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Research Article

# Effect of Various Factors (Temperature, pH and Light Intensity) on Growth of *Colletotrichum capsici* Isolated from Infected Chilli

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### ABSTRACT

Chilli is an important vegetable and a spice crop worldwide and one of the most important vegetables in India. Andhra Pradesh, Karnataka, Maharashtra, Orissa, West Bengal, Tamil Nadu, Madhya Pradesh, Gujarat, Assam, Rajasthan and Punjab are found to be important states growing chilli in India. Guntur District contributes 30% of the total chilli produced in India, where as Andhra Pradesh as a whole contributes 75% of India's chilli exports. The chief constituent of chilli, Capsaicin has antioxidant, antibacterial and anti-cancerous properties. Anthracnose caused by Colletotrichum capsici is a major problem in India and one of the more significant economic constraints of chilli production worldwide, especially in tropical and subtropical regions. Three isolates of C.capsici were collected from different chilli growing areas of Guntur District and their pathogenicity was proved under laboratory conditions. Effect of different pH levels, temperature, light intensity and seed quality tested against the growth of C. capsici under in vitro. Results indicated that the growth of C. capsici was maximum at pH range of 6.50-7.00 and temperature range of 25-30°C. Exposure of the fungus to alternate cycles of 12 hr light and 12 hr darkness resulted in the maximum mycelia growth of C. capsici compared to the 24 hr exposure to either continuous light or dark. Potato dextrose agar medium supported significantly the maximum growth of all the three isolates of C. capsici. Further, the strains were found to vary morphologically between the isolates under the study.

*Key words: Chilli, Anthracnose, pathogenicity test, seed quality test, temperature, pH and light intensity.* 

#### **INTRODUCTION**

Chilli is one of the most popular spice and widely grown vegetables in the world and the most popular in India belongs to the family 'Solanaceae'<sup>6</sup>. There are more than 400 different varieties of chillies found all over the world.

The area cultivated with chilli worldwide is about 17,00,000 ha for producing fresh chilli and around 18,00,000 ha for producing dried chilli; a total area of 37,29,900 ha with a total production of 2,00,00,000 tonnes<sup>2</sup>.

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The most important and exporters of chilli include China India, Mexico, Morocco, Pakistan, Thailand and Turkey. Chilli is an imperative cash crop and India is the largest grower, consumer and exporter of dry chillies and other products to over 90 countries around the world14. Andhra Pradesh, Karnataka, Maharashtra, Orissa, West Bengal, Tamil Nadu, Madhya Pradesh, Gujarat, Assam, Rajasthan and Punjab are found to be important states growing chilli in India. Guntur District contributes 30% of the total chilli produced in India, where as Andhra Pradesh as a whole contributes 75% of India's chilli exports. About 35 varieties of chillies are cultivated in Guntur which includes 'Wonder Hot Chilli' (Hottest Guntur Chilli), '334 Chilli' (Premium export quality). 'Teja Chilli' (Fine variety of Guntur chilli), '273 Chilli' (Common wrinkled chilli), 'Guntur Sannam-S4 Type' (Most famous type and has a huge demand throughout the world).Other Guntur chillies are Phatki, Indo-5, Ankur, Roshni, Bedki, and Madhubala. Phatki and Teja could be too spicy for some people's taste while a few others are used just to add color to the dish. Chilli plants are been attacked by more than 100 different types of pathogens during their growth and development. Chilli is prone to number of fungi, bacteria, virus, nematode mycoplasma like organisms, which and significantly affect its production and quality. Fortunately, only a few of them cause economic losses. Three species of Colletotrichum including C. capsici, С. acutatum and C. gloeosporioides have been identified as the most important pathogens causing anthracnose of chilli. However, C. capsici is predominant and appeared to be virulent compared to C. gloeosporioides<sup>7</sup>. Anthracnose, derived from a Greek word meaning 'coal', is the common name for plant diseases characterized by very dark, sunken lesions, containing spores<sup>4</sup>. Colletotrichum capsici is most adhesive that adhere to the plant surface and remain latent until such physiological changes occur in the fruit and cause economic losses to the farmers due to low fruit quality and is our marketability many

