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Research Report

A Combination of Chitosan and Chemical Fertilizers Improves Growth and Disease Resistance in *Begonia* × *hiemalis* Fotsch

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Abstract. Chitosan has been proposed to elicit defense responses in plants. In this study, we evaluated the potential roles of chitosan as a fertilizer supplement to stimulate *Begonia* × *hiemalis* Fotsch 'Schwabenland Red' growth and resistance to gray mold caused by the fungus *Botrytis cinerea*. We evaluated the effect of treatment with fertilizer containing various ratios of N, P, and K in combination with different concentrations of chitosan on plant growth and disease resistance. Of the sixteen treatments examined, the treatment consisting of an N:P:K ratio of 2.8:1.0:1.4 and chitosan concentration of 0.10 $g \cdot L^{-1}$ had the most positive effect on plant height, crown development, and other horticultural traits (i.e., flowering time, flower diameter, and flower quantity) at 20, 40, and 60 days after treatment, and significantly reduced the severity and incidence of gray mold compared to the controls and other treatments at 60 days after treatment. Furthermore, this treatment markedly increased superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and phenylalanine ammonia-lyase (PAL) activities. Based on these findings, we suggest that the chemical composition of the fertilizer and concentration of chitosan used affect the degree to which *Begonia* × *hiemalis* growth is stimulated and pathogen resistance is improved.

***Additional key words*:** enzyme activities, morphological characteristics, pathogens

Introduction

Begonias are versatile, temperate plants belonging to the *Begoniaceae* family (Hickey and King, 1981). *Begonia* × *hiemalis* Fotsch 'Schwabenland Red' or Elatior Begonia is a winter flowering begonia that originated from crosses between *B. socotrana* Hook and *B.* × *tuberhybrida* (Sandved, 1968). *Begonia* × *hiemalis* Fotsch plants do not produce seed and are mainly propagated through asexual means (Pierik and Tetteroo, 1987). As *Begonia* × *hiemalis* plants are susceptible to numerous ubiquitous fungi (Awal et al., 2013), especially gray mold caused by *Botrytis cinerea* (Hausbeck and Moorman, 1996), there is interest in improving the disease resistance of these plants.

The antifungal activity of chitosan (β-1,4-linked glucosamine polymer) has been documented in several in vitro studies (Ait Barka et al., 2004; Trotel-Aziz et al., 2006; Meng et al., 2008; Boonlertnirun et al., 2012). Chitosan, derived from crustacean shells, has been shown to stimulate plant growth (Boonlertnirun et al., 2012) and enhance the germination index, increase the shoot and root dry weight, and reduce the time to flowering in several ornamental plants (Ohta et al., 2004). Chitosan treatments improve the growth of *Zea mays* (maize; Mondal et al., 2013) and increase the germination rate of *Arachis hypogaea* (peanut; Zhou et al., 2002) and *Oryza sativa* L. (rice; Ruan and Xue, 2002). Importantly, chitosan has been shown to induce resistance to diseases in many plant species (Nandeeshkumar et al., 2008). Controlling plant pathogens with chitosan has been extensively explored, and the level of success was found to depend on the pathosystem and the derivative, concentration, degree of deacetylation, viscosity, and formulation of chitosan used (El Hadrami et al., 2010). Chitosan activates defense responses in numerous plant species by inducing various defense-related compounds. For instance, chitosan increases the activities of lipoxygenase (LOX), phenylalanine ammonia-lyase (PAL), and chitinase in *Vitis vinifera* (grapevine) leaves (Trotel-Aziz et al., 2006), induces phytoalexin in *Pisum sativum* (pea) pods (Hadwiger and Beckman, 1980), stimulates the production of pathogenesis-related (PR) proteins in the

leaves of rice seedlings (Agrawal et al., 2002), and stimulates lignification in *Triticum aestivum* (wheat) leaves (Barber et al., 1989), thereby inhibiting fungal growth and promoting protection against further infection. Chitosan application may accelerate the plant's response to pathogen infection by stimulating chitinase and glucanase production (Benhamou, 1996). Therefore, there is increasing interest in using chitosan as a fertilizer supplement to enhance protection against pathogens and stimulate plant growth, especially in crops such as rice (Boonlertnirun et al., 2008) and ornamental plants. Although the exact mechanisms by which chitosan induces defense responses in plants are poorly understood, the roles of chitosan in preventing pathogen infection and stimulating plant growth are well known (Bautista-Banos et al., 2006; Nge et al., 2006).

No studies have hitherto characterized the relationship between the elicitation of defense responses by chitosan and the establishment of disease resistance in *Begonia* × *hiemalis* Fotsch 'Schwabenland Red'. Thus, we aimed to determine the effects of chitosan as a fertilizer supplement on the growth and flowering of this Begonia cultivar, and also on the activities of various enzymes implicated in the plant defense response and on the incidence of gray mold.

Materials and Methods

Plant Materials and Growth Conditions

Vegetative propagation of *Begonia* × *hiemalis* Fotsch 'Schwabenland Red' was carried out according to the method of Xu et al. (2007) in a greenhouse at Sichuan Agricultural University in Ya'an, China. Leaves from young *Begonia* × *hiemalis* plants were excised as explants. After sterilization, leaf explants were cut using aseptic techniques and placed on a solid nutrient medium. For shoot and root induction, these plantlets were transferred onto solid Murashige and Skoog (MS) medium with different proportions of 6-benzylaminopurine (6-BA, 0.1-1.2 mg·L^{-1}) and 1-naphthalene acetic acid (NAA, 0.1-1.2 mg·L^{-1}) for twelve weeks. Rooting occurred over the first six weeks followed by six weeks of shoot elongation. After twelve weeks, the rooted plantlets were transferred into pots containing a sterilized soil mix as follows. First, the agar was washed off with tap water. The roots were then soaked a few times in the fungicide carbendazim (5%) in a shaded greenhouse to protect against fungal infection. Finally, plantlets were sown in plastic pots containing a medium composed of peat and perlite (3:1, v/v), which had been previously autoclaved at 121°C for 30 min. During the first two weeks, the plants were kept under plastic cover to increase the relative humidity and reduce irradiance. More than 95% of the plants survived transplanting. After three weeks, *Begonia* × *hiemalis* plants were selected based on uniform color, size, and the absence of blemishes or disease.

