한국식물보호학회지 제18권 제1호
Korean J. Pl. Prot. 18(1) : 35~41(1979)

Ginseng anthracnose in Korea

Factors affecting primary inoculum, growth of the pathogen, disease development and control

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人蔘·炭疽病에 關한 硏究

傳染源, 病原菌의 生態, 發病要因 및 防除

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ABSTRACT

Four to 17% of the seeds of ginseng (*Panax ginseng* Meyer) collected from seemingly healthy plants carried *Colletotrichum panacicola* Nakata et Takimoto whereas the seeds from the plants with anthracnose sympotoms carried 42% of the same fungus. Prevalent organisms isolated other than *C. panacicola* from seeds of both kinds of plants were *Fusarium*, *Alternaria*, *Phoma*, *Trichoderma* and others, and in that order on acidified potato sucrose agar. *C. panacicola* also was isolated from 18 months old herbarium specimens. The fungus in the infected tissues also survived during the Korean winter months either on the soil surface or in the soil at 10 and 30 cm in depth.

When conidial suspensions of *C. panacicola* were inoculated on detached ginseng leaves, anthracnose symptoms occurred from 25 to 35°C. No symptoms occurred at temperatures below 17°C. Direct sunlight increased significantly the number of anthracnose lesions over those obtained in leaves inoculated in darkness or in 400 lux of fluorescent light. The lesions decreased as age of the leaves increased or as the number of conidia applied decreased. Optimum temperature for mycelial growth and conidial formation of *C. panacicola* was 25°C. Optimum pH for the mycelial growth was at pH 2.8 ~4.6 while the most conidial formation occurred at pH 5.2~5.8.

When fungicides were applied in the field to ginseng plants with a conidial suspension of *C. panacicola*, the most effective control of the anthracnose disease was by spraying with difolatan, and followed by maneb, zineb, captan and phaltan; Bordeaux mixture and ferbam were significantly less effective but significantly better than the inoculated control plants.

INTRODUCTION

The demand for ginseng (*Panax ginseng* Meyer) and its products at home and abroad has been increased due to the efficacy of the root that has proved scientifically (7). It is necessary to increase in cropping land or yield in order to fufill demand. However, it is very difficult to expand the cropping area in Korea because the plant can not be raised continuously on same land for a long time. Therefore the

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most efficient way to increase the yield of the root is by controlling the major disease problems of ginseng.

Among the ginseng root diseases, damping-off and root rot complex are the most serious problems. The aerial shoots also are attacked by several pathoginic fungi of which the anthracnose fungus, *Colletotrichum panacicola* Nakata et Takimoto, is one of the most important ones since it causes seedling blight in the nursery bed and leaf spot in the permanent beds, resulting in early defoliation of the plants and retarding the growth of roots.

Anthracnose of ginseng was first reported by Nakata and Takimoto (10) in 1922 describing the symptoms, physiology and ecology of the pathogen and control measures. The disease was named leaf blight at that time to avoid the confusion with leaf anthracnose which had been reported by Whetzel et al (13). Few reports on ginseng anthracnose are available except that the disease was found in ginseng seeds or on plants obtained from north Korea by Russian scientists (3, 7) after the World War II.

According to Lee et al. (8), 50% of 3 to 5 year-old plants in Buyeo, Korea were defoliated in the early part of August. Chung (5) also reported that 50% of plants older than 3 years in Kwachon and Yangchi, Korea were infected in early August.

To establish effective control measures of a disease it is urgent that both the basic and the applied fields of the disease association should be investigated. Therefore, this study was conducted to investigate the physiology, epidemiology and chemical control of the fungus. An abstract of this study was reported earlier (5, 6). The authors wish to express special thanks to a former graduated student, Miss Ha-ja, Choi for her help in this study and to Dr. Seung-Hwan Ohh and Dr. Oscar Calbert for preparation of the manuscript.

Materials and Methods

The fungus used in this study was isolated from an infected leaf of the ginseng plants obtained from Yangchi, Korea and was maintained on potato-sucrose agar (PSA). Unless otherwise specified, all laboratory trials were conducted as completely randomized experiments and field plots were grown in a randomized block design with three replicates for each treatment. Results were analyzed statistically and the means were compared using Duncan's multiple range tests (11).