post harvest diseases of fruit exhibit the phenomenon of quiescence in which symptoms do not develop until fruit ripen<sup>1</sup>. Anthracnose causes extensive pre and post harvest damage to chilli fruits causing anthracnose lesions. Chilli anthracnose usually develops under high humid conditions when rain occurs after the fruits have started to ripen, losses of up to 84%<sup>15</sup>.In anthracnose, the ripe fruits turning red get affected by this disease, whereas green fruits are not generally attacked. Symptoms on the fruit first appear as sunken, water-soaked lesions that expand rapidly on the fruit<sup>16</sup>. Fully expanded lesions are soft, sunken and range in color from dark red to tan black, generally described as anthracnose disease<sup>17</sup>. A small, black, circular spot initially appears on the skin of fruits and it then spreads in the direction of the long axis of the fruit, thus becoming more or less elliptical. As the infection progresses, the spots get either diffused becoming black, greenish or grey in color or they are markedly delimited by a thick and sharp black outline enclosing a lighter black or straw colored area. On this discolored area numerous black acervuli are found scattered. When a diseased fruit is cut open the lower surface of the skin is found with minute, spherical, covered black stromatic masses or sclerotia of the fungus. Seeds are also covered by a mat of fungal hyphae. Such seeds turn rusty in color. Affected fruits are deformed in shape and lose their pungency. Ultimately the diseased fruits shrivel and dry up. Once the lesion develops, the fungus produces the conidia within 3 to 5 days at 30 °C and at 90% relative humidity. A dark growth of the fungus may be visible in these lesions with tan to pink concentric circles of spores evident in some cases. Disease symptoms occur only on ripened fruits occasionally appears on leaf as leaf spot and also on mature green fruits. Colletotrichum capsici is an air- borne, seed-borne, and also soil-borne pathogen. It can survive in moist soil and plant debris for several years. The fungus can spread by rain splash and irrigation water. It can also spread by infected soil, farm tools, and shoes. The fungus can be carried by

intraembryonally. chilli seeds Enzymes produced by C. capsici can disrupt seed tissues. Conidial masses generated from the acervuli can serve as primary inoculums source. The fungus can spread from the seed to the placenta of the fruit, then penetrating the developing ovules or young seed. Infection of seeds can also occur directly from the mother plant. Conidia can remain dormant on the surface of the testa until seed germination. The disease has been observed to occur in three phases, which includes seedlings blight or damping off stage, leaf spot or die back and anthracnose or fruit rot. During die-back the fungus causes necrosis of tender twigs from the tip to backwards<sup>13</sup>. Infection usually begins during flowering stage and flowers dry up. This drying up spreads from the flower stalks to the stem and subsequently causes die-back of the branches and stem. The entire branch or the entire top portion of the plant may withers away and partially affected plants bear fruits which are few and are of low quality. The dead twigs look water soaked to brown, becoming gravish white or straw colored in advanced stage of the disease. A large number of black dots (acervuli) are seen scattered all over the necrotic surfaces of the affected twigs. Sometimes the necrotic areas get separated from the healthy area by a dark brown to black band. Die-back usually appears after the stoppage of rain and when there is prolonged deposition of dew on the plants<sup>9,12</sup>. Conidia from acervuli and microsclerotia get dispersed in water splash and thus spread to the foliage and fruit<sup>1</sup>.

#### MATERIALS AND METHODS Sample collection and storage:

Healthy and diseased ripen chilli fruits were randomly collected from the fields of Guntur region, which is an hot spot region of Andhra Pradesh in chilli production. The collected samples were sun dried and then kept in brown paper bags with proper lable and stored in the refrigerator at 4°c for subsequent studies.

#### Isolation and maintenance of pathogen :

The pathogens were isolated on potato dextrose agar (PDA) medium from the

diseased specimen showing typical symptoms. The infected portion of the fruit was cut in to small bits, surface sterilized in 0.01% sodium hypochlorite solution for 30 seconds, washed in repeated changes of sterile distilled water and plated on to PDA medium. The plates were incubated at temperature  $(28 \pm 2^{\circ}C)$  for five days and were observed for fungal growth<sup>10</sup>. The fungi were purified by single spore isolation technique. The purified isolates were maintained on PDA plates and preserved in refrigerator for subsequent studies.

