

Use of Paraquat to Aid Detection of Fungi in Soybean Tissues

R. F. Cerkauskas and J. B. Sinclair

Graduate research assistant and professor, respectively, Department of Plant Pathology, University of Illinois, Urbana 61801. Supported in part by the Illinois Agricultural Experiment Station. Accepted for publication 1 May 1980.

ABSTRACT

CERKAUSKAS, R. F., and J. B. SINCLAIR. 1980. Use of paraquat to aid detection of fungi in soybean tissues. *Phytopathology* 70:1036-1038.

Soybean stems and pods were surface disinfested, then immersed in a solution of commercial paraquat and incubated for 4 days under continuous light at 25 C. A greater number of lesions with fruiting structures and conidia of *Phomopsis* spp., *Cercospora kikuchii*, and *Fusarium* spp. were formed on plant parts immersed in paraquat than on nonimmersed tissues. There was no increase in the occurrence of *Alternaria*

spp. Nontreated plant parts incubated for an additional 6 days failed to develop lesions in numbers equal to those of the paraquat-treated tissues. Field application of paraquat resulted in greater numbers of pycnidia of *Phomopsis* spp. and acervuli of *Colletotrichum dematium* var. *truncata* on stems of Bonus and Wells soybeans than on nonsprayed plants.

Additional key words: pod and stem blight, purple seed stain, *Diaporthe phaseolorum* var. *sojae*, *Glycine max*, herbicides.

Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride, Chevron Chemical Co., Fresno, CA 93705), an herbicide used as a harvest aid for many crops (7,10,15) acts as a desiccant and defoliant on soybeans; it increases the rate of moisture loss of seeds, desiccates green weeds, and permits an early harvest by increasing the rate of senescence (9). We have shown that an application of paraquat on soybeans reduces seed quality (1) and increases the occurrence of fruiting structures of *Phomopsis* spp. and *Colletotrichum dematium* var. *truncata* on stems of paraquat-treated soybean plants (3). Spraying with Roundup or with a mixture of sodium chlorate:sodium borate (1:1, w/w) did not increase *Phomopsis* or

C. dematium var. *truncata* fruiting structures (3). Paraquat affects the sporulation of certain fungi (5,6,13,14) and increases the ability of certain fungi to colonize plant tissues by altering the outcome of competition with saprophytic fungi (16). Paraquat suppresses spore germination and inhibits mycelial growth and spore production of *Septoria nodorum* on detached wheat leaf segments (6). Paraquat sprayed onto volunteer barley plants in the field increases spore production of *Rhynchosporium secalis* for up to 3 wk after spraying (14). Other herbicides, such as 2,4-D ester and 2,4-D amine, inhibit sporulation of *R. secalis* in agar culture and in barley leaf tissue (13).

Soybean stem tissues may be colonized early in the growing season by *Phomopsis* spp. (*Diaporthe phaseolorum* var. *sojae*) without the production of symptoms or fruiting structures until the

end of the growing season (8,11). We report on the use of paraquat as an aid for the detection of fungi in soybean stem and pod tissues. A portion of the data was reported in abstracts (2,3).

MATERIALS AND METHODS

Laboratory studies. Pods and/or stem pieces were harvested from Amsoy, Bonus, Hawkeye, or Hitatsa cultivars of soybean (*Glycine max* [L.] Merr.) grown in a field plot at the University of Illinois Plant Pathology Research Center at Urbana in 1977 and 1978. Several surface disinfection methods were applied to the tissues to determine if disinfection had an effect on results.