Experimental Design and Treatment

Orthogonal array testing was carried out using the software Orthogonal Design Assistant II v3.1 for four factors (Table 1) (Peng et al., 2006). The experiment was conducted as a randomized complete block design during the 2012/03 and 2013/05 seasons in the greenhouse at Sichuan Agricultural University. The experimental scheme consisted of 16 treatments (T1 to T16) of chitosan combined with N, P, and K fertilization regimes, plus two controls (C1 and C2; Table 2). The 16 treatments differed in chitosan concentration and in N, P, and K ratios. Nitrogen, P, and K were applied as urea, calcium superphosphate, and potassium chloride, respectively. The controls consisted of fertilizer with no chitosan (C1) and fertilizer with the fungicides Carbendazim (1% WP) and Mancozeb (63%) (Fawole et al., 2010) with no chitosan (C2). Deacetylated chitosan from crab shell was purchased from Sigma (Cat. No.448877). All treatments were administered via drip irrigation in a 200 ml mixture of chitosan and fertilizer. The samples were harvested on the 20^{th}, 40^{th}, and 60^{th} day after treatment. Each treatment consisted of approximately 35 plants and each experiment was independently replicated three times.

Determination of Enzyme Activity

Plant leaf samples (7 g fresh weight (FW)) were collected for analysis on the 20^{th}, 40^{th}, and 60^{th} day after treatment. For extraction of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), 0.5 g of leaf tissue was homogenized in 10 mL extraction buffer solution containing 0.05 M phosphate buffer (pH 7.8) in an ice bath. The hom-

Table 1. The factors and levels of orthogonal array testing

Test no.	Factors			
	N (mM)	P_2O_5 (mM)	K_2O (mM)	Chitosan (mg L^{-1})
1	8.65	0.33	2.00	0.01
2	17.30	0.65	4.00	0.10
3	25.95	0.98	6.00	0.50
4	34.60	1.30	8.00	1.00

Table 2. Treatment regimen for *Begonia* × *hiemalis* Fotsch 'Schwabenland Red' plants Nitrogen was provided with Urea

Treatment	Urea ($mg \cdot L^{-1}$)	$Ca(H_2PO_4)_2$ ($mg \cdot L^{-1}$)	KCl ($mg \cdot L^{-1}$)	Chitosan ($mg \cdot L^{-1}$)
C1	521.98	184.60	752.00	0.00
C2	521.98	184.60	752.00	0.00 (with fungicides)
T1	260.99	92.30	376.00	0.01
T2	260.99	184.60	752.00	0.10
T3	260.99	276.90	1128.00	0.50
T4	260.99	369.20	1504.00	1.00
T5	521.98	92.30	752.00	0.50
T6	521.98	184.60	376.00	1.00
T7	521.98	276.90	1504.00	0.01
T8	521.98	369.20	1128.00	0.10
T9	782.97	923.00	1128.00	1.00
T10	782.97	184.60	1504.00	0.50
T11	782.97	276.90	376.00	0.10
T12	782.97	369.20	752.00	0.01
T13	1043.97	92.30	1504.00	0.10
T14	1043.97	184.60	1128.00	0.01
T15	1043.97	276.90	752.00	1.00
T16	1043.97	369.20	376.00	0.50

Phosphate was provided with $Ca(H_2PO_4)_2$
Potassium was provided with KCl

ogenates were centrifuged at 4°C for 20 min at 12,000 rpm and the supernatants were used to assay the activities of the three enzymes. POD activity in the extract was determined via the method of De Azevedo Neto et al. (2006) by measuring the rate of change in absorbance at 470 nm with a spectrophotometer. The SOD activity was determined according to the method of Stewart and Bewley (1980) by measuring the ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). CAT activity was determined according to the method described by Patra et al. (1979) as the decline in absorbance at 240 nm due to H_2O_2 consumption. Extraction and measurement of phenylalanine ammonia-lyase (PAL) was performed according to the method of Trotel-Aziz et al. (2006). PAL activity was determined based on the production of *trans*-cinnamate, as measured by the absorbance change at 290 nm.

Analysis of Morphological Characteristics

To determine the optimal treatment for growth in *Begonia* × *hiemalis* Fotsch 'Schwabenland Red', we measured plant height and crown area with a meter measuring tape on day zero (just before treatment) and on the 20^{th}, 40^{th}, and 60^{th} day after treatment, according to the method of Bohlman and O'Brien (2006). Furthermore, the relative rates of growth (in plant height and crown area) were determined over the 60-day period. Additionally, the phenotype, flowering time, and diameter and quantity of flowers were assayed 60 days after treatment.

