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Effect of film-forming polymers on control of lily leaf blight caused by *Botrytis elliptica*

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Abstract

The effectiveness of film-forming polyelectrolytes for the control of lily leaf blight caused by *Botrytis elliptica* was evaluated using laboratory a leaf disk assay, greenhouse tests and field trials. Among the six polyelectrolytes, 400 ppm FO4240SH, FO4490SH and FO4550SH reduced the disease severity of lily leaf blight in leaf-disk tests. Both FO4240SH and FO4490SH also suppressed sporulation of the pathogen on leaf disks. In greenhouse tests, the number and size of lesions on leaves of *Lilium oriental* hybrid cv. Star Gazer were markedly reduced by FO4490SH and FO4550SH. Field trials showed that the effectiveness of FO4490SH was similar or better than that of procymidone on the reduction of lily leaf blight disease severity. The polymers had no harmful effects on the lily plants. The cationic polyelectrolytes FO4240SH, FO4490SH and FO4550SH reduced the percentage of conidial germination, inhibited germ-tube growth, and also suppressed the esterase production by germ tubes of *B. elliptica*. All the above evidence indicates that the disease control achieved with polyelectrolytes is due, at least in part, to the reduction of esterase secretion by *B. elliptica*.

Introduction

Epidermis-coating antitranspirants, such as film-forming polymers and polyelectrolytes, have been reported to provide protection against several plant diseases such as anthracnose, early blight, leaf spot, leaf blight, downy mildew, powdery mildew, rust and grey mould, etc. (Elad et al., 1990; Gale and Poljakoff-Mayber, 1962; Han, 1990; Hsieh and Huang, 1997; Kamp, 1985; Zekaria-Oren et al., 1991; Ziv, 1983; Ziv and Frederiksen, 1983, 1987; Ziv and Hagiladi, 1984, 1993; Ziv and Zitter, 1992). The effects of antitranspirants on the control of diseases appear similar to those of the natural cuticle layer in defending plants against pathogens. *Botrytis elliptica* (Berk.) Cooke, the causal agent of lily leaf blight, enters host plants either by direct penetration or indirectly through stomata on abaxial surfaces or damaged tissues (Doss et al., 1988). Therefore, application of film-forming materials to lily plant surfaces may have a prophylactic effect and thus reduce lily leaf blight. The purpose of the present work was to evaluate the effects of six polyelectrolytes on control of the disease. In addition, the possible mechanisms involved in the reduction of lily leaf blight produced by polyelectrolytes were studied.

Materials and methods

Isolates of B. elliptica

Two single-spore isolates (B061 and B066) of *B. elliptica* were obtained from diseased lilies in 1993 and maintained on potato dextrose agar (PDA; Difco Laboratory) at 4 °C. These isolates sporulated vigorously under near UV light (Black Light Blue, F10T8BLB, Sankyo Denki, Japan). To maintain

pathogenicity, surface-sterilized lily leaves were inoculated with conidia and then the pathogen was reisolated by harvesting freshly-formed spores from diseased tissues (Doss et al., 1984).

Inoculum preparation

To prepare fungal inoculum, *B. elliptica* was grown on autoclaved lily leaves or petals for 7–10 days at 20 °C under near UV light. Conidia were harvested with sterile forceps and suspended in sterile water in a microcentrifuge tube. The spore suspension was centrifuged at $3000 \times g$ for 10 min. The supernatant was discarded and sterile water was added to the spore pellet. Shaking and centrifugation was repeated three times. Spores were counted under a haemocytometer and the spore concentration adjusted to 10^5 conidia per ml.

