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Multiyear Study on the Yield of Soybean as Affected by Humic Biostimulation at Uruguay

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Abstract: Soybean (*Glycine max* L.) is of considerable importance in Uruguay as an export crop, however, significant variations in the production occur due to the impact of prolonged droughts and/or extreme temperatures mostly during the grain filling period. Biostimulant Substances (BS) as the humics cause effects on crops as better efficiency in nutrient absorption, increases yield and root growth as well as tolerance to abiotic stress. Data from farm trials during six years on the effect of an humic bio stimulant applied at 4 L ha^{-1} rate (treated) vrs (untreated check) at the R2-R3 development stage on crop strips across the farm field was collected at 85 localities of 15 Departments of Uruguay. The observed yield (g/plant) and the yield components (pods/plant and grains/plant) were significantly increased by the application of the humic substance in all years and localities tested where soybean crops have endured a range of different types of climates from water and hight temperature stresses, excessive rainfall and years with very favorable conditions. The historical behavior of the humic biostimulation technique shows that it can be adopted by soy producers under the evidence that the yield per plant depends on the amount of pods and grains per plant that are increased in all cases by the application of humic. The exogenous application of humic SB as part of the present agronomic management for extensive soybean field crops can be used to promote sustainable intensification. Further development of this technology requires incorporating the topic of biostimulants within the research programs of national agricultural R&D institutions focusing on the mechanisms of action, forms and times of application. This technology can be pursued in legume crops as a short term and low cost improvement to be adopted by soy farmers.

INTRODUCTION

Soybean (*Glycine max* L.), the global sixth most important crop (in terms of tonnage) is of considerable importance in Uruguay as an export crop where the area of cultivation and production have increased steadily since 2006-2013 (288 and 373%, respectively), reaching 966.000 ha with a yield of 2927 kg ha^{-1} at 2019 and positioning Uruguay as the eighth largest world producer (FAOSTAT 2019). Significant variations in yield occur due to the impact of prolonged droughts and/or extreme temperatures mostly during the grain filling period[1]. Kobraei *et al*.[2] reported a 43% decrease in soybean yield due to drought stress compared to normal cultivation conditions. Although, the crop presents plasticity against stresses in the vegetative growth phase that is reduced during the reproductive stages[3].

As reviewed by Du Jardin[4], Biostimulant Substances (BS) effects on crops are earliness, better efficiency in nutrient absorption, increases in yield and quality, increase and maintenance of leaf pigments (chlorophyll and carotenoids) and greater root growth as well as improving the efficiency in plant water use and tolerance to abiotic stress and yields with or without stress. The effects of BSs including the Humic Sustances (HS), measured by bioassays, immunological tools and molecular genomics are largely explained by the positive, integrated and induced regulation (signaling) of endogenous genes responsible for the biosynthesis of protective compounds that allow attenuating oxidation processes caused by water stress, high temperatures or against pathogens[5].

Legume crop yields tend to vary more than cereal crops, largely due to environmental constraints such as drought which limits symbiotic nitrogen fixation[6]. Changes in temperature, rainfall and CO_2 rate can influence soybean growth and the final grain yield, however, most researches have considered till now only the impacts of planting date, drought stress, irrigation regimes and variety on growth and yield of soybean[7].

The HSs including fulvic and humic acids) are supramolecular associations of small, heterogeneous molecules extracted from soils, sediments, composted biomasses of agro-industrial residues. Extracting HS from agro-industrial residues is environmentally important because it recycles precious C which would be otherwise burnt or landfilled[8]. This can be pursued in the soybean field crop as a short term and low cost improvement to be adopted by farmers after proper validation at farm level.

