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Biocontrol Activity of *Acremonium strictum* BCP Against Botrytis Diseases

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Biological control activity of *Acremonium strictum* BCP, a mycoparasite on *Botrytis cinerea*, was examined against six plant diseases such as rice blast, rice sheath blight, cucumber gray mold, tomato late blight, wheat leaf rust, and barley powdery mildew in growth chambers. The spore suspension of strain BCP showed strong control activities against five plant diseases except against wheat leaf rust. On the other hand, the culture filtrate of *A. strictum* BCP was effective in controlling only cucumber gray mold and barley powdery mildew. Further *in vivo* biocontrol activities of *A. strictum* BCP against tomato gray mold were investigated under greenhouse conditions. Control efficacy of the fungus on tomato gray mold increased in a concentration-dependent manner. Treatment of more than 1×10^6 spores/ml significantly controlled the disease both in tomato seedlings and in adult plants. The high disease control activity was obtained from protective application of the strain BCP, whereas the curative application did not control the disease. Foliar infections of *B. cinerea* were controlled with 1×10^8 spores/ml of *A. strictum* BCP applied up to 7 days before inoculation. In a commercial greenhouse, application of *A. strictum* BCP exhibited the similar control efficacy with fungicide procymidone (recommended rate, 500 μg/ml) against strawberry gray mold. These results indicate that *A. strictum* BCP could be developed as a biofungicide for *Botrytis* diseases under greenhouse conditions.

Keywords : *Acremonium strictum*, biocontrol agent, biofungicide, *Botrytis cinerea*, mycoparasite

Botrytis cinerea Pers ex Fr is a well-known plant pathogenic fungus with a wide host range that causes heavy yield losses in onion, potato, strawberry, table grapes, and the wine industry (De Curtis et al., 1996). Conventional method of controlling Botrytis diseases involves frequent applications of fungicides and these repeated applications may lead to development of resistance. Widespread resistance against benzimidazole fungicides such as benomyl, carbendazim, and thiophanate-methyl has been reported in many countries (Gullino and Garibaldi, 1986; Kim et al., 1993; Moorman and Lease, 1992; Staub and Diriwaechter, 1986). In some greenhouse resistance to dicarboximides such as iprodione, vinclozolin, and procymidone has also been reported (Katan, 1982; Leroux and Clerjeau, 1985; Lorenz and Eichhorn, 1982; Pezet, 1982). Furthermore, *B. cinerea* strains have also developed resistance to the mixture of carbendazim and diethofencarb which have a specific antifungal activity against benzimidazole-sensitive and -resistant isolates, respectively (Moyano et al., 2004). In addition, an increasing concern about the effects of pesticide residues on the environment and human health leads to the increased restrictions on the use of pesticides (Jansma et al., 1993). Biological control offers an environment friendly supplement or alternative to chemical control.

Mycoparasite, organism parasitizing fungi, has frequently been used as biological control agents to control plant fungal disease. *Ampelomyces quisqualis* Ces. and *Trichoderma harzianum* Rifai are typical mycoparasites and have been commercialized as tradenames AQ-10 and Trichodex, respectively. *A. quisqualis* was shown to colonize hyphae and conidiophores of powdery mildew fungi belonging to the genera *Oidium*, *Erysiphe*, *Sphaerotheca*, *Podosphaera*, and *Uncinula* and formed pycnidia within the conidiophores of its hosts (Falk et al., 1995; Hashioka and Nakai, 1980; Kiss, 1997). On the other hand, *T. harzianum* is a mycoparasite of several economically important plant pathogenic fungi. Trichodex®, a preparation of *T. harzianum* T39, has effectively controlled Botrytis diseases in greenhouse crops in many countries (Elad et al., 1993; Elad et al., 1995).