#### Seed quality test

Collected untreated seed samples were subjected to initial seed health testing by Standard Blotter Method. Seeds of each variety obtained from different locations were tested by employing the standard blotter method with three replicates. Three pieces of blotting paper of 90 mm size were moistened with distilled water and placed in 90 mm sterilized peri plates after draining exess water. Untreated seeds were placed at the rate of 25 seeds per petri plate at equal distance in each petri plate. The plates were incubated at (28±2°c) under alternate cycles of 12 hours UV light and darkness. After 8 days of incubation, the seeds were examined for the associated fungi.

#### Pathogenicity test

The pathogenicity of each isolated fungi was tested by pin-prick (PP) inoculation method under in vitro conditions, on detached semiripe fruits (turning red) of chilli. The spore suspensions of different isolates were obtained by adding 10 ml of sterilized distilled water to 14 day old culture grown on PDA in 30 ml test tube, maintaining the spore concentration of  $10^7$  spores / ml. The chilli fruits collected from the field were surface sterilized with 0.01% of sodium hypochlorite solution for 30 seconds and washed thrice with sterile distilled water to remove any trace of sodium hypochlorite. These chilli fruits were air dried by placing on sterilized blotting paper and subsequently inoculated with spore suspension of isolated fungus. In pin prick (PP) method, the sterilized needle was used and two pricks were given on the fruit, prior to the inoculation through spray

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of spore suspension. Sterile distilled water was used as control instead of the conidial suspension. The inoculated fruits were placed in a plastic container lined with four layers of paper towel moistened with sterile distilled water to produce a humid environment and later sealed with plastic sheet and incubated at  $28\pm2^{0}$ C. The disease development was recorded by measuring lesion length of the diseased portion after 8 days of inoculation. Disease severity was scored on a 0–5 scale.

Table-2	2:	Disease	severity
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Disease grade	Disease reaction
0.0-1.0	Resistant (R)
1.1-2.0	Moderately resistant (R <sup>+</sup> )
2.1-3.0	Moderately susceptible (S')
3.1-4.0	Susceptible (S)
4.1-5.0	Highly susceptible (S <sup>+</sup> )

#### Morphological characterization

Each *C. capsici* isolate was cultured on PDA for eight days. The cultures were grown under dark 12/12 hr light cycles at 28 °C in an incubator. Three replications were performed by taking three pieces of 5 mm diameter plugs from the culture edge of each isolate and placing each of them onto a new PDA plate (9 cm diameter). The new cultures were grown under the same conditions as described above. Morphological characteristics of colony, conidia and appressoria were investigated.

#### Colony growth rate and characteristics

Colony diameter of each isolate was measured at 8 days after inoculation (DAI), and mean colony growth rate was determined. Colony characteristics including surface mycelium, color and mass conidia color was recorded.

#### Conidia shape and size

Conidia suspension was made from 8-day-old colony of each isolate. To investigate its conidia shape and size, 15 conidia were randomly selected from each replicate to measure their length and width under compound microscope with 100x magnifier.

# Appressoria shape and size

Appressoria shape and size were investigated using modified slide culture technique. A culture was prepared by placing a piece of 10x10 mm WA and dropping conidia suspension of each isolate at the edge of the WA. The slide culture was covered with a sterile cover slip and was held in a petri dish to serve as a moisture chamber for 24 hrs at room temperature. 15 randomly chosen appressoria length and width were measured from each replicate.

### **Temperature:**

The effect of temperature on growth of the pathogen was studied. Different temperatures maintained for the growth of pathogen on PDA were 5, 10, 15, 20, 25 and 30°C. Mycelial disc of 8 mm was used to inoculate Petri plates. Three replications were maintained for each treatment. Inoculated plates were kept in incubator and temperature was adjusted to required level. The mycelia growth was recorded on the seventh day after inoculation.

