한국식물병리학회지 3(4): 285-290(1987)
Korean J. Plant Pathol. 3(4): 285-290(1987)

EFFECTS OF LEAF MATURITY ON THE DISEASE PROGRESS OF SEPTORIA BROWN SPOT IN SOYBEAN

Jeung Haing Oh

Department of Agronomy, College of Agriculture, Dankook University, Cheonan 330, Korea

大豆잎의 成熟度가 갈색무늬병의 進展에 미치는 影響

吳 正 行

檀國大學校 農科大學 農學科

ABSTRACT

Septoria brown spot caused by *Septoria glycines* Hemmi is one of the serious fungal diseases in soybean. Since little has been known about the disease progress in the field, the present study was conducted to determine the factors affecting the disease progress in the soybean plant. Disease severity and pattern of the progress of the Septoria brown spot were different with varieties. Susceptibility of soybean plants increased with increase of plant age and leaf maturity in order from the primary leaf to the newly expanded leaf. It seemed to be related with conidial germination on the leaves. Germination and germtube elongation were more inhibited by the diffusates obtained from upper leaves than those from lower leaves and they were higher in a susceptible variety than in a moderately resistant one.

Key words : epidemiology, soybean, septoria brown spot.

要 約

大豆 갈색무늬병의 진전은 한 식물체에서 上位葉으로의 진전속도(垂直感染率 ; vertical progress)과 밀접한 관계가 있다. 실제로 圃場에서의 갈색무늬병 진전은 주로 下位葉에서 發病하기 시작하여 上位葉에 비하여 罹病程度도 높은 것이 보통이다. 이러한 현상의 원인을 구명하기 위하여 수행한 본 실험에서 갈색무늬병의 감염율은 잎의 성숙도와 正의 相關을 보였으며 이러한 幼葉抵抗性은 接種源의 分布와는 無關한 것으로 보였다. 또 上位葉의 葉表面溢出液(leaf diffusate)은 病源菌 胞子發芽 및 菌糸伸張의 억제 정도가 하위잎의 일출액에 비하여 높았으며 이러한 경향은 抵抗性品種에서 더욱 현저하여 葉溢出液의 어떤 發芽抑制物質이 갈색무늬병의 幼葉抵抗性에 관여 하는 것으로 推定되었다.

INTRODUCTION

More attention is given to soybean prodution in recent years by developing new beverages and foods made of soybean. However, the average yield per unit area is relatively low as compared to those of other staple crops(13). Soybean disease is one of the responsible stress factors for yield reduction. Among several diseases, Septoria brown spot caused by *Septoria glycines* Hemmi(2, 3, 4) is one of the serious fungal diseases, occurring in most of the soybean growing area(8). It appears early in the rainy season on the primary leaves of soybean plants. Heavily infected leaves gradually turn yellow and fall prematurely. Greatest damage occurs where soybeans are consecutively grown in the same field. Remarkable yield reductions due to Septoria brown spot epidemics were reported(6, 7, 9, 12). Since the pathogen spreads from lower to upper leaves in warm humid weather conditions as the soybean plants grown, an attempt was made to determine relationships between the disease progress and leaf maturity at present study.

MATERIALS AND METHODS

Evaluation of Septoria brown spot. Disease assessment was made five times with the varieties, KAS 604-24, KAS 320-4, KAS 639-8 and KEX-2 from a week after inoculation in the field. Twenty plants selected at random in each plot were rated and the ratings were averaged to obtain the percentage diseased leaf area. The Septoria brown spot severity was calculated by using the method of Young and Ross(12) as follows : (% defoliation + % remaining leaves) x proportion of remaining leaf area diseased. Diseased leaf area was determined by using a modified Horsfall and Barrat scale(5). Percent defoliation was derived from comparing total nodes to the number of nodes defoliated on the main stem. The progress of Septoria brown spot based on the number of infected leaves to total nodes of main stem was determined on the same plants in the soybean growth stage(1).

Preparation of soybean seedlings for inoculation. Two soybean varieties, KAS 320-4 and KAS 639-8, were used as differential hosts. The former is susceptible to Septoria brown spot whereas the latter is moderately resistant with slow progress of the disease in the field. Planting dates were staggered at one week intervals to obtain the plants 3-, 4- and 5- week-old at the time of inoculation. Three plants per pot were grown in 6 inch Wagner pots containing silty loam soil. When the oldest planting was 5-week-old, all plants were inoculated with spore suspension containing 2×10^5 conidia/ml. The inoculum was prepared by rinsing 2-week-old cultures with sterile water and screening through 3 layers of cheesecloth. Conidial suspension was atomized on each plant to the point of run-off, inoculated plants were incubated in a moist chamber for 48 hours, then removed to a greenhouse bench at 18-27°C. Two weeks after inoculation, disease severity on each leaf was rated by the modified scale of Horsfall and Barrat(5).

