Effect of chitosan foliar application on the performance and yield of soybean and maize under field conditions

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Chitosan is a natural heterogeneous polymer, non-toxic and find many potential applications in agriculture. The search of a biopolymer to replace the synthetic agrochemicals, the effects of foliar application of chitosan solution on agronomic performance under field experiments have been investigated in two consecutive harvests (2014/2015 and 2015) of soybean and maize. Two chitosan concentrations (0.5 and 1%) were sprayed four times during the culture development and the evaluation was based on morphological characteristics, growth and yield components. Seeds were collected to determine the content of macro- and micronutrients. Results revealed that most of the morphological and growth parameters (plant height, leaf number per plant) and yield attributes were not increased with the chitosan treatment in soybean and maize. The soybean treated with foliar application of chitosan 0.5% increased the accumulation of nitrogen by 9,14% when compared with the water treatment, but the effect was not consistent in both seasons and cultivars. The values of the other nutrients did not change the content. Maize plants treated with chitosan (0.5% or 1%) significantly decreased the boron concentration by 66,7% in the grains analyzed and may have influenced the plant development (height) without altering the other agronomic parameters. Therefore, whereas the climatic conditions in these two seasons were stable and biotic or abiotic stresses were not observed, foliar application of chitosan solution did not present differences that could establish a pattern of behavior in field experiments conducted by two harvests.

Keywords: chitosan application; plant growth; field experiments.

1. Introduction

Chitosans, a family of partially deacetylated chitin derivatives, are among the few natural polycationic polymers. They are used as raw materials for multifunctional applications in medicine, cosmetics, food packaging (Aranaz et al., 2018; Zhao et al., 2018; Kumar et al., 2020; Joseph et al., 2021), agriculture (Chandra et al., 2015; Malerba & Cerana, 2019; Yu et al., 2021), and other fields. As a versatile functional biopolymer family, chitosans which are considered to be biocompatible in the human body and biodegradable in the environment, are predicted to play significant roles in the future bioeconomy (Muxika et al., 2017). Still, chitosans are a generally under-utilized bioresource (Muñoz et al., 2018) even though due to their non-toxicity, they are approved as food supplements (Morin-Crini et al., 2019) and as a 'basic substance' that may be used for agricultural applications in Europe without the need for regulatory approval (EU, 2014; EU 2015). Chitosan polymers and oligomers or their derivatives exhibit a number of agriculturally relevant biological activities, including plant growth-promoting activities (Hidangmayum et al., 2019; Sharma et al., 2020), plant resistance-inducing elicitor and priming activities (Vander et al., 1998; Trouvelot et al., 2014; Malerba & Cerana, 2018), and antimicrobial activities (Sahariah & Másson, 2017; Verlee et al., 2017; Kucharska et al., 2019). These bioactivities have been shown to be dependent on the structure of the chitosans used (Bellich et al., 2016; Cord-Landwehr et al., 2020; Wattjes et al., 2020), i.e., their degrees of polymerization and acetylation and, as shown more recently, also on their pattern of acetylation (Basa et al. 2020).

Chitosans can exert a number of positive influences on crop production, acting either as a biopesticide protecting plants from disease resistance, or as a biostimulant or biofertilizer to increase food production through stimulation of growth and development, improvement of nutrient and water use efficiency, increase of abiotic stress tolerance, and improvement in crop productivity and quality of the harvest (Trouvelot et al., 2014; Malerba & Cerana, 2016; Sharif et al., 2018; Hidangmayum et al., 2019; Li et al., 2019; Sharma et al, 2020). Such uses as biostimulants or biopesticides are becoming increasingly attractive to decrease the input of agro-chemicals through a partial replacement by agro-biologics in conventional agriculture (Gozzo and Faoro, 2013).

