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BRIEF COMMUNICATION

Effect of foliar application of chitin and chitosan oligosaccharides on photosynthesis of maize and soybean

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Abstract

On the first day after foliar application, chitosan pentamer (CH5) and chitin pentamer (CHIT5) decreased net photosynthetic rate (P_N) of soybean and maize, however, on subsequent days there was an increase in P_N in some treatments. CH5 caused an increase in maize P_N on day 3 at 10^{-5} and 10^{-7} M; the increases were 18 and 10 % over the control plants. This increase was correlated with increases in stomatal conductance (g_s) and transpiration rate (E), while the intercellular CO_2 concentration (C_i) was not different from the control plants. P_N of soybean plants did not differ from the control plants except for treatment CH5 (10^{-7} M) which caused an 8 % increase on day 2, along with increased g_s, E, and C_i. On days 5 and 6 the CHIT5 treatment caused a 6-8 % increase in P_N of maize, which was accompanied by increases in g_s, E, and C_i. However, there was no such increase for soybean plants treated with CHIT5. In general, foliar application of high molecular mass chitin (CHH) resulted in decreased P_N, particularly for 0.010 % treated plants, both in maize and soybean. Foliar applications of chitosan and chitin oligomers did not affect ($p > 0.05$) maize or soybean height, root length, leaf area, shoot or root or total dry mass.

Additional key words: dry mass; elicitor; *Glycine*; intercellular CO_2 concentration; stomatal conductance; transpiration rate; *Zea*.

Among the promising approaches for inducing plant disease resistance and reducing damage from fungal pathogens, an exciting strategy is the use of elicitor molecules (Ward *et al.* 1991). Biotic elicitors are generally macromolecules, originating either from the host plant or from plant pathogens, which induce structural and/or biochemical responses associated with expression of plant disease resistance (Dixon *et al.* 1994). A large number of compounds, including oligosaccharides (Yoshikawa *et al.* 1993), have been suggested to play a key role in mediating the induction of plant defense reactions. Microbial compounds, including chitin and chitosan, are potential as powerful biocontrol agents in agricultural systems (Hadwiger *et al.* 1988).

Chitin, a high molecular mass polymer of β-1,4-*N*-acetylglucosamine is the second most abundant natural polymer on earth, after cellulose. Chitin is a major structural component of the shells of crustaceans, exoskeletons of insects, and cell walls of fungi and some algae (Shahidi *et al.* 1999). Chitosan, a deacetylated form of chitin, improves the ability of plants to protect themselves from pathogens such as fungi (Reddy *et al.* 1999).

Major plant defense responses that can be induced by chitin include lignification and induction of phytoalexin production (Barber *et al.* 1989, Yamada *et al.* 1993). Oligosaccharide fragments of chitin induce defense responses in plant cells, although, at least in the induction of lignification in wheat, it is uncertain whether the chitin oligosaccharides can provoke the same activity as the intact polysaccharide (Barber and Ride 1994). Chitosan promotes plant and root growth (Hirano 1988, Tsugita *et al.* 1993, Harada *et al.* 1995, Ohta *et al.* 1999). Ohta *et al.* (1999) have suggested that the growth promotion might be a nitrogen effect because chitosan contains about 8.7 % N. However, they did not rule out the possibility that the growth promotion was due to an elicitor effect as observed by Suzuki and Shinshi (1998).

One of the most sensitive physiological plant variables, with respect to biotic and abiotic stress, is photosynthesis. Little is known about the effects of chitin and chitosan on the biochemistry of photosynthesis. To the best of our knowledge there has also been no previous

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Abbreviations: C_i – intercellular CO_2 concentration; CHH – high molecular mass chitin; CH5 – chitosan pentamer; CHIT5 – chitin pentamer; g_s – stomatal conductance; P_N – net photosynthetic rate.

report regarding the effects of foliar applied chitin and chitosan oligomers on gas exchange in soybean and maize. Therefore, the objective of this work was to determine the effect of foliar applications of chitin and chitosan on plant photosynthetic rate and related growth variables.

