Pathogen Physiology, Epidemiology and Varietal Resistance in White Rot of Apple

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조원대 · 김충회 · 김승철 : 사과 흰빛썩음병(白腐病)의 病原菌 生理, 圃場에서의 傳染 및 品種抵抗性

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ABSTRACT Severity of incidence of white rot on apple fruit ranged from 5 to 16% and averaged 9% over major apple growing area in 1981.

An isolate of *Botryosphaeria ribis* obtained from rotted apples developed lesions on leaves, branches and fruits of apple, pear, peach and grape in a series of wound inoculation test. *B. ribis* grew well on both potato sucrose agar and oatmeal agar. The best condition for vegetative growth on these two media was at 25～30°C, pH 4, and 10～15% sucrose content under light illumination. Rot development on fruit was first observed in the orchard at early August when sugar content in fruit reached 9.0%. Thereafter, number of rotted apples increased as sugar content increased. There was no correlation between the pH of juice of fruit and rot incidence. Infection on fruit began to occur as early as mid-June when young fruits were formed and infections were continued until harvest. When apple fruits were collected at 10-day intervals from the orchard beginning from early June and were wound-inoculated with *B. ribis*, rot lesion developed regardless of the stage of fruit growth. Incidence of white rot in the orchard was severe on Golden-delicious and Yukou, intermediate on Aoli, Fugi and Indo, and least on Jonathan and Red-delicious.

INTRODUCTION

Apple white rot caused by an ascomycetous fungus *Botryosphaeria ribis* Gross. & Dug. (syn. *B. dothidea* Ces. & de Not) is becoming a major problem in apple production. The fungus causes soft light-colored rot on fruit and produces canker or wart on stem, branch and twig.

Seriousness of this disease in Korea was previously reported (5). Under no control measure, fruit infection often reaches 100% at harvest. The fungus also attacks wide variety of woody plants of 34 genera and 20 families including pear, peach and grape (6, 8). Some researchers (2,4,7) have reported that apple fruits were not rotted until fruits reached a certain point of maturity. Drake (2) found that primary infection occurred soon after fruits are formed. Sugar content in fruit was considered to be important for white rot incidence (3). The pH of juice of fruits was not correlated with lesion development (3).

The major objective of this research is to determine when fruits become infected and to examine relationships between rot development and maturity of fruit in relation to pH and sugar content. Host range and physiology of *B. ribis* and varietal resistance were also examined.

MATERIALS AND METHODS

Disease survey. Six major apple growing areas; Kyŏngsan, Gunwi, Andong, Yesan, Chungju and Ŭmsŏng were surveyed to examine severity of incidence of white rot during Aug. 22 to Aug. 24 in 1981. Three to 4 orchards were chosen from each area and percentage of rotted fruits was examined on the total of 85 to 150 trees randomly chosen in

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the selected orchards. A total of 125 to 310 fruits was examined at three directions in each tree. Fruits having light colored rot lesion (diam. 1.0 cm or above) or showing irregular bound lesion were considered as infected fruits. Fruit lesions having concentric ring with dark color were not considered as white rot.

Pathogen physiology. Vegetative growth of *B. ribis* was examined on potato sucrose Agar (PSA, potato 500g, sucrose 7g, agar 12g, distilled water 1*l*) and oatmeal agar (OMA, oatmeal 60g, agar 12g, distilled water 1*l*) at 15, 20, 25, 30 and 35°C. An inoculum consisting of a 0.5cm diameter disc of *B. ribis* culture was placed in the centre of 9cm diameter petri dishes containing PSA and OMA. After inoculation, the cultures were incubated at the desirable temperature. Diameter of *B. ribis* colony was measured 72 hrs after inoculation. Each treatment was replicated five times.

In order to examine effect of pH on growth of *B. ribis*, pH of PSA and OMA media were adjusted at 4, 5, 6, 7, 8, and 9 prior to hardening of the media using 1N HCl or 1N NaOH. After adjusting pH, media were plated into 9cm petri dishes and inoculated with *B. ribis* in the same way described above. The culture media were incubated at 25°C. Colony diameter was measured 2 days after inoculation. Each treatment was replicated five times.

To study the effect of sucrose levels on the growth of *B. ribis*, concentration of sugar in the media was prepared to 1, 5, 10, 15, 20 and 30%(w/v). The pH of media was adjusted 5.7 using 1N HCl or 1 N NaOH. A disc of *B. ribis* was introduced into petri dishes containing the media. The culture was incubated at 30°C for 3 days. Colony diameter and degree of aerial mycelia were then recorded. The treatment was replicated five times.