Inoculation and Measurement of Disease Progress

Botrytis cinerea strain 630 was isolated from grapevine leaves and maintained on potato dextrose agar (PDA). Conidial suspensions (10^5 conidia/mL) were obtained by flooding the fungal culture with sterile distilled water (SDW) containing 0.03% Tween-20. Plants were inoculated 24 h after treatment and then incubated for 60 days at 22°C and 95% relative humidity in incubation chambers. For disease assessment, 20 plants from each treatment or control group were selected on the basis of uniform growth and the absence of disease symptoms at day zero. After 60 days, the severity of disease was evaluated on a nominal scale of 0-4, with: 0 = healthy plants; 1 = 25% of the leaf area shows symptoms; 2 = 50% of the leaf area shows symptoms; 3 = 75% of the leaf area shows symptoms; 4 = 100% of the leaf area shows symptoms. In addition, disease incidence (Disease IN) was calculated 60 days after inoculation with *B.cinerea* by scoring the proportion of plants that showed characteristic symptoms, according to the method of Bell et al. (1998) and as follows:

$$\text{Disease IN} = (\Sigma \text{ the number of diseased plants/total number of plants investigated}) \times 100$$

Twenty plants were evaluated per treatment and experiments were repeated twice with similar results.

Statistical Analysis

Statistical analyses were performed using the statistical software SPSS 19.0. Data were subjected to One-way analysis of variance (ANOVA) using Duncan's Multiple Range Test. Differences were considered to be significant at $p < 0.05$. Error bars in the figures represent the standard deviation of the means. The disease incidence was subjected to arc sine transformation.

Results

Plant Growth

Plant height and crown area of *Begonia* × *hiemalis* Fotsch 'Schwabenland Red' were significantly influenced by the different treatments (T1 to T16; C1 and C2 served as controls; Table 2). Plants subjected to the T11 treatment were tallest on the 20th, 40th, and 60th day after treatment (ANOVA; $p < 0.05$) (Fig. 1A). At day 60, plants subjected to the T11 treatment were 10.47 cm tall (versus 1.65 cm for C1 and 2.23 cm for C2). Furthermore, we calculated the relative rate of growth and crown development in *Begonia* × *hiemalis* plants exposed to the treatments after 60 days compared with day zero (Fig. 1C and 1D). Plants subjected to the T11 treatment had the highest relative rate of growth (in plant height; 50%) on the 60th day compared with the 40th day after treatment (ANOVA; $p < 0.05$) (Fig. 1C). The relative rate of plant growth (height) for the T7 treatment was 21.82% on the 60th day compared with the 40th day and significantly lower than that of C1 (the first control) (ANOVA; $p < 0.05$).

For treatment T11, crown area after 60 days was 175.08 cm^2, significantly greater than that of plants subjected to the other treatments and the control plants (ANOVA; $p < 0.05$) (Fig. 1B). The crown of plants subjected to the T11 treatment was also significantly larger at 20 and 40 days after treatment than was that of the C1 and C2 plants (ANOVA; $p < 0.05$). As shown in Fig. 1B, the crown area of the control (C1) plant did not differ significantly at 0, 20, and 40 days after treatment (ANOVA; $p > 0.05$). Compared with the two controls (C1 and C2), all chitosan treatments significantly increased the relative rate of crown development (ANOVA; $p < 0.05$) after 60 days, especially in treatments T4 and T11 (Fig. 1D).

The Effects of Chitosan on Flower and Plant Phenotypes

We next evaluated differences in flowering time and in the diameter and quantity of the flowers 60 days after treatment (Table 3). After 60 days, most of the treatments resulted in significant changes in the three parameters as compared to the two controls (C1 and C2) (ANOVA; $p < 0.05$). Flowering occurred primarily within 40-50 days of treatment. Treatments T11, T2, and T16 had significantly longer flowering times and T16, followed by T11 and T13 had significantly greater flower diameters (2.78, 2.69, and 2.68 cm, respectively) compared to the other treatments (e.g., 2.14, 2.15, and 2.45 cm for C1, C2, and T1, respectively). The T11 treatment resulted in the most flowers per plant, with an average production of 17 flowers per plant. Treatment T3 had the poorest results in all flowering parameters examined after 60 days. We also characterized the whole plant morphology of *Begonia* × *hiemalis* Fotsch 'Schwabenland Red' exposed to different treatments (Fig. 2). For this part of the study, we compared plants subjected to the T3, T6, T16, and T11treatments with those in the control group (C1 and C2). As shown in Fig. 2, *Begonia* × *hiemalis* plants subjected to the T11 and T16.

The Effects of the Treatments on the Activity of Four Enzymes

Defense responses of *Begonia* × *hiemalis* Fotsch 'Schwabenland Red' subjected to the treatments were studied by measuring the activity of POD, SOD, CAT, and PAL, which are known to be involved in the plant's defense response. Non-uniform changes in the activities of four enzymes were seen for all treatments (Fig. 3). On the 20th day, changes in POD activity were variable among the treatments as compared with the two control plants (Fig. 3A). By 40 and 60 days after treatment, POD activity was significantly higher in plants subjected to nearly all treatments than in the controls (ANOVA; $p < 0.05$). The greatest POD activity (35.92 mM H_2O_2 $min^{-1} \cdot g^{-1}$ FW) was observed in plants subjected to the T11 treatment.

Furthermore, CAT activity varied at different time points (Fig. 3B). On the 40th day, all chitosan treatments significantly increased CAT activity in the *Begonia* × *hiemalis* plants (ANOVA; $p < 0.05$) and the T11 treatment resulted in the greatest increase in CAT activity (Fig. 3B), with levels at 60 days after treatment being the highest among all treatments (ANOVA; $p < 0.05$).

On the 40th day, SOD activity showed no significant increase for many of the treatments (ANOVA; $p > 0.05$) (Fig. 3C). However, on the 20th and 60th days, SOD activity in most of the treatments was higher than that of the two controls. On the 60th day, SOD activity was greatest for the T2 treatment (430.8 U $min^{-1} \cdot g^{-1}$ FW), followed by T11 (387.88 U $min^{-1} \cdot g^{-1}$ FW).

