FACTORS AFFECTING THE IDENTIFICATION OF RACES OF UROMYCES PHASEOLI (PERS.) WINT. VAR. PHASEOLI

by

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INTRODUCTION

Bean rust, caused by Uromyces phaseoli (Pers.) Wint. var. phaseoli, has been a recurring problem in Oregon for many years. In some years damage is severe while in others, such as 1961, little loss was attributed to bean rust. Following the introduction, about 1950, of the variety F.M. 1, the incidence and severity of bean rust decreased. Within a few years, however, this variety was being damaged by the disease. Several workers (6, 10, 13, 19, 33) have established that many races of the fungus are present in the United States. Hikida (15, p. 45) concluded that two races were present in the Willamette Valley of Oregon in 1959-60, both being identified as new races.

The identification of races of U. phaseoli var. phaseoli is based on the reaction of several differential bean varieties to infection by the fungus. The system used at present, as described by Harter and Zaumeyer (13, p. 721) in 1941, utilizes a 0 through 10 reaction scale. Grade 0 denotes immunity and grade 1 a necrotic flecking reaction. Grades 2 through 10 are determined by the relative size of the spore-bearing pustules. Grades 0 and 1 are well defined and very usable, but no distinct pustule size limits have been assigned to grades 2 through 10. Moreover, a collection of all identified races of U. phaseoli var.

phaseoli has not been maintained for comparison with new collections. With no comparative material available it is difficult to determine with consistency the exact numerical grade that should be assigned to a particular host-parasite reaction.

Difficulties encountered in the identification of races of U. phaseoli var. phaseoli indicated the desirability of a revised method of race identification which would be more reliable and easier to use. The principal objective of this study was to develop a revised method for race identification and to investigate some of the factors influencing the host-parasite relationships which could affect the identification of races. Attempts were made to determine the effects of temperature, inoculum density, and source of inoculum, and to compare the reactions of primary and trifoliate leaves to infection.

REVIEW OF LITERATURE

Symptoms of Bean Rust

U. phaseoli var. phaseoli attacks primarily the leaves, but on occasion also the petioles, stems and pods. The first symptoms are small spots on the underside of the leaf which are slightly raised and nearly white in color. Under favorable conditions these appear four to five days after inoculation. The spots enlarge and the leaf epidermis ruptures, exposing rust-colored urediospores within about ten days. The uredia are of various sizes and may be surrounded by a chlorotic halo on some varieties. Sporulation may be slight or abundant, depending on the race of the fungus and variety of host. Black-colored teliospores gradually replace the urediospores near the end of the growing season except in tropical areas where they are rarely observed (32, p. 37).

Urediospores and teliospores are the most commonly observed stages of rust in the field in summer and fall. Aecia may appear in the spring. The aecia are off-white to grayish in color and produce abundant aeciospores.

Life Cycle

U. phaseoli var. phaseoli is an autoecious obligate parasite with the entire life cycle confined to a single host on which are produced stages 0, I, II, and III.

Andrus (2, p. 560) reported the occurrence of the aecial stage in the greenhouse. Milbrath (17, p. 282), Jones (16, p. 809), Eastman (5, p. 60) and Brien and Jacks (4, p. 282) have reported its occurrence in the field. Urediospores and teliospores are produced abundantly in the field in most areas but Townsend (23, p. 25) and Harter, Andrus and Zaumeyer (11, p. 739) reported that teliospores are rarely produced in Florida and California.

Hosts

The principal host of Uromyces phaseoli var. phaseoli is Phaseolus vulgaris L. Arthur (3, p. 296) reported that rust also occurs on P. lunatus L., P. multiflora Willd., P. polystachyus (L.) B.S.P., P. perennis Walt., and P. sinuatus Nutt. Fromme (9, p. 71) reported that additional hosts are P. adenanthus G. Meyer, P. anisotrichus Schl., P. atropurpureus Moc. and Sesse, P. coccineus Jacq., P. disophyllus Beth., P. obvallatus Schlect., and P. retussus Benth. U. S. Department of Agriculture Handbook No. 165 (24, p. 266) reported that P. acutifolius A. Gray and P. angularis (Willd.) W. F. Wright are also hosts for this fungus.

Systems of Rating Susceptibility or Resistance of Varieties

Various systems have been used to determine varietal susceptibility or resistance. Fromme and Wingard (7, p. 8)

classified bean varieties according to their susceptibility to U. phaseoli var. phaseoli under field conditions. Four classes were recognized -- rust-free, rust-proof, rust-enduring and rust-susceptible. A few years later the same authors (8, p. 385-404) modified the earlier method by using a susceptible variety as a standard and expressing the relative susceptibility of other varieties in terms of that standard. They used the average size of the sorus as an index of susceptibility. Wingard (28, p. 38) a number of years later grouped several bean varieties roughly into three classes based on their reaction to infection: (1) varieties that were immune, (2) those exhibiting severe flecking as a result of hypersensitivity, and (3) those on which numerous fertile sori were produced.