Acremonium species have also been known as mycoprasites against various plant pathogenic fungi such as *Aspergillus*, *Colletotrichum*, *Mucor*, *Alternaria*, *Puccinia*, *Uromyces*, *Sphaerotheca*, etc (Chaturvedi et al., 1990; Malathrakis, 1985; Pon et al., 1959; Simay, 1988; Singh et al., 1978; Srivastava et al., 1981). In addition, plant-epiphytic and -endophytic *Acremonium* isolates that produce antifungal compounds have been reported (Goodman and

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Burpee, 1991; Janisiewicz, 1988; McGee et al., 1991). *Acremonium* species have been used as biocontrol agents of various plant diseases because of their mycoparasitic and antagonistic activities against plant pathogens (Bettiol, 1996; Goodman and Burpee, 1991; Janisiewicz, 1988; Janisiewicz and Martinsburg, 1990; Jayapal Gowdu and Balasubramnian, 1992). Especially, *Acremonium alternatum* Linc: Fr. and *Acremonium persicinum* Nicot and Gams being mycoparasites on *Catacauma torrendiella* Batista and *Coccostroma palmicola* (Speg.) Von Arx & Muller were commercialized for the control of coconut tar spot caused by both fungal pathogens and successfully reduced development of the disease in Brazil (Bettiol, 1996).

We isolated a strain BCP of *Acremonium strictum* W. Gams, a mycoparasite on *B. cinerea* (Choi et al., 2008). In addition, the strain possessed desirable traits as a biocontrol agent such as its quick sporulation, massive spore production in a wide range temperature, the aggressive parasitism on *B. cinerea*, and production of strong antifungal compound, verlamelin (Kim et al., 2002). Thus, to investigate that *A. strictum* BCP could be developed as a biofungicide for Botrytis diseases under greenhouse conditions, we tested biocontrol activity of strain BCP against six plant diseases that include rice blast, rice sheath blight, cucumber gray mold, tomato late blight, wheat leaf rust, and barley powdery mildew under growth chamber conditions. Biocontrol potential of *A. strictum* BCP against some Botrytis diseases such as gray mold diseases of tomato and strawberry was also examined under greenhouse and field conditions.

Materials and Methods

Microorganisms, media and culture conditions. *A. strictum* BCP was inoculated in Erlenmeyer flask containing potato dextrose broth (PDB; Difco, Beckton and Dickinson Co., USA) by placing mycelium plugs cut from the margins of actively growing cultures on potato dextrose agar (PDA; Difco, Beckton, Dickinson, and CO, Sparks, MD. USA). The flask was incubated for 10 days on a rotary shaker at 150 rpm and 25°C, and then the fermentation broth was filtered with cheese clothes to eliminate mycelia. After centrifuging the culture filtrate at 10,000×g for 10 min, the pellet (spores) was washed three times with sterile distilled water to remove medium compounds. Required concentration of each spore suspension was made by diluting with sterile distilled water and then added with Tween 20 (a nonionic surfactant, Samchun Pure Chemical Co.) at a concentration of 250 μg/ml.

Fungal plant pathogens were incubated on PDA for *B. cinerea*, on oatmeal agar (OA; Becton and Dickinson Co.) medium for *Magnaporthe grisea* (Hebert) Barr and *Phytophthora infestans* (Mont) de Bary, and in wheat bran solid medium (wheat bran 200 ml, rice bran 100 ml, distilled water 100 ml in a 1-liter Erlenmyer flask) for *Corticium sasaki* Matsu as previously described (Kim et al., 2001). Two obligate parasitic pathogens *Puccinia recondita* Rob ex Desm and *Blumeria graminis* (DC.) Golovin ex Speer f. sp. *hordei* Em. Marchal were maintained by periodical transfer on each host plant wheat and barley, respectively.

Biocontrol of six plant diseases in growth chambers. Spore suspension and culture filtrate of *A. strictum* BCP were tested for their *in vivo* biocontrol activity against six plant diseases: rice blast (*M. grisea*), rice sheath blight (*C. sasaki*), cucumber gray mold (*B. cinerea*), tomato late blight (*P. infestans*), wheat leaf rust (*P. recondita*), and barley powdery mildew (*B. graminis* f. sp. *hordei*). Plants such as rice (*Oryza sativa* L., cv. Nakdong), cucumber (*Cucumis sativus* L., cv. Hausbaekdadagi), tomato (*Lycopersicon esculentum* Mil., cv. Seokwang), barley (*Hordeum sativum* Jessen, cv. Dongbori), and wheat (*Triticum aestivum* L., cv. Chokwang) plants were grown for 1 to 4 weeks in a controlled greenhouse at 25±5°C and used for *in vivo* biocontrol activity. The seedling plants were sprayed with spore suspension and culture filtrate of the strain BCP to run-off and maintained for 24 h. Bioassays for *in vivo* antifungal activity were performed as previously described by Kim et al. (2001).