# pH:

The effect of pH on the growth of the pathogen was studied as per the method followed by Kiryu using PDA medium. Different pH levels viz., 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 were used. The pH levels of the medium were adjusted in a digital pH meter using 0.1 N Hydrochloric acid and 0.1 N Sodium hydroxide. The media with different pH levels were sterilized, cooled and poured in the sterilized Petri plates in 20 ml quantities and allowed to solidify. The eight mm disc of pathogen was placed on the centre of the Petri plates. The plates were incubated at room temperature  $(28 \pm 2^{\circ}C)$  for ten days. The diameter of the mycelia growth was recorded. Three replications maintained for each treatment.

#### **Light Intensity:**

The effect of light on the growth of pathogen was studied by exposing the inoculated culture

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to alternate cycles of 24 h light, 24 h dark and 12 h light and 12 h dark in an environment chamber maintained at room temperature ( $28 \pm 2^{\circ}$ C). Mycelial disc of eight mm was used to inoculate Petri plates. Three replications were maintained for each treatment. Inoculated plates were kept in environment chamber and light intensity was adjusted to required level. The mycelia growth was recorded on eight day after inoculation.

# RESULTS

#### Isolation

The isolation of *C. capsici* from the infected fruits (Plate-1) was made as described in material and methods. The pure culture obtained was again sub cultured on potato dextrose agar slants and kept in the refrigerator at  $5^{\circ}$ C for further studies.



Fig. 1: Isolation of pathogen from infected chilli

### Seed quality test

Of twenty chilli seed samples tested only five seeds get germinated under moist conditions provided. Seeds with black colored fungal mycelia were not germinated as a result of infection by pathogen showed in plate-2.



Fig. 2: Chilli seeds on blotters colonized by Colletotrichum capsici. Chilli seeds germinated indicated by arrows

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Isolate Lesion size on chilli (Cm±SE)					
SVUCC1	1.34±0.3				
SVUCC2	0.71±0.5				
SVUCC3 2.79±0.4					

Values have been taken in triplicates

The isolates were grouped into resistant  $(R,R^+)$ and susceptible (S') categories to distinguish the isolates. Isolate SVUCC3 maximum size of lesion with an average length of  $2.79\pm0.4$ cm in fruits and in the remaining isolates it varied from  $0.59\pm0.2$  cm to 2.79 cm. The data on disease grade and disease reaction indicated differential interaction between host and isolates of pathogen. Isolate SVUCC3 was distinct from the remaining isolates as it gave susceptible reaction.

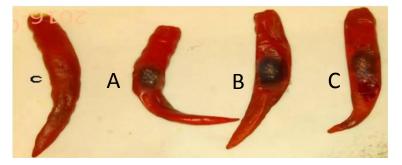


Fig. 3: Pin Prick method. C, control; A, SVUCC1; B, SVUCC2; C, SVUCC3

Table-5b). Reaction of child it duty to Conclot ichild cupsici					
Isolate	Disease grade and Reaction				
SVUCC1	$(\mathbf{R}^{+})$				
SVUCC2	(R)				
SVUCC3	(S')				

Table-3b): Reaction of chilli fruits to Colletotrichum capsici

(R) – Resistant,  $(R^+)$  - Moderately Resistant, (S') – Moderately suceptable

# Morphological characteristics of *C.capsici* isolates

The morphological characteristics of three C.capsici isolates were studied to characterize.

#### Colony growth rate and characteristics

Colony characteristics were classified based on 1) colony growth rate 2) Surface mycelium description 3) Colony colour 4) Conidia mass colour

#### **Colony growth rate**

Growth rate of three *C.capsici* isolates on PDA was ranged from 6.5 to 10.2 mm/day. The three isolates were divided into two groups according to colony growth rate, the growth rate less than 7.5 mm/day and the growth rate of 7.5 mm/day.

#### Surface mycelium

The *C.capsici* isolates could be divided in to 2 groups according to the surface of mycelium: Uniform (U) and Concentric rings (C). Surface mycelium of 2 C.capsici isolates were uniform, and 4 were concentric rings.

