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Effect of Temperature, Relative Humidity, and Free Water Period on Lesion Development and Acervulus Formation of *Colletotrichum gloeosporioides* on Red Pepper

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고추 탄저병균 *Colletotrichum gloeosporioides*의 병반 및 분생자층 형성에 미치는 온도, 상대습도 및 수분 지속기간의 영향

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ABSTRACT : Effect of temperature, relative humidity (RH) and free water period on anthracnose development by *Colletotrichum gloeosporioides* was examined on red pepper fruits. Mycelial growth of *C. gloeosporioides* was best at 28℃, but greatly retarded at 32℃. Minimum inoculum density required for lesion development varied with isolates, but was mostly above 1.0×10^5 conidia/ml. Optimum temperature for lesion development on fruits was 31℃. Percentage of lesion development was decreased as incubation temperature decreased. Similar trend of temperature response was observed for acervulus formation on the developed lesions. Acervuli were not developed on the lesion as low as at 19℃. Lesion development and acervuli formation tended to increase as increasing RH, but were greatly inhibited at the RH lower than 88%. More than 2 hours of free water period after inoculation were required for lesion development. Lesion development was increased as free water period increased. This study indicates that anthracnose development by *C. gloeosporioides* favors the conditions of high temperature above 28℃, high humidity above 90% RH, and requires free water period longer than 2 hours.

Key words **: Red pepper, *Capsicum annuum*, anthracnose, *Colletotrichum gloeosporioides*, lesion development.**

Anthracnose has been a great problem for red pepper production in Korea. Since the disease occurs usually on mature red fruits around harvesting time, its damage directly causes yield loss. Causal organisms were identified to five species of genus *Colletotrichum* (7). In the previous study (6) some of the authors reported that *C. gloeosporioides* among other species was a predominant species responsible for frequent anthracnose epidemics in Korea. In order to develop efficient control measures of this disease, more understanding on ecology of the pathogen, especially *C. gloeosporioides*, is required.

Most studies (9) on red pepper anthracnose conducted in Korea are concentrated on evaluation of fungicides and varietal resistance to the pathogen. Some researchers (1, 2) studied etiology of the pathogen, but little studies have been conducted on ecology of the pathogen. For instance, there is little information available on how the climatic conditions affect anthracnose development by *C. gloeosporioides*.

This study was conducted to examine the effect of temperature, relative humidity and free water period on anthracnose development on red pepper fruits by *C. gloeosporioides*.

MATERIALS AND METHODS

Isolates. Six isolates of *C. gloeosporioides*, namely 603, 615, 621, 625, 630, 645, were used in this study. Those isolates were obtained from diseased red-pepper fruits in the previous study (6) and have been

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preserved in the Department of Plant Pathology, Agricultural Sciences Institute. Method of isolation and identification of the isolates have been described previously (6).

Measurement of *in vitro* growth. Mycelial growth of three *C. gloeosporioides* isolates, 615, 645, and 621, were examined at 20, 24, 26, 28 and 32℃ on potato sucrose agar (PSA). A 0.5 cm diam. disc of the 7-day-old culture was seeded in the center of petri dish containing PSA with 5 replications. The dishes were placed at the pre-set temperature regimes, and colony diameter was measured 7 days after incubation.

Inoculum preparation and inoculation. Conidial suspension of the 7-day-old PSA culture of each isolate of *C. gloeosporioides* was used as inoculum. Conidia were harvested, and then the concentration was adjusted to 1.2×10^5 conidia/ml, unless otherwise stated. Mature fresh red fruits of cultivar Hanbyul (about 12 cm long, maximum diam. 2 cm) that was grown in the greenhouse were inoculated. The fruits were washed several times in the tap water, surface-disinfected in 1% clorox for 3 min, and put into plastic boxes (25×15×5 cm) moistened with several layers of wet filter paper so as to have 5 to 8 fruits per box. Inoculation was performed by placing one drop (0.02 ml) of the conidial suspension on the surface of center portion of each fruit. Treatment in each experiment was replicated three times. The inoculated fruits were incubated at 28℃ for 7 days for lesion development.