Leaf disk preparation, inoculation and film-forming polymers

Oriental hybrid lily (cv. Star Gazer) grown from bulbs in a greenhouse for 1.5–2 months were used to prepare leaf disks. For leaf disk assays, the fifth to tenth leaves below the apex were surface-sterilized by dipping entire excised leaves in 0.1% sodium hypochlorite three times and rinsing in three changes of sterile water. Leaf disks (15 mm diameter) were cut from surface-sterilized leaves with a cork borer and fully submerged in diluted polyelectrolyte solutions. Six polyelectrolytes including poly(acrylamide/dimethylamino ethyl-methacrylate cationic monomer) (FO4240SH, FO4490SH, and FO4550SH) (SNF, St-Etienne cedex, France), poly(acrylamide/sodium acrylate) (AN910SD, FA920SD and AN934SD) (SNF, St-Etienne cedex, France) were used. The polyelectrolytes were diluted to concentrations of 250, 333, 400 and 500 ppm. The disks (8 per replication) were put upside down in petri dishes (90 mm in diameter) each containing a sterilized filter paper (60 mm in diameter). Sterile water (2 ml) was added to the filter paper. A spore suspension was sprayed (Sigma spray unit, Sigma Chemical Co, St. Louis, MO, USA) over the disks to make the inoculation drop size about 0.3 mm in diameter. The disks were then incubated at 20 °C in darkness. Disease severity was assessed after 3 days and the percentage of lesion areas on leaf disks was recorded. After recording, plates containing inoculated leaf disks were placed under near UV light at 20 °C to induce pathogen sporulation. After incubation for a further 5 days, leaf disks were placed in test tubes containing 10 ml sterile water. Spore concentrations were counted using a haemocytometer.

Greenhouse and field assays

The effect of six polyelectrolytes (400 ppm) on the control of lily leaf blight was measured under greenhouse conditions. Ten lily bulbs were grown in a plastic pot ($60 \times 20 \times 15$ cm) with non-sterilized nursery soil. The plants were put on the bench in a greenhouse kept at 24 ± 4 °C. Each treatment had four replicates. After growth for 1.5–2 months, lily plants were inoculated with *B. elliptica* by spraying a spore suspension over whole leaves until run off. The inoculated plants were then covered with plastic bags to keep 100% relative humidity and moved into a growth chamber maintained at 20 °C. Disease severity was recorded over the following 7 days. Lesion numbers were counted and lesion diameters were measured.

In field trials, lily plants grown for 1.5 months in a row were blocked into 16 plots during February to March of 1997 and 1998. Each plot was 5×0.6 m. The diseased lily plants, *L. oriental* hybrid cv. Macro Polo, Acapulco and Casa Blanca, were individually treated with 333 ppm of FO4490SH, or 250 ppm of procymidone (Sumilex, Sumiotmo Chemical Taiwan Company, Taipei), or both, three times at one-week intervals. The controls were treated with tap water. Lily plants were naturally infected and disease severity was counted 7 days after the third treatment. Disease severity of each treatment was classified on a scale of 0–4, 0 = no lesions observed, 1 = 1–10% leaf area infected, 2 = 11–25% leaf area infected, 3 = 26–50% leaf area infected, and 4 = over 50% leaf area infected.

Effect of polyelectrolytes on spore adhesion, germination and esterase production

Sterile cover glasses (18×18 mm) were submerged in solutions of polyelectrolytes (400 ppm), air-dried and kept at room temperature for further use. Twenty-five microlitres of spore suspension (1×10^5 ml^{-1}) were placed on the cover glasses, which had been coated with various polyelectrolytes, and then incubated at 100% relative humidity at 20 °C. After 120 min incubation, inoculation sites were washed by delivering 30 drops of water from a height of 5 cm onto the inoculated surface held at an angle 45 ° (Braun and Howard, 1994). After washing, spores remaining on the cover

glass were immediately counted, determining the percentage of spores still adhered to the cover glasses. At the same time, percentages of spore germination and lengths of germ tubes were measured under an Olympus microscope (BHSM-F, Olympus Optical Co. Ltd., Tokyo, Japan). The degree of wettability (hydrophobicity/hydrophilicity) for these polyelectrolytes was determined by measuring the contact angle of a sessile droplet of distilled water placed on the testing material surface (Neumann and Good, 1979). All experiments were repeated twice.

The presence of esterase activity on the germ tube of *B. elliptica* was assessed by a method which uses indoxyl acetate as the substrate of nonspecific carboxylic acid esterases (Barnett and Seligman, 1951). Substrate hydrolysis results in the accumulation of pigmented crystals of indigo blue. Twenty-five microlitres of spore suspension was placed on a cover glass coated with polyelectrolytes and incubated at 20 °C. After 6 h incubation, 25 µl of indoxyl acetate solution (25 ml 2 M sodium chloride, 10 ml 0.1 M phosphate buffer pH 7.8, 0.25 g calcium chloride in 14 ml distilled water, 20 mg indoxyl acetate dissolved in 1 ml acetone) was added. At least 100 spores and germ tubes per treatment were observed under light microscope. Esterase index was rated on a scale of 0–4, 0 = no blue crystal materials observed in germ tubes, 1 = 1–10% areas of germ tubes with blue crystal materials, 2 = 11–25% areas of germ tubes with blue crystal materials, 3 = 26–50% areas of germ tubes with blue crystal materials, and 4 = over 50% areas of germ tubes with blue crystal materials. The experiment was repeated twice.