Bonus soybean plants were harvested at random at weekly intervals beginning 40 days after planting (V₈ stage; ie, eight nodes on the main stem) (4) through 81 days after planting (R_{5.5} stage; ie, mid-pod stage). Stem pieces 4 cm long were cut from each plant, washed in tap water for 5–6 hr, immersed in 95% ethanol for 3–4 sec, immersed in 0.05% NaOCl (10% Clorox) for 4 min, and then rinsed in sterile distilled water for 1 min. Twelve randomly selected stem pieces were immersed for 45–60 sec in the paraquat test solution (11.64% paraquat = 1:40 dilution of formulated Paraquat 29.1 L with sterile distilled water and filter sterilized. Twelve nontreated stem pieces served as controls. The treated and nontreated stem pieces were placed separately on moist filter paper

TABLE 1. Effect of paraquat treatment of Bonus soybean stem pieces on subsequent colonization with mycelium of *Fusarium* and *Phomopsis* spp.

Age of stem pieces in weeks after planting (replicates)	Percent of stem surface covered by mycelium of <i>Fusarium</i> and <i>Phomopsis</i> spp. ^a		
	Paraquat ^b	Nontreated	Nontreated + 6 days ^c
6 (7)	67* ^d	4	32* ^d
7 (5)	65*	0	44*
8 (10)	68*	2	0*
9 (13)	86*	8	23*
10 (10)	69*	7	22*
11 (8)	90*	6	48*

^a Means based on 12 stem pieces for each replicate.

^b Stem pieces immersed for 45–60 sec in a 11.64% solution of formulated Paraquat (29.1 L), and incubated for 4 days.

^c Incubated for a total of 10 days (4 days of incubation + 6 additional days).

^d *Differs significantly ($P = 0.05$) from the nontreated control.

‡ Differs significantly ($P = 0.05$) from the paraquat treatment.

(Whatman No. 1) in 9-cm diameter culture plates and kept for 4 days in an incubator programmed for continuous light (800 μ Ein/m²/sec), 100% relative humidity and 25 C. Mycelial growth of *Fusarium* spp. and *Phomopsis* spp. was rated using a scale of 1 to 5, in which 1 = 0 to 5%, 2 = 6 to 25%, 3 = 26 to 75%, 4 = 76 to 95%, and 5 = 96 to 100% of stem pieces covered with mycelium.

Twenty-four pods from Bonus plants in the same field plot were harvested at the same time intervals as for stem pieces and treated as previously described. The mean rating for coverage with mycelium of *Fusarium* and *Phomopsis* spp. and the mean number of lesions caused by *Cercospora kikuchii* (Matsumoto & Tomoyasu) Gardner were recorded. The experiment was done five times.

Twenty-four pods and stem pieces also were harvested as previously described from a field plot of Amsoy and Hitatsa soybeans that had been inoculated with a spore suspension of *C. kikuchii* (5,000 conidia per milliliter). The tissues were surface disinfested as previously described with a 15-min tap water wash, but no ethanol immersion, and incubated as previously described. The total number of lesions on pods and stem pieces caused by the test fungus was recorded after 4 days.

Twenty-four pods and stem pieces also were harvested as previously described from a field plot of Hawkeye soybeans. The tissues were surface disinfested as previously described for Bonus except without the tap water wash and plated and incubated as previously described for the other cultivars. The plant parts were rated for the occurrence of mycelium and spores of *Alternaria* spp., determined by culture characteristics and conidia production after 4 days. The experiment was done five times.

Field studies. Bonus and Wells soybeans at the R_{5.5} (mid-pod) to R₆ (full-pod) stages or at the R₇ stage (eg, 50% of the leaves yellow) were sprayed with commercial paraquat (29.1 L) at 2.34 L/ha. Ten stem pieces were harvested from the mid-region of plants from each of five replications from sprayed and nonsprayed plots at one or more of the following times: 5, 10, 18, 20, 21, 32, or 36 days after spraying. The stem pieces were rated by using a scale of 1 to 5 for the percent stem coverage by pycnidia of *Phomopsis* spp. and acervuli of *C. dematium* var. *truncata* in which 1 = 0%, 2 = trace to 5%, 3 = 6 to 25%, 4 = 26 to 50%, and 5 = more than 50%. The proportion of the stems with 26% or more colonization by pycnidia or acervuli were grouped together for statistical analysis (12).