Inoculum density study. For studying effect of inoculum density on the disease development, conidial concentrations of the six isolates of *C. gloeosporioides* were adjusted to 5.0×10^2, 5.0×10^3, 5.0×10^4, 1.0×10^5, and 1.0×10^6 conidia/ml. Red pepper fruits in the moistened plastic boxes were inoculated with each conidial concentration by the method mentioned above. Percentage of lesion development was measured after 7 days incubation at 28℃.

Temperature study. Red pepper fruits were inoculated with the isolate 615 by the method described above. The inoculated fruits were incubated at 19, 22, 25, 28 and 31℃. Lesion development was examined at 48, 72 and 96 hr after inoculation. Formation of acervuli on the developed lesions was examined under the microscope.

Free water period study. Period of free water condition required for successful lesion development was examined with the isolate 621. Condition of the plastic box that was moistened with several layers of wet filter paper and that was covered with cling wrap usually gave 100% relative humidity, and this condition was regarded as free water condition. Free water period was controlled by withdrawing the inoculated red pepper fruits from the plastic box at 2 hr intervals starting the inoculation time until 12 hr after inoculation. The withdrawn fruits were washed five times with sterile distilled water and incubated at 28℃ for 7 days. Percentage of lesion development and acervuli formation on the developed lesion was then examined.

Relative humidity study. Relative humidity in the plastic box mentioned above was adjusted to 67, 72, 82, 88, 92 and 100% following the method described by C.M.I.(3). Red pepper fruits were placed on a metal rack in the plastic boxes so as not to contact directly with acid solution that was used for humidity control. The plastic boxes were incubated at 28℃ for 7 days, and lesion development and acervuli formation were examined.

RESULTS

Effect of temperature on mycelial growth. Vegetative growth of *C. gloeosporioides* isolates tested was greatest at 28℃ and least at 32℃, regardless of isolates (Table 1). The pathogen also grew well at 26℃, but its growth rate was decreased at the lower temperatures.

Effect of inoculum density on lesion development. Inoculum density greatly affected lesion development on fruits (Table 2). All isolates except the isolate 621 failed to develop lesion at the concentrations of below 1.0×10^5 conidia per ml, even though virulence of the isolates differed with isolates, hi-

Table 1. Effect of temperature on mycelial growth of three isolates of *Colletotrichum gloeosporioides* obtained from diseased red pepper fruits

Isolates	Colony diameter (mm)[a]				
	20℃	24℃	26℃	28℃	32℃
615	41.0± 2.2	54.0± 2.1	59.0± 2.1	64.0± 4.3	29.0± 1.4
645	51.8± 1.0	54.0± 1.2	57.0± 1.3	58.0± 0.9	29.0± 1.2
621	47.0± 2.3	50.0± 0.9	57.0± 1.6	58.0± 0.8	30.0± 1.7

[a] Values are means of 5 replications and their standard deviations after 7-day-growth on PSA.

ghest with the isolate 621 and lowest with the isolate 645. Isolate 621 developed lesions at as low as 5.0×10^4 conidia/ml, and number of developed lesions was increased as the inoculum density increased.

Effect of temperature on lesion development and acervulus formation. Lesion development and acervuli formation on the developed lesions were greatest at 31℃, and gradually decreased as incubation temperature decreased (Table 3). Both the development of lesions and acervuli formation on the developed lesions were greatly retarded at the lower temperature as 19℃ and 22℃. No acervuli were formed at 19℃ within 96 hr incubation period. Development of lesions tended to increase as incubation period prolonged. At the higher temperatures such as 28℃ and 31℃, percentage of lesion formation reached 62 to 73% within 4 days of incubation, and more than half of the developed lesions produced acervuli.