MATERIALS AND METHODS

In this multiyear study, data from 85 farm trials during six years (2015-2020), on the effect of an humic bio stimulant applied at 4 L ha^{-1} rate (treated) vs. (untreated check) at the R2 (full flowering)-R3 (pod formation) plant development stage on crop strips across the farm field, was collected at localities of 15 Departments of Uruguay. In each farm trial, crop sectors (treated and check) with similar conditions in terms of slope, type of soil, plant density and state of the crop were chosen at harvest. The sampling consisted of randomly extracting 30 plants from the sectors and carried out by walking in the crop and changing rows taking 10 steps without looking the furrow to extract plants in the middle of a chosen row, without missing plants and whose stem base were of medium size. Subsequently, in the laboratory, the pods/plant and the individual grains/plant counts and the weighing (g) of each individual plant sampled are carried out. Finally, the weight of a thousand grains and the number of grains/pod retained were calculated. The yield and the yield components at each location/year/treatment (original data available on request) was analyzed by the paired t-student test, 95% with the STHDA program. The humic biostimulant was produced from vermicomposting by the worm *Einsenia fetida* of mounds of wheat straw and cow manure under controlled conditions and as part of a licensed process. The composition of the humic biostimulant was as follow: total humic extracts 5.72% P/V; humic acids 4.05% P/V; fulvic acids 1.22% P/V; boron 0.1% W/V; auxins (AIA, AIP) 0.1-0.05 mg L^{-1}; gibberellins (GA3) 0.5-2 mg L^{-1}; cytokinin (Adenine) 0.01-0.05 mg L^{-1}; amino acids 7-9.5 mg L^{-1}; Enzymatic Activity: oxidase and transpeptidase. Density: +/- 0.003 g mL^{-1}; pH: 6.8.

RESULTS AND DISCUSSION

The observed yield (g/plant) and the yield components (pods/plant and grains/plant) were significantly increased by the application of the HS applied in R2-R3 in the 6 years and over all the localities tested (Table 1).

In general terms and at national level the climate during the reproductive phase of the soybean growing season at Uruguay (February, March and April) can be summarized, from the farmers point of view as:

- 2015 year with drought at March and early April
- 2016 year with excessive rainfall at the end of the cycle (April)
- 2017 good year
- 2018 very dry year
- 2019 very good year with national yield performance record
- 2020 year with hydric and high temperature stresses

Meteorological data of rainfall, evapotranspiration and maximum temperatures during the reproductive phase of the soybean crops (Fig. 1) shows over the years six contrasting climatic situations. The results were consistent and independent of the climate variation year-to-year.

The component grain/pod was not affected with the exception of the year 2015. The weight of the 1000 grains was slightly but not significantly increased on 2015, 2018, 2019 and 2020.

In all the years and localities as compared with the non treated check, the HS increased the yields in a range

Table 1: Soybean yield and yield components as affected by an humic biostimulant treatment foliar-sprayed on R2-R3 on soybean farms, since, 2015 to 2020 at different locations in Uruguay

Year and general climatic condition	Farm trials	Paired samples (1)	Treatment	Pods/plant	Grains/plant	Grains/pod	Yield g/plant	Weight 1000 grains g
2015	20	678	HUMIC	44.4	91.6	1.73	12.42	141.1
			CHECK	38.8	78.1	1.68	10.71	137.9
			t(2)	9.5554	11.015	4.5909	7.9706	2.2268
			p-value	2.215e-20	4.547e-26	5.261e-06	6.716e-15	0.02629
2016	11	354	HUMIC	45.1	85.9	-	14.1	167.7
			CHECK	43.2	80.2	-	13.1	167.3
			t	1.8799	3.0048	-	3.2874	0.2956
			p-value	0.06095	0.002847	-	0.001113	0.7677
2017	17	510	HUMIC	73.2	154.9	2.15	24.3	156.4
			CHECK	62.7	132.6	2.13	20.8	156.9
			t	10.9905	10.6701	-0.5883	10.0708	-0.5174
			p-value	2.331e-25	4.006e-24	0.5566	7.094e-22	0.6051
2018	24	433	HUMIC	33.7	72.5	2.13	10.13	142.6
			CHECK	31.1	62.8	2.14	8.78	140.3
			t	3.7753	7.3579	0.2591	7.5482	2.0038
			p-value	0.0001822	9.487e-13	0.7957	2.643e-13	0.04571
2019	7	168	HUMIC	78.8	167.4	2.19	28.17	178.3
			CHECK	69.1	132.5	2.22	24.36	174.6
			t	3.1085	6.3118	-1.7436	4.1719	2.1856
			p-value	0.002211	2.393e-09	0.08331	4.846e-05	0.03024
2020	6	199	HUMIC	49.1	109.3	2.20	15.64	136.9
			CHECK	42.2	96.4	2.23	13.91	139.6
			t	4.4745	3.9323	-1.6708	3.5551	-0.9724
			p-value	1.287e-05	0.0001161	0.09673	0.0004721	0.3323