Effect of light on growth of *B. ribis* was examined under the conditions of continuous illumination, continuous darkness and 12 hr-period alteration of the two conditions. Two fluorescent lamps (BLB) were illuminated 30 cm above the culture. Dark treatment was obtained by wrapping the petri dishes with aluminum foil. All cultures of three treatments were in the same incubator that was at 28°C. Three days after incubation, colony size and degree of aerial mycelia were examined. Each treatment was replicated five times.

Pathogenecity test. An isolate (80A1) obtained from an infected apple fruit was used to determine host range of *B. ribis*. The isolate was previously sent to the Common Wealth Mycological Institute (CMI) in England for identification and has been identified as *B. ribis* Gross. and Dug. The isolate was maintained on OMA in the laboratory. Pathogenecity of the isolate was checked at 4 wks intervals on apple fruits. Inoculum was prepared by macerating pycnidia of *B. ribis* harvested from 4 wks old culture with small volume of water on a hole slide glass. Concentration of the inoculum was adjusted to 50~70 conidia in an 100x microscopic field. Pathogenecity of *B. ribis* was tested on pear (Changsiprang), peach (Baekdo) and grape (Neomascut). Leaves, branches and fruits were collected from an orchard in Kumkok farm near Suwon and were separately inoculated with *B. ribis*. Prior to inoculation, wounds were made on leaves and fruits with a bunch of pins and made on branches with a knife. Inoculation was achieved by placing a drop of spore suspension of *B. ribis* on wound sites. In case of branch, inoculated site was wrapped with water-soaked cotton plug. Inoculated leaves, branches and fruits were incubated at 28°C. There were five replications in each test. Two weeks after inoculation, formation of lesion, lesion size and pycnidial formation on lesions were examined.

Field epidemiology. To examine relationship between pH and sugar content in apple fruit and white rot incidence, fruits samples were taken at 10 day intervals from Golden-delicious tree beginning May 20, when young fruits were formed. A total of 30 fruits was sampled at one time to make three replications with 10 apples in each replication. Fruits were washed first with mild detergent solution and then, washed with distilled water. Juice was extracted from these fruit samples using a garlic press. The pH of the extract was determined using pH meter (Fisher, Model 140). Sugar content in the extract was measured by a hand refractometer (Mc Cormick's Fruit Tree Co.,). Progress of white rot incidence was continuously monitored during the sampling period in the Kumkok orchard where fruit samples were taken. A total of ten trees of Golden delicious was randomly chosen and marked with garden stakes. Percentage of rotted fruits was examined at 9～10 day inter vals based on 100 fruits per tree. To examine whether the apple fruits can be infected with artificial wound inoculation, fruits samples were taken over the growth stage of fruits and were inoculated in the laboratory in the same way described in the pathogenecity test section. Development of lesion on the inoculated sites on apple fruits was examined 3 days after incubation at 27°C.

Time of infection in the field condition was examined using the method exposing the apple fruits for only certain period of time in the growing season. Fruits in a Golden-delicious tree were wrapped individually with grease paper bags just after fruits formed. There were eleven different periods for exposing the fruits. A total of thirty wrapped fruits was exposed at one time for 10 days by removing paper bags. After 10 day exposure, the fruits were wrapped again with the paper bags. A series of exposing and wrapping fruits was continued at 10 day intervals beginning May 20 to September 20. Presence or absence of white rot lesion on the fruits was examined on October 10 after removing all the paper bags. Percentage of rotted fruits was then calculated.

Varietal resistance. Degree of infections of white rot on eight apple varieties; Jonathan, Aoli, Red-delicious, Yukou, Fugi, Kukwang, Golden-delicious and Indo was examined by artificial inoculation in the laboratory and/or by field observation. A total of five mature apple fruits of each variety was taken from the Kumkok orchard on mid-September and was inoculated with *B. ribis* in the same way described in the pathogenecity test section. The inoculated fruits were incubated at 25°C for five days. Size of lesion was then examined. Degree of white rot incidence in the orchard was examined based on total of 250 apples on each variety. Five trees were randomly chosen from each variety. Percentage of rotted fruits was determined on September, 14 based on 50 apples per tree.

