Mode of Occurrence and Control of Purple Speck of Soybean

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The purple speck is an important constraint to soybean production, because it spoils marketability of the product rather than decreases grain yields. Therefore, the author attempted to develop effective control measures, i.e., fungicidal control based on an appropriate cultural method, by making clear the mode of occurrence of the disease.

Mechanism of infection

The spores are spread by the dispersion in air. The spores which reached soybean plants begin to germinate when water droplets exist on the surface of the plants. After the germ-tubes elongated, they can survive even when the water droplets dry up. They cease to grow when no water is available, but they resume to grow when water becomes available . They intrude into leaves, stems, and pods through stomata and wounds, and into seeds through cuticule.

The leasions hardly expand on leaves and stems until the plants become old, because they are obstructed by bascular bundles. In pods too, the lesions remain unexpanded in the assimilatory tissue beneath the outer epidermis until the pods begin to mature, because the pathogen in that assimilatory tissue can not enter into the inner portion due to the presence of the mechanical tissue at the border between the outer and

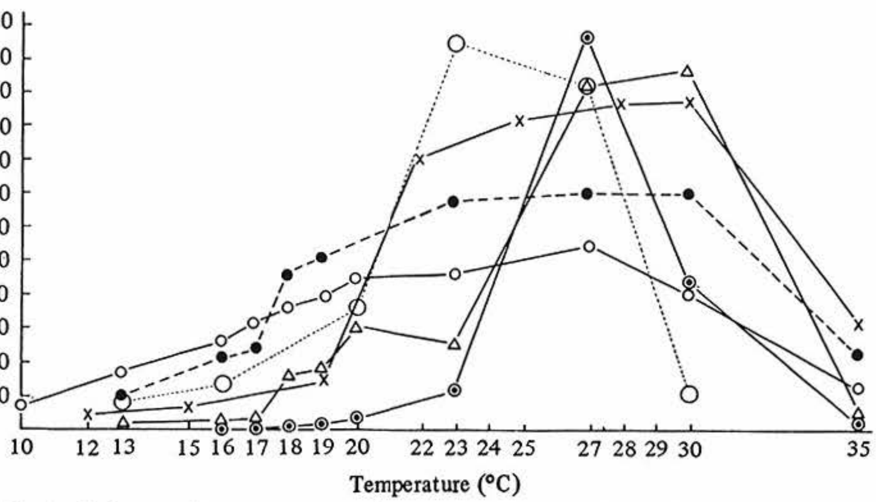

Fig. 1. Influence of temperature on the sporulation, spore germination, development of pathogen and infection of seeds from diseased pods

△—△ : Sporulation (from diseased seed)—no. of spores formed/0.16 mm²
⊙—⊙ : Sporulation (from lesion of leaf)—no. of spores formed/3 mm²
×—× : Spore germination—percent germination (%)
○—○ : Development of pathogen inside plant tissue—elongation during 7 days (cm)
●—● : Seeds infected from diseased pods—percent diseased seeds (%)
◯—◯ : Purple color secreted by pathogen—purple color extracted from pathogen cultured for 11 days (taking the value at 27°C as 100)

inner assimilatory tissues. The entry into the inner tissue occurs after the beginning of pod maturation. The seeds become purple when they come into contact with the pathogen during the course of their development. However, the purple seed stage comes at the maturity stage, because the lesions and hyphae do not spread up to the inner epidermis of the pods until that stage. The purple color emerges in cytoplasm of seed coats, but it also emerges in the hyphae themselves spreading in tissues. This pigment is secreted from the fungi, and has a property of anthocyan. The color begins to emerge one day after infection, and becomes deeper two days after infection. The infection process is sporulation—germination—spreading in tissues–infection to seeds from infected pods–purple color development of seeds. Response of the process to temperature is shown in Fig. 1. The optimum temperature is between 20 and 28°C, although slight differences exist among stages of the process. Rain favors the fungi, particularly for the infection and color development of seeds. When the lesions on pods are large enough to infect seeds during 15 days before harvest, the more the rain the more the infected seeds observed in fields under the temperature range permitting the infection.