Effect of polyelectrolytes on mycelial growth of B. elliptica

Six filter papers treated with polyelectrolytes were put on PDA in 9-cm-diameter petri dishes and spore suspension (50 µl) was placed on the centre of the filter paper. After 5 days incubation at 20 °C, the filter papers were removed and the diameter of mycelial colony which had penetrated through the filter paper, grown on PDA plates was recorded. The filter paper treated with sterile water was used as a control. Each treatment had five replicates. The experiments were done in twice.

Effect of polyelectrolytes on spore behaviour

Submerged lily leaf disks treated with the solution of FO4490SH (400 ppm) were inoculated on the abaxial surface with spore suspension of *B. elliptica* and incubated at 20 °C. Leaf disks treated with distilled water were served as controls. After 16 h of inoculation, inoculated plant tissues (15-mm diameter of leaf disks) were placed into a 1 N KOH solution for 30 min, and then autoclaved in the solution for 15 min at 121 °C to clear the leaf tissues. After three rinses in deionized water, cleared samples were mounted in the stain solution (0.05% aniline blue in 0.067 M K_2HPO_4 at pH 9.0) and examined using UV fluorescence according to the method described by Hood and Shew (1996). Percentage of conidial germination and infection of leaf disks were examined under an Olympus light/fluorescent microscope (BX50+BX-FLA, Olympus Optical Co. Ltd., Tokyo, Japan).

Results

Effect of polyelectrolytes on severity of Botrytis *leaf blight*

Concentrations of 333 and 400 ppm of FO4240SH, FO4490SH and FO4550SH were sufficient to reduce disease development of lily leaf blight on leaf disks (Table 1). At 400 ppm, FO4240SH, FO4490SH and FO4550SH also reduced lesion number and lesion areas in leaf-disk assays (Table 2). These results were consistent with those from greenhouse tests in which lesion number and lesion size on leaves were markedly reduced when whole plants were sprayed with these polyelectrolytes (Table 2). FO4490SH and FO4550SH reduced disease development on leaf-disk assays irrespective of the method of inoculation (Table 3). All polyelectrolytes significantly reduced the sporulation of *B. elliptica* on leaf disks (Table 4). FO4240SH and FO4490SH were considered to be the best polyelectrolytes for controlling lily leaf blight. The results of field trials showed that the effect of FO4490SH was greater than or similar to that of procymidone (Table 5).

Effect of polyelectrolytes on spore adhesion, germination and esterase production

A higher percentage of spores became attached to glass surfaces which had been treated with the cationic polyelectrolytes (Table 6). There was no correlation ($r = 0.035$, $p > 0.05$) between the wettability of substrata and spore adhesion. Cationic polyelectrolytes were more effective in reducing the percentage of spore

Table 1. Effect of diluted polyelectrolytes on severity of lily leaf blight using a leaf-disk assay

Polyelectrolyte	Concentration of polyelectrolytes (ppm)						Coefficient of determination[2]
	0	250	286	333	400	500	
AN910SD	92[1]	96	100	100	98	88	$R^2 = 0.19$, $p < 0.0084$
FA920SD	89	86	90	100	93	83	$R^2 = 0.03$, $p < 0.5173$
AN934SD	91	85	89	95	90	81	$R^2 = 0.04$, $p < 0.3693$
FO4240SH	89	81	73	49	40	76	$R^2 = 0.24$, $p < 0.0018$
FO4490SH	91	71	23	4	8	25	$R^2 = 0.60$, $p < 0.0001$
FO4550SH	90	83	28	10	13	18	$R^2 = 0.61$, $p < 0.0001$

[1]Lesion area per leaf disk (%).
[2]Data were evaluated by analysis of variance and nonlinear regression model statistical procedures with the SAS/STAT system for personal computers (SAS Institute, Inc., Cary, NC). Data were the mean of five replicates and recorded 4 days after inoculation at 20 °C.