Pots were arranged in a completely randomized design with three replicates per treatment. The estimates were converted into the control percentage by the following equation. Control value (%)=100 (1−B/A), where A=area of infection (%) on leaves or sheaths sprayed with Tween 20 solution alone and B=area of infection (%) on treated leaves or sheaths. Values were expressed as a percentage of the control (±standard deviation).

Biocontrol of tomato gray mold under greenhouse conditions. In order to investigate further biocontrol activities of *A. strictum* BCP against *B. cinerea*, tomato seedlings (the second-leaf stage) and/or tomato plants grown for 10 weeks in a greenhouse maintained at 20±5°C were used. Disease control activities of strain BCP by 1-h and 1-day protective, and 1-day curative applications, the control efficacy depending on concentration of spores, and duration of protective effect were tested under greenhouse conditions (20±5°C and 70-100% RH). For the other tests except for curative effect, foliar applications of the strain BCP were made protectively.

To examine the control efficacy depending on spore concentration, the strain BCP was applied at several concentrations of 1.0×10^4, 1.0×10^5, 1.0×10^6, 1.0×10^7 and

1.0×10^8 spores/ml on tomato seedlings and adult plants. After 24 h, the treated plants were inoculated with spore suspension of *B. cinerea* (1×10^6 spores/ml for seedlings and 5×10^6 spores/ml for adult plants). To evaluate 1-day curative activity, the fermentation broth (5×10^6 spores/ml) of *A. strictum* BCP was sprayed on seedling plants 20 h after inoculation of the pathogen (5×10^5 spores/ml). In contrast, in order to test the control efficacy by 1-day and 1-h protective applications, the tomato plants were treated with the same fermentation broth of strain BCP 1 day and 1 h before inoculation of *B. cinerea* (5×10^5 spores/ml), respectively. To examine duration of protective effect of *A. strictum* BCP on tomato gray mold, the spore suspension of strain BCP (at concentrations of 1×10^7 and 1×10^8 spores/ml) was sprayed on tomato plants 1, 3, 5, and 7 days prior to inoculation of *B. cinerea* and kept under greenhouse conditions temperature of 20±5°C and relative humidity of 50-80% until inoculation of the fungal pathogen (1×10^6 spores/ml). The inoculated plants were placed in a greenhouse (20±5°C and 70-100% RH).

Pots were arranged as a randomized complete block design with three replications per treatment and the other procedures were conducted as described above. Disease rating was carried out 4-5 days after inoculation as area of infection (%) and three estimates were converted into the percentage of control (±standard deviation) by comparing with the controls.

Biocontrol activity of BCP in the field. Experiment was carried out in a commercial strawberry (*Fragaria ananassa* Duch., cv. Janghee) greenhouse near Nonsan City, Korea. Cold-stored strawberry runners kept at −2°C until the day before planting, were transplanted in the beginning of November. General N, P, K, and Mg fertilizer mixture were applied twice a week and fungicide active to *B. cinerea* was not applied. Experiment was conducted in the strawberry production condition under natural disease occurrence.

We used a randomized complete block design, with three rows, each containing three treatments. Each treatment plot was 4 m long, but we only recorded data in the middle 3 m, leaving 0.5 m on both sides as separation between treatment plots. The three treatments were: (1) control, (2) *A. strictum* BCP (3×10^7 spores/ml), (3) fungicide procymidone (Sumilex®; Dongbang-Agro Co.; 500 μg/ml, recommended rate). The treatments were sprayed according to farmer's conventional practice, approximately every 1 week, and spraying was done three times with a hand-held sprayer until run-off. Prior to every spraying, ripe fruits from each plot were harvested and the number of total and diseased fruits per plot was counted. The percentage of diseased fruits for each treatment was converted into the control percentage when compared with the controls. Analysis of variance (ANOVA) was performed on the data with the PROCGLM procedure (SAS Institute, Cary, NC, USA). If P>F was less than 0.01, means were separated with Duncan's multiple range test at the $P = 0.05$ level.