Similarly, PAL activity was statistically higher in plants subjected to chitosan applications on the 40th and 60th day, respectively, compared with their corresponding controls.

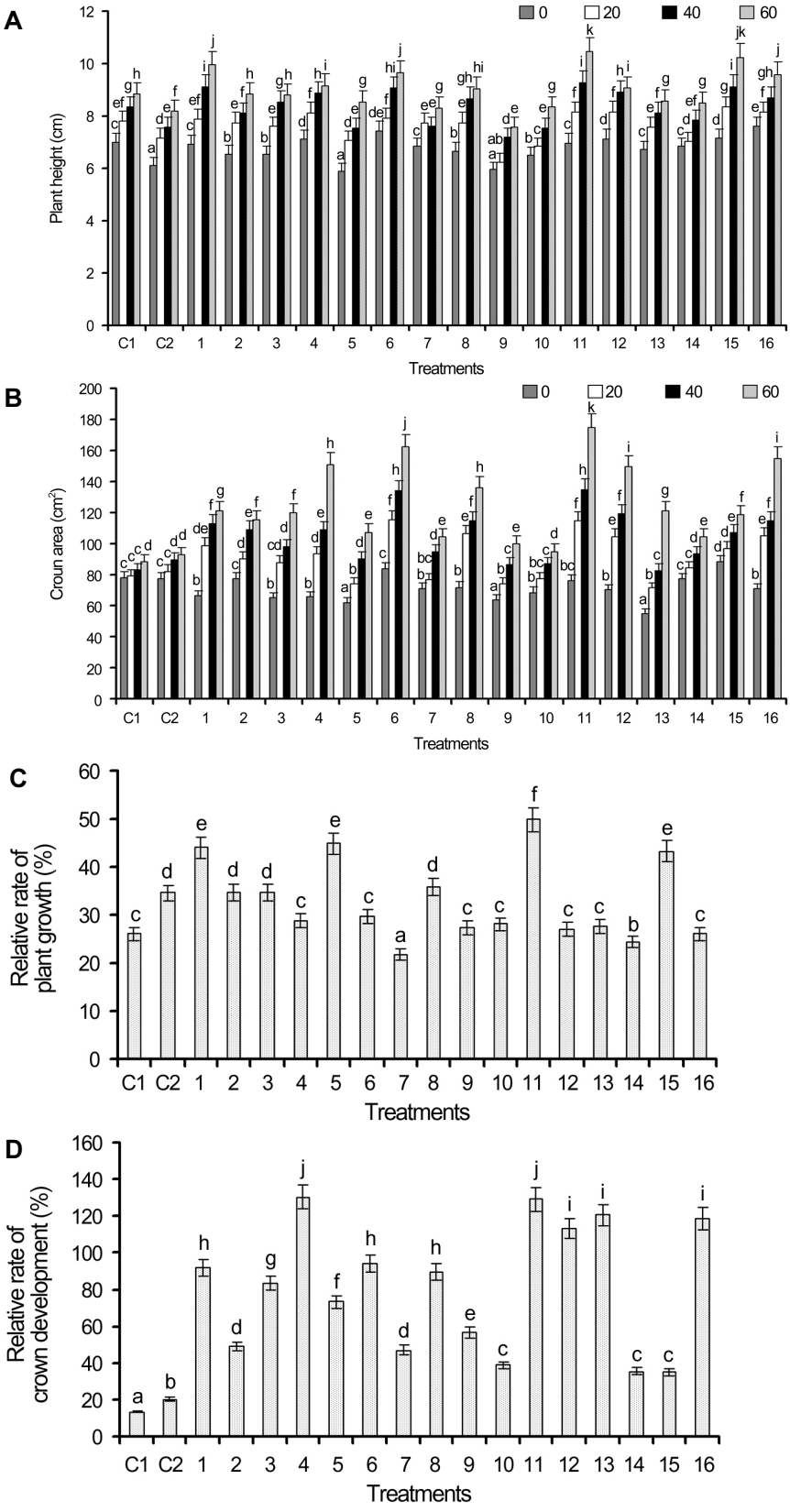

Fig. 1. Effects of chitosan treatments in chemical fertilizer on plant growth. Plant height (A) and crown area (B) were measured on the 0, 20th, 40th and 60th day after treatment (T1 to T16 versus the C1 and C2 controls; Table 2), and on the relative rate of plant growth (in height) (C) and crown growth (in area) (D) rates were determined over the 60-day period. Bars represent standard deviations of the means and different letters indicate significant difference according to Duncan's Multiple Range Test at $p < 0.05$. Control (C1 and C2) and treatment (T1 to T16) regimens are described in Table 2.

Table 3. Effects of chitosan and chemical fertilizer treatments on flower characteristics after 60 days in *Begonia* × *hiemalis* Fotsch 'Schwabenland Red'

Experimental group	Flowering time (days)	Flower diameter (cm)	Flower quantity
C1	44.00±3.74c	2.14±0.05b	9.00±0.52cd
C2	41.50±3.88b	2.15±0.03b	8.32±0.36b
T1	45.00±1.01c	2.45±0.04e	9.25±0.56d
T2	51.25±4.07e	2.60±0.03fg	9.40±0.36d
T3	37.00±1.25a	2.13±0.04b	7.20±0.67a
T4	41.75±2.15b	2.63±0.04fg	8.25±0.21b
T5	46.00±1.09c	2.32±0.02cd	9.33±0.41d
T6	47.25±1.38d	2.63±0.03fg	11.50±0.11fg
T7	49.00±1.52d	2.03±0.04a	11.00±0.12ef
T8	46.00±1.96c	2.60±0.02fg	8.75±0.21bc
T9	48.00±8.83d	2.31±0.02cd	9.20±0.43d
T10	44.75±2.87c	2.25±0.02bc	10.75±0.36e
T11	52.75±4.18e	2.69±0.05gh	17.00±0.88hi
T12	48.00±2.33d	2.57±0.01f	10.50±0.23e
T13	46.50±2.04c	2.68±0.06gh	16.50±0.38h
T14	46.81±3.48cd	2.15±0.02b	9.25±0.35d
T15	45.25±3.12c	2.35±0.04cd	10.00±0.41de
T16	51.00±3.82e	2.78±0.05i	11.20±0.58fg

The data are means of three replicates and expressed as the mean ± standard deviation (SD). The means within the same column followed by different letters are significantly different according to Duncan's Multiple Range Test at $p < 0.05$.

