# **Epidemiological Investigations to Optimize the Management of Pepper Anthracoose**

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An understanding of anthracnose (Colletotrichum acutatum) infections, including the infection of flowers and latent infection early in the season, is necessary to achieve successful control by means of properly timed spraying with a curative fungicide. In the present study, latent anthracnose infection of chili was investigated under greenhouse and field conditions in 2007-2008. Flowers on greenhouse-grown seedlings were infected and 11% of the young fruits subsequently showed symptoms of anthracnose. Apparently healthy-looking green peppers obtained from unsprayed fields or an organic market also exhibited symptoms of anthracnose after 4 days of incubation under high moisture conditions at 25°C; less than 1% of the peppers were found to be latently infected. To determine the natural timing of infection in the field, 3,200 fruits were wrapped in paper bags and then selectively unwrapped and examined for signs of infection. Field experiments were conducted at Suwon (cvs. Yokkang, Manitta, Olympic) and Asan (cv. Chunhasangsa) in 2008. The 7- to 10-day wrapping periods were July 25-31, July 31-August 7, August 7-15, August 15-24, and August 24-September 3. The 1- to 2month wrapping periods were from July 4, July 31, and August 15 until harvest (Sept. 3). The controls consisted of 1,712 field-grown non-wrapped fruits. The rates of infection on the various cultivars were Yokkang 55%, Manitta 37%, Olympic 55%, and Chunhasangsa 20%. A distinct period in which anthracnose infection suddenly increased could not be identified; however, attempts to guess the approximate timing of field infection showed that 0-39% of the plants had latent infections, while depending on the cultivar, 8-14% of the plants examined in August and 4-13.5% of the those examined during May-July showed symptoms of infection. Delaying fungicide spraying by 24 and 48 h after artificial infection decreased the rates of infection by 10% and 25-30%, respectively. Chemical control of anthracnose based on a forecasting model should be considered starting from the transplanting stage, with spraying within a day after warning and care being taken not to latently infect apparently healthy pepper fruits.

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Anthracnose is one of the most important pathogens of chili pepper, both in Korea (Kim et al., 2008) and worldwide (Pakdeevaraporn et al., 2005). Damage to the plants results in sunken necrotic tissues and concentric rings of acervuli (Manandhar et al., 1995), and fungicide spraying is the most common and practical method to control anthracnose. Since the pathogen frequently affects green or red peppers, chemical spraying is concentrated in July and August, when these fruits are formed. However, pepper anthracnose also can infect young seedlings (Manandhar et al., 1995) as well as small immature fruits (Lewis et al., 2004). Thus, to be effective, fungicides including those that are curative, must be applied before infection is established.

Latent infection by Colletotrichum spp. of apparently healthy fruits has been reported since 1913 (Shear and Wood, 1913). Subtropical fruits, such as orange, bananas, mango, papaya, and tomato, are vulnerable to latent infection by Glomerella spp. (Prusky and Plumbley, 1992; Verhoeff, 1974). Colletotrichum acutatum was reported to latently infect almond (Dieguez-Uribeondo, 2008; Forster and Adaskaverg, 1999), orange (Agostini et al., 1992), and strawberry (Freeman, 1997; King et al., 1997). Although quiescent infections have been suggested to occur in chili (Than et al., 2008), latent anthracnose infection of chili pepper is not common. Since symptoms of infection do not become macroscopically visible until the fruits have ripened (Kim et al., 2008), latent infection can cause major losses, even during dry harvest periods (Witting et al., 1997). Thus, spraying cannot be restricted to the fruit development stage: instead, the longer the period of potential latent infection, the longer the required period of fungicide application.

Susceptibility to latent infection with respect to developmental stage differs, with periods of low- and high-level risks of disease (Luo and Michailides, 2001a). For example, the risk of latent infection by *Monilinia fructicola* at the bloom stage is moderate and reaches a minimum in early June before increasing again in parallel with fruit development and maturity (Luo and Michailides, 2001b). More-

over, the risk of latent infection was shown to be highly dependent on the duration of the moisture period and on the inoculum density (Hong and Hwang, 1998). Thus, in addition to the developmental stage of the host, susceptibility to latent infection involves seasonal patterns and environmental conditions (Emery et al., 2000; Grove, 1998). These different risk conditions for latent infection play an important role in decision-making processes in anthracnose management.