1 = treated vs. non treated check; 2 = Paired student t test (95%)

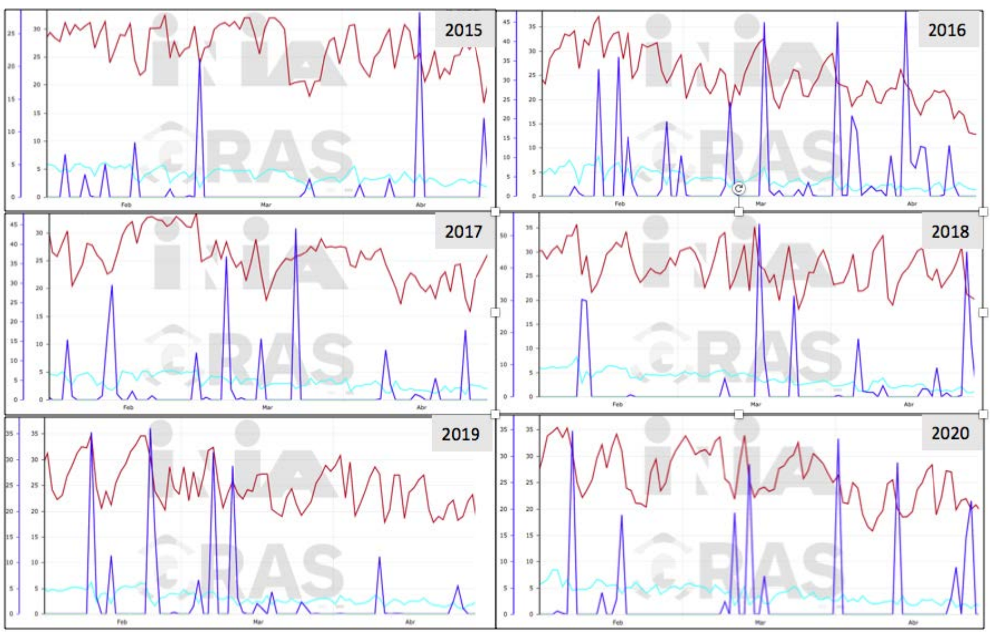

Fig. 1: Penman evapotranspiration (mm) (light blue); maximum temperature°C (red) and effective precipitation (mm) (violet) in the months of February, March and April, since, 2015 to 2020. Data: INIA GRAS, La Estanzuela Experimental Station, Uruguay

from 6.87-16.82% (Fig. 2a-c). This was a consequence of sustained significant increases in the number of pods retained at harvest and in the number of grains per plant. The weight of the 1000 grains was slightly increased over the check while the number of grains per pod (very constant variable in soybeans) was not significantly affected (Fig. 2d, e). In the last year (2020) an +12.43% (1.73 gr pl^{-1}) increase in yield over the control was found based on more grains/plant (+13.38%) which in turn is closely related to +16.35% in pod retention emplyingan estimated yield increase of 311 kg ha^{-1} by the HS assuming a plant density of 180.000 harvested plants per ha.

Current evidence suggests that the effects of humic substances on crop plants are characterized by both structural and physiological changes in roots and stems related to the absorption, assimilation and distribution of nutrients (characteristics of efficiency in the use of nutrients). Humic vermicompost extracts promoted in rice, the activation of the antioxidant enzymatic function and the increase of ROS-scavenging enzymes necessary to inactivate the toxic oxygen radicals produced in plants under drought and salinity stress[9]. In addition, they can induce changes in the primary and secondary metabolism of plants related to tolerance to a biotic stress. In support of this, Rose *et al*.[10] using a random effects meta-analysis showed that the dry weights of roots of different plant species increased approximately 22% in response to exogenous application of humics. The same humic reported here applied on barley resulted in a significant increase in root dry weight (+184% over the non treated check) (unpublished) (Effect of the moment of application and the dose of two humic biostimulants on the yield of brewing barley. January 2021 DOI: 10.13140/RG.2.2.16612.40321).