Table 2. Comparison of a leaf-disk assay and greenhouse tests as a means of estimating the prophylactic effect of polyelectrolytes on control of *Botrytis* leaf blight of lily

Polyelectrolyte (400 ppm)	Leaf-disk assay[1]		Greenhouse test[2]	
	Lesion number per leaf disk	Lesion area (%) per leaf disk	Lesion number per leaf	Lesion size (mm)
AN910SD	7.8 ± 4.7 ab	56.9 ± 15.6 a	30 ± 12 b	2.0 bc
AN934SD	6.5 ± 3.5 bc	56.5 ± 21.0 a	26 ± 11 bcd	1.9 bc
FA920SD	5.5 ± 2.3 c	40.6 ± 16.8 b	29 ± 13 b	3.1 a
FO4240SH	1.8 ± 1.7 d	35.6 ± 14.1 bc	28 ± 11 bc	1.4 c
FO4490SH	1.5 ± 1.7 d	30.0 ± 12.4 cd	17 ± 6 d	1.6 c
FO4550SH	1.6 ± 1.7 d	25.4 ± 11.8 d	19 ± 6 cd	1.9 bc
Check (water)	8.4 ± 3.2 a	55.0 ± 15.7 a	40 ± 13 a	2.3 b

[1]Lesion numbers and lesion areas on leaf disks were recorded 1 day and 3 days after inoculation and incubation at 20 °C.
[2]Lesion numbers and lesion sizes on leaves were recorded 7 days after inoculation and incubation at 20 °C in greenhouse, respectively.
Data followed by the same letter in each column do not differ significantly ($p = 0.05$) according to Duncan's multiple range test.

Table 3. Effect of polyelectrolyte on severity of lily leaf blight 4 days after inoculation with *B. elliptica*

Polyelectrolyte (400 ppm)	Lesion area per leaf disk (%)		
	Pre-inoculation[1]	Simultaneous inoculation[1]	Post-inoculation[1]
AN910SD	60.0 ab	61.3 a	65.0 ab
FA920SD	63.8 ab	52.5 ab	60.0 b
AN934SD	67.5 a	57.5 a	55.0 b
FO4240SH	55.0 ab	35.0 bc	60.0 b
FO4490SH	41.3 bc	13.8 d	7.0 d
FO4550SH	30.0 c	20.0 cd	23.8 c
Check (water)	70.0 a	55.0 a	77.5 a

[1]Pathogen was inoculated 24 h before and after or simultaneous treatment with polyelectrolytes.
Data followed by the same letter in each column do not differ significantly ($p = 0.05$) according to Duncan's multiple range test.

Table 4. Effect of polyelectrolytes on sporulation of *B. elliptica* on leaf disks[a]

Polyelectrolyte (400 ppm)	No. of spores per leaf disk ($\times 10^3$)
AN910SD	206 ± 12[b]
FA920SD	67 ± 13
AN934SD	83 ± 20
FO4240SH	7 ± 3
FO4490SH	23 ± 7
FO4550SH	87 ± 8
Check (water)	320 ± 9

[a]The inoculated leaf disks were placed under near UV light at 20 °C for 5 days for inducing sporulation.
[b]Means ± deviation.

Table 5. Effect of polyelectrolyte (FO4490SH) and procymidone on severity of lily leaf blight caused by *B. elliptica* in field trials in 1997 and 1998

Treatment[1]	Disease severity (%)[2]			
	Marco Polo	Marco Polo	Acapulco	Casa Blanca
	Mar. 12, 1997	Mar. 12, 1998	Mar. 12, 1998	Mar. 12, 1998
Check (water)	40.9 a	31.0 a	19.8 a	71.0 a
FO4490SH	28.4 b	14.3 b	4.3 b	49.0 b
Procymidone	23.1 b	20.3 b	10.5 ab	60.0 ab
FO4490SH + procymidone	20.9 b	16.5 b	3.5 b	56.0 ab

[1]Final concentrations of FO4490SH and procymidone were 333 and 250 ppm, respectively.
[2]Disease severity was assessed on a scale of 0–4, 0 = no lesion observed, 1 = 1–10% leaf area infected, 2 = 11–25% leaf area infected, 3 = 26–50% leaf area infected, and 4 = over 50% leaf area infected.
Data followed by the same letter in each column do not differ significantly ($p = 0.05$) according to Duncan's multiple range test.