Table 4. The severity and incidence of gray mold caused by *Botrytis cinerea* on the 60th day after treatment with different concentrations of chitosan in combination with chemical fertilizer

	C1	C2	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16
Incidence (%)	60^{k}	50^{j}	25^{e}	30^{f}	45^{i}	35^{g}	25^{e}	15^{c}	30^{f}	25^{e}	30^{f}	15^{c}	0^{a}	20^{d}	15^{c}	40^{h}	15^{c}	10^{b}
	(50.77)	(45.00)	(30.00)	(33.21)	(42.13)	(36.27)	(30.00)	(22.79)	(33.21)	(30.00)	(33.21)	(22.79)	(0.00)	(26.57)	(22.79)	(39.23)	(22.79)	(18.44)
Severity	2.64i	2.58i	1.21e	1.52f	2.36h	1.93g	1.15e	0.63c	1.47f	1.18e	1.43f	0.58c	0.17a	0.87d	0.69c	2.12g	0.66c	0.47b

Figures in parentheses are arc sine transformed values.
Different lowercase letters indicate significant differences among treatments according to Duncan's Multiple Range Test at $p < 0.05$.

(ANOVA; $p < 0.05$) (Fig. 3D). PAL activity was greatest in plants subjected to the T11 treatment after both 40 and 60 days of treatment (161.79 and 198.32 U·g^{-1} FW, respectively). On the 20th day, PAL activity was noticeably lower in plants subjected to the T3 treatment than in the two controls (ANOVA; $p < 0.05$).

Disease Analysis in *Begonia* × *hiemalis* Fotsch 'Schwabenland Red'

There were significant differences with respect to disease severity and incidence with the different treatments (ANOVA; $p < 0.05$) (Table 4). Compared with the two controls at 60 days, chitosan treatment significantly decreased disease severity and incidence. The C1 and C2 controls were severely infected with gray mold caused by *B. cinerea*, with over 50% of the leaf area of these plants showing symptoms (stage 2; see Materials and Methods). Treatment T11 showed the greatest inhibition of *Botrytis cinerea* growth. Gray mold disease severity and incidence were 0.17 and 0.00 according to the nominal scale of 0-4 on the 60th day in treatment T11, respectively. Plants subjected to the T3 treatment exhibited the worst incidence and severity of gray mold 60 days after treatment.

Discussion

This study revealed that combined treatment with chemical fertilizer and chitosan resulted in significant differences in plant growth, flowering characteristics, and disease resistance. Consistent with these findings, previous studies indicated that chitosan promotes growth in various plants, such as *Eustoma grandiflorum* (Ohta et al., 1999) and rice (Boonlertnirun et al., 2012). In this study, all treatments with 0.10 g·L^{-1} chitosan induced an increase in plant height and crown area,

Fig. 2. Differences in phenotype 60 days after treatment with chitosan in combination with chemical fertilizer in *Begonia* × *hiemalis* Fotsch 'Schwabenland Red'. Six representative treatments were selected according to Table 3. (A) C1, (B) C2, (C) T3 treatment, (D) T6 treatment, (E) T16 treatment, and (F) T11 treatment.

especially in treatment T11 (Fig. 1). Treatment with 0.10 $g \cdot L^{-1}$ chitosan was more effective than treatment with higher or lower concentrations. Relative to the two control regimens, the chitosan treatments resulted in a longer flowering period and an increase in flower number and flower diameter (Table 3). Similar results have been found in *Passiflora edulis* (passion fruit) exposed to chitosan treatments (Utsunomiya and Kinai, 1994). Treatment T3 had the smallest effect on flowering time, flower number, and flower diameter on the 60^{th} day. The following reasons might account for this: (1) the chemical fertilizer itself may play a role in plant growth, (2) the concentration of chitosan is key and high chitosan concentrations may have a negative effect on plant growth.

Chitosan, as a natural fungicide, has been used to control diseases or reduce their spread and to enhance plant defenses. Some previous studies showed that chitosan treatments could directly inhibit spore germination, germ tube elongation, and mycelial growth of many phytopathogens (Ben-Shalom et al., 2003; Xu et al., 2007). Furthermore, studies examining the influence of chitosan and its derivatives on plant resistance against pathogens indicated that chitosan treatments result in significant changes in the activities of resistance-related enzyme of different plant species during plant defense responses (Khan et al., 2003; Trotel-Aziz et al., 2006; Zeng et al., 2010; Wang and Gao, 2013). To determine the effects of chitosan in combination with chemical fertilizer on the activities of such enzymes, we measured the POD, SOD, CAT, and PAL activities of plants subjected to the various treatments.