RESULTS

Disease survey. In 1981, severity of white rot incidence averaged 9.3% over the major growing area (Table 1). Among six major growing areas, Yesan area was most severe and Andong area was relatively less with 4.8% severity. Majority of apple varieties in the surveyed area was Fuji, Golden-delicious, Jonathan and Kukwang.

Vegetative growth. Vegetative growth of *B. ribis* was generally better on oatmeal agar than on potato sucrose agar (Table 2). Optimum temperature for growth was 25 to 30°C. Below or above this range, the rate of vegetative growth was reduced. Aerial mycelia of *B. ribis* were also best at 25 and 30°C.

Mycelial growth was best on pH 4 in both

Table 1. Severity of white rot incidence on apple fruit at six major apple growing areas in 1981

Area surveyed	Number of orchards [a] examined	Total number of [b] trees examined	% infected fruits	Standard deviation
Kyŏngsan	4	124	8.7	2.08
Kunwi	4	150	6.5	2.82
Andong	4	145	4.8	1.58
Yesan	4	120	15.6	6.64
Chungju	3	85	10.8	4.50
Ŭmsŏng	3	108	9.4	1.30

[a] Examined on August 22 to August 24, 1981.
[b] Majority of apple variety in the examined areas was Fuji, Golden-delicious, Jonathan and Kukwang.

media and generally became reduced as pH of media increased (Table 2).

Illumination of fluorescent light over the culture increased growth rate of *B. ribis*. Aerial mycelia were also also abundant under the presence of light. Mycelial linear growth under the condition of light and darkness every 12 hr was intermediate between continuous light and continuous dark treatments.

Table 2. Effects of temperature, pH, photoperiod and sugar content on the vegetative growth of *Botryosphaeria ribis* on potato sucrose agar (PSA) and oatmeal agar (OMA)

Treatment[a]		Growth rate (mm/day) on		Degree of [c] aerial mycelia
		PSA	OMA	
Temperature	15°C	8	12	+
	20	17	20	+
	25	19	26	++
	30	17	27	++
	35	12	19	+
pH	4	20	21	++
	5	14	15	+
	6	14	17	+
	7	18	16	+
	8	10	15	+
	9	10	12	+
Photoperiod	light	25	30	++
	dark	20	23	+
	diurnal	22	26	+
Sugar content	1%	22	23	++
	5	25	23	++
	10	26	27	+
	15	26	27	+
	20	26	27	−
	30	24	25	−

[a] Temperatures used in the pH, photoperiod and sugar content studies were 25, 28 and 30°C, respectively.
[b] Values are averages of five replications.
[c] − (little), + (moderate), ++ (good).

The rate of mycelial linear growth was best at 10 to 20% of sugar content in the both media (Table 2). Above or below this range, growth rate was decreased. As sugar content increased, aerial mycelia of the culture were decreased, particularly at above 20% of sugar content.

Pathogenecity. *B. ribis* isolate (80A1) obtained from a rotted apple fruit also infected leaves, branches and fruits of pear, peach, and grape in the wound inoculation tests (Table 3). Lesions on fruits of apple and peach was usally light yellow in color, irregularly bounded, and quickly spread around the fruits showing typical white rot symptom. Lesions on pear fruits were usually circular and darkish brown in color. Rate of lesion enlargement was somewhat slower compared to those of apple and peach. In case of leaf inoculation, lesions were dark-brown in color and were mostly circular regardless of host species inoculated. In case of branch, small dark brown sunken lesion was developed on inoculated sites. Size of lesion on leaves and branches was much smaller than that on fruits. Pycnidia of *B. ribis* usually formed around lesions. Formation of ascocarp on developed lesions was occasionally observed. Development of lesion on grape was unsuccessful in many cases. Only 10~30% of inoculations resulted

Table 3. Pathogenecity of *B. ribis* on leaves, branches and fruits of apple, pear, peach and grape shown in a series of artificial wound inoculation tests in the laboratory

Host[a] (Common name)	No. of samples developed lesion/total no. of samples inoculated[b]					
	leaf		branch		fruit	
	inoculated	check	inoculated	check	inoculated	check
Malus pumila var. *domestica* (apple)	9/9	0/9	9/9	0/9	15/15	0/15
Pyrus sinensis (pear)	9/9	0/9	8/9	0/9	15/15	0/15
Prunus persica (peach)	9/9	0/9	8/9	0/9	15/15	0/15
Vitis vinifera (grape)	2/9	0/9	3/9	0/9	5/15	0/15

[a] The varieties of apple, pear, peach and grape used in the study were Golden-delicious, Changsiprang, Baekdo and Neomascut, respectively.
[b] Development of lesion on the inoculated sites was examined after 14 days incubation at 28°C.

lesion formation. No lesions developed in control treatment inoculating with sterile water, regardless of host and plant materials inoculated.