There are countless examples in literature describing beneficial effects of chitosan treatments such as foliar spray, seed coating, or root dipping in different crop species. The recent literature describes effects of chitosans and chitosan nanoformulations towards relevant crop plants such as tomato (Suarez-Fernandez et al., 2020), wheat (Zhang et al., 2018), soybean (Yang et al., 2019; Mehmood et al., 2020), maize (Choudhary et al., 2017; Choudhary et al., 2019), and beans (Chatelain et al., 2014). In maize plants, e.g., seed treatment and foliar spray of chitosan nanoparticles led to physiological and biochemical alterations including changes in gene expression, enhanced growth, and yield increases (Sharma et al., 2020).

In spite of so many positive reports, there are a number of problems remaining with the use of chitosans as agro-biologics, in particular the often-observed fact that the results are poorly reproducible (Raafat & Sahl, 2009; Bellich et al., 2016). Different possible reasons for this lack of reproducibility have been proposed, i.a. batch-to-batch differences when using poorly characterized chitosan samples, differences in chitosan-reactivity between species, particularly between monocot and dicot species, and even between cultivars of the same crop species, or different environmental and climatic effects in different seasons and at different locations. In our systematic study, we have used a well-characterized chitosan in two consecutive seasons at two field locations on two cultivars of both soybean and maize, evaluating the effects of chitosan foliar application on growth, yield, and agronomic performance.

2. Material and Methods

2.1. Plant Material

Seeds of the commercial soybean (*Glycine max* (L.) Merrill) cultivar BMX Potência RR and maize (*Zea mays* L.) hybrid Pioneer 30F53 HX were used in the 2014/2015 harvest. In the second harvest (in 2015), the soybean seeds were of the commercial cultivar CD 2630 RR, and maize seeds were from DKB 330 PRO2.

2.2. Preparation of chitosan

The chitosan used was a commercially available chitosan preparation (produced from shrimp shell chitin by Mahtani Chitosan Pvt. Ltd. India obtained by partial acetic acid-catalyzed depolymerization of a microcrystalline chitosan polymer. We characterized the polymer as having a medium degree of polymerization (number average DP = 400), a reasonably low dispersity ($Đ$ = 2.7), and a medium degree of acetylation (average DA = 20%), and the hydrolysate as being composed of equal amounts of a polymer fraction having an average DP of 90 and partially acetylated chitosan oligomers of DP 2-15, both, with a DA of 20%, using state-of-the-art analytical techniques (see below). The chitosan was provided as a stock solution (10% chitosan w/v) in slight stochiometric excess of acetic acid (pH 4.3).

DA was analyzed using ^{1}H-NMR spectroscopy (AV300 MHz spectrometer, Bruker, USA) according to Kumirska et al. (2009) in D_2O/DCl and evaluated according to Hirai et al. (1991) and Lavertu et al. (2003). Recorded spectra were transformed using the 1st Fourier function of the MesteReNova software (v. 9.0, Mestrelab Research, Spain); a manual phase correction and a full polynomial baseline adjustment were performed. The chemical shifts were adjusted using the deuterium oxide signal at 4.79 ppm, and peaks were manually integrated.

DP and $Đ_{DP}$ were analyzed using HP-SEC-RID-MALLS (Schatz et al., 2003). Briefly, the Mw distribution was determined using an Agilent HPLC system equipped with three TSKgel columns (G6000PWXL-CP 13 µm, G5000PWXL-CP 10 µm, G300PWXL-CP 7 µm, 7.8 mm diameter, 30 cm length) coupled online with a refractive index detector (Agilent Series 1200 RID) and a multiangle laser light scattering detector (PSS SLD 7000 MALLS). Elution was done using a 50 mM ammonium acetate buffer (pH 4.5). For Mw determination, data was evaluated using WinGPC software by PSS (GPC-MALS_1), using dn/dc values deduced from a polynomial based on previous studies relating the dn/dc with the DA (Schatz et al. 2003). On the basis of Mw and DA, the corresponding DP was calculated.

