg/ha)
Herbicide with CropBooster OP	**15,785**	**8,101**	**16,505**	**12,698**
Glyphosate + tembotrione (Laudis) + atrazine (Aatrex)	15,894	8,125	16,672	12,786
Mesotrione/s-metolachlor/atrazine/bicyclopyrone (Acuron) + glyphosate	16,427	8,003	16,571	12,858
S-metolachlor/mesotrione/glyphosate (Halex GT) + atrazine	15,034	8,175	16,273	12,448
Herbicide without CropBooster OP	**12,390**	**7,487**	**16,094**	**12,039**
Glyphosate + tembotrione (Laudis) + atrazine (Aatrex)	10,211	—	16,518	12,152
Mesotrione/s-metolachlor/atrazine/bicyclopyrone (Acuron) + glyphosate	15,821	7,802	15,620	12,327
S-metolachlor/mesotrione/glyphosate (Halex GT) + atrazine	13,861	7,173	16,144	11,647

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Traitement	1	8995514.355	8995514.355	7.771923578	0.006719979
Traitementherbicide	2	4557692.492	2278846.246	1.968872281	0.1467643666
Annee_Site	2	1319227604	659613802.2	569.8915992	4.43E-46
Traitement:Traitementherbicide	2	563533.3052	281766.6526	0.243440097	0.784543447
Residuals	75	86807798.59	1157437.315	NA	NA

5.4. Herbicides + biostimulant trial in soybean

The objective of this trial was to test the effect of the Oligo Prime® technology on soybean yield, when used in a mixture with herbicides generating high levels of ROS. The yield obtained when these herbicides were applied with the CropBooster OP was higher than when these herbicides were applied alone. The difference observed was 167 kg/ha (2.5 bu/acre). This difference is significant.

These positive differences were repeated in 2021 and 2022, but not in 2023.

The comparison of yields obtained when subjected to different herbicide programs is significant. This analysis shows that some herbicide programs have resulted in higher yields. The interaction between herbicides and biostimulant treatments is not significant.

Soybean

	2021 (kg/ha)	**2022** (kg/ha)	**2023** (kg/ha)	**Average** 2021-2023 (kg/ha)	
Herbicide without CropBooster OP	**4,347**	**3,130**	**4,822**	**4,181**	
Herbicide with CropBooster OP	**4,594**	**3,364**	**4,751**	**4,349**	
Effect of the CropBooster OP technology	**247**	**233**	**-72**	**167**	***

Soybean

	2021 (kg/ha)	**2022** (kg/ha)	**2023** (kg/ha)	**Average** 2021-2023 (kg/ha)	
Herbicide with CropBooster OP	**4,594**	**3,364**	**4,751**	**4,349**	
S-metolachlor/metribuzin (Boundary LQD) + glyphosate	4,222	—	—	4,222	
Fomesafen/glyphosate (Flexstar)	4,732	3,296	4,713	4,333	
Chlorimuron-ethyl (Classic) + glyphosate	4,734	3,431	4,301	4,203	
2,4-D choline (Enlist)	—	—	5,277	5,277	
Herbicide without CropBooster OP	**4,347**	**3,130**	**4,822**	**4,181**	
S-metolachlor/metribuzin (Boundary LQD) + glyphosate	4,194	—	—	4,194	
Fomesafen/glyphosate (Flexstar)	4,492	3,130	4,784	4,171	
Chlorimuron-ethyl (Classic) + glyphosate	4,316	3,130	4,180	3,890	
2,4-D choline (Enlist)	—	—	5,523	11,647	
Effect of the CropBooster OP technology	**247**	**233**	**-72**	**167**	**

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Traitement	1	685634.2325	685634.2325	8.279121954	0.005056829
Traitementherbicide	3	12068772.14	4022924.046	48.57732768	1.84E-18
Annee_Site	4	53164980.56	13291245.14	160.4935025	5.04E-39
Traitement:Traitementherbicide	3	607333.7567	202444.5856	2.44454453	0.069449722
Residuals	86	7122076.994	82814.84877	NA	NA

CONCLUSION 6

According to the impact assessment of biotic and abiotic stresses carried out by Buchanan et al. (2000), crop yields, on average, only reach 18% of their theoretical maximum yield. Since yield losses associated with *abiotic* stresses range between 66% and 82% compared with 5 to 10% for *biotic* stresses, increasing crop yields cannot disregard the impact abiotic (environmental) stresses has on productivity.[5]

Of course, farmers cannot control the frequency and magnitude of the environmental stresses affecting plants and crops.

The relatively recent introduction of biostimulants such as those offered by the Oligo Prime® technology makes it possible to complate a reduction in the negative impacts brought on by abiotic stress on yields.

The results obtained in the various trials carried out on corn and soybean fields allow us to draw these main conclusions.

- **Effectiveness**. Trials conducted between 2018 and 2023 demonstrated the effectiveness of the Oligo Prime® technology when used in the CropBooster OP.
- **Impact of the Oligo Prime® technology**. The observed differences in yield are explained by the impact of the technology has on photosynthesis and on the increase in the concentration of proteins and enzymes needed to reduce the concentration of ROS in crops.

Trials will continue in 2024.

The Oligo Prime® technology is therefore an effective tool available to farmers. And let's never lose sight that the goal is to improve the ability of crops to tolerate abiotic stresses by using biostimulants that will reduce the impact of abiotic stresses on crop yields. And an increase in crop yields will make it possible to better respond to the increase in average food demand per person of 40% by 2030.[1]

Isn't that a goal we all share...

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