Effect of relative humidity on lesion development and acervulus formation. Percentage of lesion development was increased as relative humidity increased (Fig. 1). Only 3 to 6% of the inoculated fruits developed lesions at RH 67 to 82%, but it increased to 26 and 51% at RH 88 and 92%, respectively. About 65% of inoculated fruits developed lesions at saturated humidity condition. Development of acervuli on the developed lesions showed similar patterns in response to temperature. Acervuli for-

Table 2. Influence of inoculum density of *Colletotrichum gloeosporioides* on lesion development on detached red pepper fruits

Isolates	% lesion development[a]				
	5.0×10^{2}[b]	5.0×10^{3}	5.0×10^{4}	1.0×10^{5}	1.0×10^{6}
603	0	0	0	0	73.3± 9.4
615	0	0	0	0	93.3± 11.5
621	0	0	13.3± 9.9	33.3± 10.3	100± 0.0
625	0	0	0	0	33.3± 9.4
630	0	0	0	0	86.6± 9.8
645	0	0	0	0	20.0± 3.0

[a] Values are means of 3 replications and their standard deviations. One drop of conidial suspension was placed on the surface of the center portions of detached red pepper fruits in the moistened plastic box.
[b] Number of conidia per ml of the inoculum suspension.

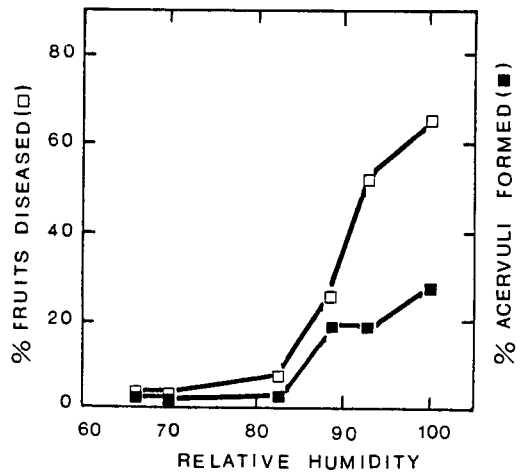

Fig. 1. Effect of relative humidity on lesion development as expressed by % diseased fruits and acervulus formation on detached red pepper fruits inoculated with *Colletotrichum gloeosporioides* and incubated at 28 ℃. Each data point is means of 3 replications that are consisted of 5 fruits per replication.

Table 3. Effect of temperature on lesion development and acervulus formation of *Colletotrichum gloeosporioides* on detached red pepper fruits

Temperature (℃)	No. fruits inoculated	% Lesions formed[a]			% Acervulus developed[b]		
		48 hr	72 hr	96 hr	48 hr	72 hr	96 hr
19	36	0	5.0	16.0e	0	0	0d
22	40	5.0	10.0	30.0d	0	0	20.0c
25	38	10.5	28.9	47.0c	2.6	15.7	42.1b
28	32	18.5	40.6	62.5b	6.2	25.0	53.1a
31	42	33.3	52.4	73.8a	12.9	40.5	59.5a

[a] Red fruits in the moistened plastic box were point-inoculated with a drop of conidial suspension and incubated at 28℃. Values are means of 3 replications. The means followed by the same letter in the same column are not significantly different based on the least significant difference (LSD) test at $p=0.05$.
[b] Formation of acervuli was examined on the developed lesions.

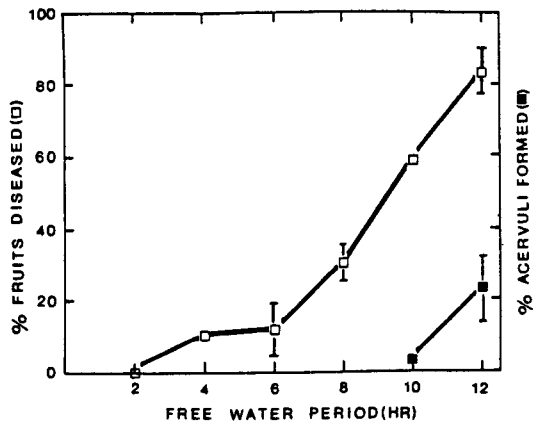

Fig. 2. Effect of free water period on lesion development as expressed by % diseased fruits and acervulus formation on detached red pepper fruits inoculated with *Colletotrichum gloeosporioides* and incubated at 28 ℃. Bracket at each data point represents standard deviation of the means of three replications that are consisted of 9 fruits per replication.

mation was increased as relative humidity increased. Less than 3% of the developed lesions formed acervuli at RH 82% or less, but it increased to 19% to 27% at RH higher than 88%.