2.3. Field experiments

The study was conducted in the field at two locations in the Western Region of Paraná (PR) State, Brazil. During the 2014/2015 harvest, the experiments were carried out in Pérola District, PR, and they were repeated in 2015 in the municipality of Palotina, PR. The soil is classified as an eutroferric red Oxisol of clay texture (Santos et al., 2018) and according to the Köppen classification, the predominant climate type in the areas is the humid subtropical (CFA).

The four treatments included the liquid chitosan solution at concentrations of 0.5 and 1% (i.e., 0.05 and 0.1% active ingredient (a.i.)), as well as the fungicides cyproconazole + azoxystrobin (rates of 60 g + 24 g a.i. ha^{-1}) (Syngenta®). Control plants were treated with tap water.

Solutions were sprayed on the aerial parts of plants four times (every ten days) during the crop development at 2 L / plot. The treatments occurred at post-emergency of soybean (V4) and in maize (V5/V6) by using a pressurized CO_2 backpack sprayer with a bar equipped with six spray nozzles (AI 110.015) at a constant pressure of 2 bar, at a speed of 1 m s^{-1}, providing a spray volume of 200 L ha^{-1}.

Each plot was 3 m wide by 5 m long and contained six rows of plants (0.45 m spacing). Discounting one row on each side as a border and considering 2 m linear of usable area, the total experimental plot area was 3.6 m^2. The experiments were carried out in a randomized block design with four treatments and four replicates (total of 16 plots). The trial area was 60 m^2 and the total experimental area was ca. 240 m^2.

2.4. Evaluation of agronomic traits and yield

For soybean, the plant height (cm), number of pods per plant, number of pods of 2 and 3 grains, 100-seed weight, and yield were evaluated. Macro- and micronutrient content (N, P, K, Ca, Mg, S, Cu, Fe, Zn, Mn, B) were determined in the grains for both years. The parameters were evaluated at the R8 stage (full maturity), i.e. 95% of the pods had the typical mature pod color (Fehr et al., 1971). The number of pods per plant was evaluated by manually counting the number of pods in ten plants randomly chosen in the used area of each plot. Pods were threshed in a thresher for experiments, cleaned with sieves, and packed in paper bags for further elemental analysis.

For maize plants, the evaluations were plant height (m), weight of 1,000 seeds (g), yield (kg ha^{-1}), basal stem diameter (mm), and height of first ear insertion (m). Macro- and micronutrient content (N, P, K, Ca, Mg, S, Cu, Fe, Zn, Mn, B) were determined in the grains only for the first harvest. These data were collected after the point of physiological maturity of the crop (R6), when the grains had moisture at the harvest point (Ritchie et al., 1993). Plant height evaluation was carried out by measuring the stalk length of ten randomly selected plants per plot before harvest, measured with a wooden millimeter ruler.

For yield determination, plants were harvested from the useful area of each plot, the humidity corrected to 13%, and the results expressed in kg ha^{-1}. For 100-grain mass, two sub-samples with 13% moisture corrected mass were measured. For other variables, the average value was determined, with ten plants evaluated in the area of each plot.

The mineral nutrient (macro- and micronutrients) of the grain samples were analyzed by the Laboratory of Soil (Pirasolo in Piracicaba, São Paulo, Brazil) according to Malavolta et al. (1997).

2.5. Statistical analysis

All data were submitted to analysis of variance F-test ($p \leq 0.05$) and the means of the treatments were compared using Tukey test at 0.05 significance level. The values were analyzed using the SISVAR® software according to Pimentel-Gomes & Garcia (2002).

3. Results

3.1. Field experiments with soybeans

Foliar spraying of 0.5 and 1% chitosan solution did not improve physiology and agronomic traits of soybean (Table 1) in the first (2014/2015) or second (2015) season of the experiment. The values of pods with two or three grains per plant, total number of pods per plant, plant height, weight of 100-seed, or yield (in kg ha^{-1}) were not significantly different between the treatments (chitosan, fungicide, or water control).