CAT is a common enzyme found in nearly all living organisms that are exposed to oxygen and catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani et al., 2004). POD and SOD are important oxyradical detoxification enzymes in plant tissues. SOD protects cells from oxidative stress by dismutating super oxide anions to H_2O_2, and POD is a group of essential enzymes involved in the formation and metabolism of ROS, thereby creating a highly toxic environment to pathogens (Torres et al., 2006). In the present study, we found that the activities of CAT, POD, and SOD were generally greater in *Begonia* × *hiemalis* Fotsch 'Schwabenland Red' plants exposed to chitosan treatments than in the control plants, especially in plants subjected to the T11 treatment (Fig. 3A-C). This suggests that chitosan induces the activities of some antioxidant enzymes such as POD, SOD, and CAT, thereby promoting protection against pathogens. Our results were in agreement with those of Ali et al. (2011) and Hong et al. (2012), which indicated that chitosan had a direct effect on

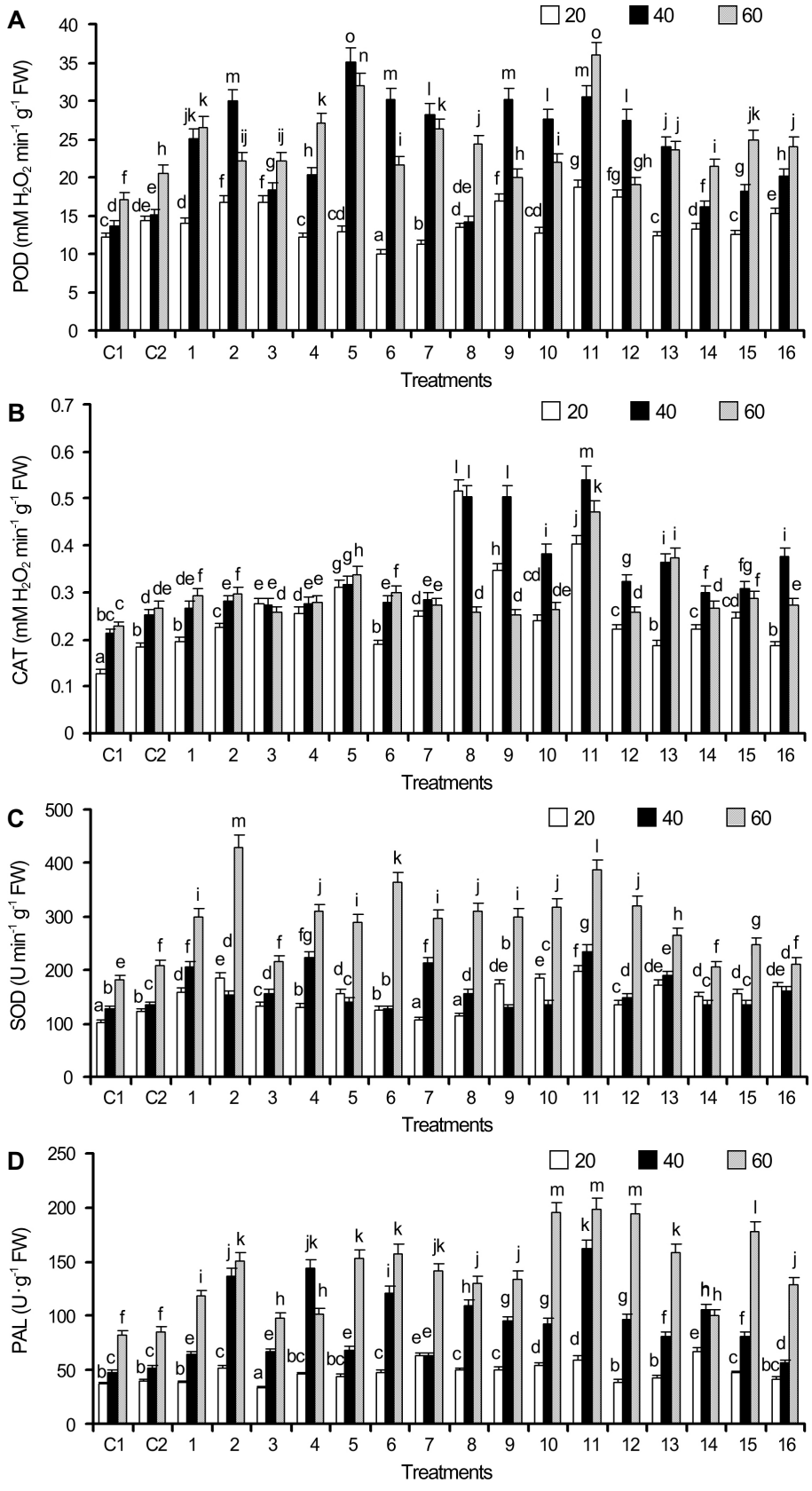

Fig. 3. Changes in POD, SOD, CAT, and PAL activities in *Begonia* × *hiemalis* Fotsch 'Schwabenland Red' on the 20th, 40th, and 60th day after treatment. Each value is the mean of three replicate samples ± SD. Different lowercase letters above the bars indicate significant differences according to Duncan's Multiple Range Test between treatments at $p < 0.05$. Control (C1 and C2) and treatment (T1 to T16) regimens are described in Table 2.

the induction of disease resistance by enhancing the activities of POD and SOD.

PAL is a key enzyme in the synthesis of secondary metabolites, especially phytoalexins and salicylic acid (SA), which were proposed to associated with resistance (Reymond and Farmer, 1998; Jeandet et al., 2002). Increases in PAL activity have been found to be one of the earliest responses to the onset of infection by pathogens in plants (Khan et al., 2003). In this study, we observed that PAL activity was higher in all of the chitosan-treated plants than in the two controls 40 and 60 days after treatment (ANOVA; $p < 0.05$) (Fig. 3D). The increase in PAL activity was tightly associated with the concentration of chitosan and the ratio of chemical fertilizer, in agreement with a report in grapevine leaves (Trotel-Aziz et al., 2006).