Source of inoculation and disease development

1) *Wintering of the pathogen and primary infection*

The pathogens winter in dry diseased seeds and diseased plants, though a few of them can winter at wet conditions in fields. The primary infection takes place in cotyledons of diseased seeds, and hypocotyls of diseased plants scattering in the fields.

2) *Disease occurrence in stems and leaves*

When the lesions on cotyledons spread, they attack hypocotyls and seldom cause wilt. The diseased area by purple speck expands upwards to the simple leaf and to the first compound leaf. Then, until late July–early August the disease occurrence is temporarily suspended. However, the early lesions hold their ability of sporulation for about 30 days after the leaf-fall, serving as the source of inoculation for the disease occurrence in leaves, stems, and leaf petioles in the later growth stages.

3) *Disease occurrence in pods and seeds*

The disease development in pods and seeds in fields is given in Table 1. The disease begins to occur in pods ca. 30 days after flowering and in seeds ca. 60 days after flowering (at the stage of beginning of pod yellowing).

Table 1. Development of the disease on pods and seeds observed in a field

Date	DAF*	Percent of diseased pods	Percent of diseased seeds
Aug. 21	21	0%	—%
26	26	4	—
31	31	15	—
Sept. 4	35	23	—
10	45	39	—
15	50	46	0
22	57	100	0
24	59	—	9
28	63	—	32
Oct. 11	75	—	41
22	86	—	59

* DAF: Days after flowering

4) *Harvesting time, method of drying, and infected seeds*

As shown in Table 2, the later the time of harvest, and the poorer the post-harvest drying, the more was the disease occurrence. Storage of seeds not sufficiently dried increases the number of infected seeds.

Fungicidal control

1) *Seed disinfection*

Result of seed disinfection by thiram benomyl and thiram thiophanate methyl, which are registered fungicides, are shown in Table 3. Early and uniform germination, high percentage

Table 2. Percent of diseased seeds as influenced by time of harvest and post-harvest drying

Date* of observation of disease	Percentage of infected seeds	Post-harvest drying methods			
		Drying on ground in a fiield	Hanging outdoors	Drying on ground in a closed greenhouse	Hanging in an open greenhouse
15 days before	—%	—%	—%	—%	—%
10 days before	0.2	—	—	—	—
5 days before	1.9	—	—	—	—
0 (Oct. 10)	23.5	—	—	—	—
5 days after	41.3**	39.2	34.6	28.5	29.1
10 days after	57.6**	48.5	37.5	30.1	28.7
15 days after	69.7**	53.6	37.9	31.7	29.3

* Date of observation is expressed by no. of days before or after the proper harvest day (Oct. 10).

** Observed with plants left standing in the field after Oct. 10.

Table 3. Effect of seed disinfection to control purple speck

Treatment	Seed germination percentage	No germination caused by disease			Diseased cotyledon			Diseased hypocotyl	
		Purple speck	Anthracnose	Unknown	Purple speck	Anthrac-nose	Brown spot	Purple speck	Anthrac-nose
Disinfection	99.3	0.2	0.1	0.4	2.9	1.0	0.6	0	0
No disinfection	87.9	1.7	2.7	7.7	64.4	12.8	1.6	2.6	6.0

Dry smear with wettable powder of thiophanate-methyl 0.4% per weight of dry seeds

of germination, markedly reduced infection in cotyledons and hypocotyls and hence no sporulation there were observed as the result of the seed treatment. The disease-preventive effect was apparently recognized up to the second compound leaf. In addition, the seed treatment was effective to prevent infection from diseased plants on the soil surface, though not fully, and to control other seed-borne diseases.

2) *Application of fungicides at the growth stage*

Thiophanate methyl fungicide, benomyl fungicide, and Bordeaux mixture are registered chemicals. As given in Table 4, the most effective application time for thiophanate methyl and benomyl fungicides was 20–40 days after flowering, and that for Bordeaux mixture was 14–30 days after flowering. These dates are irrespective of earliness or lateness of soybean varieties. In general, wettable form shows higher effect than dust form. They are applied 1–2 times at the rate of 180 *l*/10 a for liquid, and 4 kg/10 a for dust. The fungicide must be applied so as to cover the whole plant, particularly the inner portion below the plant canopy, if the good control is to be done.