Table 6. Assessment of *Botrytis* spore adhesion and germination on a glass surface coated with polyelectrolytes 2 h after treatment at 20 °C

Polyelectrolyte (400 ppm)	Adhesion[1] (%)	Germination (%)	Wettability[2] (degree)
AN910SD (−)	34.5 c	22.9 a	18.3 c
FA920SD (+/−)	20.3 d	12.4 b	19.9 c
AN934SD (−)	20.0 d	12.8 b	13.8 d
FO4240SH (+)	87.8 a	5.7 c	11.0 d
FO4490SH (+)	94.0 a	5.4 c	26.0 b
FO4550SH (+)	73.8 b	8.0 bc	33.5 a
Check (water)	26.5 cd	24.5 a	26.8 b

[1]Washing procedure as described by Braun and Howard (1994).
[2]The degree of wettability for the glasses coated with polyelectrolytes was determined by measuring the contact angle of a sessile droplet of distilled water placed on the test surface.
Data followed by the same letter in each column do not differ significantly ($p = 0.05$) according to Duncan's multiple range test.

germination and at shorting the length of germ tubes compared to anionic polyelectrolytes (Table 7). In addition, the efficiency of cationic polyelectrolytes on coagulating enzymes released by the pathogen was assessed. Cationic polyelectrolytes strongly suppressed esterase activity in germ tubes (Table 7, Figure 1). Moreover, esterase concentration in germ tubes was highly correlated ($r = 0.769$, $p < 0.01$) with lesion numbers appearing on leaf disks (Figure 2). These data suggest that cationic polyelectrolytes inhibit spore germination and germ-tube elongation and that this results in reduction of esterase production and, consequently, in suppression of disease development.

Table 7. Effect of polyelectrolyte coating on a cover glass surface on spore germination, germ-tube length and esterase activity in germ tubes of *B. elliptica* after 6 h at 20 °C

Polyelectrolyte (400 ppm)	Spore germination (%)	Germ-tube length (μm)	Esterase index[1] ($n = 50$)
AN910SD	83 ± 3 a	98 ± 28 a	2.65
AN934SD	87 ± 2 a	79 ± 24 b	2.05
FA920SD	83 ± 4 a	79 ± 28 b	2.55
FO4240SH	38 ± 16 b	40 ± 15 c	1.65
FO4490SH	47 ± 9 b	33 ± 22 cd	0.73
FO4550SH	42 ± 5 b	26 ± 6 d	1.13
Check (water)	85 ± 3 a	68 ± 24 b	2.88

[1]Esterase index was rated on a scale of 0–4, 0 = no blue crystal materials observed in germ tubes, 1 = 1–10% areas of germ tubes with blue crystal materials, 2 = 11–25% areas of germ tubes with blue crystal materials, 3 = 26–50% areas of germ tubes with blue crystal materials, and 4 = over 50% areas of germ tubes with blue crystal materials.
Data followed by the same letter in each column do not differ significantly ($p = 0.05$) according to Duncan's multiple range test.

Effect of polyelectrolytes on mycelial growth of B. elliptica

B. elliptica penetrated filter papers treated with polyelectrolytes or sterile water and underneath grew into the agar. However, fungal growth and penetration was delayed through filter paper treated with the polyelectrolytes AN910SD, FO4240SH, FO4490SH and FO4550SH. Both FO4490SH and FO4550SH effectively decreased mycelial penetration and expansion

