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Effect of Plant Age on Infection of Soybean by *Calonectria ilicicola*

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*Calonectria ilicicola*의 감염에 대한 콩 식물체 나이가 미치는 영향

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ABSTRACT : A series of greenhouse tests was conducted to evaluate infection of *Calonectria ilicicola* on soybean plants of different ages at time of inoculation. Lesion length and number of perithecia were determined on cultivars Braxton, Deltapine 726, and Riverside 699 that were 10~40 days old and 4-10 days old at time of inoculation. Quadratic and linear relationships were described between plant age at inoculation and lesion length or perithecia production in greenhouse studies. Soybean seedlings exhibited low susceptibility to *C. ilicicola* regardless of cultivar susceptibility. On 8- or 10-day-old Braxton, lesion length and perithecia numbers were reduced. Lesion lengths were longest on plants 30 days old whereas perithecia production was greatest on plants 20~30 days old at time of inoculation. Differences in lesion length and perithecia production that were observed on young plants (4~10 days old) were similar to relative levels of susceptibility in soybean cultivars in greenhouse and field tests, suggesting that reaction to *C. ilicicola* in soybean cultivars may be determined early in plant development.

***Key words* : *Calonectria crotalariae, C. ilicicola, Cylindrocladium crotalariae, Glycine max*, red crown rot**

The soilborne fungus *Calonectria ilicicola* Boedijn & Reitsma [anamorph: *Cylindrocladium parasiticum* Crous, Wingf. & Alfenas (7), syn. *C. crotalariae* (Loos) Bell & Sobers (2)] has been reported to cause red crown rot on soybean (*Glycine max* (L.) Merr.) (3-6) and Cylindrocladium black rot on peanut (1, 2, 10). In Louisiana, total yield loss per year to all diseases during 1988~1993 averaged about 16%, and was valued at $37 million (18, 19, 21). Although no specific statewide figures for yield loss to red crown rot have been published, this disease is widespread and important in the alluvial soils along the Mississippi River in Louisiana (4, 6) and portions of Mississippi (16). Actual field incidence of the disease may be as high as 20~30% on susceptible cultivars (4), which would be expected to cause 10~15% yield loss (6). In Mississippi, yield loss estimates of 25~30% have been reported (16).

Recommended control strategies for this disease in Louisiana include delayed planting and use of less susceptible cultivars (3, 5). Delayed planting frequently is not an option because of weather and other constraints. Planting less susceptible cultivars is more attractive, but limited information exists on reactions of soybean cultivars to red crown rot. Therefore, a greenhouse technique was developed to allow rapid screening of soybean cultivars to this disease (12). This technique involves inoculation of young seedlings (10 days old); however, no study has addressed the role of plant age at time of inoculation on infection by *C. ilicicola* and symptom development. Peanut plants that were young (14 days old) at time of inoculation had less severe disease by *C. ilicicola* than plants that were older (50~90 days old) (1). On soybean, reduced disease incidence following delayed planting (4, 6) may result from plants that are younger and thus less susceptible to the disease at time of infection by *C. ilicicola*. Therefore, a series of greenhouse tests was conducted to evaluate infection of *C. ilicicola* on soybean plants of different ages at time of inoculation.

MATERIALS AND METHODS

Study 1. Test 1 was conducted on soybean cultivars Braxton and Deltapine 726 which were shown in

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previous greenhouse studies to have low and moderate susceptibility, respectively, to *C. ilicicola* (11). In the greenhouse, soybean seeds were planted in 22-cm-diameter plastic pots at 10-day intervals so that plants were 10, 20, 30, or 40 days old at time of inoculation (June 30 and July 20 for tests 1 and 2, respectively). The pots contained a mixture of fumigated sandy loam soil (80% sand, 5% silt, 15% clay), peat moss, and perlite (3:2:1, v/v/v). Virulent isolate SG915 of *C. ilicicola* (11) was used in all experiments. A disc of mycelium (10 mm in diameter) in potato dextrose agar (PDA) cut from the advancing margin of a culture (10 days old) was placed in contact with each stem base and covered carefully with soil. The plants were watered daily with care for 3~5 days to prevent dislodging inoculum discs from crowns. Control plants were inoculated with PDA discs without fungus. Length of typically necrotic lesions on stems was measured above the soil line 52 days after inoculation. This experiment was established as a completely randomized design with 20 replicates.