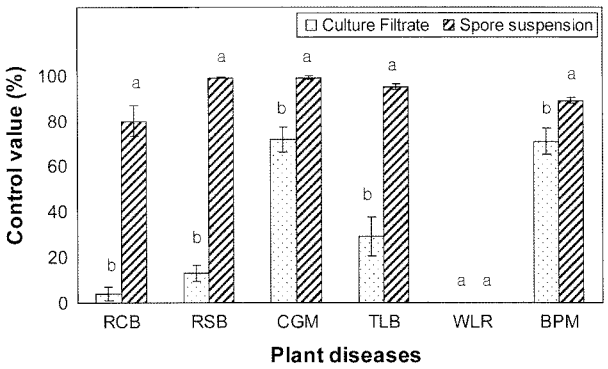

Fig. 1. Control efficacy of spore suspension and culture filtrate of *Acremonium strictum* BCP against six plant diseases in growth chambers. RCB, rice blast; RSB, rice sheath blight; CGM, cucumber gray mold; TLB, tomato late blight; WLR, wheat leaf rust; BPM, barley powdery mildew. The plant seedlings were inoculated with spores or mycelial suspension of the test pathogens 1 day after spore suspension and culture filtrate of the strain BCP were sprayed to run-off on the plants. The disease severity of untreated control plants was 30% for RCB, 95% for RSB, 53% for CGM, 70% for TLB, 15% for WLR, and 30% for BPM. Bars represent standard deviations of the means and each mean followed by a different letter differs significantly at P=0.05.

Results

Biocontrol activity of strain BCP against six plant diseases. Spore suspension and culture filtrate prepared from the 10-day-old PDB culture of *A. strictum* BCP were investigated for *in vivo* biocontrol activities against six plant diseases: rice blast, rice sheath blight, cucumber gray mold, tomato late blight, wheat leaf rust, and barley powdery mildew. The spore suspension (1×10^8 spores/ml) of the strain BCP showed strong disease control efficacy against five plant diseases except wheat leaf rust (Fig. 1). On the other hand, the culture filtrate of the fungus potentially controlled cucumber gray mold and barley powdery mildew, but it was not effective to control rice blast, rice sheath blight, tomato late blight, and wheat leaf rust. Especially, the spore suspension and the culture filtrate controlled the development of cucumber gray mold by 99% and 72%, respectively.

Biocontrol activity against tomato gray mold under greenhouse conditions. Biocontrol efficacy of *A. strictum* BCP on tomato gray mold was depending on the spore concentrations (Fig. 2). The control activities of the strain BCP were similar in both tomato seedlings (the second-leaf

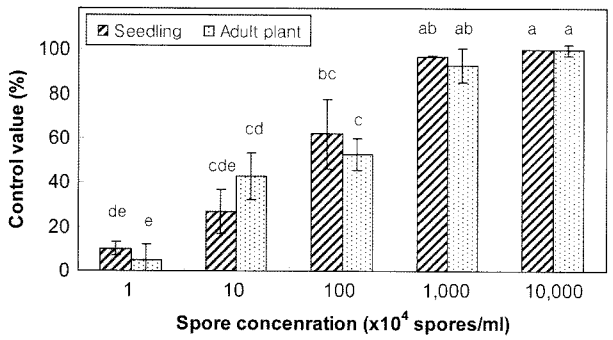

Fig. 2. *In vivo* biocontrol activity of *Acremonium strictum* BCP against tomato gray mold depending on spore concentration in a greenhouse. Tomato seedlings and adult plants were inoculated with spore suspension of *B. cinerea* (1×10^6 spores/ml for seedlings, 5×10^6 spores/ml for adult plants) 1 day after the spore suspension of strain BCP were sprayed to run-off. Untreated controls of seedlings and adult plants had 89±2.5% and 40±8.2% affected area, respectively. Bars represent standard deviations of the means and each mean followed by a different letter differs significantly at P=0.05.

Fig. 3. Control effect of *Acremonium strictum* BCP against tomato gray mold. Tomato plants were inoculated with spore suspension of *B. cinerea* (5×10^6 spores/ml) 1 day after the spore suspension of strain BCP (1×10^7 spores/ml) were sprayed to run-off (Left, untreated control plant; right, plant treated with *A. strictum* BCP).

stage) and tomato plants of flowering stage. Sprays of spore suspensions (more than 1×10^7 spores/ml) gave near perfect control against gray mold disease in both stages of tomato plants (Fig. 3). *A. strictum* BCP sprayed 1-day and 1-h protectively on tomato plants showed good control activities against tomato gray mold (Table 1). In contrast, 1-day curative application did not reduce the development of tomato gray mold.