A. Primary sources of inoculum isolated from seed

Seeds harvested in July 1969 were used to determine the mycoflora. The seeds were stratified and obtained from the Kwacheon Gingeng Experiment Station. Seeds from Yangchi, Korea were harvested from both seemingly healthy plants and anthracnose diseased plants. All seeds were treated in 1% NaOCl for 3-5minutes and plated on PSA and incubated for 7-10 days at room temperature. The pH of the medium was adjusted to pH 4.0 with lactic acid before use. Frequency and kind of fungi that grew from the seeds were determined and recorded.

B. Factors affecting disease development

Effects of age of ginseng leaves, and light and temperature on disease development were investigated by using detached leaflets from three year old plants grown at Yangchi. The leaflets were washed with sterile water and placed on water saturated filter paper in petri plates for each experiment. The leaflets were inoculated with a conidial suspension (5×10^3 conidia /m*l*) and incubated for 7 days at room temperature and the percentage of infected leaf area was determined for each treatment.

C. Factors affecting growth and sporulation of the pathogen

Mycelial growth was investigated using 100m*l* aliquots of potato sucrose broth (PSB) in 250m*l* Erlenmeyer flask inoculated with a conidial suspension (8×10^5 conidia/m*l*) and incubated for 8 days. Mycelial weights were determined by drying the mycelial mat in an oven at 40°C for 48 hours. For the studies of temperature relationships PSB was used and for the studies of pH, PSA petri plates were flood-seeded with a conidial suspension. The number of conidia/mm^2 were counted with the help of a hemocytometer.

D. Chemical control of anthracnose of ginseng

Seven different chemicals listed (Table 1) were used and each were sprayed three times on 2 year-old ginseng plants every other week beginning from May 31, 1969. The plants were inoculated with co-

nidial suspensions (25×10^3 conidia/ml) on July 26 and the percentages of infected leaf areas were determined on August 20. Each treatment consisted of two rows with 4 plants in each row and was replicated three times. Detailed explanations will be given in the results where it is felt to be necessary.

Table 1. Chemicals and the dilutions used as sprays to control ginseng anthracnose.

Fungicides	Active ingredient	Dilution
Bordeaux mixture	$CuSO_4 \cdot {}_xCu(OH)^2\ {}_yCa(OH)_{2z}H_2O$	10-10-150
Zineb 75%	Zinc ehtylenebis (dithiocarbamate)	1 : 500
Maneb 70%	Manganese ethylenebis(dithiocarbamate)	1 : 500
Difolatan 80%	N-Tetrachloroethylthio-4-Cyclohexene-1,2-dicarboximide	1 : 900
Captan 50%	N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide	1 : 500
Phaltan 50%	N-(trichloromethythio) phtalimide	1 : 500
Ferbam 76%	Ferric dimethyl dithiocarbamate	1 : 800

Results

Isolation of primary inoculum from seed

The number and kind of fungi isolated from seeds collected from diseased and non-diseased plants were variable and diversified(Table 2). Fourty-two percent of seeds obvioushy infected plants carried the anthracnose fungus but the seed from seemingly healthy plants had only 4% to 17%. *Fusarium* sp. also were isolated in high frequency but from the healthy seeds the percentage varied widely (8-51%), again by source and treatment. *Fusarium* sp. was the species most frequently isolated from the stratified seed. *Phoma* and *Alternaria* also were present in relatively high counts. Thirty-one percent of non-stratified seeds from Yongin carried no microorganisms. Except for *C. panacicola*, species of *Fusarium*, *Alternaria* and *Phoma* that were isolated were not identified even though there were possibilities of their being pathogenic to ginseng plants (Table 2).

Table 2. Percentage of organisms associated with ginseng seeds obtained either from seemingly healthy or *Colletotrichum panacicola* infected plants

Organism isolated	Seeds from seemingly healthy plants		Seeds from anthracnose infected plants at Yongin[b]
	Kwachon[a]	Yongin[b]	
Colletotrichum	(%)	(%)	(%)
panacicola	4	17	42
Fusarium	51	8	40
Alternaria	11	2	5
Phoma	16	—	—
Trichoderma	8	2	—
Mucor	trace	8	3
Geotrichum	—	11	1
Yeast	—	16	2
Stemphyllium	trace	—	—
Aspergillus	trace	—	—
Unknown	6	5	2
Organism free seed	4	31	5

a. Based on 500 stratified seeds
b. Based on 500 non-stratified seeds

Survival of *C. panacicola*

Infected ginseng leaflets were put into a vinyl bag containing 20 grams of field soil with a moisture content of 30% (w/w). The bags were put on the open surface, or in soil at 10 and 30 cm in depth on December 5. Survival of the fungus was determined by plating the leaflets on PSA after 3 months exposure. Herbarium specimens of leaflets infected with *C. panacicola* were examined for survival of the fungus.