Preparation of soybean leaf diffusate. Leaf diffusates of each location of the 5-week-old plants were prepared by shaking 3.5g leaves with 100ml deionized water in a 300ml flask for 30 min at 20°C on a reciprocating shaker(50 cycle/min). The diffusates were reduced in vacuum evaporator to a volume which corresponds to half the amount of the water required to wet the leaves to run-off (approximately 2μ liter/cm^2). This value was determined by spraying to the leaves of a known leaf area. All diffusates were stored at 5°C until needed. Conidial germination in the diffusates was determined by the slide germination test. Free sugar content of the leaf diffusates was determined by Somogy's method(10), and leaf chlorophyll of the 5-week-old plants as analysed by Yoshida *et al*(11).

RESULTS

Disease severity of four different varieties, as expressed by $\log\frac{X}{1-X}$, when X is percentage of diseased leaf area, was determined to evaluate the

disease resistance in the field. Each variety showed a different response to Septoria brown spot. KAS 320-4 was the highest disease severity and KAS 639-8 was lowest(Fig. 1). The progress of Septoria brown spot was also different with the varieties. In general, the higher the disease severity, the higher was the disease progress from lower leaves to upper leaves at the late state of growth.

Because numerous studies have considered the possible involvement of leaf diffusates in plant resistance, the relationship between the disease progress and conidial germination on the soybean leaf diffusates was determined with leaves inserted at different positions in a susceptible variety and a moderately resistant vaiety. Percent diseased leaf area of Septoria brown spot on the plants at different intervals after seeding was significantly different in both vaieties(Table 1). As the soybean plants aged, susceptibility to Septoria brown spot was significantly increased. Diseased leaf area in the susceptible variety KAS 320-4 was 33.6%, 32.2% and 20.9% on the plants in 5, 4 and 3 weeks after seeding, respectively. On the moderately resistant variety KAS 639-8, 11.6% in the 5-week-old plants and 10.5% in the 4-week-old plants appeared, but the diseased leaf area dropped to 9.5% for plants inoculated at 3 weeks after seeding. There was a statistical difference in susceptibility with leaf position on main stem in both varieties. Disease severity was increased in order from primary leaf to newly expanded top leaves and average severity was 69.3% to 4.2% in susceptible variety and from 27.9% to 0.6% in moderately resistant variety.

Since a loss of chlorophyll may be a classical symptom in leaf maturity of aging, chlorophyll contents of soybean leaves were analysed(Table 2). Although visual difference was not observed between the leaves with different position on the main stem, total chlorophyll was slightly higher in the resistant variety than susceptible one and consistently increased in upper leaves in both varieties. Increase in

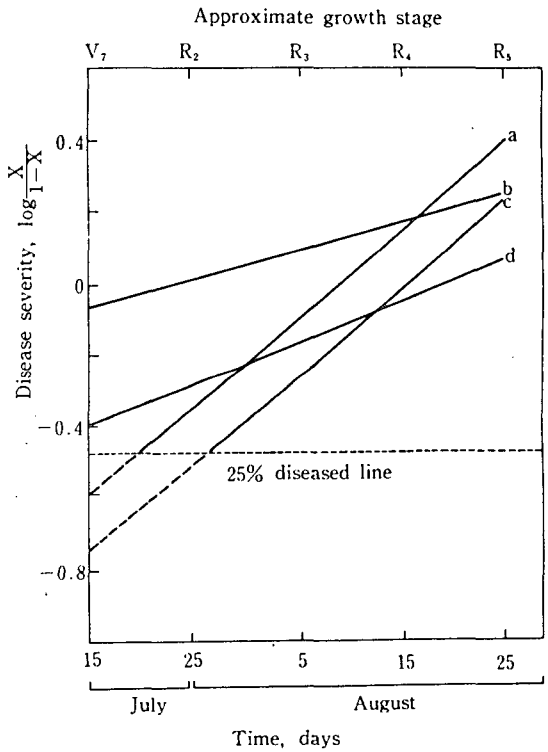

Fig. 1. Disease progress of Septoria brown spot on the varities KAS 604-24(a), KAS 320-4(b), KAS 639-8(c) and KEX-2(d) based on the portion of diseased leaf area per plant in field.