Table 1. Effect of chitosan solution applied four times by foliar spraying on growth parameters in soybean.

	First Season (2014/2015)						**Second Season (2015)**					
Treatment	Pods with 2 grains/ plant	Pods with 3 grains/plant	Pods per plant	Plant height (cm)	100-seed weight (g)	Yield (kg ha^{-1})	Pods with 2 grains/ plant	Pods with 3 grains/ plant	Pods per plant	Plant height (cm)	100-seed weight (g)	Yield (kg ha^{-1})
Control	7.0	25.4	32.4	60.3	17.8	2,978	14.2	17.0	31.2	81.1	13.2	1,613
Chitosan 0,5%	6.2	20.7	26.8	57.7	18.0	2,790	15.1	17.2	32.3	80.6	13.5	1,944
Chitosan 1%	6.8	25.3	32.2	59.8	18.8	2,988	12.3	16.3	28.5	81.8	12.6	1,836
Fungicide[1]	7.5	23.9	31.4	58.0	18.9	3,048	14.2	18.2	32.4	78.0	12.6	1,902
Mean	6.9	23.8	30.7	59.0	18.4	2,951	13.9	17.2	31.1	80.4	13.0	1,824
SD	11.0	10.1	8.7	3.8	6.1	11.5	15.2	12.4	11.9	3.8	4.1	21.9
Test F	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1]Cyproconazole + azoxystrobin (60 + 24 g of active ingredient/hectare)
ns: differences not significant by F-test ($p \leq 0.05$)

The results obtained from the first season (2014/2015) using cultivar BMX Potência RR showed that the amount of all grain nutrients analyzed except for Mg, i.e. the concentrations of N, P, K, Ca, S, Cu, Fe, Zn, Mn, and B, did not change regardless of the concentration of chitosan applied. Only the Mg content was slightly reduced by the 0.5%, but not by the 1% chitosan treatment (Table 2).

Table 2. Macro- and micronutrient contents in soybean (*Glycine max* cv. BMX Potência RR) grains under four foliar sprays of chitosan during the first season (2014/2015).

Treatment	N	P	K	Ca	Mg		S	Cu	Fe	Zn	Mn	B
Control	62.0	5.2	16.7	4.1	2.4	a	3.4	10.5	93.0	49.3	38.5	29.5
Chitosan 0,5%	61.3	4.8	16.0	4.0	2.2	b	3.0	9.5	113.0	48.0	41.0	37.3
Chitosan 1%	61.4	5.3	16.9	4.2	2.4	a	3.5	9.5	81.0	56.8	42.5	36.5
Fungicide[1]	60.4	5.2	17.5	4.1	2.3	ab	3.0	10.0	85.8	56.8	40.3	36.5
Mean	61.3	5.1	16.8	4.1	2.3		3.2	9.9	93.2	50.9	40.6	34.1
SD	4.5	4.6	4.5	2.8	3.3		7.8	8.4	34.1	17.0	9.1	28.0
F	ns	ns	ns	ns	*		ns	ns	ns	ns	ns	ns

[1]Cyproconazole + azoxystrobin (60 + 24 g of active ingredient/hectare)
ns: differences not significant by F-test ($p \leq 0{,}05$)
*Values followed by the same letter in the column are not significantly different by F-test ($p \leq 0{,}05$)

In the second season (2015), the experiments with soybean used the cultivar CD 2630 RR and the amounts of P, K, Mg, S, Cu, Fe, Zn, and B that accumulated in the reproductive structures did not change by the foliar treatments. On the other hand, we observed differences between the treatments for the contents in N and the micronutrients Mn and Ca (Table 3). Nitrogen accumulation increased by almost 10% in the plants treated with 0.5% chitosan compared with water treatment, but it was not significantly different between the two chitosan (0.5 or 1%) and the systemic fungicide treatments. Manganese content was reduced by the 0.5% chitosan treatment when compared to fungicide, but did not change significantly compared to the control plants. Application of fungicide decreased the calcium values, but these values were not changed by the chitosan treatments.