In a previous study, we proposed an anthracnose forecasting model consisting of fungicide spraying in response to an infection event (Ahn et al., 2008). According to this model, successful control of anthracnose is achieved using a curative fungicide that is sprayed during the time that it is known to be effective. However, even a curative fungicide is effective for only 3-8 h after the start of, for example, C. acutatum infection of strawberry, when symptoms of infection are not yet visible (Turechek, 2006). If latent anthracnose infection of the flowers or young fruits of pepper is confirmed, management strategies should be initiated early in the season. In most commercial pepper-growing fields in Korea, fungicide control of anthracnose is concentrated in August, after visible symptoms have been detected in the field. However, the risk of latent infection must be considered (Xu et al., 2000). Some farmers spray periodically, every 7-10 days, to control anthracnose even before the transplant stage, when the flowers have just formed. This approach is not environmentally friendly as it entails 10 or more rounds of spraying in a growing season, underlining the need for alternative control strategies.

The objectives of this study were to identify latent infection by pepper anthracnose of flowers and young fruit in greenhouse-grown plants; quantify latent infection on several commercial cultivars in the field; broadly estimate the timing of anthracnose infection such as May-July, August, and according to observed incidence in the field; and quantify the curative effect of fungicides and determine the maximum allowed spraying interval by delaying the timing of spraying after artificial infection.

#### **Materials and Methods**

Artificial infection of flowers and young fruits in the greenhouse: Four- to 6-week-old pepper plants of the cultivar Chunhajangsa were grown in plastic pots (25 cm diameter) containing a commercial soil preparation. Each pepper plant had 20-30 flowers and 15-25 young fruits that were less than 5 cm long. A suspension of anthracnose spores ( $1 \times 10^5$  spores/ml) was sprayed on both the flowers and the fruits of each plant. The effects of four treatments combining anthracnose infection and a 24-h moisture period after infection were evaluated: infection followed by a moisture period; infection without a subsequent moisture

period; no infection, then moisture; and neither infection nor moisture. For each treatment, 5-7 plants, bearing at least 100 flowers and 50-70 young fruits, were investigated, with each experiment replicated three times. Infected flowers developed healthy fruits, diseased fruits, or dropped without further development. Infected young fruits developed into either healthy or diseased fruits. Anthracnose infection was assessed 28 days after treatment by culturing some of the dropped flowers on potato-dextrose agar (PDA) medium containing 100 mg streptomycin after surface sterilization with 10% sodium hypochlorite. After 7 days of incubation at 25°C, the pathogen was isolated, or not.

Curative effects of fungicides in the greenhouse: Ninety-day-old pepper plants were grown in pots (25 cm diameter) containing a commercial soil preparation. Each plant bore 10 or more fully grown, green pepper fruits. These were infected with a suspension of anthracnose spores and the infected fruits were subsequently sprayed with the fungicides tebuconazole and propineb either immediately or 1 or 2 days after fungal infection. As a positive control, distilled water was sprayed on the infected fruit immediately after infection. The negative control was non-infection. For each treatment, 100 fruits from 10 plants were used. Infection was confirmed by placing all of the infected plants in a humidified chamber for 24 h. The presence of anthracnose on the fruits was assessed until 2 weeks after infection with the pathogen.

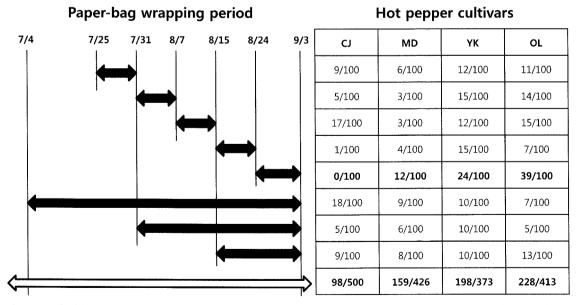
Visible symptoms of latently infected pepper fruits under field condition: To study latent infection in the field, plants were placed in either plastic or paper bags to accelerate visible symptom or to block out natural infection, respectively. In addition, apparently healthy green fruits from a commercial organic farm and from a non-sprayed experimental field were incubated in 4-cm-wide test tubes to visualize symptoms of latent anthracnose infection. An apparently healthy green fruit under observation for infection was placed in a 4-cm-wide test tube with sufficient moisture (2-3 days, 100% room humidity, RH). In addition, a green fruit still attached to the plant was placed in a plastic bag with wet kimwipes for a week and likewise observed for symptoms of latent infection. The experiment was conducted on July 11 and again on July 30, 2007, at Asan and Yesan fields with 100 apparently healthy fruits from the cultivars Manitta (Asan) and Daong (Yesan) obtained from a non-sprayed plot in each field. Anthracnose assessments were conducted twice, immediately after removal of the plastic bag on July 18 and at harvest on September 12.