Table 1. Effect of leaf position on susceptibility of soybean to Septoria brown spot

Variety	Weeks after seeding	Diseased leaf area(%)					LSD(.05)*
		Primary	1st	2nd	3rd	Avr.	
KAS 320-4	5	67.9	42.8	14.3	9.5	33.6	19.509**
	4	82.5	25.7	17.4	3.3	32.2	18.351**
	3	57.7	24.5	1.4	0	20.9	15.213*
	Avr.	69.3	31.0	11.0	4.2	29.0	
KAS 639-8	5	31.1	8.1	5.0	2.0	11.6	5.925**
	4	23.9	12.3	5.6	0	10.5	4.881**
	3	28.9	9.1	0.1	0	9.5	3.061**
	Avr.	17.9	9.8	3.5	0.6	10.5	

*and** indicate statistical significance at 5% and 1% level, respectively.

Table 2. Relationships between brown spot severity and chlorophyll contents(mg/gfw) of the soybean leaves at different maturity

Leaf location	Variety KAS 320-4				Variety KAS 639-8			
	Disease severity	Chlorophyll a	Chlorophyll b	Total	Disease severity	Chlorophyll a	Chlorophyll b	Total
Prim. L	67.9	5.635	6.555	12.191	31.1	5.767	7.092	12.857
1st L	42.8	5.713	6.508	12.221	8.1	5.962	6.892	12.852
2nd L	14.3	5.778	6.550	12.399	5.0	5.960	7.002	12.960
3rd L	9.5	5.820	6.772	12.550	2.0	6.018	7.018	13.034
FLSD(.05)[a]	19.51**	0.06*	NS	NS	5.93**	0.15*	NS	NS
r[b]		−0.99*	−0.57	−0.91		−0.96	−0.61	−0.67

[a]*and** indicate statistical significance at 5% and 1% level, respectively based on Fisher's Least Significant Difference Test. NS means non-significant.

[b]r indicates correlation coefficient between chlorophyll contents and disease severity.

Table 3. Free sugar contents, hydrogen ion concentration of diffusates from the soybean leaf at different maturity and effect of the diffusates on germination of conidia of *Septoria glycines*

Variety	Leaf location	Spore germination(%)	Germtube[a] length(μ)	Free sugar[b] content(%)	pH
KAS 320-4	Prim. leaf	85	65	0.105	6.5
	1st leaf	83	72	0.109	6.8
	2nd leaf	85	61	0.116	7.0
	3rd leaf	75	57	0.115	7.1
	4th leaf	70	46	0.133	7.0
	FLSD(.05)	NS	NS	NS	NS
KAS 639-8	Prim. leaf	79	42	0.133	6.5
	1st leaf	76	75	0.169	6.7
	2nd leaf	73	67	0.169	6.6
	3rd leaf	58	35	0.169	6.7
	4th leaf	52	15	0.187	6.5
	FLSD(.05)[c]	18.24*	7.82*	NS	NS

[a]Spore germination and germ tube length were measured 24 hrs after treatment.

[b]Free sugar contents were analysed from the diffusates of 18 leaves.

[c]*and** indicated statistical significance at 5% and 1% level, respectively.

Table 4. Effect of diffusates obtained from basal or apical leaves of two different soybean varieties on germination of conidia and germtube length of *Septoria glycines*

Treatment	Variety	Leaf location	Germination[a]	Germtube length(μ)
Diffusate alone	KAS 320-4	Apical	72 a[b]	48 a
		Basal	86 b	65 b
	KAS 639-8	Apical	55 c	17 c
		Basal	80 ab	45 a
Diffusate amended[c]	KAS 320-4	Apical	76 a	69 a
		Basal	92 b	81 b
	KAS 639-8	Apical	68 c	36 c
		Basal	83 ab	68 a

[a]Germination percent was derived from 30 spores tested with 3 replicates in 24 hrs.

[b]The same letter are not significantly different according to Duncan's multiple range test at P=0.05.

[c]Diffusates were amended with 0.1 mol glucose.

chlorophyll content was responsible for content of chlorphyll-a rather than chlorophyll-b. The content of chlorophyll-a was negatively correlated to the disease severity with statistical significance(P =0.05). The conidial germination in soybean leaf diffusates was different with leaf position(Table 3). Conidial germination was significantly greater in diffusates from lower leaves than upper leaves of both varieties. Also, germtube length was significantly reduced in diffusates from upper leaves as compared to those from lower leaves. It was more remarkable in susceptible variety than resistant

variety. Free sugar content in the leaf diffusates had a tendency to be greater in resistant variety rather than susceptible one, and slightly higher in upper leaves than lower leaves of both variety.