In case of duration of protective effect, foliar infection of *B. cinerea* was near perfectly controlled with 1×10^8 spores/ml of *A. strictum* BCP applied until 7 days before

Table 1. Biocontrol efficacy of *Acremonium strictum* BCP against tomato gray mold[a]

Treatment	Control value (%)[b] ± SD
1-Day protective	88±7.2 b
1-Hour protective	100±0.0 a
1-Day curative	0±2.4 c

[a]To evaluate 1-day curative activity, the fermentation broth (5×10^6 spores/ml) of strain BCP was sprayed on tomato seedlings 20 h after inoculation of tomato gray mold pathogen, *Botrytis cinerea* (5×10^5 spores/ml). In contrast, in order to test the control efficacy by 1-day and 1-h protective applications, the tomato plants were treated with the same fermentation broth of *A. strictum* BCP 1 day and 1 h before inoculation of the pathogenic fungus (5×10^5 spores/ml), respectively. Disease rating was carried out four days after inoculation and control plants had 60-85% affected area.

[b]Each value represents the mean of disease controls (%) of three replicates ± standard deviation. Means in a column followed by a different letter differ significantly at P=0.05.

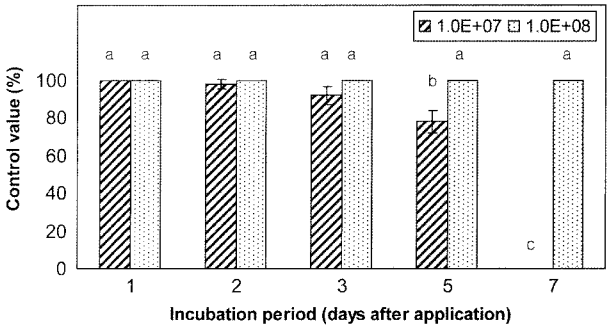

Fig. 4. Duration of protective effect of *Acremonium strictum* BCP against tomato gray mold. The strain BCP was sprayed at 1×10^7 and 1×10^8 spores/ml onto tomato plants 7, 5, 3, and 1 days before inoculation and then kept under greenhouse conditions (20±5°C and 50-80% RH) until inoculation of *Botrytis cinerea* (1×10^6 spores/ml). Disease rating was carried out four days after inoculation and untreated control plants showed disease severity of more than 50%. Bars represent standard deviations of the means. And each mean followed by a different letter differs significantly at P=0.05.

challenging with the pathogen (Fig. 4). On the other hand, the strain BCP treated at a concentration of 1×10^7 spores/ml displayed good control efficacy on gray mold disease of tomato plants sprayed up to 5 days before inoculation. However, it did not show control activity in 7-day protective application.

Biocontrol activity of strain BCP in the strawberry production condition. In our field experiment performed in the Nonsan farmhouse, the biocontrol efficacy of the strain BCP against *B. cinerea* was confirmed (Table 2). *A. strictum* BCP (3×10^7 spores/ml) treated three times at one-week-intervals showed statistically similar control efficacy against strawberry gray mold to procymidone, a chemical fungicide, applied at 500 mg/ml. This result was similar to

Table 2. Biocontrol activity of *Acremonium strictum* BCP on gray mold of strawberry fruits in a commercial greenhouse[a]

Treatment	Diseased fruits (%)[b]	Control value (%)[b]
A. strictum BCP	2.7±4.6 a	75±39 a
Procymidone	4.0±3.6 ab	67±30 ab
Control	12.0±4.4 c	0±36 c

[a]Each treatment was sprayed three times, at an interval of about 7 days, in a commercial greenhouse to run-off on strawberry plants. Prior to every spraying, ripe fruits from each plot were harvested and the number of total and diseased fruits was counted.
[b]Each value represents the mean of three replicates ± standard deviation. Means in a column followed by a different letter differ significantly at P=0.05.

that obtained in the growth chamber and greenhouse that utilized artificial infection of the fungal pathogen.