In addition, healthy-looking green fruits were collected either from non-sprayed experimental plots or a commercial organic farm and observed for signs of latent infection according to the following procedure. A detached fruit was suspended on wires in a test tube 4 cm in diameter and 15 cm high, with a kimwipe soaked with 10-ml sterilized water placed in the bottom to provide moisture. Fruit to be tested was first surface-sterilized with 70% ethanol for 2 min and then left to hang in the tube for 12 days at 25°C. During 2007-2008, 600 fruits from non-sprayed plots and 468 fruits from the organic farm were tested. To confirm whether the test-tube incubation was able to show anthracnose latent infection on fruits, artificial infection was conducted, in which 100% of the infected fruits displayed visible symptoms after 12 days of incubation.

Blocking natural infection of pepper fruits under field condition: The results of the plastic-bag field study carried out in 2007 showed that latent infection of attached fruits was rare, whereas unwrapped fruits showed symptoms typical of a latent infection. Paper-bag treatment blocked natural infection. Assuming that natural anthracnose infection mainly occurs within a certain period of time in the field, such as directly after a heavy rain, the incidence of infection would certainly differ between fruits that were wrapped or unwrapped at that time. In addition, the longer the wrapping period is, the lower the incidence of anthracnose infection will be in the field. This conclusion was confirmed by wrapping 100 apparently healthy fruits in paper bags for various amounts of time. Prior to wrapping, the selected fruits were sprayed with tebuconazole to eliminate existing latent infection. The wrapping periods were July 25-31, July 31-August 7, August 7-15, August 1524, and August 24-September 3. After 7-10 days of wrapping, the paper bag was removed. Red-ripened peppers were wrapped during the last two periods and were harvested on September 3. The effects of a longer wrapping period prior to harvest was also examined, i.e., July 4-September 3, July 31-September 3, and August 4-September 3 (Fig. 1). The 3,200 wrapped fruits were assessed immediately after removal of the paper bag and again at harvest. The pepper fruits were from the Asan and Suwon fields, which had not undergone chemical control in 2008. Cultivars Chunhajangsa (Asan) and Manitta, Yunkkang, and Olympic (Suwon) were used. At least 350 fruits of each cultivar (total of 1,712 fruits), all from the same field and without wrapping, were assessed to determine the natural infection rate.

#### Results

Diseased fruits developed on 11% of the infected flowers (Table 1). Fungus could be re-isolated on 90% of the dropped flowers of plants that had undergone the infection treatment. These results confirmed floral infection by anthracnose on hot peppers. In addition, 91% of the infected young fruits had symptoms of anthracnose. Moisture treatment after artificial infection increased the symptoms on flowers and young fruits by about 50% compared to treatments without moisture. Except for the infection/moisture treatment, 51-54% of the flowers dropped regardless of the treatment; in plants subjected to the infection/moisture treatment, 10% more flowers eventually dropped. This higher rate associated with the latter treatment may have been due



**Fig. 1.** Anthracnose incidence according to wrapping periods at Asan and Suwon fields. Fruits were wrapped in paper bags to prevent their natural infection by anthracnose in the field. Apparently healthy fruits were selected and treated with tebuconazole before wrapping to eliminate preinfection. After August 15, selected pepper fruits were allowed to ripen until they were red and then used in the experiments. CJ: cv. Chunhajangsa, MD: cv. Manitta, YK: cv. Yukkang, and OL: cv. Olympic.