Following lesion measurement, plants were removed from soil and lower stems and roots were washed immediately with water. A section of stem extending from the soil line to 1 cm above the uppermost margin of each lesion was excised and incubated under continuous fluorescent light at 25°C in a petri plate that contained moistened filter papers. Sections (5 cm) from control stems were excised and incubated similarly. Numbers of perithecia on three sites (2×10 mm) on each stem section were counted 14 days after incubation.

Test 2 was a duplicate of test 1 with the following changes. Soybean cultivar Riverside 699 was added to represent a cultivar with high susceptibility to red crown rot (11). Lesion lengths were measured 35 days after inoculation because the disease developed faster than that of test 1.

Study 2. Soybean cultivars Braxton, Deltapine 726, and Riverside 699 were planted in the greenhouse as previously described at two-day intervals so that plants were 4, 6, 8, or 10 days old at time of inoculation (July 24 and August 3 for tests 1 and 2, respectively). Procedures for inoculation, lesion measurement, perithecia counts, and experimental design were as described for Study 1, except that production of perithecia was determined 21 days after incubation. Lesion lengths on plant stems were measured 35 days after inoculation. This test was conducted twice with 16 and 20 replicates, respectively. For both studies, statistical analyses were conducted using PROC GLM of SAS (17). Relationships between dependent variables were examined using PROC CORR.

RESULTS

Study 1. In test 1, both lesion length and perithecia production were influenced by plant age at time of inoculation, but not by cultivar. No significant cultivar × plant age interactions were detected (Table 1). Quadratic relationships were determined between plant age at inoculation and lesion length or perithecia production (Fig. 1). Lesions were longest on plants 30 days old whereas perithecia production was greatest on plants 20 days old at time of inoculation (Table 1 and Fig. 1).

In test 2, both lesion length and perithecia production were influenced by plant age but not cultivar as observed in test 1. Significant cultivar × plant age interactions were detected for both lesion length and production of perithecia in test 2 (Table 1). Lesion length

Table 1. Lesion length[a] and production of perithecia[b] by SG 915 isolate of *Calonectria ilicicola* on soybean cultivars (10~40 days old)

Test/Factor	Level	Lesion length (mm)	Perithecia/cm^2
Test 1			
Cultivar	Deltapine 726	38.6	23.7
	Braxton	36.3	29.6
Plant age	10	28.6	20.7
	20	34.9	35.7
	30	48.2	31.0
	40	36.3	18.8
Source			
Cultivar		F=0.78 P=0.3794	F=2.51 P=0.1155
Plant age		F=18.75 P=0.0001	F=3.68 P=0.0139
Cultivar × Plant age		F=2.20 P=0.0906	F=0.74 P=0.5301
Test 2			
Cultivar	Riverside 699	30.4	43.5
	Deltapine 726	33.2	37.6
	Braxton	32.0	37.1
Plant age	10	22.5	29.7
	20	36.9	55.5
	30	39.1	37.5
	40	30.0	36.5
Source			
Cultivar		F=2.46 P=0.0883	F=1.06 P=0.3495
Plant age		F=55.30 P=0.0001	F=8.56 P=0.0001
Cultivar × Plant age		F=3.80 P=0.0013	F=6.57 P=0.0001

[a] Lesion length was measured 52 and 35 days after inoculation in tests 1 and 2, respectively.
[b] Perithecia were determined 14 days after incubation under continuous fluorescent light at 25°C.