Paraquat can be used without hazard to operators with precautions to avoid contact of the concentrated solution with the skin and eyes, and care to avoid inhaling spray mist.

TABLE 2. Proportion of Bonus and Wells soybean stems with 26% or more of the surface showing pycnidia of *Phomopsis* spp. and/or acervuli of *Colletotrichum dematium* var. *truncata* from nonsprayed plants or from plants sprayed with paraquat

Cultivar	Plant stage at time of spraying ^a	Sampling time after spraying (days)	Proportion of heavily colonized (>26%) stems ^b	
			Paraquat- sprayed	Nonsprayed
Bonus	R _{5.5} (mid-pod)	21	0.62* ^c	0.02
Wells	R _{5.5}	10	0.72*	0.10
	R _{5.5}	15	0.95*	0.00
Bonus	R ₇ (50% of leaves yellow)	5	0.40*	0.12
	R ₇	10	0.66*	0.20
	R ₇	18	0.66*	0.28
	R ₇	36	0.58*	0.18
Wells-Plot 1	R ₇	5	0.24 NS ^d	0.15
	R ₇	10	0.66*	0.30
	R ₇	15	0.70*	0.28
	R ₇	20	0.88*	0.54
Wells-Plot 2	R ₇	5	0.22*	0.04
	R ₇	10	0.72*	0.28
	R ₇	15	0.96*	0.24
	R ₇	20	0.86*	0.66
	R ₇	32	0.76 NS	0.80

^a Based on the plant growth stages designated by Fehr et al (4).

^b Based on 50 stems per treatment per sampling period.

^c * = differs significantly ($P = 0.05$) from nonsprayed.

^d NS = no significant difference.

RESULTS

Laboratory studies. Immersion of Bonus stem pieces in the paraquat solution resulted in increased numbers after 4 days with mycelium of *Fusarium* and *Phomopsis* spp. (Table 1). When the control stems were incubated for an additional 6 days, the number of stems with mycelium was still significantly below that of the paraquat-immersed stems. Immersion of pods from the same field plot in the paraquat solution significantly ($P = 0.05$) increased colonization by mycelium of *Fusarium* and *Phomopsis* spp. in all experiments. The overall mean ratings of mycelial coverage for paraquat-treated pods for the five experiments was 2.08 and for the control it was 0.21.

In the five experiments the overall mean number of *C. kikuchii* lesions on Bonus pods that had been immersed in the paraquat solution was significantly ($P = 0.05$) higher at 18.94 than the overall mean number for the control (0.68 lesions).

The total numbers of lesions on pods and stems of Amsoy and Hitatsa soybeans inoculated with *C. kikuchii* and immersed in the paraquat solution were 180 and 114, respectively, and significantly ($P = 0.05$) higher than the numbers on pods and stems of the control, which were 21 and 23, respectively.

There were no significant differences in the occurrence of *Alternaria* spp. on Hawkeye pods and stems immersed in the paraquat solution when compared to the control in four of five experiments involving pods and three of four experiments involving stems. The mean rating for paraquat-treated pods was 4.6 and for the control was 4.1. The mean rating for paraquat-treated stems was 5.0 and for the control stems was 4.6.

Field studies. *Phomopsis* spp. produced significantly more pycnidia and *C. dematium* var. *truncata* produced significantly more acervuli on stem pieces of Bonus and Wells soybeans sprayed with paraquat either in the R_{5.5} to R₆ or in the R₇ stages compared to nonsprayed plants at all but two sampling times (Table 2). Stem samples taken from the two separate Wells soybean plots yielded similar results.