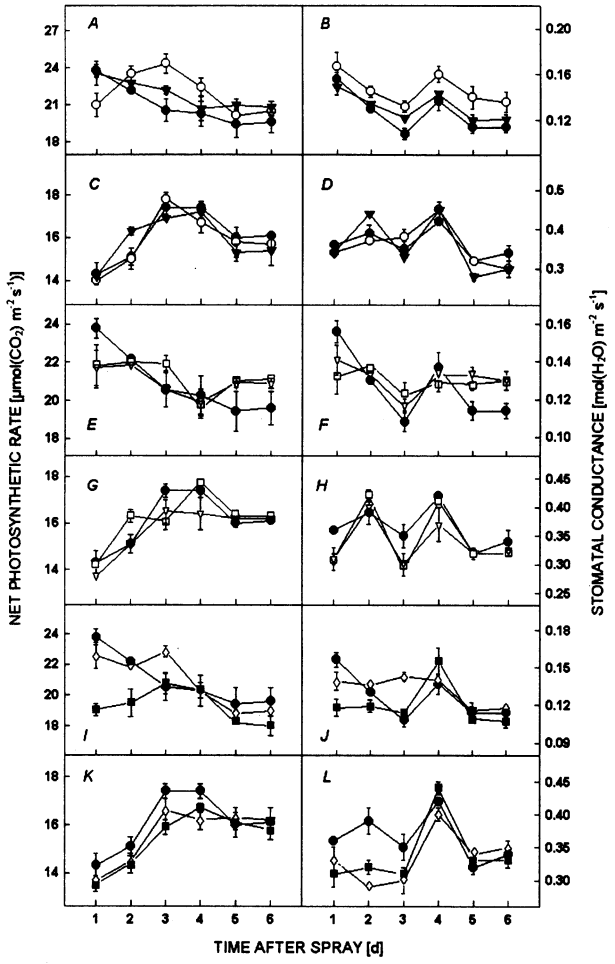

Fig. 1. Effect of foliar application of chitosan pentamer, CH5 (*A-D*), chitin pentamer, CHIT5 (*E-H*), and high molecular mass chitin, CHH (*I-L*) on net photosynthetic rate (*A, C, E, G, I, K*) and stomatal conductance (*B, D, F, H, J, L*) of maize hybrid Poineer 3921 (*A, B, E, F, I, J*) and soybean cv. OAC Bayfield (*C, D, G, H, K, L*). Error bars indicate ± SE. • (control), ○ (CH5, 10^{-5} M), ▼(CH5, 10^{-7} M), □ (CHIT5, 10^{-5} M), ▽ (CHIT5, 10^{-7} M), ■ (CHH, 0.010 %), ◊ (CHH, 0.001 %).

The experiment was conducted in a research greenhouse of the Plant Science Department of McGill University, Ste Anne de Bellevue, QC, Canada. Soybean (a C_3 plant) cv. OAC Bayfield was germinated and grown in trays containing sterile vermiculite until the VC stage (unifoliate leaves sufficiently unfolded that the edges are not touching) (Fehr and Caviness 1977). Seedlings in the tray were watered when necessary. Uniform and healthy soybean seedlings were selected from the tray and transferred to small pots (15.5 cm diameter and 15.0 cm depth) containing sterilized soil (*Promix, Premier Tech*, Rivier de Loup, QC), whereas maize plants were grown directly in sterilized soil. Following transplanting into pots, the plants were watered regularly. The greenhouse growth conditions were 24/16 °C (day/night) air temperature and 75 % relative humidity. The light-dark cycle was 16-8 h. Supplemental lighting was supplied using high-pressure sodium lamps (*Phillips*, Montreal, QC).

CH5 and CHIT5 were purchased from *Seikagaku Kogyo Co.*, Tokyo, Japan; CHH was purchased from *Aldrich Chemicals*, Milwaukee, WI, USA. CHH is not water-soluble, therefore a stock solution (10 kg m^{-3}) was prepared by dissolving the powder in 0.25 M HCl with constant stirring. The pH was adjusted to between 6.0-6.5 with 2 M NaOH. The solution was dialyzed overnight against distilled water at 4 °C. The dialyzed solution was then used to make the 0.010 and 0.001 % dilutions. Maize and soybean plants were sprayed until dripping either with CHIT5 or CH5 pentamer at concentrations of 10^{-5} and 10^{-7} M prepared in water containing 0.02 % *Tween 20* (polyoxyethylenesorbitan monolaurate, *Sigma Chemicals*, St. Louis, MO, USA). CHH was sprayed at 0.010 and 0.001 %. The control plants were sprayed with water containing 0.020 % *Tween 20*. All the spraying was done using a hand atomizer (*Nalgene*, USA) and each plant required about 5 cm^3 of spray solution. The plants were arranged following a completely randomized design with 3 replicates. The entire experiment was conducted twice, with similar results, and the data from the two experiments were pooled.

Gas exchange was measured with a portable open-system photosynthesis meter equipped with the standard leaf chamber (encloses 6 cm^2 of leaf area) and CO_2 injection system (model *6400-01*, *Li-Cor*, Lincoln, NE, USA). Irradiance for all measurements was 800 μmol m^{-2} s^{-1}, provided by a red-blue radiation source (model *6400-02*, *Li-Cor*, Lincoln, NE, USA). P_N, g_s, and E were measured daily for six days following the spray treatments. P_N was determined between 10:00 and 13:00 h. Ten days after spraying, data on plant growth variables (plant height, leaf area, shoot dry mass, root length, and root dry mass) were collected. The plant material was dried at 70 °C for 48 h prior to weight determinations. Data were analyzed with the Statistical Analysis System (*SAS*, NC, USA, 1989). Comparisons of multiple means were conducted with an *ANOVA* protected LSD test (Steel and Torrie 1980).