Relationship between white rot incidence and pH and sugar content in fruit. The pH of juice from apple fruits was about constant with pH 3.2 to 3.3 throughout the stages of fruit growth (Table 4). Sugar content in fruit increased gradually as fruits became mature. Sugar content was 7.0% in young fruit at early season but was 13.0% at harvest. In the orchard, rotted fruit by *B. ribis* was frist found on August 5 when sugar content of fruits reached 9.0% (Table 4). Thereafter, number of rotted apple increased rapidly as sugar content increased. At mid-September, almost all the fruits in the field were rotted. The sugar content in fruits was 13% at this time. Significant positive correlation was found between white rot incidence and % sugar content in fruits. Correlation coefficient between these two factors was 0.9861 and was statistically significant at 99% level.

Table 4. Relationship between white rot incidence and pH and sugar content of juices from apple fruits of Golden-delicious sampled at intervals over the growing season in Suwon in 1981

Time of sampling	pH of juice[a]	Sugar content (%) in apple[b]	% rotted apple in field[c]
June 4	3.20	7.0	0
15	3.25	7.0	0
24	3.25	7.8	0
July 5	3.30	7.3	0
16	3.20	8.1	0
25	3.20	8.5	0
August 5	3.20	9.0	1.9
14	3.20	9.0	11.8
25	3.20	11.0	37.5
September 6	3.20	12.5	84.5
16	3.20	13.0	97.7

[a] Values are average of 3 replications
[b] Values are average of 3 replications
[c] Percentage of rotted fruits was examined based on total of 450 fruits in five trees of Golden-delicious.

Table 5. Percentage of fruit infections occurred at different stage of fruit growth when determined by sequentially exposing fruits for certain period of time during the growing season in Suwon in 1981

Period of fruit exposure	No. fruits rotted/no. fruit exposed (% infection)[a]		No. fruits developed lesion/no. fruits inoculated[b]
Jun. 1～Jun. 10	0/30	(0)	3/3
Jun. 11～Jun. 20	2/30	(6.1)	3/3
Jun. 21～Jun. 30	3/19	(15.8)	3/3
Jul. 1～Jul. 10	4/20	(20.0)	15/15
Jul. 11～Jul. 20	3/25	(12.0)	15/15
Jul. 21.～Jul. 31	4/27	(14.8)	15/15
Aug. 1～Aug. 20	4/30	(13.3)	15/15
Aug. 11～Aug. 20	4/27	(11.1)	15/15
Aug. 21～Aug. 31	1/23	(4.3)	15/15
Sep. 1～Sep. 10	1/24	(4.2)	15/15
Sep. 11～Sep. 20	0/26	(0)	15/15

[a] Rot incidence was examined at September 23, 1981.
[b] The result came from arfiticial wound inoculation with fruits sampled from the same tree used in fruit exposure study.

Table 6. Severities of white rot on eight different apple varieties determined at artificial wound inoculation with sampled fruits or determined by field observations in the orchard in Suwon in 1981

Variety	Lesion length [a] (mm) on fruits		% rotted fruits in the field
	inoculated	check	
Jonathan	23	0	8.0
Aoli	14	0	18.0
Red-delicious	13	0	12.0
Yukou	19	0	32.3
Fuji	—	—	22.3
Kukwang	22	0	—
Golden-delicious	21	0	29.0
Indo	—	—	21.3

[a] Determined 5 days after incubation at 25°C. Values are average of 5 replications. In case of check, stetile distilled water was used in the inoculation.
[b] Based on a total of 250 fruits of 5 trees in each variety with 5 replications.

Time of fruit infection. Fruits were infected with *B. ribis* as early as mid-June, just after fruits formed(Table 5). Thereafter, frequency of infections rapidly increased to about two folds, continued at this rate until mid-August, and then decreased at the end of season. In 1981 season, highest infection took place at first part of July. When fruits were sampled over the growth periods and wound-inoculated with *B. ribis* in the laboratory, all inoculated fruits developed lesion regardless of the growth stage of fruit sampled (Table 5).