Table 3. Macro- and micronutrient contents in soybean (*Glycine max* cv. CD 2630 RR) grains under four foliar sprays of chitosan during the second season (2015).

Treatment	N		P	K	Ca		Mg	S	Cu	Fe	Zn	Mn		B
Control	53.6	b	4.5	16.2	2.9	a	2.1	3.3	13.0	86.0	53.8	25,0	ab	35,0
Chitosan 0.5%	58.5	a	4.2	15.9	2.7	ab	2.0	3.1	10.5	87.0	54.0	26,5	a	32,3
Chitosan 1%	54.8	b	4.6	16.1	2.7	ab	2.0	3.1	14.0	90.0	51.8	21,5	bc	28,5
Fungicide[1]	56.2	ab	4.5	16.4	2.3	b	2.0	3.1	15.0	115.0	54.0	18,5	c	39,8
Mean	55.7		4.4	16.1	2.6		2.0	3.1	13.1	94.5	53.4	22.9		33.9
SD	2.9		5.1	4.4	7.8		4.8	9.9	16.1	26.1	6.8	9.01		17.4
F	*		ns	ns	*		ns	ns	ns	ns	ns	*		ns

[1]Cyproconazole + azoxystrobin (60 + 24 g of active ingredient/hectare)
ns: differences not significant by F-test ($p \leq 0.05$)
*Values followed by the same letter in the column are not significantly different by F-test ($p \leq 0.05$)

3.2. Field experiments with maize

Regarding the parameters related to agronomic performance (Table 4), treatments with both chitosan concentrations (0.5 and 1%) slightly reduced the plant height (by less than 3%) while none of the other parameters, i.e. stem diameter, ear insertion height, weight of 100 seeds, and yield, were affected by chitosan or fungicide treatment in either season.

Table 4. Effect of chitosan solution applied four times by foliar spraying on growth attributes in maize.

Treatment	**First Season (2014/2015)**						**Second Season (2015)**	
	Stem diameter (mm)	Ear insertion height (cm)	Plant height (cm)		100-seed weight (g)	Yield (kg ha^{-1})	100-seed weight (g)	Yield (kg ha^{-1})
Control	22.8	124.1	241.8	a	37.7	10.140	36.5	10,750
Chitosan 0.5%	23.0	121.6	235.5	b	37.8	10.626	35.6	9,444
Chitosan 1%	22.7	121.6	235.6	b	38.1	10.349	36.5	10,958
Fungicide[1]	23.7	120.3	238.2	ab	38.6	9.881	35.9	9,860
Mean	23.0	121.9	237.8		38.0	10.249	36.1	10,253
SD	2.8	3.7	1.1		2.0	8.3	6.3	10.1
F	ns	ns	*		ns	ns	ns	ns

[1]Cyproconazole + azoxystrobin (60 + 24 g of active ingredient/hectare)
ns: differences not significant by F-test ($p \leq 0.05$)
*Values followed by the same letter in the column are not significantly different by F-test ($p \leq 0.05$)

Considering the contents in macro- and micronutrients (Table 5), treatment with both chitosan concentrations significantly decreased the boron concentration by two thirds in the maize grains when compared with water control plants, though the chitosan values were not significantly different from those of the fungicide treatment which itself was one third lower than the control, but this difference was not significant. All other values, i.e. N, P, K, Ca, Mg, S, Cu, Fe, Zn, and Mn, were not affected by the treatments.

Table 5. Macro- and micronutrients content in maize grains (hybrid Pioneer 30F53 HX) under four foliar spray of chitosan during the first season (2014/2015).