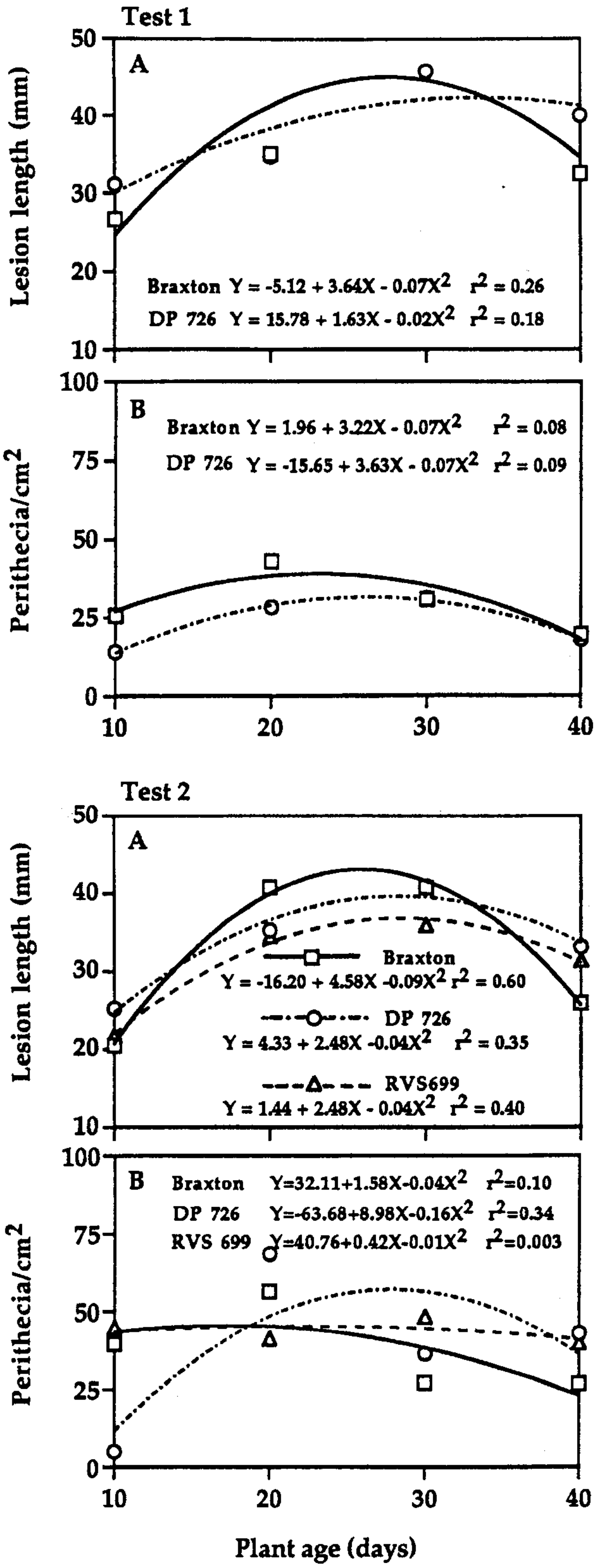

Fig. 1. Relationship between plant age and (A) lesion length and (B) production of perithecia by SG915 isolate of *Calonectria ilicicola* on soybean plants (10~40 days old). DP 726=Deltapine 726 and RVS 699=Riverside 699.

and perithecia production on all three cultivars were greatest following inoculation of plants 20-30 days old (Table 1 and Fig. 1). Production of perithecia was greatest on Deltapine 726 and Riverside 699 30~40 days old at time of inoculation. However, perithecia production on Braxton decreased on older plants (Fig. 1). Lesion length and perithecia production were not correlated in either test (r=0.04, P=0.64 for test 1 and r =0.001, P=0.99 for test 2).

Study 2. Lesion length and perithecia production were affected by plant age but not cultivar in both tests (Table 2). Significant interactions between cultivar and plant age at time of inoculation were detected only for production of perithecia in both tests (Table 2). Linear relationships were described between plant age and both lesion length and production of perithecia in both tests (Fig. 2). Little disease development was detected when plants were inoculated at 4 days old regardless of cultivar susceptibility. On 8- or 10-day old plants,

Table 2. Lesion length[a] and production of perithecia[b] by SG 915 isolate of *Calonectria ilicicola* on soybean cultivars (4~10 days old)

Test/Factor	Level	Lesion length (mm)	Perithecia/cm^2
Test 1			
Cultivar	Riverside 699	19.2	76.2
	Deltapine 726	20.6	68.9
	Braxton	19.0	64.2
Plant age	4	13.2	47.2
	6	15.4	48.0
	8	23.4	82.4
	10	26.6	98.0
Source			
Cultivar		F=2.85 P=0.0603	F=1.22 P=0.2969
Plant age		F=109.50 P=0.0001	F=33.54 P=0.0001
Cultivar × Plant age		F=2.02 P=0.0659	F=2.47 P=0.0260
Test 2			
Cultivar	Riverside 699	18.7	87.0
	Deltapine 726	19.8	88.5
	Braxton	18.8	71.7
Plant age	4	12.2	56.4
	6	16.4	69.3
	8	20.3	87.6
	10	27.3	112.3
Source			
Cultivar		F=0.61 P=0.5432	F=4.85 P=0.0088
Plant age		F=88.78 P=0.0001	F=29.75 P=0.0001
Cultivar × Plant age		F=0.35 P=0.9086	F=2.50 P=0.0236

[a]Lesion length was measured 35 days after inoculation.
[b]Perithecia were determined 21 days after incubation under continuous fluorescent light at 25°C.

