CULTURAL CHARACTERISTICS AND A DETERMINATION OF THE DIFFERENT RACES OF *PHYTOPHTHORA FRAGARIAE* HICKMAN IN ILLINOIS

B. G. TWEEDY AND DWIGHT POWELL
University of Illinois, Urbana

INTRODUCTION

Red stele, caused by the fungus *Phytophthora fragariae* Hickman, is an extremely important root rot of strawberries in Illinois. This disease is the cause of as much or more loss to Illinois strawberry growers than any other disease. The disease is capable of complete annihilation of a field or area of strawberry production. In many areas in Illinois, fields have become so heavily infested that farmers can no longer grow strawberries.

Up to this time, no practical method of chemical control for this disease is known, but sources of resistance have been found. The use of resistant varieties is complicated by the fact that there are at least six strains of the pathogen in the United States (personal conversation with R. Converse, U.S.D.A., Beltsville, Maryland). A variety may be completely resistant in one field or area and susceptible in another. Therefore, the knowledge of the different races present in a given area and the prevalence of each race is essential to an adequate breeding program for resistance. Since there are commercially acceptable resistant varieties available, this information is essential in deciding which of the resistant varieties should be planted in a given area.

The purpose of this investigation was to determine the races of the pathogen present in Illinois fields and to study the life habits of the pathogen in an attempt to develop a simpler means of race identification.

Anderson reported the first occurrence of this disease in the United States in 1935, but at that time the disease was well known in England. Wardlaw (1927) reported the disease in England but was uncertain as to its cause. Alcock (1936) was the first to assign the cause of the disease to *Phytophthora*. However, she was unable to isolate the incitant after more than 4,000 attempts. Pathogenicity was proven in a crude manner.

Shortly after the disease was reported in Illinois, it was reported at Federalsburg, Maryland (Bain and Demaree, 1938) and at present it has been reported in most of the important strawberry producing areas in the northern two-thirds of the United States.

Varietal resistance to *P. fragariae* was first reported by Alcock (1929) and has since been reported by many authors. Scott, *et al.* (1940) later demonstrated that there was more than one race of the pathogen. They found Scottish varieties resistant in some soils, but susceptible in others. They also found that Aberdeen va-

rieties were susceptible in soils where the Scottish varieties were resistant. They concluded that at least two strains (strain A and S) were present in Maryland. In a later report (1953) they presented evidence that strawberry fields in Maryland were infested with three races.

McKeen (1958) from Canada, using eight different American and Canadian varieties, presented evidence which indicated that at least six races of the pathogen were present in Canada. He based his results on the relative degree of susceptibility of the host.

Converse, *et al.* (1958) in a recent publication reported the presence of five races, although one was recognized only in the greenhouse. Since the authors have subscribed to the key to races of *Phytophthora* presented by Converse, a reproduction of this key is given in Table 1.

Materials and Methods

Pathogenicity tests were conducted in a temperature controlled room. The temperature was adjusted to approximately 10°C. Twelve hours of illumination were supplied daily using fluorescent bulbs.

Strawberry plants were collected from 43 different locations in Illinois, most of which were large commercial plantings. Eighteen samples of soil infested with red stele were also collected. These collections were used in determining the number of races and the prevalence of each race.

To make isolations, roots were thoroughly washed with tap water, rinsed in sterile water and surface sterilized in 5% clorox solution for five minutes. The root cortex was peeled away and a small piece of the diseased stele was placed on cornmeal agar in a Petri plate and incubated at 20°C. After four days, hyphal tips were transferred to lima bean agar.

Test plants were propagated from runner plants and grown two to three weeks in sterilized soil before being inoculated. The isolates were used to inoculate the host tester varieties, Stelemaster, Vermilion, Blakemore and Md. 683. Artificial inoculation was accomplished primarily by (1) growing healthy test plants in naturally infested soils; and (2) placing mycelial cultures in sterilized soil in which healthy test plants were growing.