3) *Measures against the development of fungicide-tolerant fungi*

Application of mixtures of fungicides differing in ingredients or alternative application of different fungicides are effective to inhibit the development of tolerant fungi. Alternative or mixture application of mineral copper fungicide, benomyl fungicide, and TPN fungicide are as effective as the single application of each of them. The sequence in alternative application is mineral copper followed by thiophanate methyl or benomyl fungicide.

4) *Method of disease-forecasting*

Dispersing spores are trapped by glass slides,

Table 4. Time and frequency of fungicidal application in growing season

Application frequency	Application time	Percent of diseased seeds			Remarks
		Thiram benomyl*	Thiophanate methyl**	Bordeaux mixture***	
Seed disinfection only		43.3%	—%	—%	
1 time	Flowering time	57.7	—	14.0	
	Days after flowering 7	42.5	—	12.0	Start of pod enlargement
	11	15.8	—	—	Enlargement of pod
	14	—	2.2	1.0	
	16	2.3	—	—	Maximum length of pod
	21	—	0.5	0.7	
	28	0.2	0.1	1.0	End of pod growth and start of seed enlargement
	35	—	0.1	7.7	
	42	—	1.0	9.7	
	49	—	1.7	—	
	56	—	4.7	—	
	63	—	6.4	—	Just before defoliation and start of seed disease
	70	—	22.3	—	Defoliation and increase of seed disease incidence
2 times	Days after flowering 11 and 16	2.0	—	—	
	21 and 35	—	0.1	—	
	28 and 42	—	0.0	—	
	35 and 49	—	0.1	—	
Seed disinfection plus application 11 and 16 days after flowering		4.7	—	—	
No application		50.0	20.5	16.1	

* Experiment in 1979, ** in 1980, and *** in 1981.

horizontally installed at the height of 20 cm for 24 hr from 9:00 A.M. The number of spores trapped every day was averaged for a half-decade of a month, and was shown in comparison with the disease severity in Fig. 2. It was found that yearly fluctuation in transitional pattern of disease occurrence in leaves and stems was closely related to the transition of spore dispersion: severe occurrence of disease at an early growth stage gives a large number of dispersing spores, which cause severe occurrence of disease after the 4th half-decade of August and an increased number of dispersing spores. On the contrary, less disease at an early growth stage with less number of dispersing spores resulted in less disease in late August with less spores, which caused less diseased seeds. Particularly in the year, in which spore dispersion occurs every day and it increases with time, more diseased seeds were produced. As already mentioned, the rainfall during 15 days before harvest is closely related to the occurrence of diseased seeds, effect of the number of dispersing spores and rainfall at the middle growth stage was compiled as follows:

Year	No. spores dispersed	Rainfall	Percent diseased seeds
1979	Many	Much	50
1980, 82, 83	Less	Less	14
1981	Less	Much	14

This result indicates that the rainfall apparently promotes the infection of seeds whenever the