Harter, Andrus and Zaumeyer (11, p. 737-759) used an infection rating system of 0 to 10 in testing varieties of beans for resistance and susceptibility with 0 representing immune varieties and 10 the most susceptible.

Wei (25, p. 1091) used a type-0, -1, -2, -3, -4 and -X scale for rating varieties as to resistance or susceptibility. Zero (0) denoted immunity when the fungus was unable to establish itself and which resulted in various sized necrotic spots. Type-1 was used when the uredia were small and were surrounded by necrotic tissue. In type-2 the uredia were 150 to 300 microns in diameter,

with or without faintly perceptible chlorotic rings. Uredia 250 to 500 microns in diameter often associated with a chlorotic halo were typical of type-3 reaction. In type-4 the uredia were large with the production of secondary sori six to eight days after the appearance of the primary sori. Also included was type-X in which more than one type of infection was produced by a single strain of rust on one leaf blade. Types-0, -1 and -X were further subdivided on the basis of size of the necrotic areas.

Races of U. phaseoli var. phaseoli

Fromme and Wingard (8, p. 403) in 1921 reported the probable occurrence of two strains of the bean rust fungus, one strain from the United States with which they were working and one reported from South America which they were unable to obtain for comparison with the United States strain. In 1935 Harter, et. al. (11, p. 737-759) reported two physiologic races of U. phaseoli var. phaseoli. In 1939 Harter (12, p. 9) reported the isolation of 13 distinct physiologic races. Harter and Zaumeyer (13, p. 723) in 1941 proposed a method for race identification and distinguished 20 races. Identification was based on the size of the pustules occurring on the hosts 14 days after inoculation. The eleven grades in the system are outlined below.

Grade 0 - totally immune, no lesions or other evidence of infection.

Grade 1 - necrotic flecks without spores. There is considerable variation in the general characteristics of the flecks on several of the differential varieties, caused by the various races; some are very small and round, while others are angular in shape and vary greatly in size.

Grade 2 differs from grade 1 largely in that, although the sori are small, some spores are produced. The infection centers may or may not be surrounded by a necrotic area. Plants falling into this class are highly resistant.

Grades 3 to 10 are differentiated on the basis of the size of the spore-bearing pustules. Grades 3, 4, 5, and to a less extent grade 6 are regarded as commercially resistant, and higher grades up to 8 are regarded as possessing some degree of tolerance.

Harter and Zaumeyer state that very close agreement among race determination experiments must not always be expected inasmuch as environmental conditions are known to influence the degree of infection, and mesothetic types sometimes confuse the results. Several tests may be required, but usually only one or two are necessary according to the authors.

Using the system of identification devised by Harter and Zaumeyer, Fisher (6, p. 104) identified races 21 through 30, Sappenfield (19, p. 282) race 31, and Zaumeyer (33, p. 460) race 32. Hikida (14, p. 388) identified race 33 and Goode (10, p. 691) race 34.

Environmental Factors Affecting Host-Parasite Relationships

A. Effects of Temperature

Wei (25, p. 1093-1095) found that in varieties of beans which are either highly susceptible or highly resistant to a given race of rust, temperature does not influence the type or amount of infection. However, in the case of mesothetic hosts which are intermediate in their response, various temperatures brought about changes in the type of infection. The normal pustule type occurred around 20°C which Wei feels is optimum for the host. Larger pustules were formed at temperature of 16° and 28°C indicating increased susceptibility.

Schein (21, p. 486-488) described the occurrence of necrotic spots in place of rust pustules on Pinto 111 when the plants were grown for seven days at 32°C. Large rust pustules normally develop on this variety. Schein did not indicate the race of bean rust used in the experiments.

From other experiments he reported that the optimum day-night temperature for disease development was 27° and 21°C. This is in contrast to the findings of Sempio (22, p. 48-49) who reported that 19-20°C was optimum for disease development. Harter, et. al. (11, p. 757) obtained best infection at about 17°C and best germination of urediospores at 14.5°C.