The anthracnose fungus was recovered in each treatment varying between 4 to 6% among the treatments. Twenty tissue particles were plated from each treatment. The fungus also can survive more than 14 and 18 months on a culture medium and on

diseased leaves, respectively.

Environmental factors affecting anthracnose development

1. Temperature

Three year old detached ginseng leaflets were placed in petri plates and inoculated with a conidial suspension. The plates thus treated were incubated at 8, 13, 17, 25, and 30°C. Of these temperature treatments, only the leaves kept at 25 and 30°C became infected and they showed 27 and 35% of the leaf area to be diseased, respectively. There was no disease development in leaflets held at temperatures below 17°C.

2. Light

Leaflets were inoculated in mid August and kept in the dark petri plates or were illuminated with daylight fluorescent lamps with 400, 1200 lux light intensity or exposed to direct sunlight where the temperature was similar to that of laboratory conditions.

The precentage of infected leaf area was determined to be 4 to 5% when the leaflets were kept in the dark or at 400 lux, and was 26% and 75% when exposed to 1200 lux or to direct sunlight, respectively (Table 3).

The differences were significantly different at the 5% level among low and high light intensity treatments.

Table 3. Effect of light on the development of anthracnose disease of detached ginseng leaflets[a]

Treatment	Percentage of infected leaf area
Dark	4 x[b]
Fluorescent light 400 lux	5 x
Fluorescent light 1200 lux	26 y
Direct sunlight	75 z

a. Average of 3 leaflets with 3 replicates
b. Different letters following each datum indicate significant differences at the 5% level by Duncan's multiple range test.

3. Effects of age of plants and inoculum concentration

Leaflets developing anthracnose symptoms varied widely in percent of the leaf area infected and rate of development from two, three, four and five year old ginseng plants that were placed in petri plates ad ninoculaetd with spore suspensions containing 10^4, 5×10^4 and 10^5 conidia/ml (Fig.1). As shown in Fig. 1, the younger leaflets were the most susceptible to the fungus and the results were significantly different at 1% level. The rate of disease development was highest in the leaflets from three year old plants. There were no interaction between the inoculum concentrations and the ages of the plants.

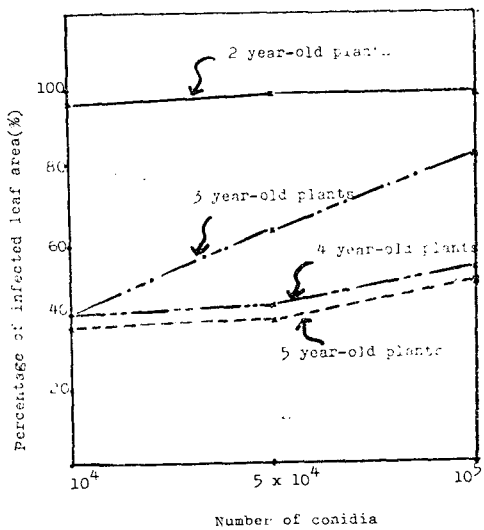

Fig. 1. Effects of age of plants and inoculum concentration on disease development

Factors affecting growth and sporulation of the pathogen

1. Effect of temperature

Potato sucrose broth (PSB) was adjusted to pH4.0 with lactic acid and used for the experiment. For the spore counts, the mycelial mat was mixed in a

Table 4. Effect of temperature on mycelial growth and sporulation of *Colletotrichum panacicola*[a]

Temperature(°C)	Dry weight(mg)	Number of conidia 10^5/ml
10	137[b] wx	0[b]
15	228 y	11 x
20	266 yz	48 y
25	320 z	123 z
30	193 xy	4 x
35	76 w	0

a : Based on the averege of three replicates
b : Different letters following each datum indicate significant difference at 5% level by Duncan's multiple range test.