In order to know whether the inhibition of conidial germination and germbube growth in leaf diffusates was attributed to nutrient competition, the leaf diffusates were amended with equal volume of 0.1 mol glucose. Conidial germination and germtube growth were increased in amended diffusates as compared to those in diffusate alone, but they were still greater in diffusate from basal leaves than apical leaves as similar to those on diffusate alone (Table 4).

DISCUSSION

The differential response of soybean varieties to Septoria brown spot was accentuated by evaluating the relationship between the progress of Septoria brown spot and plant development. The soybeans with higher disease progress from lower leaves to upper leaves was higher in disease severity, even though it was not always coincide with each other. This relationship enables to obtain quantitative informations concerning Septoria brown spot epidemics during the growing season. Lim(6) suggested that one should give a particular attention to quantitative measurement for evaluating Septoria brown spot reactions. Disease severity, defoliation and density of pycnidia were mentioned as some quantifiable characters that might be used. Accordingly, vertical disease progress from lower leaves to upper leaves in a plant seemed to be one of the factors available for evaluating Septoria brown spot resistance.

Susceptibility of soybean plant to Septoria brown spot was likely associated with age of the plants. Since soybean leaves on main stem develop acropetally, it can be considered that lower leaves are more aged than upper trifoliolate leaves. In all cases of the inoculation study, the primary leaves were more susceptible to infection than upper trifoliolate leaves regardless of soybean varieties. This indicates that primary leaf state was adequate for measuring Septoria brown spot severity to screen the disease resistance, even though the flowering stage was optimum growth stage for screening.

Conididal germination on leaf surface would be important process in conferring host resistance because, if their germination do not occur, a potential source of infection may be warded off temporarily or permanently. In the present study, leaf diffusates conditioned the host for resistance by inhibiting conidial germination. If free sugar content in the diffusates were responsible for the demonstrated inhibition of the disease development, it must present more amounts in the exudates from the mature leaves than the juvenile leaves, which were more resistant compared to matured one. However, free sugar was rather greater in a juvenile leaves than mature leaves. Therefore, the inhibition of conidial germination and hyphal growh in the leaf diffusates of upper juvenile leaves as considered not due to the insufficient carbon source for spore germination, but due in part to the presence of preformed fungistatic substance on the leaf surface. However, identification of the inhibitory substance will more clarify the role of the leaf diffusates in the resistance of soybean plants.

REFERENCES

1. Fehr, W. R., Caviness, C. E., Burmood, D. T. & Pennington, J. S.(1971). Stage of development descriptions of soybeans, *Glycine max* (L) Merril.(Abstr.) *Crop Science* 11 : 929–931.
2. Fucikovesky, L. A.(1972). Pigments of soybean leaves infected by *Septoria glycines* and *Pseudomonas glycinea*. *Trans. Brit. Mycol. Soc.* 59 : 506–508.
3. Hemmi, T.(1915). A new brown spot disease of the leaf of *Glycine hispida* Maxim. caused by *Septoria glycines* sp. *Sapporo Nat'l. Hist. Soc. Trans.* 6 : 12–17.
4. Hemmi, T.(1940). Studies on Septorioses of

plants. VI. *Septoria glycines* Hemmi causing the brown spot disease of soybean. *Mem. Coll. Agr. Kyoto Imp. University* 47 : 1-4.

5. Horsfall, J. G. & Barrat, R. W.(1945). An improved grading system for measuring plant diseases(Abstr.). *Phytopathology* 3 : 655.
6. Lim, S. M.(1979). Evaluation of soybean for resistance to Septoria brown spot. *Plant Dis. Reptr.* 63 : 242-245.
7. Lim, S. M.(1980). Brown spot severity and yield reduction in soybean. *Phytopathology* 70 : 974-977.
8. Nakata, K. & Takimoto, K.(1934). A list of crop diseases in Korea. *Agr. Expt. Sta. Govt. Central Chosen Res. Reptr.* 15 : 1-146.
9. Pataky, J. K. & LIM, S. M.(1980). Effect of Septoria brown spot on the yield components of soybeans. *Plant Disease* 13 : 28-30.
10. Somogy, M.(1954). A new reagent for the determination of sugars. *J. Biol. Chem.* 160 : 61-68.
11. Yoshida, S., Forno, D. A., Cook, J. H. & Gomez, K. A.(1972). Laboratory manual for physiological studies of rice. IRRI Pub.
12. Young, L. D. & Ross, J. P.(1979). Brown spot development and yield response of soybean inoculated with *Septoria glycines* at various growth stages. *Phytopathology* 69 : 8-11.
13. Year book of agriculture and forestry statitics, (1981). USDA.