**Table 1.** Anthracnose infection on flowers and young fruits of hot peppers growing in a greenhouse. The four treatments combined infection of 4- to 6-week-old hot pepper cv. Chunhajangsa plants with spore suspensions of *Colletotrichum acutatum* and 24-h periods of moisture post-infection. The infected flowers developed either healthy or diseased fruits or the flowers dropped such that fruits did not develop

Treatment	Flower infection			Young fruit infection	
	Developed to diseased fruits	Dropped flowers	Re-isolation of anthracnose from the dropped flowers	Diseased fruits	
Infection/Wetness	11.0±1.15	63.0±2.52	70/79	91.3±1.67	
Infection/None wetness	$2.7 \pm 0.33$	$53.7 \pm 4.37$	28/71	$49.3 \pm 5.36$	
None infection/Wetness	$0.0\pm0.00$	$52.0 \pm 5.03$	2/79	$0.0 \pm 0.00$	
None infection/None wetness	$0.0 \pm 0.00$	$51.3 \pm 7.51$	0/69	$1.3 \pm 1.33$	

<sup>1.</sup> Numbers with standard errors indicate the percentage of each treatment. Each treatment was carried out in triplicate, with 80-100 flowers or fruits infected each time. 2. Re-isolation numbers denote the number of flowers from which anthracnose fungus was isolated following culture in PDA medium.

to anthracnose; otherwise, the main reason for flower dropping was development of the peppers. Among the noninfected plants, 1.3% of the fruits were nonetheless infected and in 2.5% (2/79), anthracnose was re-isolated from noninfected flowers, most likely due to having been contaminated by touch inside the greenhouse or from the dew chamber used for moisture. These occurrences were regarded as experimental artifacts.

Symptoms of anthracnose infection were noted in 86-90% of the fully grown green fruits (Table 2). Infection-control values for plants treated with the curative fungicide tebuconazole were 81%, 70%, and 39% immediately, 1, and 2 days after infection, respectively, whereas the values obtained with the protective fungicide propineb were 61%, 48%, and 25%, respectively. Curative treatment 1 day after infection resulted in a 10% decrease in the infection-control value and a 35-40% decrease 2 days after infection. Propineb also has some curative effects although it was about 20% less effective than tebuconazole.

Wrapping the infected fruits in plastic bags did not seem to accelerate the appearance of latent infection. After a 7-day moisture period, most of the wrapped fruits showed no symptoms, except those treated on July 11 at Asan (Table 3). However, wrapped fruits exposed to natural infection after moisture treatment were found to be infected severely at harvest, with a 70-90% rate of anthracnose infection at

**Table 2.** Anthracnose incidence and the infection-control values obtained following spraying with either a curative (tebuconazole) or a protectant (propineb) fungicide. The fungicides were sprayed immediately, or 1 or 2 days after artificial infection

Fungicides	Tebucor	nazole	Propineb	
Treatment	Disease incidence	Control value	Disease incidence	Control value
Infection	90.2%	_	86.0%	_
Right after infection	16.7%	81.3%	34.8%	60.9%
1 day after infection	26.5%	70.3%	46.0%	48.4%
2 days after infection	54.0%	39.4%	67.3%	24.5%

**Table 3.** Attached green peppers on plants in an experimental field in 2007 were covered with plastic bags to show symptoms arising from latent anthracnose infection. Of the 100 covered peppers, only a few were apparently healthy without any fungicide spray

Humid period	Fields	Anthracnose investigation		
		Right after treatment	Harvest	
7/11~7/18	Asan	3/100	89/100	
	Yesan	0/100	51/100	
7/30~8/7	Asan	0/100	70/100	
	Yesan	0/100	26/100	

**Table 4.** Diseased fruits arising from latently infected but apparently healthy green peppers from an organic farm and an unsprayed experimental field. Anthracnose symptoms were induced by placing the peppers in a test tube with 100% room humidity for 4 days. The numbers indicate the diseased fruits among the fruits tested each time

Source	Date of experiment	Anthracnose
Commercial organic fruits	2007-05-08	2/9
	2007-05-20	0/9
	2007-05-23	0/30
	2007-06-03	0/30
	2007-06-08	3/30
	2008-03-14	0/60
organic nuits	2008-04-01	0/60
	2008-05-15	0/60
	2008-05-26	0/60
	2008-06-19	0/60
	2008-07-02	0/60
	2007-07-11	0/100
A £ .1.J	2007-07-30	25/100
Asan field	2008-06-30	0/100
	2008-07-21	0/100
Yesan field	2007-07-11	0/100
	2007-07-30	0/100

Asan and a 25-50% rate at Yesan. These values were similar to those of the non-treated control plot (data not shown).