Waring Blendor after adding 30m*l* water to each flask. The fungus did not grow at all at 5°C, and very little growth was noticeable at 35°C (Table 4). The most abundant growth and sporulation of the fungus was at 25°C. No sporulation was detected at 10 and 35°C. The cardinal temperatures for conidial production were observed to be 15, 25 and 30°C, respectively (Table 4). These results were significantly different at 5% level.

2. Effect of pH

Mycelial dry weight were determined by growing the fungus in 50 m*l* of PSB in 250 m*l* Erlenmyer flasks and 50m*l* of citrate phosphate buffer solution which were sterilized separately. Hydrogeni on concentration of the medium was adjusted to 2.8 to 8.0 as shown Table 5.

The optimal pH for mycelial growth of the fungus was pH 2.8 to 4.6; however, the best growth obtained at pH 3.4 (Table 5). The growth was sharply decreased at pH 5.2 and only varied slightly (not significantly) from pH 5.8 to pH 8.0.

Conidial production was determined using PSA with citrate phosphate buffer incorporated into the 9 cm diameter petri dishes and incubated at 25±°C and illuminated for4 days. Best sporulation occurred at pH 5.2 to 5.8 and decreased sharply below and above these concentrations (Table 5). Below pH 3.4, only mycelial growth was abundant and there were no conidia produced at pH 2.8. Above pH 4.0, the culture showed a salmon color which is the typical color characteristic of this anthracnose fungus when producing abundant conidia. The results obtained were significantly different at 5% level (Table 5).

Table 5. Effect of pH on mycelial growth and condial production of *C. panacicola*

pH	Mycelial dry weight[a] (mg)	Number or conidia[b] (10^5/mm^2)
2.8	166 x[c]	0
3.4	173 x	7 w
4.0	154 x	22 x
4.6	130 x	24 x
5.2	70 y	56 y
5.8	7 z	57 y
6.4	19 z	35 z
7.0	40 z	33 z
7.6	39 z	33 z
8.0	30 z	32 z

a : Based on the average of three replicates
b : Based on the average of three replicates and each replicate consisted of three 5mm^2 mycelial disks
c : Different letters following each datum indicate significant difference at 5% level by Duncan's multiple range test.

Chemical control of anthracnose of ginseng

The average number of leaf spots per plant in the control (unsprayed) was 20 and almost of all the aerial shoots of the plant were dead (Table 6). Bordeaux mixture, which has been used widely by ginseng growers, showed a similar degree of control as ferbam in controlling the anthracnse of ginseng. However, it was significantly less effective than difolatan, maneb, zineb and phaltan. The plants showed small white of brown spots on the leaves when treated with chamicals other than the Bardeaux mixture. The results were significantly different at 1% level (Table 6).

Table 6. Effect of various fungicides in control of anthracnose of ginseng

Fungicides		Dilution	Number of lesions per plant[a]
Ferbam	76%	1 : 800	5.0 y[b]
Bordeaux Mixture		10-10-150	4.8 y
Phaltan	50%	1 : 500	2.0 z
Captan	50%	1 : 500	1.5 z
Zineb	50%	1 : 500	1.0 z
Maneb	70%	1 : 500	0.9 z
Difolatan	80%	1 : 900	0.8 z
Control (unsprayed)			20.0 x

a : Based on the average of 3 replicates and each replicate consisted of 2 rows of 4 plants per row.
b : Different letters following each datum indicate significant difference at the 5% level by Duncan's multiple range test.

Discussion

The anthracnose of ginseng, as described before, was first reported in 1922 describing symptoms in detail and named the fungus(10). In order to avoid confusion between the Pestalozzia leaf anthracnose and the *Colletotrichum* anthracnose, the latter was called leaf blight at the time. However, the name has also been changed in USA (1) and there is no

reason to call it as leaf blight. Therefore, it is reasonable to call it anthracnose disease by adopting general practice in naming plant disease since all the diseases caused by *Colletotrichum* belonging to Melanconiaceae have been called anthracnose.