Treatments	N	P	K	Ca	Mg	S	Cu	Fe	Zn	Mn	B
Control	14.2	2.8	3.1	0.6	0.9	1.6	2.5	18.0	22.5	3.9	4.5 a
Chitosan 0.5%	15.2	3.2	3.2	0.5	1.0	1.5	2.0	20.0	23.5	4.1	1.5 b
Chitosan 1%	13.7	3.0	3.3	0.5	0.9	1.4	2.0	20.0	22.8	4.0	1.5 b
Fungicide[1]	14.4	3.3	3.2	0.6	1.0	1.3	2.5	16.0	23.3	4.0	3.0 ab
Mean	14.4	3.1	3.2	0.5	0.9	23.5	2.3	18.5	23.00	4.00	2.63
SD	6.2	7.6	4.8	15.1	7.3	1.4	33.1	14.0	4.4	0.1	31.8
F	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*

[1]Cyproconazole + azoxystrobin (60 + 24 g of active ingredient/hectare)
ns: differences not significant by F-test ($p \leq 0.05$)
*Values followed by the same letter in the column are not significantly different by F-test ($p \leq 0.05$)

4. Discussion

Chitosans can have different effects when applied to crop plants as a foliar spray (Malerba & Cerana, 2016; Rabêlo et al., 2019). Initially, the action as a biopesticide protecting plants from disease was investigated (Sahariah & Másson, 2017), but lately, the action as a biostimulant or biofertilizer increasing the plants' tolerance to abiotic stress and/or promoting their growth and development, e.g. by stimulating photosynthesis or increasing water or nutrient use efficiency, is gaining increasing interest (Hidangmayum et al. 2019; Mehmood et al., 2020). This is accompanied by ongoing changes in the legislation for the registration of agro-chemicals and agro-biologics. In the European Union, the category of 'biostimulants' has been defined in the framework of the fertilizer regulation, and similar actions were taken, e.g. in India and Brazil (where they are named 'biofertilizers') (MAPA, 2020), or are in the process of being implemented, e.g. in the USA. Chitosan hydrochloride has been approved as a 'basic substance' in the EU which may be used in agriculture without the need for registration (EU, 2014; EU 2015), and chitosan is about to be included in the list of low risk biopesticides in the USA, with similar implications. Unlike the old categories of 'plant strengtheners' which existed in different legislations in the past, the new categories are supposed to be better defined, based on empirical evidence and a basic understanding of modes of action (Cord-

Landwehr et al., 2020; Wattjes et al., 2020). This will require systematic studies such as the one presented here, including field trials under different environmental conditions and on different cultivars of different plant species.

The current study was designed to test the effect of chitosan treatments on plant performance under natural field conditions, using as model species soybean and maize. We chose a dicot and a monocot crop as it is well documented that monocot and dicot species differ in their responses to elicitors and priming agents involved in triggering induced disease resistance (Ortmann et al., 2006; Balmer et al., 2012). Both soybean and maize are crops of global economic importance, driving the economy of many countries, mostly being used as animal feed or biogas production, but some also for human consumption. At the same time, large scale soybean and maize production has a strong environmental impact (Fearnside, 2001), including through irrigation, fertilization, and the use of pesticides for plant protection. Chitosan used as biostimulant and/or biopesticide could help mitigate this negative influence.

While a positive influence of chitosan or chitosan derivatives on soybean growth and metabolism has been described repeatedly, studies using well-defined chitosans and reporting on field trials are rare. Treatment with chitosan oligomers (DP ca. 20; DA ca. 10 %) increased the germination rate of soybean seeds (Hai et al., 2019). Several genes involved in isoflavone accumulation, including phenylalanine ammonia-lyase (PAL) and isoflavone synthase (IFS), were up-regulated and the contents of flavonoid and total phenolics were increased during soybean seed germination upon treatment with chitosan oligomers (DP 3-7, DA unknown) (Jia et al., 2019). Chitosan (DP and DA not specified) modified biochar (from rice straw) treatment influenced physiological and biochemical parameters and the growth of soybean seedlings grown in Hoagland nutrient solution and increased the plants' tolerance to saline stress (Mehmood et al., 2020). Foliar application of chitosan pentamer (DP 5, DA not specified, probably 0 %) did not significantly affect soybean height, root length, leaf area, shoot/root mass ratio, or total dry mass in greenhouse experiments (Khan et al., 2002).