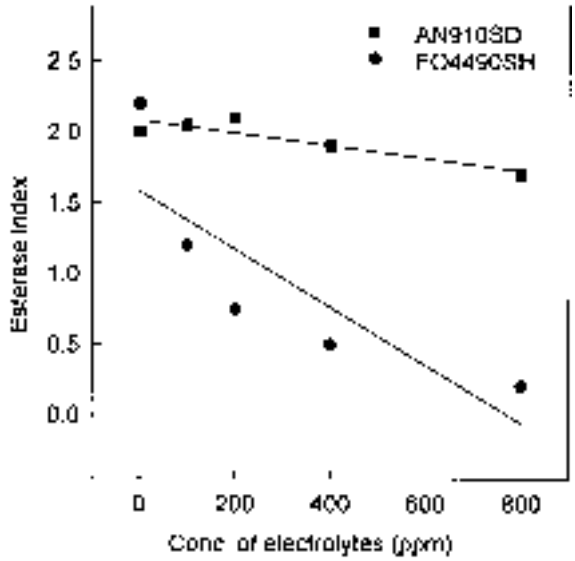

Figure 1. Correlations between concentration of polyelectrolytes and esterase production by germ tubes of *B. elliptica*. Twenty-five microlitres of spore suspension (1×10^5 ml^{-1}) were dropped on the cover glasses that were coated with different concentrations of anionic polyelectrolyte AN910SD and cationic polyelectrolyte FO4490SH and then incubated at 100% relative humidity at 20 °C. After 6 h incubation, 25 µl of indoxyl acetate solution was added. Blue crystal materials were observed in germ tubes and the amounts present scored on a scale of 0–4.

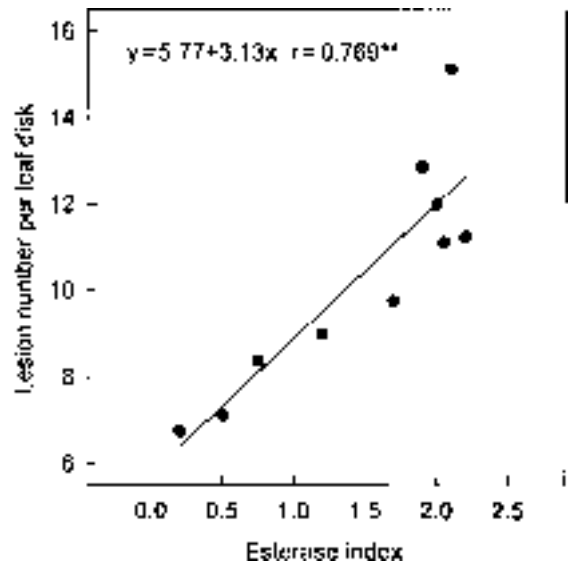

Figure 2. Correlations between concentration of esterase and lesion number on leaf disks.

Table 8. Effect of polyelectrolytes on mycelial growth of *B. elliptica* at 20 °C

Polyelectrolyte (400 ppm)	Mycelial growth[1] (mm in diameter)
AN910SD	35 ± 5 cd[2]
FA920SD	45 ± 9 bc
AN934SD	58 ± 1 a
FO4240SH	34 ± 5 cd
FO4490SH	25 ± 1 d
FO4550SH	27 ± 7 d
Check (water)	48 ± 3 ab

[1]Diameter of mycelial colony, that had penetrated through the filter paper, grown on PDA was recorded 5 days after spore suspension of *B. elliptica* was dropped on a sterile filter paper treated with polyelectrolytes and covered on PDA plate.
[2]Means ± deviation. Data followed by the same letter do not differ significantly ($p = 0.05$) according to Duncan's multiple range test.

into agar (Table 8). These results showed that the polyelectrolytes had a small effect as a mechanical barrier to fungus infection.

Effect of polyelectrolytes on spore behaviour

Germination of conidia on the abaxial surface of leaf disks treated with distilled water was observed 2–4 h after inoculation; up to 75% of the conidia germinated within 6 h. However, only about 45% of conidia germinated on leaf disks treated with 400 ppm FO4490SH within 6 h. The percentage of conidal germination in both FO4490SH-treated and untreated leaf tissues, 16 h after inoculation, was similar to that of conidial germination at 6 h after inoculation. Percentage of germination in both of the treatments was 46% and 75%, respectively. On the surface of distilled water-treated leaf disks, 22% of germinated conidia failed to penetrate into the epidermal cells. The rest of the germinated conidia penetrated plant tissues through epidermal cells (direct infection), guard cells or stomata. The percentage of infection was 21%, 25% and 8%, respectively. However, on the surface of FO4490SH-treated leaf disks, a high percentage of germinated conidia failed to penetrate into plant tissue. In the 46% of germinated conidia, only 8% of infections were successfully shown by the appearance of disease symptoms. Direct infection via epidermal cells was less than that via guard cells and stomata (Table 9).