#### Colony and Conidia mass colour

Colour colour was divided in to three groups whitish to grey, whitish to brown. Conidia mass colour was divided into two groups 1) dark to grey and 2) brown to orange. Conidia are hyaline and fusiform.

### Appressoria

Clavate forms were commonly observed.

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Table-4: Cultural characteristics of isoates							
Isolate	Setae	Sclerotia	Conidia shape	Colony colour	Appressoria shape	Growth rate mm/day	
SVUCC1	Present	Absent	Falcate, fusiform	Greyish orange	Clavate	8.5	
SVUCC2	Present	Absent	Falcate, fusiform	Whitish Darkbrown	Clavate	6.6	
SVUCC3	Abundant	Absent	Falcate, fusiform	Whitish orange	Clavate	10.2	

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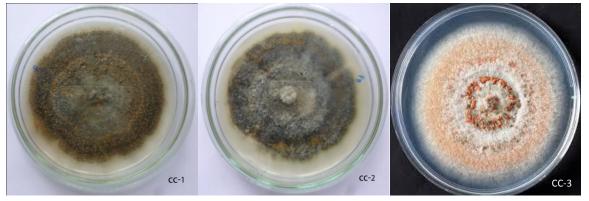


Fig. 4: Different strains of Colletotrichum capsici on PDA

# Effect of pH on the growth of *C. capsici:* Effect of pH on the mycelial growth of different isolates of C. capsici was studied. The results revealed that the maximum mean growth of 78.8±0.4 mm was observed at pH-

6.5 in SVUCC3 isolate followed by SVUCC1

(75.5±0.5 mm) and SVUCC5 (69.5±0.4mm). The lowest mean growth was recorded in SVUCC2 at pH-3 (19.2±0.6mm). The pH below six and above seven was detrimental to the growth of pathogen (Table-5).

Isolate			Colony diameter (mm $\pm$ SE) at different P <sup>H</sup>							
	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5
SVUCC1 24	24.4±0.4	28.4±0.2	36.5±0.4	42.0±0.5	48.2±0.3	52.8±0.8	60.9±0.5	75.5±0.5	65.6±0.2	44.6±0.3
SVUCC2 19	9.2±0.6	26.8±0.6	34.3±0.1	44.2±0.8	46.8±0.5	50.0±0.2	55.0±0.8	69.4±0.2	60.2±0.5	50.4±0.1
SVUCC3 21	21.4±0.5	28.7±0.2	36.9±0.3	41.5±0.4	52.2±0.4	54.6±0.5	58.2±0.5	78.8±0.4	65.4±0.4	51.0±0.2

Table-5: Effect of pH on the mycelia growth of different isolates of C.c.	apsici*
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\*Values are taken in triplicates

# Effect of temperature on the growth of C. capsici

Effect of different temperatures on the mycelial growth of different isolates of C.capsici was studied. The results revealed that a highest mean growth of (72.4±0.2 mm) was observed at 25°C in SVUCC5 which was followed by 30°C (71.3±0.2 mm). Mean growth was found to be the lowest in SVUCC1 at 5°C as it recorded 17.2±0.4 mm (Table-6).

Table-6: Effect of temperature on the growth of different isolates of C. capsici\*

Isolate	Colony diameter (mm ± SE) at different temperature						
Isolate	5°C	10°C	15°C	20°C	25°C	30°C	35°C
SVUCC1	17.2±0.4	30.5±0.4	41.6±0.6	55.6±0.5	70.6±0.3	62.4±0.5	40.2±0.2
SVUCC2	17.2±0.8	28.2±0.2	38.2±0.2	50.2±0.8	70.4±0.5	60.2±0.4	38.0±0.4
SVUCC3	19.4±0.6	31.4±0.3	41.3±0.8	55.9±0.3	66.8±0.2	62.4±0.2	40.6±0.8

Values have been taken in triplicates

# Effect of light intensity on the Growth of *C. capsici*

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Effect of light intensity on the growth of different isolates of *C. capsici* was studied. The results showed that all the isolates grew well when they were exposed with alternate

cycles of 12 h dark and 12 h light with mean growth of 72.6 $\pm$ 0.2 mm followed by 24 h light exposure (48.3 $\pm$ 0.4 mm) and the lowest growth of all isolates was found when exposed to 24 h dark (32.1 $\pm$ 0.2 mm) (Table-7).