DISCUSSION

Paraquat stimulated the appearance of fungal mycelium and induced the formation of lesions and fruiting structures of at least four soybean pathogens on surface-disinfested pods and stem pieces: *C. kikuchii*, *C. dematium* var. *truncata*, *Fusarium* spp., and *Phomopsis* spp. It did not affect a saprophytic *Alternaria* spp. The use of paraquat as a field spray or a laboratory dip induced the formation of these fungal structures about 2 wk before symptoms appeared on nontreated tissues. This may be due, in part, to the inducement of senescence (9). The reduction of the latent period of infection and the enhancement of the formation of fruiting structures of certain soybean pathogens indicates that field-grown soybeans sprayed with paraquat may serve as an important source of inoculum for the following crop. Similar results were obtained with increased spore production by *R. secalis* as a result of paraquat application on volunteer barley plants (14), but is in

contrast to the antifungal activity of paraquat against *S. nodorum* (6).

Detection of infection by these pathogens by dipping surface disinfested tissues in paraquat and then incubating them on moist filter paper is easier and more accurate than plating tissues on an agar medium (11). The technique can be used to assess infection levels, predict potential disease loss, and to decide whether to use a fungicide. The technique also can be used to study the epidemiology of these fungi.

LITERATURE CITED

1. CERKAUSKAS, R. F., S. R. FOOR, and J. B. SINCLAIR. 1978. Effects of paraquat used as a harvest aid on soybean seed quality. (Abstr.) Phytopathol. News 12:85.
2. CERKAUSKAS, R. F., J. B. SINCLAIR, and S. R. FOOR. 1979. The use of paraquat to detect latent colonization by fungi of soybean stem and pod tissues. No. 85 in: Abstracts, World Soybean Res. Conf. II. Westview Press, Boulder, CO.
3. CERKAUSKAS, R. F., and J. B. SINCLAIR. 1979. Effect of three dessicants on soybean stem colonization by *Phomopsis sojiae* and *Colletotrichum dematium* var. *truncata*. (Abstr.) Phytopathology 69:539.
4. FEHR, W. R., C. E. CAVINESS, D. T. BURMOOD, and J. S. PENNINGTON. 1971. Stage of development descriptions for soybean (*Glycine max* L.) Merr. Crop Sci. 11:929-931.
5. GROSSBARD, E., and D. HARRIS. 1976. The action of Gramoxone W and Roundup on cereal pathogens. Meded. Fac. Landbouwwet. Rijksuniv. Gent 41:693-702.
6. HARRIS, D., and E. GROSSBARD. 1979. Effects of the herbicides Gramoxone W and Roundup on *Septoria nodorum*. Trans. Br. Mycol. Soc. 73:27-33.
7. HOLE, C. C., and R. C. HARDWICK. 1978. Chemical aids to drying seeds of beans (*Phaseolus vulgaris*) before harvest. Ann. Appl. Biol. 88:421-427.
8. KMETZ, K. T., C. W. ELLETT, and A. F. SCHMITTHENNER. 1979. Soybean seed decay: sources of inoculum and nature of infection. Phytopathology 69:798-801.
9. OSBORNE, D. J. 1968. Defoliation and defoliants. Nature 219:564-567.
10. SANDERSON, J. F. 1976. Pre-harvest desiccation of oilseed crops. Outlook Agric. 9:21-25.
11. SCHILLER, C. T. 1978. Studies on selected microorganisms associated with soybean seeds and plants and their interactions. Ph.D. Diss., University of Illinois, Urbana-Champaign, 71 pp.
12. SKOL, R. R., and F. J. ROHLF. 1969. Biometry. W. H. Freeman and Co., San Francisco, CA. 776 pp.
13. SKOROPAD, W. P., and W. W. KAO. 1965. The effect of some herbicides on sporulation of *Rhynchosporium secalis*. Phytopathology 55:43-45.
14. STEDMAN, O. J. 1977. Effect of paraquat on the number of spores of *Rhynchosporium secalis* on barley stubble and volunteers. Plant Pathol. 26:112-120.
15. WHIGHAM, D. K., and E. W. STOLLER. 1979. Soybean desiccation by paraquat, glyphosate and ametryn to accelerate harvest. Agron. J. 71:630-633.
16. WILKINSON, V., and R. L. LUCAS. 1969. Influence of herbicide on the competitive ability of fungi to colonize plant tissues. New Phytol. 68:701-708.