Schein (20, p. 674-680) reported that day-night

temperatures of 80-70°F in controlled environment rooms were optimum for bean rust development on the variety Pinto 111. Higher and lower temperatures retarded first expression of symptoms and also the rate of symptom development. He also observed that a temperature regime of 90°F during the day and 80°F at night prevented symptom expression. The fungus remained alive for ten days and resumed development at lower temperatures. A local necrosis developed when infections of certain ages were exposed to a continuous temperature of 90°F for five to seven days. Infections 96 and 120 hours old developed the local necrosis, those 72 hours old were killed and those over 120 hours old became static without evidence of local necrosis. The threshold for the local necrosis reportedly lies between 72 and 96 hours. When exposed to 90°F during the threshold period, the host-parasite interaction was affected adversely in proportion to the time it endured the condition. A 4-hour endurance delayed attainment of full sporulation by about 1 day; 8 and 15 hours exposure caused a 2-day delay; and 24 hours, a 3-day delay.

Sempio (22, p. 48-49) reported that temperatures of 34° to 36°C for two and one half days killed well developed mycelium in the leaves without appreciable injury to the plant. The treatment was applied four days after inoculation.

B. Effects of Light

Wei (25, p. 1104) found that light was essential during the infection period for successful entrance of the fungus. Reduction in light intensity increased the incubation period and if sufficiently prolonged induced necrosis in the largest type infection (type-4). Lower light intensity increased the proportion of type-3 infections on X-type (mesothetic) hosts.

C. Effects of Host Nutrition

Wei (25, p. 1097-1099) reported that excess nitrogen increased the amount of infection per unit area while nitrogen deficiency decreased the amount of infection. Low potassium levels resulted in more infection while high levels reduced infection. He reported that the effect of phosphorus was not clear. Wei reported that the nutrient supply does not change the type of pustule on resistant and very susceptible varieties but that a low N-K ratio may increase the number of type-3 pustules on a mesothetic host. He also observed that anything that hastened senescence encouraged the development of established infections but reduced the number of new infections.

D. Effect of Host Age

Wei (25, p. 1104) reported that aging of host tissue generally reduced the amount of infection. The type of infection was not materially affected on most varieties, but

the proportion of type-3 pustules was increased on the mesothetic host until it replaced the normal type-X pustule.

Effects of Inoculum Concentration

Petersen (18, p. 607-614) found that as the concentration of wheat rust urediospores in the inoculum preparation was increased, the number of uredia produced per cm^2 of leaf surface increased linearly to a maximum of 0.7 per 100 viable urediospores. There was a distinct tendency for the reaction class observed at low inoculum densities to assume the characteristics of less susceptible infection types with high inoculum densities. The differences in degree of chloronemia were insignificant among inoculum densities. Yarwood (30, p. 540) also reported that when adequate dosages of urediospores of U. phaseoli were placed on sunflower leaves before inoculation of the leaves with Puccinia helianthi, or along with the inoculum, the sunflower leaves were protected from infection by P. helianthi. Similarly, spores of P. helianthi protected bean leaves from infection by U. phaseoli.

Yarwood (29, p. 376) observed that established bean rust infections reduced later infections by the same race of rust in an area up to 50 mm beyond the first infection. The reduction was in number but not size of pustules. The greater the age of the first infection, the greater the area of inhibition of the second. Based on other

experiments, Yarwood concluded that the local immunity was at least in part due to a gas formed by the rust fungus which was toxic to rust spores. He concluded that the effect was on the infection process, not on pustule development. In another paper Yarwood (31, p. 24) reported that with increasing concentration of bean rust urediospores, the percentage of the spores that germinated decreased but the length of the germ tubes increased. The results were based on the concentration of spores per unit area of leaf or per unit volume of agar substrate. Stimulation of germ tube growth was apparent throughout most of the germination period. Spore germination was greater at 25°C than at lower temperatures. This contrasts with the results of Harter, et. al. (11, p. 757) who obtained the best germination at 14.5°C.

Yarwood (29, p. 374-377) reported that, on water agar in closed petri dishes, there was a 3.35-fold increase in urediospore germination when the number of spores was reduced from 19,000 to 1,100 spores per cm^2.

Wilson (27, p. 596) in similar experiments found more than a 4-fold increase in germination when spore numbers were reduced from 43.5×10^4 (435,000) to 6.66×10^4 (66,600) per cm^2 at temperatures of 16°, 19.5°, and 22.0°C. In other experiments with urediospores of bean rust and wheat rust, inhibition through increased numbers of

urediospores was not observed.

Allen (1, p. 259) reported that the rate of germination of urediospores of Puccinia graminis f. tritici was inversely related to the quantity of spores present. Solutions on which urediospores had been floated for a few hours or more contained a substance or substances highly active in preventing the germination of other urediospores.

Wilson (26, p. 229) found that aqueous diffusion products from urediospores, sprayed on bean leaves, protected against rust infection. Results showed an 81 percent control of rust at the highest concentration of urediospore diffusion products. Wilson concluded that the diffusion products inhibited germination of urediospores.