Fruits obtained from commercial organic farms and non-controlled fields and then incubated in test tubes rarely showed evidence of latent infection (Table 4). Among 468 fruits from organic markets, only 5 showed symptoms of anthracnose. Except for a 25% rate of infection of field fruits treated on July 30 at Asan, none of the other fruits showed symptoms of infection.

Field anthracnose infection rates of peppers left unwrapped throughout the season in 2008 at Asan and Suwon were 19.6% for the cultivar Chunhasangsa, 37.3% for cv. Manitta, 53.1% for Yukkang, and 55.2% for Olympic (Fig. 1). Infection was more severe in the cultivars Yukkang and Olympic than in the cultivars Chunhasangsa and Manitta. Of the plants subjected to five 7- to 10-day wrapping periods, 1-17% of cv. Chunhasangsa, 3-6% of cv. Manitta, 12-15% of cv. Yukkang, and 7-15% of cv. Olympic showed symptoms of infection. Thus, with a few exceptions, all of the cultivars had similar levels of infection during the fruit-forming season. Nonetheless, identifying the critical period when the rate of anthracnose infection dropped suddenly was difficult. Depending on the cultivar, 9-18% of fruits wrapped in paper bags for 2 months were infected with anthracnose. The infection rates of fruits wrapped for 7-10 days were never lower than those of fruits wrapped for 40 or 18 days. Except for cv. Chunhasangsa, 12-39% of anthracnose infections involved fruits wrapped between August 24 and September 3.

### Discussion

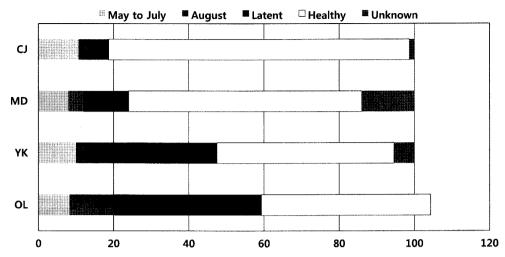
In the plants analyzed in this study, anthracnose infected 11% of control pepper flowers as they developed into young fruits, whereas 90% of young fruits were easily infected by the pathogen under similar but artificial conditions. Flower infection was confirmed by re-isolation of the fungus from the infected dropped flowers. In an earlier study, the rate of citrus flower infection was found to be 10-40% (Tokunaga and Ohira, 1973). Witting et al. (1997) reported that M. fructicola infects flower blossoms, with infection between full bloom and shuck fall causing considerable fruit losses. Since both pepper flowers and young fruits can be infected by anthracnose, control beginning at the flower stage should be considered. Similarly, strawberry plants are susceptible to C. acutatum during all stages of development, including flowers and green and ripe fruits (Freeman, 1997). Charigkapakorn (2000) suggested controlling chili anthracnose beginning at the first bloom stage. In addition, a positive correlation was observed between flower infection and the incidence of diseased fruits following M. fructicola infection of strawberry (Witting, 1997).

The rate of natural infection of the green fruits from organic products and a non-sprayed field was less than 1%. In the experiment involving detached green pepper fruits, less than 1% of the organic fruits were infected. Since natural infections were rare in this study, in future investigations, the sample size should be increased to more than 100 fruits to quantify natural infection in the field. Kwon and Lee (2002) did not detect anthracnose infection in a field of plants covered with plastic film, whereas an infection rate of 12% was found in a parallel open field. In an earlier study of latent infection with Colletotrichum coccodes, less than 1% of the plants were infected but paraquat treatment may have increased latent infection (Cerkauskas, 1988; Nam et al., 2004). Although latent infection is not easily quantified, note that natural infection can occur and that an initially low rate of infection can exponentially increase.

The infection-control values obtained with curative and protective fungicide treatment were 80% and 60%, respectively, when plants were sprayed immediately after infection. Propineb is a protective fungicide that also has slight curative properties, although it is 10-15% less effective than tebuconazole, a curative fungicide. If the timing of infection control was delayed to 1 and 2 days postinfection, the curative effect was 10% and 35-40%, respectively. In carrying out integrated pest management (IPM), the curative fungicide must be properly applied following signs of an anthracnose infection. Pyraclostrobin should be sprayed on strawberry plants within 1 day postinfection with strawberry anthracnose, *C. acutatum* (Turechek et al., 2006), because a 24- or 48-h delay was found to decrease infection-control by as much as 20%.