Effect of free water period on lesion development and acervulus formation. Free water condition after inoculation significantly affected lesion development by *C. gloeosporioides* (Fig. 2). No lesions were developed within 2 hrs of free water period after inoculation, but about 10 to 12% of the inoculated fruits developed lesions within 4 to 6 hrs of free water period. Percentage of lesion development was rapidly increased at longer than 6 hrs of free water period, and reached above 80% at 12 hrs of free water period. Acervulus formation on the developed lesions was first observed at 10 hrs of free water period and was increased to about 20% at 12 hrs of free water period (Fig. 2).

DISCUSSION

In Korea, rainy season begins usually from late June, and ends in late July or early August. Weather during this period is characterized by hot and humid conditions with frequent rainfalls. About 60% of the annual precipitation in Korea are during this period. Anthracnose of red pepper also begins to occur at this time mainly on mature green and red fruits, and progresses rapidly when hot and humid weather continues. Farmers have often been noticed that anthracnose development varies greatly with year. Epidemic year of anthracnose was reported to have longer rainy period and high temperature, particularly in July and August (2). In consistence to the pattern of seasonal progress in fields, the present study revealed that *C. gloeosporioides*, the major pathogen of red pepper anthracnose, favors high temperature above 28℃, and humid and long free-water period conditions for successful lesion development and subsequent inoculum production. Hot and humid summer days with frequent rains in the rainy period would give favorable conditions for anthracnose development.

The data on the relationship between anthracnose development and some key climatic variables such as temperature and RH in this study could be used for forecasting the disease. Daily index of weather based on temperature, RH, rainfall or free water period could be used to predict anthracnose progress in field, as used in another research (2).

Although there were some variations among isolates, the lowest inoculum concentration required for successful infection appeared to be above 1.0×10^5 conidia per ml. The variations seemed to be mostly from the differences in inherent virulence among isolates, since the most virulent isolate 621 required the least inoculum concentration for lesion development.

The fungal pathogen *C. gloeosporioides* grew less on artificial media when temperature exceeded 30℃. However, lesion development and acervulus formation were found better at over 30℃. It is common to observe that optimum temperature for vegetative growth of a pathogen is not always to be optimum for lesion development, since it involves penetration process, such as conidial germination and appressorium formation. The additional studies on the influence of some key climatic factors in penetration process would be of value for elucidating the ecology of this universal pathogen.

요 약

고추 탄저병균 *C. gloeosporioides*의 병반 및 분생자층 형성에 미치는 온도, 습도, 수분 지속기간의 영향을 고추의 붉은 과실을 가지고 실내에서 조사하였다. 인공배지(PSA)에서의 탄저병균의 균사생장

은 28℃에서 가장 좋았으며 32℃의 고온에서는 급격히 억제되었다. 병반형성에 필요한 접종원의 농도는 균주에 따라 다소 차이는 있으나 대부분의 경우는 1×10^5 conidia/ml 이상이었다. 고추 과실에의 병반 형성은 31℃에서 가장 좋았으며 온도가 낮을수록 병반 형성율도 감소하였다. 병반상의 분생자층 형성율도 이와 비슷한 경향을 보였으며 19℃의 저온에서는 분생자층이 형성되지 않았다. 병반 및 분생자층 형성은 습도가 높을수록 증가하였으나 상대습도 88% 이하에서는 급격히 감소하였다. 병반형성에는 접종 후 2시간 이상의 수분 지속기간이 필요하였으며 그 후 수분 지속기간이 길어질수록 병반 형성율도 증가하였다. 이상의 결과 *C. gloeosporioides*에 의한 고추 탄저병은 28℃ 이상의 고온, RH 90% 이상의 고습도 및 2시간 이상의 수분 지속기간이 병반 형성에 좋은 환경으로 생각된다.

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