A Rapid Technique for Studying Fusarium Wilt of Peas

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ABSTRACT

A laboratory method for studying the host pathogen relationship of pea wilt employing a rotary shaker is described. Wilt symptoms were evident after a 10-day incubation period, and results were similar to those obtained in the greenhouse after 6 weeks. Phytopathology 61:342-343.

Additional key words: Fusarium oxysporum f. *pisi,* race 5, inoculum concentrations, differential hosts.

Resistance of peas (*Pisum sativum* L.) to isolates of *Fusarium oxysporum* Schlecht. f. sp. *pisi* (Linf.) Snyd & Hans. was studied in the laboratory using a technique similar to one described by Wensley & McKeen (7) that saved both time and space.

Seed of test varieties were surface disinfested (5) and germinated in rolled, moist paper towels in an incubator set at 21 ± 1 C. Isolates of *F. oxysporum* f. *pisi* race 1 and 2 were obtained locally; the isolate of race 4 was obtained from W. A. Haglund and was originally from Bolton et al. (1). The culture of race 5 (3) was isolated by the authors from wilt infected peas, which were resistant to races 1, 2, and 4.

All cultures were singlespored before use. Conidial inoculum of *F. oxysporum* f. *pisi* was grown in Kerr's liquid medium (4) in shake culture, for 5 days at 24 C. Mycelium was removed by straining the growth medium through a double layer of cheesecloth. Conidial concn were determined by use of a hemacytometer and diluted to a concn of 1×10^4 conidia/ml (opt for typical disease expression) with sterile water. The adjusted spore suspensions were aseptically distributed in sterile 113-g, wide mouth, French bottles (90 ml/bottle). Two 7-day-old test seedlings were aseptically placed in each bottle with cotyledons just immersed in the spore suspension (Fig. 1). The seedlings were supported by a slit styrofoam plug. The bottles, with seedlings and inoculum, were placed on a New Brunswick Gyrotory incubator shaker adjusted to 100-120 cycles/min. Fluorescent lights provided 800-1,000 ft-c during a 15-hr day. The air temp was 24-28 C. Wilt symptoms were evident 10 days after inoculation. Individual plants were rated for disease severity with a scale of 0-5 (0 = healthy and 5 = dead plant). The mean per cent wilt was calculated from the disease index as described by Ebbels (2). Each test was repeated with three replications/treatment.

Results with this technique were compared with results from greenhouse tests using the same pea cultivars and *Fusarium* isolates. Peas were germinated and grown in autoclaved vermiculite in flats (30 seeds/flat) for 1 week. The seedlings were carefully removed, and their roots dipped in a concd spore suspension (1×10^6 conidia/ml) and replanted in the vermiculite. An additional 50 ml of the same spore suspension was then poured over the vermiculite in each flat. Once every 2 weeks, nutrients were supplied by adding 200 ml of a 1 H (Hoaglund's solution) (6) to each flat. At 6 weeks, wilt symptoms were evident and the plants indexed. This test was replicated twice with 30 plants/treatment.

The differential host responses to the *Fusarium* isolates were similar in the greenhouse and laboratory tests, indicating the value of the incubator-shaker method as a rapid technique of testing host-pathogen relationships in pea wilt (Table 1). The low indices reported on resistant host varieties could result from a lack of uniform resistance in the seed samples used. Also, the low disease index of Winner in the check was due to early yellowing and dieback of the lower leaves of this early maturing cultivar.

The incubator-shaker technique is a useful tool in

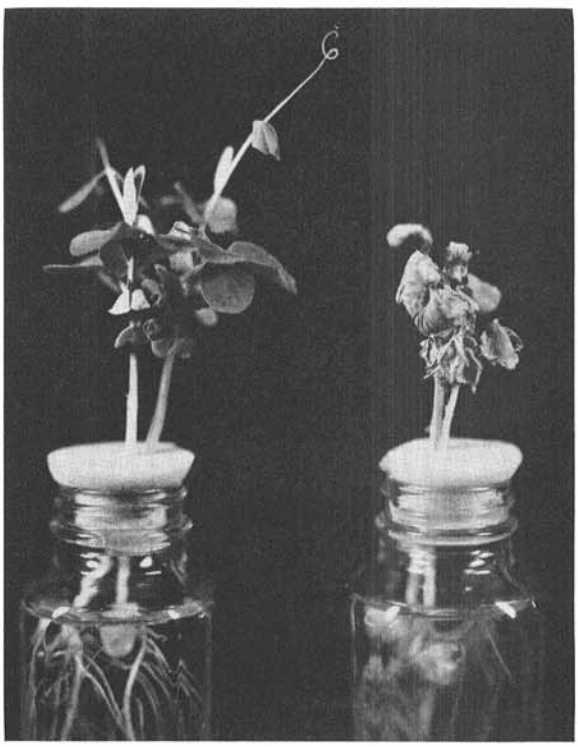

Fig. 1. Two-week-old peas, of the cultivar New Era with roots and cotyledons immersed in a conidial suspension of *Fusarium oxysporum* f. sp. *pisi* race 5 (right), and sterile water (left) after a 10-day incubation on the rotary shaker.

TABLE 1. Reactions of four pea cultivars to four races of pea wilt using the greenhouse and laboratory shaker technique

Pea cv	Resistant to:	Mean % wilt									
		Check		Race 1		Race 2		Race 4		Race 5	
		G	S[a]	G	S	G	S	G	S	G	S
Winner	None	5	2	19	23	95	50	45	40	93	83
W.R. Perf.	Race 1	0	0	1	0	20	17	27	50	100	100
New Era	Race 1, 2	0	0	4	3	0	0	12	50	100	100
New Wales	Race 1, 2, 4	0	0	0	3	0	0	4	10	100	80

[a] G = Greenhouse pathogenicity test results (average of two tests with 30 plants/treatment).
S = Laboratory incubator-shaker test results (average of two tests with three replications of two plants/treatment).

studying the host-pathogen relationships of pea wilt because: (i) results can be obtained rapidly; (ii) results agree with greenhouse tests; and (iii) only a small amount of space is required.

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