Isolate	Growth in (mm ± SE)					
Isolate	24 h light	24 h dark	12 h dark/light			
SVUCC1	54.3±0.6	39.6±0.8	70.6±0.2			
SVUCC2	42.4±0.2	36.2±0.5	69.5±0.8			
SVUCC3	43.1±0.8	34.4±0.6	72.5±0.4			

Table-7: Effect of light intensities on the growth of Colletotrichum capsici\*

Values have been taken in triplicates

#### DISCUSSION

Chilli (Capsicum annum L.) is one of the most important spices, used very widely throughout world. Anthracnose or fruit rot is the most important fungal disease of chilli in which cause chilli yield loss up to 84 per cent. Anthracnose disease is responsible for major economic losses in chilli production worldwide. especially in tropical and subtropical regions<sup>8</sup>. The assessment of plant diseases and their effects on yield normally involves five distinct process: developing a descriptive growth stage key for the particular crop species in question, developing methods to assess the incidence and severity of disease, developing statistically sound methods of sampling crop populations for assessment of the amount of disease, estimating the negative impact of particular levels of the disease on crop yield and quality and evaluating the economic benefit from various methods available for reducing the amount of disease.Hence, detailed studies were carried out on this pathogen on isolation, seed quality and proving pathogenicity. The pathogenicity study showed that the behaviour of C. capsici isolates were homogeneous with regard to disease symptoms. However, variation in virulence or the level of disease (measured quantitatively) within the isolates was observed. The results of seed health tests confirmed the seed borne nature of this feature pathogen, a known in other Colletotrichum species<sup>11</sup>, and its ability to infect internal seed tissues.Morphological characters including colony growth rate,

surface mycelium, colony colours, mass conidia colours, condo size, and aspersoria size was studied in three isolates. Each character showed different phenotypes as follows: 1) colony growth rate: slow, medium, and fast 2) surface mycelium: uniform, concentric rings, sector, and irregular 3) colony colours: whitish to grey and whitish to brown 4) mass conidia colours dark grey to brown and orange. C. capsici colonies appear as a dense white to dark grey mass that is dark brown on the reverse side, with abundant setae<sup>3</sup>.In studies on an optimum range of temperature, pH levels, light requirements for growth of fungus were 25°C to 30°C, pH of 6.5 to 7.0, respectively. Temperature is most important physical, environmental factor in regulating the growth and reproduction of fungi. However, maximum growth and sporulation of fungus was recorded at 25°C temperature and 6.5 pH. At reduced pH, the cell membrane becomes saturated with the hydrogen ions which limit the passage of cations. The reverse could be obtained when medium are alkaline and accumulated hydroxyl ions preventing the passage of essential anions. An alternate cycles of 12 hour daylight under day light tubes and 12 hours darkness supported good growth and sporulation. Light has profound effect on the mycelial growth of pathogen. Exposure of the colony to alternate cycles of light and darkness lead to maximum mycelial growth. The results agrees with findings of where in he found that alternate cycle of 12 h light and 12 h dark yielded maximum growth of fungus when

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compared to the continuous exposure of light or dark $^{5}$ .

#### CONCLUSION

This study revealed three fungal isolates were found associated with diseased chilli fruits, collected from Guntur region. By the morphological studies of the isolates growth rate was evaluated as slow, medium and fast ranging from 6.5 to 10.2 mm/ day. Abiotic factors such as temperature, pH and light intensities are also influenced the growth rate of Colletotrichum capsici.Finally concluded disease symptoms and morphological that studies of the pathogen resembles as colletotrichum capsici and are virulent as evident from pathogenecity test causes substantial reduction in fruit quality parameters of chilli.

**Conflicts of interest**: The authors declare no conflict of interest.

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