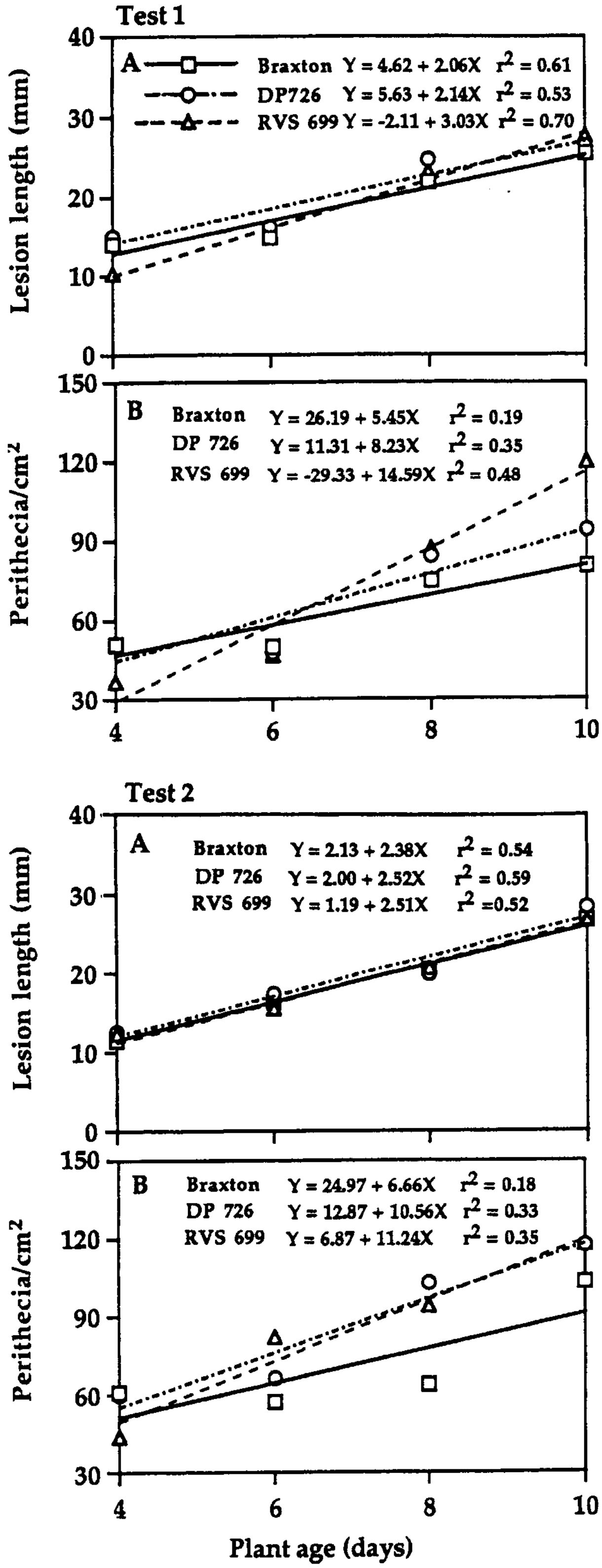

Fig. 2. Relationship between plant age and (A) lesion length and (B) production of perithecia by SG915 isolate of *Calonectria ilicicola* on soybean plants (4~10 days old). DP 726=Deltapine 726 and RVS 699=Riverside 699.

Braxton supported fewer perithecia in both tests (Fig. 2). Lesion length and perithecia production on cultivars were significantly correlated in tests 1 (r=0.476, P=0.0001) and 2 (r=0.422, P=0.0001).