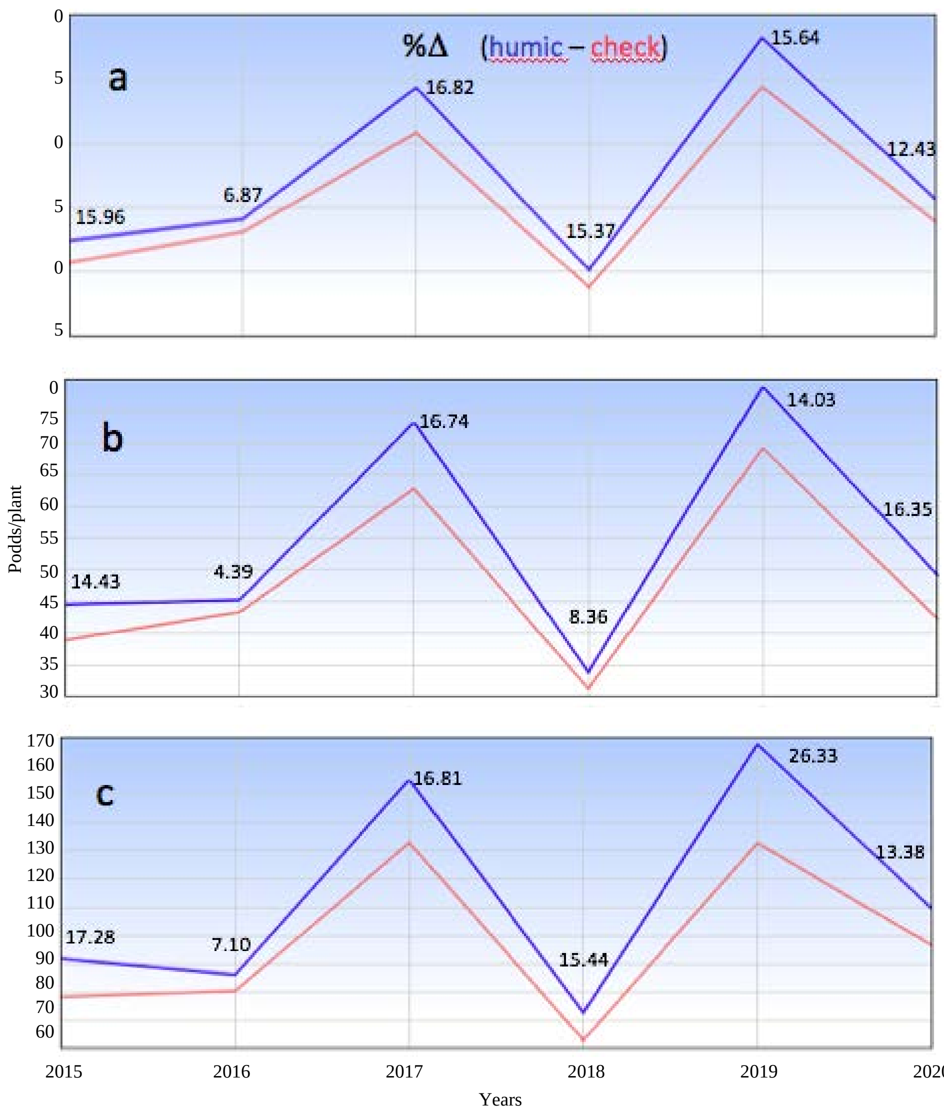

Fig. 2(a-c): Percentage of change over the non treated check for: (a) yield/plant; (b) pods/plant and (c) grains /plant of the soybean crop at multiple locations treated with humic biostimulant at R2-R3 over a six year period