Varietal resistance. In field observation, Yukou and Golden-delicious were most seriously infected with 32 and 29% of fruit infections, respectively. Jonathan and Red-delicious were least infected with 8 and 12%, respectively. Aoli, and Fuji were intermediate with 18~22% infections. In the artificial wound-inoculations, lesion enlargement on inoculated sites was less with Aoli and Red-delicious.

DISCUSSION

This study showed that fruit infection occurred as early as mid-June, just after fruits formed. Despite this early infections, rot symptom did not develop until early August. Time difference between initial infection and first symptom appearance in field was about one and half month. During this period, sugar content of fruit was increased from 7 to 9% along with an increase in fruit size. The pH of juice from fruits was not appreciably changed during this period. Although fruit infections continued to occur at similar rate regardless of the stage of fruit maturity, number of rotted apple in field was increased dramatically as the level of sugar content reached above 9%. This indicates that sugar content in fruit might be important for symptom development of *B. ribis* after infection took place. Calculated correlation between rot incidence and sugar content of fruit in this study was as high as 0.98 (r). Delay of symptom development after initial infection was previously reported by several researchers (2, 4, 7). In those studies, sugar content in apple fruit was proposed as one of the reason for delayed symptom development. The result of this study supports that there might be a threshold value of sugar accumulation in apple fruit for development of rotting lesions. This value was around 9% in our study. The pH of juice in apple fruit appeared not to be a major factor for rotting symptom development, since no correlation was found between rot incidence and pH of juice of fruit. Although rotting symptom on fruit did not develop in the field until fruit became mature, fruit samples taken at various developmental stages from the same tree developed rotting lesion in the laboratory in the artificial wound-inoculation. This suggests that wound on fruit is also important for development of white rot. Many studies (1, 8, 9) have shown that *B. ribis* is a wound pathogen. Some researcher (9) found that wound is prerequisite for infection of *B. ribis*. The fact that artificial wound inoculation produced lesions on various stages of fruit growth where rotting lesions did not occur naturally suggests that wound agents such as insects play an important role in the epidemiology of white rot.

In vitro study, maximum growth of *B. ribis* occurred at 10~20% sucrose level. This is within a range of sugar concentration in fruits where severe rot developed in the field. Mycelial growth of *B. ribis* was also best at pH 4 which is close to the pH of juice from apple fruit.

The present study showed that there is a definite point, such as 9% sugar content, in the development of rotting symptom on fruits although infections can occur as early as one and half month in advance. This information could be used in control program of white rot. Our study indicated that control program for *B. ribis* should begin as early as just after fruit formed regardless of symptom appearance. Fungicide application after rotting symptom development on fruits might be too late to control white rot. Control measures against wound agenst such as insects may be necessary to improve efficiency of the control program.

적 요

1981년도 사과 흰빛 썩음병(白腐病)의 발생은 지역에 따라 5%에서 16%에 달하였으며 평균발병 과율은 9%였다.

사과에서 분리한 병원균을 사과, 배, 복숭아, 포도의 잎, 가지, 과실에 상처 접종하였을때 접종 부위에 병반이 형성되었다.

병원균의 영양 생장은 감자설탕 한천배지와 귀리즙 한천배지에서 모두 좋았으며 특히 온도 25~30°C, pH 4, 당도 10~15%의 광조사하에서 가장 양호하였다.

사과의 이병과실은 8월 초순에 포장에서 처음 관찰되었으며 이때의 과실 당도는 9.0%였다. 그 이후 과실의 당도가 증가함에 따라 이병과실의 수도 급격히 증가하였다. 과실의 이병정도와 과일즙내 산도와는 아무런 상관이 없었다.

사과 과실의 이병시기를 과실을 일정기간동안 노출하는 방법으로 포장에서 조사한 결과, 과실의 이병은 과실이 형성되기 시작한 6월초순부터 가능하였다.

과실의 숙기별로 포장에서 과실을 채집하여 실내 유상접종 하였을때 접종 과실은 과실의 숙기와 상관없이 모두 병반을 형성하였다.

포장에서 병 발생 정도를 품종별로 조사한 결과, 골덴데리셔스, 육오가 가장 많이 이병되었고 조나단, 레드데리셔스가 가장 적었으며 아오리, 후지, 인도는 중간이었다.

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