It is a well known fact that knowledge of etiology of a given pathogen is necessary to establish effective control measures. Several seed-borne fungi incited diseases on ginseng, including anthracnose, have been reported (2,3,7,12). However, it is very surprising that 4 to 17% of seeds collected from healthy plants as well as obviously diseased plants carried anthracnose fungus. Therefore, it is necessary to be cautious when harvesting seeds and conduct laboratory tests to see if the seeds are free of organisms or not. To control such seed-borne fungal diseases, several seed treatments were tried as suggested by Bunkina et al. (2,3,12). However, several chemicals were phytotoxic and the percentage of germination was not always that much better than the untreated control (4), therefore there is a need for further sutdy.

Nakata et al. (10) reported that the conidia of the anthracnose fungus could not survive more than 7 months at room temperature. The results obtained from this study, however, reveal that the fungus can survive more than 14 and 18 months on a culture medium, and on diseased leaves, respectively. This is the first report that the fungus, *C. panacicola* can overwinter successfully on infected tissues on or in the soil.

It is generally believed that ginseng plants become older they become more vulnerable to the anthracnose fungus in the field. However, the results obtained from these experiments showed quite the opposite to be true. Because the ginseng plant is a perennial, more and more primary inoculum of *C. panacicola* is accumulated year after year in the field. Consequently more disease occurred on the older plants because there is an abundance of inoculum in close proximity to the growing plants.

Ginseng plants are favored by dense, shade and are very sensitive to direct sunlight. The plants exposed to direct sunlight, both in the seed bed and in the permanent bed, are more susceptible to the anthracnose fungus attack. In this experiment, the percentage of infected leaf areas in direct sunlight was significantly higher than that of in the dark. This phenomenon is rarely true in many plant diseases. Therefore, the mechanisms of sunlight on disease development may be determined only by studying the interaction of the pathogen and the host.

It has been known that Bordeaux mixture was very effective in controlling the anthracnose fungus by some ginseng growers. However., the results obtained here showed that there are more effective chemicals available, even though some caused very limited phytotoxicity. The phytotoxicity problem will be solved by controlling the dilution of chemicals, time and number of applications.

Generally speaking, it is believed that the favorable conditions for the vegetative growth of a fungus will also be good for growth of the reproductive state. However, the optimum temperature for the mycelial growth of *C. panacicola* found to be 20 to 25°C, while for the conidial state was 25°C. Conidial production was sharply decreased at 30°C. Furthermore, no conidial production was observed at 35°C in this experiment. This appears to be proof that no conidial production of the fungus could be obtained in a laboratory during the hot summer period. In the studies on the relationship between conidial production and the pH of the medium, it was observed that no or few conidial production occurred on an extremely acidified medium to which had been added certain acids which in the past has been a widely practiced procedure in laboratories to avoid bacterial contamination.

In conclusion, the results obtained for overwintering and the effects of several environmental factors affecting both the growth and sporulation of *C. panacicola* and chemical control of the anthracnose disease, all show the need for further extensive studies that need to be done in the future for best possible control of the anthracnose disease of ginseng.

摘　要

人蔘炭疽病에 感染된 포기의 種子에는 42%, 겉으로 健全한 포기의 種子에도 4~17%의 炭疽病菌을 保有하고 있었으며, *Fusarium*, *Alternaria*, *Phoma*, *Trichoderma* 等도 많이 分離되었다. 炭疽病菌은 室內에 있

는 잎의 組織에서 18개월간 生存하였으며, 屋外의 露地, 地下 10, 30cm에서도 越多할 수 있었다.

人蔘잎에 炭疽病菌의 分生子를 接種하였을 때에 25-35°C에서만 發病하였고 17°C以下에서는 全혀 病斑이 形成되지 않았다. 暗黑區, 400룩스區에서 炭疽病斑은 微少하였고, 1200룩스區에서 中程度, 直射光線에서는 極甚하였다. 그리고 年齡이 어린 잎일수록, 分生子數가 많을수록 病斑面積率은 높았다. 病原菌의 菌絲生長 및 分生子形成의 最適溫度는 25°C였고, 菌絲生長의 最適 pH는 2.8-4.2였으며 分生子形成은 5.2—5.8이었다.

圃場에서 殺菌劑와 人蔘·炭疽病菌의 分生子를 함께 3年生植物에 處理했을 때 防除效果가 큰 것은 difolatan 그리고 maneb, zineb, captan, phaltan의 順位였으며 ferbam과 보르도液은 若干 낮았다.

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