Table 1.—Key to the Races of *Phytophthora fragariae* Recognized in Maryland, March 1958.

Races	Varietal response of the strawberry variety or selection to *P. fragariae*. S=susceptible; Sl=slightly susceptible; R=resistant.				
	Blakemore	Md. 683	Aberdeen	Del Norte	Stelemaster
A-1	S	R	R	R	R
A-2	S	S	R		R
A-3	S	R	S	Sl	R
A-4	S	S	Sl	S	R
A-5	S	S	S	R	S

When using naturally infested soil, each soil sample was run through a pulverizer and put into metal trays. Two healthy plants of each test variety were removed from pots in which they had been grown; their roots were washed in sterile water and they were transplanted into infested soil. After an incubation period of two months, the plants were removed and examined for the presence of red stele.

When using mycelial cultures as the source of inoculum, the organism was first grown on lima bean agar slants for two weeks at 20°C. The contents of the tubes were removed and placed in the soil containing the healthy plants. Healthy plants treated with sterile lima bean agar were used as controls.

To study growth characteristics of the different strains, the following media were used:

(1) Finely ground dried lima beans, 100 g (DLB).
Bacto-agar, 20 g.
Distilled water, 1,000 ml.

(2) Frozen lima beans, 150 g (FLB).
Bacto-agar, 20 g.
Distilled water, 1,000 ml.

(3) Rolled oats, 100 g (O.A.).
Bacto-agar, 20 g.
Distilled water 1,000 ml.

(4) Fresh French beans, 150 g (FBA).
Bacto-agar, 20 g.
Distilled water, 1,000 ml.

(5) Bacto-potato dextrose agar, 40 g (PDA).
Distilled water, 1,000 ml.

(6) Bacto-cornmeal agar, 17 g (CMA).
Distilled water, 1,000 ml.

(7) Hemp seed, 3 seeds.
Distilled water, 10 ml.

In preparing formulas 1, 2, 3, and 4, the nutrients were added to the water, boiled slowly for 30 minutes and strained through four to six thicknesses of cheesecloth. Additional water was added to replenish the original volume. The agar was then added and the mixture was reheated until the agar dissolved. After tubing, the agar medium was autoclaved twice at 15 lbs pressure at 121°C for 15 minutes.

Formulas 5 and 6 were mixed and sterilized according to the manufacturer's directions. Formula 7 was prepared by placing three hemp seeds and 10 ml of water in each of several 16 X 150 mm culture tubes and autoclaving for 20 minutes.

Experiments and Results

Testing for the presence of different races. Twenty-three isolates of *P. fragariae* were made from the 43 collections. Each of these isolates was used to inoculate the host tester varieties. Suspensions of zoospores and tube cultures were used as the source of inoculum. Twelve test plants of each variety were inoculated with each isolate.

All of the isolates were pathogenic to Blakemore. None of the checks showed evidence of disease. Of the 23 isolates, four were pathogenic to Vermilion and the remaining 18 were only pathogenic to Blakemore.

Healthy plants were grown in infested soil collected from 16 different fields in Illinois. Most of the soil samples were collected from fields where isolation attempts had been unsuccessful. Two plants of each test variety were transplanted into each soil sample. The soil moisture was kept near the saturation point.

After two months of incubation the roots were examined.

Blakemore was infected in all the soil samples and Vermilion was infected in three of them. One of the soil samples was collected from the same field as an isolate that infected Vermilion. These data show that at least races A-1 and A-3 of *P. fragariae* are present in Illinois fields. Race A-3 is prevalent in several fields in Illinois and may become more important in the future. Race A-1, however, is much more prevalent than race A-3.

Effect of growth of P. fragariae on pH of culture. During the course of the investigation, frequent transfers of the organism had to be made. Cultures often would die within six months after they had been transferred. A determination of the pH of a stock culture showed that after 14 months, the medium on which the organism was growing had changed from 6.8 to 8.2. Several other cultures of various ages were tested and in each case the pH had increased from 0.2 to 1.0 above the original value.