One day after foliar application of CH5 P_N of maize leaves decreased for 10^{-5} M treated plants, however, for 10^{-7} M treated plants it did not differ from the control. In general, the CH5 caused increased P_N, numerically higher in 10^{-5} M treated plants on days 2, 3, and 4, and in 10^{-7} M treated plants on days 3, 5, and 6. However, the increases reached statistical significance ($p < 0.05$) at both concentrations only on day 3 after treatment, when 10^{-5} and 10^{-7} M CH5 treated plants had P_N 18 and 10 % higher, respectively, than the control plants (Fig. 1*A*). g_s (Fig. 1*B*)

and *E* (results not shown) of CH5-treated plants at day 3 were greater than for the control plants. In general, foliar application of CH5 to the leaves did not alter P_N of soybean leaves, the exception being 10^{-7} M CH5 which, on day 2 after treatment, showed an 8 % increase ($p < 0.05$) as compared to the control plants (Fig. 1*C*). Similar results were observed for g_s (Fig. 1*D*).

Foliar spray application of CHIT5 decreased P_N ($p < 0.05$) on the first day after application for both the 10^{-5} and 10^{-7} M treatments, compared to the control plants. However, on days 5 and 6 both 10^{-5} and 10^{-7} M treated plants had P_N 8-10 % higher than those of control plants with similar increases in g_s (Fig. 1*E,F*). In general, the foliar treatment of soybean leaves with CHIT5 did not increase any of the measured variables, the single exception being CHIT5 (10^{-5} M), which caused an increase in P_N on day two after treatment (Fig. 1*G,H*).

In maize CHH decreased ($p < 0.01$) P_N by 19 %, at 0.010 % having the greatest effect on day 1 after treatment. g_s and *E* on days 1 and 2 after foliar application were reduced for plants treated with 0.010 and 0.001 % CHH, but they increased ($p < 0.01$) in 0.001 % CHH treated plants on day 3, along with increased P_N (Fig. 1*I,J*); however, the C_i value was not different from the control (values not shown). P_N and g_s in CHH treated soybean plants were generally lower than the control plants on days 1, 2, and 3 (Fig. 1*K,L*).

Foliar application of CH5, CHIT5, and CHH after 10 d did not affect plant growth related parameters such as plant height, root length, leaf area, shoot dry mass, root dry mass, or total dry mass. They were not different from the control plants for either maize or soybean (values not shown).

In general, we observed that the reduction in P_N of CHH treated plants was primarily due to reductions in g_s, which, in turn, contributed to reduced *E*. Lee *et al.* (1999) reported that high molecular mass chitosan caused partial stomatal closure. They suggested that it might be due to the production of H_2O_2 (hydrogen peroxide) which leads to elicitor (chitosan)-induced decreases in stomatal aperture. Some pathogens enter plant tissues only through open stomata (Agrios 1997). Stomata might have the capability to sense and respond to molecules, signaling the presence of such pathogens (Lee *et al.* 1999). Stomatal closure as a defense response is closely linked with increases in cytosolic Ca^{2+}, from intracellular or extracellular sources (McAinsh *et al.* 1995). We think that similar responses occur in both maize and soybean plants following application of high molecular mass chitosan oligomers to their leaves, and that this contributed to reductions in g_s and P_N. The increase in P_N and g_s in the absence of any increase in C_i indicates that the increase in P_N is due to enhanced uptake of CO_2 within the leaf that results in improved g_s, rather than due to more open stomata leading to increased P_N. If an increase in stomatal aperture had been the primary cause of the increase in P_N, an increase in the leaf C_i would have been expected (Morison 1998). In the present study, C_4 (maize) and C_3 (soybean) plants responded differently to the applied chitin and chitosan oligomers. Chitin oligomers larger than the hexamer have strong elicitor activity for wheat leaves, however, oligomers larger than dimer or trimer have similar activity for tomato cells (Yamaguchi *et al.* 2000). This indicates that specificity for these oligo-saccharides may differ among plant species.

The foliar application of chitin and chitosan resulted in no harmful effects on development and growth of maize and soybean plants as observed 10 d after treatment. Growth related variables of maize and soybean did not differ from the control plants. We did not observe any stimulatory effect of either chitosan or chitin. However, chitosan may promote growth and yield of a number of plants (Hirano 1988, Tsugita *et al.* 1993, Harada *et al.* 1995, Ohta *et al.* 1999).

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