Finally, we also investigated the effect of chitosan in combination with chemical fertilizer on disease severity and incidence, because chitosan can trigger defensive mechanisms in plants against pathogenic infection even at low concentrations (Bautista-Banos et al., 2006). Gray mold caused by *Botrytis cinerea* is one of the most common and widely distributed diseases in many plants. A previous experiment indicated that the spread of necrotic lesions caused by *B. cinerea* was significantly reduced in grapevine leaves (Trotel-Aziz et al., 2006). In the present experiment, we demonstrated that chitosan was effective at controlling gray mold in *Begonia* × *hiemalis* Fotsch 'Schwabenland Red'. This inhibitory effect on *B. Cinerea* has not been observed in some studies with other fungal pathogens (Ma et al., 2013). Chitosan may have different effects on pathogens in different plant species. However, the incidence of gray mold was associated with the level of activity of the enzymes examined (Fig. 3). The reduction of disease severity and incidence may be ascribed to the induction of defense responses by chitosan. Furthermore, the proportion of N, P, and K in the chemical fertilizer may also affect disease severity and incidence.

In conclusion, the results from the present study indicate that, in comparison with two controls, chitosan treatments enhance antioxidant enzymes activities, stimulate plant growth, and reduce the disease incidence. However, there were significant differences in enzyme activities, plant growth, and disease resistance among plants subjected to the different chitosan treatments. These differences were related not only to chitosan concentration, but also to the ratio of N, P, and K in the chemical fertilizer, suggesting that chitosan may be a valuable chemical fertilizer supplement for stimulating growth and improving resistance to pathogens in ornamental plants.

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Literature Cited

Agrawal, G.K., R. Rakwal, S. Tamogami, M. Yonekura, A. Kubo, and H. Saji. 2002. Chitosan activates defense/stress response(s) in the leaves of *Oryza sativa* seedlings. Plant Physiol. Biochem. 40:1061-1069.

Ait Barka, E., P. Eullaffroy, C. Clement, and G. Vernet. 2004. Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. Plant Cell Rep. 22:608-614.

Ali, A., M.T.M. Muhammad, K. Sijam, and Y. Siddiqui. 2011. Effect of chitosan coatings on the physicochemical characteristics of Eksotika II papaya (*Carica papaya* L.) fruit during cold storage. Food Chem. 124:620-626.

Awal, A., A.B. Ali-Ahmed, R.M. Taha, J.S. Yaacob, and S. Mohajer. 2013. Effect of adenine, sucrose and plant growth regulators on the indirect organogenesis and on in vitro flowering in *Begonia* × *hiemalis* Fotsch. Aust. J. Crop Sci. 7:691-698.

Barber, M.S., R.E. Bertram, and J.P. Ride. 1989. Chitin oligosaccharides elicit lignification in wounded leaves. Physiol. Mol. Plant Pathol. 34:3-12.

Bautista-Banos, S., A.N. Hernández-Lauzardo, M.G. Velázquez-del Valle, M. Hernández-López, E. Ait Barka, E. Bosquez-Molina, and C.L. Wilson. 2006. Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop Prot. 25:108-118.

Bell, A.A., J.C. Hubbard, L. Liu, R.M. Davis, and K.V. Subbarao. 1998. Effects of chitin and chitosan on the incidence and severity of Fusarium yellows in celery. Plant Dis. 82:322-328.

Benhamou, N. 1996. Elicitor-induced plant defence pathways. Trends Plant Sci. 7:233-240.

Ben-Shalom, N., R. Ardi, R. Pinto, C. Aki, and E. Fallik. 2003. Controlling gray mould caused by *Botrytis cinerea* in cucumber plants by means of chitosan. Crop Prot. 22:285-290.

Bohlman, S. and S. O'Brien. 2006. Allometry, adult stature and regeneration requirement of 65 tree species on Barro Colorado Island, Panama. J. Trop. Ecol. 22:123-136.

Boonlertnirun, S., C. Boonraung, and R. Suvanasara. 2008. Application of chitosan in rice production. J. Met., Mater. Miner. 18:47-52.

Boonlertnirun, S., R. Suvannasara, P. Promsomboon, and K. Boonlertnirun. 2012. Chitosan in combination with chemical fertilizer on agronomic traits and some physiological responses relating to yield potential of rice (*Oryza sativa* L.). Res. J. Biol. Sci. 7:64-68.

Chelikani, P., I. Fita, and P.C. Loewen. 2004. Diversity of structures and properties among catalases. Cell. Mol. Life Sci. 61:192-208.

De Azevedo Neto, A.D., J.T. Prisco, J. Eneas-Filho, C.E. Braga de Abreu, and E. Gomes-Filho. 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ. Exp. Bot. 56:87-94.

EI Hadrami, A., L.R. Adam, I. EI Hadrami, and F. Daayf. 2010. Chitosan in plant protection. Mar. Drugs 8:968-987.

Fawole, O.B., M. Aluko, and T.E. Olowonihi. 2010. Effects of a Carbendazim-Mancozeb fungicidal mixture on soil microbial populations and some enzyme activities in soil. Agrosearch 10: 65-74.

Hadwiger, L.A. and J.M. Beckman. 1980. Chitosan as a component of pea-*Fusarium solani* interactions. Plant Physiol. 66:205-211.

Hausbeck, M.K. and G.W. Moorman. 1996. Managing Botrytis in greenhouse-grown flower crops. Plant Dis. 80:1212-1219.

Hickey, M. and C. King. 1981. 100 families of flowering plants. Cambridge University Press, Cambridge, New York. p. 138-140.

Hong, K.Q., J.H. Xie, L.B. Zhang, D.Q. Sun, and D.Q. Gong. 2012. Effects of chitosan coating on postharvest life and quality of guava

(*Sodium guajava* L.) fruit during cold storage. Sci. Hortic. 144:172-178.