To investigate the change in pH, both dried and frozen lima bean agar media were used. To 500 ml of each medium were added 20 ml of a phosphate buffer consisting of equal volumes of the following solutions: 11.876g $Na_2HPO_4 \cdot 2H_2O$ per liter of distilled water and 9.078g KH_2PO_4 per liter of distilled water. The pH of this solution was 6.8. Several slants of each medium were prepared in 20 x 150 mm test tubes, with and without the addition of a buffer. Transfers were made to the media from one of the isolates collected. Twelve tubes of dried lima bean agar (six with and six without buffer) were treated as the above tubes except they were uninoculated. Very little difference could be seen in the amount of mycelial development on the buffered and unbuffered media. Each month the pH was determined for each medium by the use of a Beckman pH meter. Results of the growing culture on change in pH are given in Table 2.

These data show that the cultures of *P. fragariae* caused the pH of the medium to rise 0.6 in one month and 1.1 in four months. The optimum pH for maximum growth and development of the fungus is 6.5 to 7.2. It is apparent that after one month, pH would be raised to a level adverse to normal growth and development.

Growth responses of five races on different media. The media (see above) were adjusted to pH 6.8 by the addition of a buffer. Eight transfers of each race were made to the different media (contained in Petri dishes). After 10 days of incubation (20°C), radial growth measurements (mm) were taken for each culture (Table 3).

Race 4 was found to grow much better on PDA and CMA than the other races, as the hyphae and aerial mycelium were much more dense. However, mycelial development was not as extensive as when race 4 was grown on the other agar media.

Two of the races were able to survive on hemp seed and water. It appeared to be a particularly favorable medium for race A-3 which developed a large mycelial mass around the seeds. Race 3, on the other hand, grew moderately well but mycelial

TABLE 2.—Effect of the Fungus Culture on pH Change of Medium.

Medium	Buffer	1 month	2 months	3 months	4 months
Dried Limas.....	added	pH 6.8	pH 7.0	pH 7.2	pH 7.3
Dried Limas.....	none	pH 7.4	pH 7.6	pH 7.8	pH 7.9
Frozen Limas.....	added	pH 6.8	pH 6.9	pH 7.1	pH 7.2
Frozen Limas.....	none	pH 7.3	pH 7.5	pH 7.7	pH 7.9
Check 1.....	added	pH 6.8	pH 6.8	pH 6.8	pH 6.9
Check 2.....	none	pH 6.8	pH 6.8	pH 6.8	pH 6.8

TABLE 3.—Growth Response of Different Races of *P. fragariae* on Different Media.

Races 1 through 4 isolated by Hickman and are of English origin. A-3 is an isolate of the race capable of infecting Vermilion. Each number represents an average of the 8 replications.

Race	Media						
	PDA	CMA	FBA	DLB mm growth	FLB	OA	Hemp Seed
1	7	14	21	22	22	24	none
2	7	17	21	21	22	20	none
3	6	12	22	22	23	21	fair
4	12	19	21	23	22	22	none
A-3	4	13	22	21	20	21	good

development was much less extensive in comparison with A-3. There was no visible development of the other three races on this medium. The results of this experiment indicate that even though the nutritional needs of the different races are very similar, it is possible to show distinct differences.

DISCUSSIONS AND CONCLUSIONS

The data presented above indicate that at least two strains of *P. fragariae* are present in Illinois (races A-1 and A-3). Of the two races, A-1 (common race) was found to predominate. One isolate of race A-3 was less virulent than other A-3 isolates, but it did infect several roots. If a greater number of test varieties had been used to differentiate races, perhaps this isolate would have proven to be a different race. However, with the number of test varieties employed in this experiment, it appears to be a subrace of A-3.

Strawberry growers should be concerned primarily with races A-1 and A-3 in Illinois. By using varieties resistant to both races, farmers can be relatively safe from red stele losses. It should be pointed out that collections were made only from a small percentage of the total number of infested fields.