Although studies on the effect of chitosan treatments on maize are more frequent than on soybean, the number of publications reporting field experiments is again limited. Seed treatment using chitosan (DP ca. 250-950; DA 20 %) nanofertilizer increased maize germination, and foliar application increased antioxidant enzyme activity and chlorophyll content, with synergistic effects of copper and salicylic acid, in pot and field experiments where increased growth and yield were reported (Choudhary et al., 2017; Choudhary et al., 2019; Sharma et al., 2020; Kumaraswamy et al., 2021). When used as a foliar spray, chitosan polymer (DP not specified; DA 36.5 %) increased photosynthesis under water restriction in maize plants grown in a greenhouse (Veroneze-Júnior et al., 2020). As described above for soybeans, foliar application of chitosan pentamer (DP 5, DA not specified, probably 0 %) did not significantly affect maize height, root length, leaf area, shoot/root mass ratio, or total dry mass in in greenhouse experiments (Khan et al., 2002).

In our extended field experiments, a chitosan hydrolysate (polymers DP 90, DA 20% + oligomers DP 2-15, DA 20%) applied as a foliar spray did not increase the agronomic performance, yield components, and agricultural productivity of two soybean and two maize cultivars in two growing seasons. Only subtle effects were observed, and these were not consistent across seasons and cultivars.

We had initially intended to also evaluate disease occurrence in the fields and its possible reduction by the chitosan treatments (this is why the control treatment consisted of commercial pesticides). However, climate conditions in these two seasons were not supportive of disease development so that even the non-treated control plants remained healthy, as also evidenced by the lack of an effect of the pesticide treatment over non-treated controls in any of the performance parameters investigated. In addition, temperature and rainfall were in the normal range so that no heat, cold, drought, or flooding stress occurred (Fig. S1; Fig. S2). Thus, induction of disease resistance or abiotic stress tolerance by chitosan treatment could not be evaluated in our experiment. Also, it is becoming increasingly evident that growth promoting activities of biostimulants such as chitosans are most pronounced under stress when chitosan treatments can strengthen the plants to cope with these unfavorable conditions (Veroneze-Júnior et al., 2020), and this may be the reason why it was not observed in our trials. Therefore, additional studies are needed using well-defined chitosan formulations such as the one used here, to analyze their effect under different climate conditions. Also, emphasis should be not only on fully replacing chemical inputs, but on reducing them by potentiating fungicide action, making use of synergistic effects of agro-biologics and agrochemicals (Brunel et al., 2013; Rahman et al., 2014; Ngoc & Nguyen, 2018).

Conclusion

In the present study, neither a positive nor a negative effect of the chitosan treatments was observed in either of the two crop species or of the two growing seasons. However, due to the favorable climate conditions, disease pressure was low. Consequently, the present study does not allow to draw conclusions on biopesticidal activities of the chitosan treatments. We might have assumed to observe a biostimulant/biofertilizer effect on the healthy plants, but this was also not the case, possibly due to the absence of abiotic stress. What we can conclude firmly is that the chitosan treatments, even at the rather high application rates used in our study, had no negative effect on crop performance in either of the model species. Thus importantly, a possible growth-resistance trade-off which might have been expected from a treatment aimed at stimulating disease resistance (Walters et al., 2013) was not observed upon chitosan application. The most plausible explanation for this result is the observation that chitosan treatment can stimulate photosynthetic electron transport which can be expected to deliver the extra energy required for plant strengthening (Lemke et al., 2020).

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