Jeandet, P., A.C. Douillet-Breuil, R. Bessis, S. Debord, M. Sbaghi, and M. Adrian. 2002. Phytoalexins from the Vitaceae: biosynthesis phytoalexin gene expression in transgenic plants antifungal activity and metabolism. J. Agric. Food Chem. 50:2731-2741.

Khan, W., B. Prithiviraj, and D.L. Smith. 2003. Chitosan and chitin oligomers increase phenylalanine ammonialyase and tyrosine ammonia-lyase activities in soybean leaves. J. Plant Physiol. 160:859-863.

Ma, Z.X., L.Y. Yang, H.X. Yan, J.F. Kennedy, and X.H. Meng. 2013. Chitosan and oligochitosan enhance the resistance of peach fruit to brown rot. Carbohyd. Polym. 94:272-277.

Meng, X.H., B.Q. Li, J. Liu, and S.P. Tian. 2008. Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage. Food Chem. 106:501-508.

Mondal, M.M.A., A.B. Puteh, N.C. Dafader, M.Y. Rafii, and M.A. Malek. 2013. Foliar application of chitosan improves growth and yield in maize. J. Food Agric. Environ. 11:520-523.

Nandeeshkumar, P., J. Sudisha, K.K. Ramachandra, H.S. Prakash, S.R. Niranjana, and S.H. Shekar. 2008. Chitosan induced resistance to downy milew in sunflower caused by *Plasmopara halstedii*. Physiol. Mol. Plant Pathol. 72:188-194.

Nge, K.L., N. Nwe, S. Chandrkrachang, and W.F. Stevens. 2006. Chitosan as a growth stimulator in orchid tissue culture. Plant Sci. 170:1185-1190.

Ohta, K., S. Morishita, K. Suda, N. Kobayashi, and T. Hosoki. 2004. Effects of chitosan soil mixture treatment in the seedling stage on the growth and flowering of several ornamental plants. J. Jpn. Soc. Hortic. Sci. 73:66-68.

Ohta, K., A. Taniguchi, N. Konishi, and T. Hosoki. 1999. Chitosan treatment affects plant growth and flower quality in Eustoma grandiflorum. Hortic. Sci. 34:233-234.

Patra, H.K. and D. Mishra. 1979. Pyrophosphatase, peroxidase and polyphenoloxidase activities during leaf development and senescence. Plant Physiol. 63:318-323.

Peng, J.Y., F.Q. Dong, Q.W. Xu, Y.W. Xu, Y. Qi, X. Han, L.N. Xu, G.R. Fan, and K.X. Liu. 2006. Orthogonal test design for optimization of supercritical fluid extraction of daphnoretin 7-methoxy-daphnoretin and 1 5-diphenyl-1-pentanone from *Stellera chamaejasme* L. and subsequent isolation by high-speed counter-current chromatography. J. Chromatogr. A 1135:151-157.

Pierik, R.L. and F.A.A. Tetteroo. 1987. Vegetative propagation of Begonia venosa Skan in vitro from inflorescence explants. Plant Cell Tissue Organ Cult. 10:135-142.

Reymond, P. and E.E. Farmer. 1998. Jasmonate and salicylate as global signals for defense gene expression. Curr. Opin. Plant Biol. 1:404-411.

Ruan, S.L. and Q.Z. Xue. 2002. Effects of chitosan coating on seed germination and salt-tolerance of seedling in hybrid rice (*Oryza sativa* L.). Acta Agron. Sin. 28:803-808.

Sandved, G. 1968. Flowering in *Begonia* × *hiemalis* Fotsch. as affected by daylength and temperature. Acta Hortic. 14:61-76.

Stewart, R. R. and J.D. Bewley. 1980. Lipid peroxidation associated with accelerated aging of soybean axes. Plant Physiol. 65:245-248.

Torres, M.A.J.D., J.D. Jones, and J.L. Dangl. 2006. Reactive oxygen species signaling in response to pathogens. Plant physiol. 141: 373-378.

Trotel-Aziz, P., M. Couderchet, G. Vernet, and A. Aziz. 2006. Chitosan stimulates defense reactions in grapevine leaves and inhibits development of *Botrytis cinerea*. Eur. J. Plant Pathol. 114:405-413.

Utsunomiya, N. and H. Kinai. 1994. Effect of chitosan-oligosaccharides soil conditioner on the growth of passionfruit. J. Jpn. Soc. Hortic. Sci. 64:176-177.

Wang, S.Y. and H.Y. Gao. 2013. Effect of chitosan-based edible coating on antioxidants antioxidant enzyme system and postharvest fruit quality of strawberries *(Fragaria* × *aranassa* Duch.). LWT-Food Sci. Technol. 52:71-79.

Xu, J.G., X.M. Zhao, X.W. Han, and Y.G. Du. 2007. Antifungal activity of oligochitosan against Phytophthora capsici and other plant pathogenic fungi in vitro. Pestic. Biochem. Physiol. 87:220-228.

Xu, M.L., H.Y. Zhang, and Z.X. Tang. 2007. Establishment of highly efficient regeneration system of *Rieger Begonia* and study on the sensitivity to antibiotic in vitro. North. Hortic. 7:175-178.

Zeng, K.F., Y.Y. Deng, J. Ming, and L.L. Deng. 2010. Induction of disease resistance and ROS metabolism in navel oranges by chitosan. Sci. Hortic. 126:223-228.

Zhou, Y.G., Y.D. Yang, Y.G. Qi, Z.M. Zhang, X.J. Wang, and X.J. Hu. 2002. Effects of chitosan on some physiological activity in germinating seed of peanut. J. Peanut Sci. 31:22-25.