DISCUSSION

Differential responses of plants to pathogens that are based on host age have been reported for several crops (1, 14, 15, 20). Young peanut plants were reported to be less susceptible to *C. ilicicola* but they developed severe disease when inoculated at ages of 50 days and older (1). Soybean plants in early vegetative stages (8) were less susceptible to brown stem rot caused by *Phialophora gregata* (15) but more susceptible to stem canker caused by *Diaporthe phaseolorum* var. *caulivora* (14, 20). In the current study, soybean seedlings exhibited low susceptibility to *C. ilicicola* regardless of cultivar; even the most susceptible cultivar showed resistant reaction when inoculated at 4~6 days old. Reductions in disease severity and production of perithecia as another disease index on older soybean plants (40 days old) may indicate that late growth stages of soybean may be less susceptible to *C. ilicicola.*

Differences in disease severity and perithecia production observed on young plants were similar to relative levels of susceptibility in soybean cultivars seen in greenhouse and field tests (12, 13). This suggests that reaction to *C. ilicicola* in soybean cultivars may be determined early in plant development. It has been proposed that the pathogen infects early growth stages of soybean but produces symptoms at later stages (R_3-R_4) (8). This speculation is supported by data that show cultivar Braxton among the least susceptible cultivars in greenhouse and field tests (12, 13) as well as exhibiting reduced disease severity and perithecia production in seedlings observed in the present study. It may be that if resistance is not expressed at early growth stages, the plants probably will produce disease symptoms at later growth stages.

Harris and Beute (9) reported the importance of initial defense mechanisms in Cylindrocladium black rot of peanut caused by *C. ilicicola,* which may support the idea that resistance of soybean at early growth stages determines further disease reaction. These authors (9) observed that formation of periderm (9, 10) after infection of primary branch roots suppressed further infection by germinated microsclerotia. They also found that infection with *C. ilicicola* induced less periderm in a susceptible than in a resistant peanut cultivar and this periderm was more easily destroyed by subsequent challenges with germinated microsclerotia (9). Periderm-related resistance of primary roots also has been suspected for soybean cultivars (4). This may determine the initial infection of germinated microsclerotia, which are primary inocula for red crown rot, and consequently the final symptoms on stems and leaves in the field.

Berner *et al.* (6) reported that delayed planting of the susceptible cultivar Bedford (maturity group V) as well as timely planting of maturity group VII cultivars resulted in reduced red crown rot disease incidence. These authors proposed that reduced disease incidence was due to disease escape rather than genetic resistance. Results from the current study suggest that young plants, especially stems, may be inherently less susceptible to infection and perithecia production by *C. ilicicola* than are older ones. Thus, it may be that resistance of cultivars such as Braxton is related to genetic factors but that susceptibility of cultivars such as Bedford is affected by other factors such as delayed planting, availability of infection courts, plant age, and environmental factors.

Delayed planting is the control strategy of choice because of a lack of resistant cultivars. Results reported in this study suggest a possible explanation for this disease reduction and that disease resistance of soybean may be determined at early growth stages. Additional research is needed to determine the time of fungal infection under field conditions and changes in population of fungal propagules in soil throughout the year.

요 약

*Calonectria ilicicola*의 감염에 대한 콩 식물체의 나이가 미치는 영향을 평가하기 위하여 온실 시험을 수행하였다. 식물체의 나이가 각각 다른(10~40일과 4~10일) Braxton, Deltapine 726과 Riverside 699 등의 콩 품종에 *C. ilicicola*를 접종하여 나타난 병반 길이와 자낭각의 수를 조사하였다. 식물체 나이에 대한 병반 길이와 자낭각 수는 포물선 과 직선적인 관계를 각각 보여 주었다. 콩 유묘는 품종간 감수성에 관계없이 *C. ilicicola*에 대하여 낮은 수준의 감수성을 보여 주었다. 식물체의 나이가 8~10일된 Braxton 품종에서는 접종 후 병반 길이와 자낭각의 수가 다른 품종에 비하여 낮은 수준이었다. 병반 길이는 30일된 식물체에서 가장 컸으며 자낭각의 수는 20~30일된 식물체에서 가장 많았다. 특히 어린 식물체에서 관찰된 병반 길이와 자낭각 수에서의 품종간 차이는 온실 및 포장 시험에서 관찰된 감수성의 차이와 유사하였다. 이는 *C. ilicicola*에 대한 콩 품종의 반응은 식물 발달의 초기 단계에서 결정된다는 것을 시사하고 있다.

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