Discussion

Many mycoparasites such as *Trichoderma* spp. and *Acremonium* spp. have received attention as a biocontrol agent to control plant fungal disease. Here, we investigated the potential of *A. strictum* BCP as a biocontrol agent in greenhouse and field conditions. When spore suspension and culture filtrate of *A. strictum* BCP, a mycoparasite against *B. cinerea*, were applied on leaves and/or sheaths of plant seedlings prior to inoculation with each fungal pathogen, the spore suspension showed strong biocontrol activity against economically important plant diseases such as rice blast, rice sheath blight, cucumber gray mold, tomato late blight, and barley powdery mildew (Fig. 1). On the other hand, the culture filtrate displayed potent control efficacy against only cucumber gray mold and barley powdery mildew among test plant diseases. These results suggested that application of fungal spores as well as antifungal compound produced by the mycoparasite is important for biological control of plant diseases with *A. strictum* BCP.

The spore suspension of *A. strictum* BCP potently controlled various plant pathogens: *P. infestans* classified into a pseudofungus *Oomycetes* (Kingdom Chromista) as well as some true fungi (Kingdom Fungi) such as *B. cinerea*, *M. grisea*, *C. sasaki*, and *B. graminis* f. sp. *hordei* (Fig. 1). Thus, *A. strictum* BCP can be used as a biological control agent for the control of various plant diseases occurred in the greenhouses and in the fields as the other mycoparasites. *B. cinerea* causes more severe damage under greenhouse conditions than in open fields (Bulger et al., 1987). An unfavorable environment to biocontrol agent in fields has been cited as a major reason for failure or inconsistent performance of biological control trials, since biocontrol agents are also sensitive to environmental conditions. Due to these reason, introduction of biocontrol agent in greenhouses with well controlled environment would be more effective than that in open field. Thus, we selected *B. cinerea* as a target pathogen to examine further biocontrol potential of *A. strictum* BCP, though the strain BCP showed strong *in vivo* controlling activity against various plant diseases (Fig. 1).

Although greenhouse conditions are more favorable to biological control agents than open fields, biocontrol agents may be inactivated by several environmental factors including high temperature, low humidity, leaf surface exudates and competitors (Jarvis and Slingsby, 1977; Jones and Burges, 1998; Paulitz and Belanger, 2001). *A. strictum* BCP applied at 1×10^7 and 1×10^8 spores/ml represented potent biocontrol activity against tomato gray mold disease when sprayed in foliage of tomato plants even 5 days and 7 days before inoculation, respectively (Fig. 4). This result suggested that the strain BCP successfully survives on tomato plants under greenhouse conditions (20±5°C and 50-80% RH). It would be advantageous in achieving biocontrol of gray mold disease of crops cultivated in commercial greenhouses.

With 1-day and 1-h protective applications, *A. strictum* BCP successfully controlled tomato gray mold (Table 1). It could be due to inhibitory substances produced by the fungus, or due to the mycoparasitism of strain BCP on *B. cinerea* (Choi et al., 2008; Kim et al., 2002). Because the biocontrol activity of the mycoparasite probably gets break down during incubating the treated plants under greenhouse conditions, the 1-h protective application may be more effective to control the disease than 1-day protective application (Table 1). In contrast, 1-day curative treatment did not reduce the development of the disease (Table 1). In case of *Acremonium obclavatum* W. Gams being a mycoparasite on *Puccinia arachidis* Speg, protective and curative applications of the mycoparasite caused the similar decreased infection of the pathogen on detached leaves of groundnut (Jayapal Gowdu and Balasubramnian, 1992). It was different from the 1-day curative activity of *A. strictum* BCP against tomato gray mold. Thus, application of *A. strictum* BCP for the control of Botrytis diseases must be protectively performed.

This study has shown that *A. strictum* BCP strongly reduced the development of Botrytis diseases on several crops such as cucumber, tomato, and strawberry in the field as well as in the growth chambers and greenhouses. The biocontrol agent showed a potent control activity by protective application due to their good survival under greenhouse conditions. These results indicate that *A. strictum* BCP could be developed as a biofungicide for the control of Botrytis diseases. Accordingly, further studies are required for mass-production and formulation of *A. strictum* BCP for commercialization.

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