Table 9. Infection behaviour of *B. elliptica* on leaf disks of lily coated with a thin film of polyelectrolyte (FO4490SH) 16 h after inoculation at 20 °C

Treatment	Spore germination and infection[1]				
	Germination (%)	Non-infection (%)	Direct infection (%)	Guard cells (%)	Stomata (%)
FO4490SH (400 ppm)	46 b	38 a	1 b	5 b	3 b
Check (water)	75 a	22 b	21 a	25 a	8 a

[1]Percentages of spore germination and infection were evaluated 16 h after inoculation. The inoculated plant tissues (15-mm diameter of leaf disk) were cleared, stained and examined with UV fluorescence according to the method of Hood and Shew (1996).
Data followed by the same letter in each column do not differ significantly ($p = 0.05$) according to Duncan's multiple range test.

Discussion

Lily leaf blight is a severe disease during the low temperature seasons in Taiwan and many Mediterranean countries (Hsieh and Huang, 1998; Doss et al., 1984). The causal agent, *B. elliptica*, has been reported to develop resistance to dicarboximide and benzimidazole fungicides (Chastagner and Riley, 1990; Hsiang and Chastagner, 1991; Migheli et al., 1990; Skrzypczak, 1992). Therefore, there is an urgent need to find an alternative means for controlling the disease. Plant pathologists have demonstrated that antitranspirants can produce prophylactic effects in control of plant diseases due to the plastic film formed on the plant surface. For example, Gale and Poljakoff-Mayber (1962) found that powdery mildew of sugar beet was decreased after the use of epidermal-coating antitranspirants, and for the first time proved that antitranspirants performed a prophylactic effect for disease control.

Polyelectrolytes have been used in water purification, oil recovery, paper-making and mineral processing (Mortimer, 1991). Our study has demonstrated that polyelectrolytes may be an alternative means to protect lilies against *B. elliptica*. The disease was effectively controlled by cationic polyelectrolytes under both laboratory and greenhouse conditions. On leaf disks and on intact plants under 100% relative humidity, FO4490SH was the most significantly effective substance in reducing the disease. In field trials, FO4490SH was also effective. Combinations of procymidone, which is a moderately effective fungicide, with the polyelectrolyte FO4490SH gave no statistically significant additive effect.

The effects of film-forming antitranspirants may be similar to those of the natural cuticle layer in defense against pathogens. This layer has the following properties: 1. increasing water repellency; 2. producing a mechanical barrier (Gale and Poljakoff-Mayber, 1962); 3. suppressing the growth of pathogens (Elad et al., 1990; Han, 1990; Zekaria-Oren et al., 1991); and 4. misdirecting the pathogen germ tubes from penetration (Zekaria-Oren et al., 1991). The cationic polyelectrolytes used in our study were not able to repel free water on the surface of leaves. On the other hand, the polyelectrolytes are hydrophilic and may act as a sticker (Table 6). In relation to being a possible barrier, the polyelectrolytes, such as FO4490SH and FO4550SH, effectively delayed mycelial penetration through treated filter paper and reduced expansion onto agar plates (Table 8). Apparently, the films formed by those polyelectrolytes on the filter papers might serve as a little mechanical barrier to the pathogen. The cationic polyelectrolytes, FO4240SH, FO4490SH and FO4550SH, significantly decreased the percentage of conidial germination and inhibited growth of germ tubes of *B. elliptica* (Tables 6 and 7). These results are similar to those obtained with *B. cinerea* (Elad et al., 1990); the reduction of conidial germination, germ-tube elongation and mycelial growth were due to fungistatic effects of the polymers. However, antitranspirants, such as bio-film, folicote and vapor gard, did not suppress the germination of rust spores, but they effectively suppressed the ability of germinated conidia to form appressoria (Zekaria-Oren et al., 1991). In our studies, the germination of *B. elliptica* was decreased on the surfaces of FO4490SH-treated leaf disks, and a high percentage of germinated conidia failed to penetrate into the cationic polyelectrolyte-treated lily leaves. Subsequently, direct infection was nullified (Table 9).