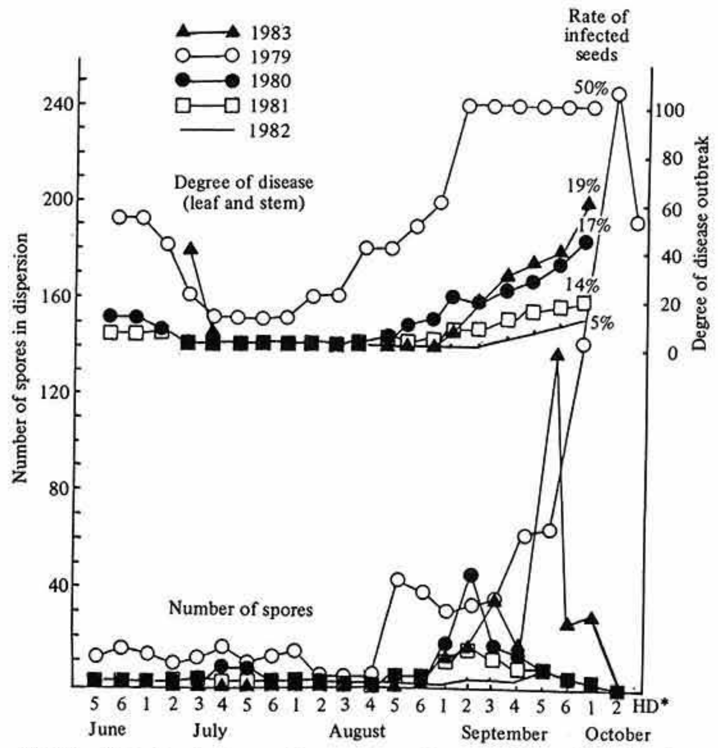

Fig. 2. Relation between the number of spores disperising in plant canopy and degree of disease outbreak

* HD=half-decade (5-day period) of a month. Numerals, 1,2,3…6, signify the first, second, third, …sixth HD of each month.

condition of diseased pods is able to infect seeds, while the rainfall effect is small when there is no such condition. Thus, the seed disease can be forecasted from the number of spores dispersing after 25th of August.

Conclusion

This disease occurs when diseased seeds are sown or diseased plant residues are brought into fields. The infected portion gradually expands from cotyledon to single leaf, and to lower compound leaf, increasing the source of inoculation to pods, and finally from pods to seeds. In the years of less incidence, the amount of source of inouclation is more influencial to seed infection than climatic conditions which are less favorable to the disease, while in the years of severe outbreak, climatic conditions which favor the disease outbreak are more determinative than the source of inoculation for the disease of seeds. This fact implies that in years of less incidence control measures at an early growth stage mainly seed disinfection, are effective to control seed disease, whereas fungicide application at the growth stage is more important than seed disinfection in years of severe outbreak. However, as the seed disinfection can prevent other diseases like anthracnose, it should be better used by considering that fact.

Of the chemicals to be applied at the growth stage, Bordeaux mixture was found to be effective in preventing the disease, while thiophanate methyl and benomyl fungicides have preventing and curative effects. Therefore, the best time of the application for the former is before pod infection, i.e. 14–30 days after flowering, while that for the latter is the initial stage of pod infection, i.e. 20–40 days after flowering. As the latter has a wide range of the optimum time of application, these fungicides can be used one time to the fields where varieties with different maturing time are grown together. They can also be used in mixture with pesticides, aiming at simultaneous control of disease and insects.

As the fungi easily become tolerant to thiophanate or benomyl, cares must be taken. Excessive application should be avoided, and different fungicides with different ingredients must be used either in their mixture or by alternative application. By taking account of the difference of the optimum time of application among chemicals, and the possibility of forecasting whether fungicide application is needed or not in late August, it may be better to use inorganic copper fungicide for the first application and thiophanate methyl or benomyl fungicide for the second one.

As the disease occurrence is dependent on climate, agronomic control composed of soils, rate of fertilizer application, cultural methods, varieties to be used, etc. is hardly effective. As to the effect of temperature on the disease, it is known that the sporulation is markedly inhibited by the temperature below 17°C, and hence no spore dispersion occurs. Therefore new infection is not observed. However, spore germination, hyphal elongation, and transmission from diseased pods to seeds are not inhibited even at 13°C, though hyphal elongation becomes slow. Therefore, lesions which exist already can expand, and diseased pods can transmit the disease to seeds even at the temperature that inhibits sporulation. In fields, purple speck occurs on seeds when daily mean temperature during 15 days before harvest is above 10°C. This means that the maturation of soybean is also delayed at the low temperature, resulting in the similar condition to the disease occurrence at the optimum temperature. Consequently, even late-maturing varieties suffer from the disease when the plants are exposed to rains or wet conditions for a long period due to delayed harvest. The most important point as an agronomic control is to harvest the crop at the right time and to carry out post-harvest drying quickly.

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