Another important factor in strawberry culture is the importing

of new races on both infested and infected nursery stock. It is very probable that such stock is being shipped in to Illinois at the present time, as evidenced by the large number of one-year-old plantings which have succumbed to red stele.

New mutants of this organism capable of infecting resistant varieties of strawberries may prove a constant threat.

A study of growth reaction on different media was an effort to devise a better system for race identification. This could save time and possibly would be more accurate than the intricate, complicated process of inoculating different host tester varieties. Since different races manifest varietal responses, it seems logical to assume that they would have different nutritional requirements.

The reduced amount of growth of races 1, 2, 3, and A-3 on PDA and CMA media may have been caused by several factors, such as (1) the presence of a toxic element; (2) the absence of an essential element, or (3) an unbalanced ratio of elements. Race 4 grew vigorously enough on these media to be easily distinguished from the other races. Races A-3 and 3 were definitely distinguished from the other races by their growth on hemp seed and water. No distinction was made between races A-3 and 3, nor between races 1 and 2 when grown on hemp seed and water. The possibility of finding differential characteristics by changing the media certainly does exist. Even though this problem has been approached in a preliminary fashion, there is sufficient evidence to warrant more research.

It is apparent from the data in Table 2 that the pathogen causes an increase in pH of the medium. According to Cochrane (1958), "pH is affected during growth by metabolic activities — raised by absorption of anions or production of ammonia from nitrogenous compounds, lowered by the formation of organic acids or absorption of cations." It can only be speculated that the organism either absorbed anions or produced ammonia from nitrogenous compounds presented in the medium. In either case, the addition of a phosphate increased the viability of *P. fragariae* in pure cultures. It was found that by adding 40 ml of a 0.1 N phosphate buffer, the viability of the cultures of *P. fragariae* could be extended from six to 24 months.

Summary

Data are presented which show that at least races A-1 and A-3 of *P. fragariae* are prevalent in Illinois. Race A-1 is more prevalent than A-3. Observations made on diseased fields show that strawberry plants infected and infested with *P. fragariae* are being shipped into Illinois from infected out-of-state nursery stock.

The growth reactions of five different races of *P. fragariae* indicate that nutritional differences exist.

Alkalinity of the medium on which *P. fragariae* was grown increased enough after one month to reduce the rate of growth. The addition of a phosphate buffer (pH 6.8) stabilized the medium and extended the viability of *P. fragariae* cultures from six to 24 months.

Literature Cited

Alcock, N. L. 1929. A root disease of the strawberry. The Gardener's Chronicle, 86:14-15.

————. 1936. The Phytophthora disease of strawberry. Scientific Horticulture, 4:25-56.

BAIN, H. F. and J. B. DEMAREE. 1938. Isolation of the fungus causing the red stele or red core disease of strawberries. Science, 88:151-152.

COCHRAN, V. W. 1958. Physiology of Fungi. John Wiley and Sons, Inc., pp. 524.

CONVERSE, R. H., D. H. SCOTT and G. F. WALDO. 1958. Two additional races of *P. fragariae* Hickman in Maryland. Pl. Dis. Reptr., 42:837-840.

MCKEEN, W. E. 1958. Races of and resistance to *Phytophthora fragariae*. Pl. Dis. Reptr., 42:768-771.

SCOTT, D. H., W. F. JEFFERS, F. M. DARROW, and D. P. INK. 1940. Occurrence of strains of the strawberry red stele fungus *Phytophothora fragariae* Hickman as shown by differential varietal response. Phytopathology, 40:194-198.

————, W. F. JEFFERS, G. F. WALDO, and D. O. INK. 1953. Resistance of strawberry varieties and selections to races of the red stele fungus. Am. Soc. of Hort. Sci., 62:306-310.

WARDLOW, C. W. 192?. Some observations on the cause of Lanarkshire strawberry disease. The Scot. Jour of Agr., 10:8-12.

Manuscript received May 11, 1961.