In a 2007 field experiment to examine latent infection of attached fruits in the field, fruits were wrapped for 7 days with plastic bags to maintain moisture. Only 0-3% of the wrapped fruits showed symptoms of anthracnose acervuli. In a previous experiment (Cerkauskas, 1988), less than 1% of latent infection was ascribed to *C. coccodes*. In the present study, quantifying latent infection either in the attached fruits of plants in plastic bags or in detached fruits placed in test tubes was difficult. However, latent infection was confirmed in the field early in the growing season of 2007. We subsequently determined that wrapping the fruits in plastic bags blocked natural infection rather than accelerate latent infection.

To block the natural infection of anthracnose, fruits were wrapped in paper bags in Asan and Suwon field experiments carried out in 2008. Infection was blocked at various periods during the season to determine the main infection time. If latent infection mainly occurs within a certain period, the incidence of anthracnose at harvest could be drastically reduced by wrapping the fruits during that time. Unfortunately, the critical time for infection could not be



**Fig. 2.** Speculated timing of latent infection based on the results of anthracnose escape experiments in the field during 2008. The proportion of healthy plants was defined with respect to the number of unsprayed pepper plants in each cultivar. Diseased plants were divided according to the time of latent infection: May-July, August, and time unknown. Fruits in the third group suddenly showed symptoms at harvest, after the paper bag was removed. Latent infections in August were infected at 10-34 days after removal of the bag. Plants latently infected in May-July showed symptoms at harvest and were not believed to be latently infected before wrapping.

determined. Furthermore, the infection rates of fruits wrapped for longer periods (1-2 months) were not much lower than those of fruits wrapped for 7-10 days. If the paper-bag wrapping had been able to completely block anthracnose infection and no latent infection of the fruits occurred, then the longer wrapping time should have resulted in less severe anthracnose infection. Note that a drastic increase in anthracnose infection was observed for fruits wrapped between August 24 and September 3, except for cv. Chunhajangsa, which had been infected latently before treatment. Infection of the other cultivars could not be blocked and when infection had occurred was unclear.

Based on the incidence of anthracnose in the 2008 field experiments, we sought to identify the timing of infection. First, cultivars were classified as infected or noninfected according to the appearance of anthracnose symptoms. Anthracnose infections in 2008 were 19.6% for cv. Chunhasangsa, 37.3% for cv. Supermanitta, 53.1% for cv. Yukkang, and 55.2% for cv. Olympic. The infections were further divided into those occurring in May-July, August, and unknown time of infection. However, in the third group, latent infection most likely occurred between August 24 and September 3 based on the average infection rate determined in fruits wrapped during July 25-31, July 31-August 7, August 7-15, and August 15-24. Those fruits were not found to be diseased immediately after unwrapping, but rather at harvest, suggesting that they had been infected in August, after removal of the paper bag. Since the treatments from July 4, July 31, and August 15 until harvest did not allow natural infection, those wrapped fruits were most likely infected during May-July. Figure 2 summarizes the speculated times of anthracnose infection at Asan of ev. Chunhasangsa and at Suwon of cvs. Manitta, Yukkang, and Olympic. The results clearly show that anthracnose infection is not restricted to August, when the fruits are growing. Although whether wrapping the fruits in paper bags completely prevents anthracnose infection is not clear, it did not prevent latent infection of peppers.

In conclusion, anthracnose was able to latently infect flowers and young fruits, with symptoms appearing as the plants developed. Although the rate of natural infection of apparently healthy green fruits in this study was at most 1%, latent infection with pepper anthracnose was also revealed. Based on the severe incidence of anthracnose in the fields in 2008, the timing of infection was most likely evenly distributed from the early to the late growing season. Environmentally friendly management of anthracnose via chemical spraying requires that a curative fungicide be sprayed within 1 day after an infection warning, as determined in an anthracnose forecasting model. In addition, anthracnose control should be considered beginning at the blooming stage, when disease arising from latent infection begins.

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