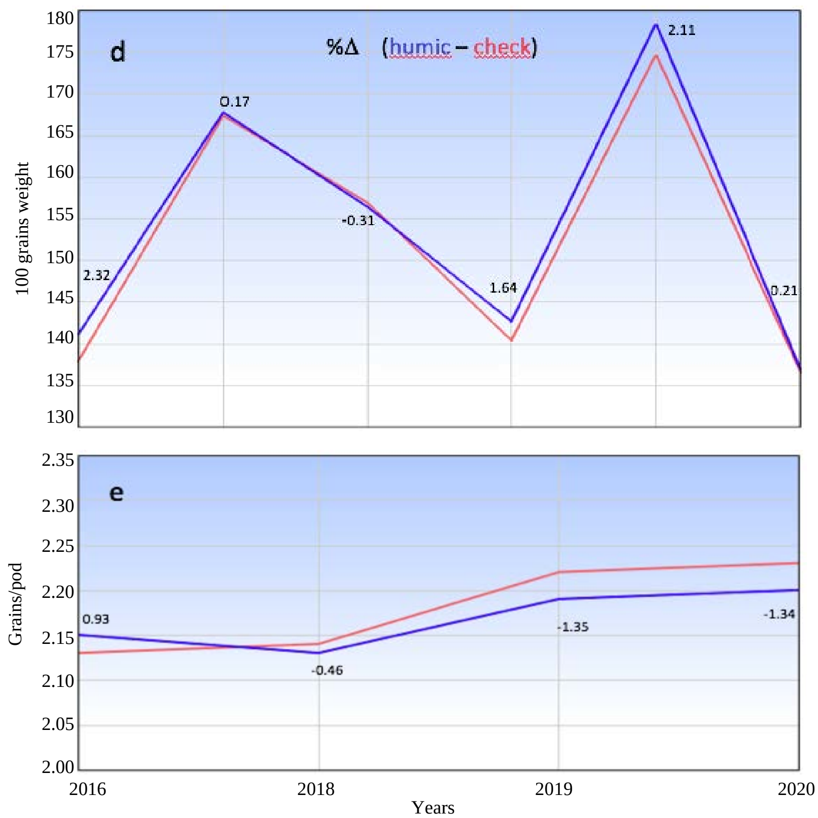

Fig. 2(d, e): Percentage of change over the non treated check for: (d) 1000 grains weight and (e) grains/pod of the soybean crop at multiple locations treated with humic biostimulant at R2-R3 over a 4 year period

Although, recent documents are focusing on the mechanisms of action of BSs few have investigated their effects on the physiology and biochemistry of soybean field plants despite the global importance as a crop. The HS are reckoned to boost crop growth and yields and protect plants from abiotic stresses by triggering specific metabolic routes studies are needed to relate HS molecular structure to specific, biostimulant-related plant traits, such those relevant to nutrient use efficiency and tolerance to abiotic stress. Further research should be devoted to the isolation of the biologically active compounds present in the HS.

In the six years, soybean crops have endured different types of climates from water and high temperature stresses (2015, 2018, 2020), excessive rainfall (2016) and years with very favorable conditions (2017 and 2019). Despite that climatic variation, the historical behavior of the HS bioestimulation technique shows good results that can be adopted by soy producers under the evidence that the yield per plant depends on the amount of pods and grains per plant that are increased in all cases by the application of humic. This results are the first of its kind in Uruguay that show that an humic substance produced in the country and applied in the early stages of the reproductive cycle of soybeans is a complementary tool to increase the sustainable crop productivity even in years with significant environmental impacts.

CONCLUSION

For environmental and economic reasons, agricultural practices are gradually evolving towards more sustainable systems, where the partial (complementation) or reduction of agrochemicals can be achieved through genetic improvement programs and/or through the identification and use of natural molecules as Humic Sustances (HS) capable of activating (signaling) related to plant metabolisms and able to induce better yields and tolerance to adverse factors. This can be pursued for the soybean crop as an improvement in crop yields in the short run of time and in a cheaper way.

Simple summary: Legume crop yields as soybean tend to vary more than cereal crops, largely due to environmental constraints such as drought. For environmental and economic reasons would be appropriate gradually evolving towards more sustainable systems through the identification and use of natural molecules as humics for inducing better yields and tolerance to adverse factors. The use of humics can be pursued as a short term and low cost improvement to be adopted by soybean farmers after validation at farm level. The yield per plant were significantly increased at 85 farm trials during 6 years irrespective of the climatic variation by the application of an humic substance produced by vermicomposting by the worm *Einsenia fetida* of mounds of wheat straw and cow manure. This results are the first of its kind in Uruguay that show that a humic produced in the country and applied at flowering is a complementary tool to increase soybean field crop productivity even in years with significant climates impacts.

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