Doss et al. (1988) reported that hydrolytic enzymes play an important role in the infection process of *B. elliptica* on easter lilies. However, esterase is

recognized as an important indicator for cuticle infection by the pathogen. In our studies, the polyelectrolytes were effective in suppressing esterase production by germ tubes of *B. elliptica* (Table 7) and coagulating enzymes released by the pathogen. Moreover, the cationic polyelectrolyte, FO4490SH, reduced spore germination and prevented germinated conidia infecting epidermal cells of leaf disks (Table 9). The amount of esterase produced by germ tubes was significantly correlated with lesion number on inoculated leaf disks. The results suggest that the possible mechanisms involved for disease control by cationic polyelectrolytes are associated with the reduction of esterase secretion by *B. elliptica*.

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References

Barnett RJ and Seligman AM (1951) Histochemical demonstration of esterases by production of indigo. Science 114: 579–582

Braun EJ and Howard RJ (1994) Adhesion of *Cochliobolus heterostrophus* conidia and germlings to leaves and artificial surfaces. Exp Mycol 18: 211–220

Chastagner GA and Riley K (1990) Occurrence and control of benzimidazole and dicarboximide resistant *Botrytis* spp. on bulb crops in western Washington and Oregon. Acta Hortic 266: 437– 445

Doss RP, Chastagner GA and Riley KI (1984) Techniques for inoculum production and inoculation of lily leaves with *Botrytis elliptica*. Plant Dis 68: 854–856

Doss RP, Christian JK and Chastagner GA (1988) Infection of Easter lily leaves from conidia of *Botrytis elliptica*. Can J Bot 66: 1204–1208

Elad Y, Ayish N, Ziv O and Katan J (1990) Control of grey mould (*Botrytis cinerea*) with film-forming polymers. Plant Pathol 39: 249–254

Gale J and Poljakoff-Mayber A (1962) Prophylactic effect of a plant antitranspirant. Phytopathology 52: 715–717

Han JS (1990) Use of antitranspirant epidermal coatings for plant protection in China. Plant Dis 74: 263–266

Hood ME and Shew HD (1996) Applications of KOH–aniline blue fluorescence in the study of plant–fungal interactions. Phytopathology 86: 704–708

Hsiang T and Chastagner GA (1991) Growth and virulence of fungicide-resistant isolates of three species of *Botrytis*. Can J Plant Pathol 13: 226–231

Hsieh TF and Huang JW (1997) Applications of antitranspirants on plant disease control. Plant Pathol Bull (Taiwan) 6: 89–94

Hsieh TF and Huang JW (1998) Factors affecting disease development of Botrytis leaf blight of lily caused by *Botrytis elliptica*. Plant Prot Bull (Taiwan) 40: 227–240

Kamp M (1985) Control of *Erysiphe cichoracearum* on *Zinnia elegans*, with a polymer-based antitranspirant. Hort Science 20: 879–881

Migheli Q, Aloi C and Gullino ML (1990) Resistance of *Botrytis elliptica* to fungicides. Acta Hortic 266: 429– 436

Mortimer DA (1991) Synthetic polyelectrolytes – A review. Polymer International 25: 29– 41

Neumann AW and Good RJ (1979) Techniques of measuring contact angles. In: Good RJ and Stromberg RA (eds) Surface and Colloid Science, Vol 11 (pp 31–91) Plenum Press, New York

Skrzypczak C (1992) Evaluation of fungicides and Atpol effect on control of *Botrytis elliptica* (Berk.) Cooke on field grown lilies. Acta Hortic 325: 793–796

Zekaria-Oren J, Eyal Z and Ziv O (1991) Effect of film-forming compounds on the development of leaf rust on wheat seedlings. Plant Dis 75: 231–234

Ziv O (1983) Control of septoria leaf blotch of wheat and powdery mildew of barley with antitranspirant epidermal coating materials. Phytoparasitica 11: 33–38

Ziv O and Frederiksen RA (1983) Control of foliar diseases with epidermal coating materials. Plant Dis 67: 212–214

Ziv O and Frederiksen RA (1987) The effect of film-forming antitranspirants on leaf rust and powdery mildew incidence on wheat. Plant Pathol 36: 242–245

Ziv O and Hagiladi A (1934) Control of powdery mildew on *Hydrangea* and *Crapemyrtle* with antitranspirants. Hort Science 19: 708–709

Ziv O and Hagiladi A (1993) Controlling powdery mildew in Euonymus with polymer coatings and bicarbonate solutions. Hort Science 28: 124–126

Ziv O and Zitter TA (1992) Effects of bicarbonates and film-forming polymers on cucurbit foliar diseases. Plant Dis 76: 513–517