Wilson (27, p. 595) determined by quantitative chromotography that aspartic and glutamic acid were present in the urediospores and tissues surrounding the pustules in quantities sufficient to account for the inhibition of urediospore germination. A gradient with the highest concentration nearest to the sorus was found in the tissue surrounding the sorus. The two amino acids protected against reinfection of tissue close to the rust pustule. By lowering the temperature of incubation to 16°C there appeared to be little, if any, protection against reinfection due to a preceding infection. Based upon the results of several experiments, Wilson concluded that the temperature and humidity at which the urediospores are produced

apparently influence the phenomenon and that in some cases the spores contain little or no inhibitor of germination. Similar results were obtained with wheat rust spores.

In determining the effect of temperature on the germination of inhibitor-depleted and inhibitor-containing spores, Wilson found that with spores of the same harvest the optimum temperature was 20°C for germination of inhibitor-depleted spores and 16°C for inhibitor-containing spores. He reported no significant variation in optimum temperature for germination of inhibitor-containing spores from one harvest to another, but there was a difference in percentage germination obtained.

METHODS, MATERIALS AND RESULTS

Inoculum Preparation

Urediospores of U. phaseoli var. phaseoli which were originally collected by Hikida (15) and identified as race 33 were increased on variety No. 650 and used as inoculum throughout these investigations. Ivory soap solution served as the basic spore carrier in all experiments. The 125 ppm soap solution was prepared by adding powdered bar Ivory soap to water and mixing in a Waring Blendor. When large quantities of inoculum were needed, 0.8 ml of a 0.1 percent Triton B1956 solution and 1.2 ml of a 1.0 percent sodium carboxymethylcellulose (CMC) solution were added to each 100 ml of the soap solution. The Triton served as a wetting agent and CMC insured an even distribution of spores throughout the preparation by slowing the settling of the rust spores. Urediospores were added to the preparation until the desired concentration was obtained as determined with a hemocytometer. The inoculum suspension was shaken repeatedly during inoculation procedures.

Inoculation Technique

Mass inoculations were made by spraying ten ml of the spore suspension on the primary leaves of five bean plants. Ten ml wet the leaves thoroughly without appreciable run-off. The inoculum was applied with an electrically driven

portable pressure-vacuum pump and a GXO artist sprayer nozzle. The nozzle was held approximately ten inches from the plants. The can containing the plants was rotated by hand and the inoculum applied as evenly as possible to both upper and lower surfaces of the leaves.

Immediately after inoculation, the plants were placed in a moisture chamber equipped with several spray nozzles (.85 80A DeLavan, Des Moines). The mist was diverted to prevent it from settling directly on the plants. After 16 to 18 hours in the moisture chamber the plants were moved to a greenhouse bench. No supplemental light was used inside the moisture chamber and no attempt was made to modify the temperature inside the chamber from that of the greenhouse.

Determination of Mean Pustule Size

The system of race identification developed by Harter and Zaumeyer (13, p. 721) was difficult to use. Pustule dimensions were not available with which to compare pustules developed on the differential varieties. As a consequence, the assignment of a grade to a particular-sized pustule has been left entirely to the judgment of the worker.

An experiment was designed to determine the average size of the pustules formed by race 33 on the various differential varieties. Hikida (14, p. 388) determined that

race 33 caused grade 10 pustules on several varieties and grade 2-3 on another differential variety. Thus, the complete range of pustule sizes should be present in these experiments. Wei (25, p. 1091) indicated that pustules of 500 microns or larger were observed on some varieties. In some preliminary measurements it was found that pustules larger than 500 microns were present on one or more varieties infected by race 33.

Nine bean seeds of each variety were planted one inch deep in number 10 cans containing a mixture of six parts sandy loam and two parts peat moss. This same mixture was used throughout the greenhouse studies. The stand was thinned to five plants per can at the time of inoculation. Four replicates of each treatment were used, arranged in four randomized blocks. Each replicate was composed of five plants in a number 10 can. Inoculum suspensions containing 20,000, 40,000 and 80,000 spores per ml were used to inoculate the primary leaves.

Fourteen days after inoculation a primary leaf was taken from each plant and the five leaves from each replicate were placed in a small plastic bag and stored at 36°F until data were taken. The diameters of ten pustules on each leaf were measured with an ocular micrometer. To prevent possible error induced by cold storage the data from all leaves in a block were taken before starting on

the next replicate. The diameter of the actual eruption through the epidermis was measured rather than the diameter of the mass of spores protruding from it (Figures 1 and 2). The masses of protruding spores were removed by blowing them from the surface of the leaf. All measurements were made on the upper surface of the leaf except for variety No. 643, on which the sori develop only on the lower leaf surface.

The data were analyzed by the analysis of variance method.

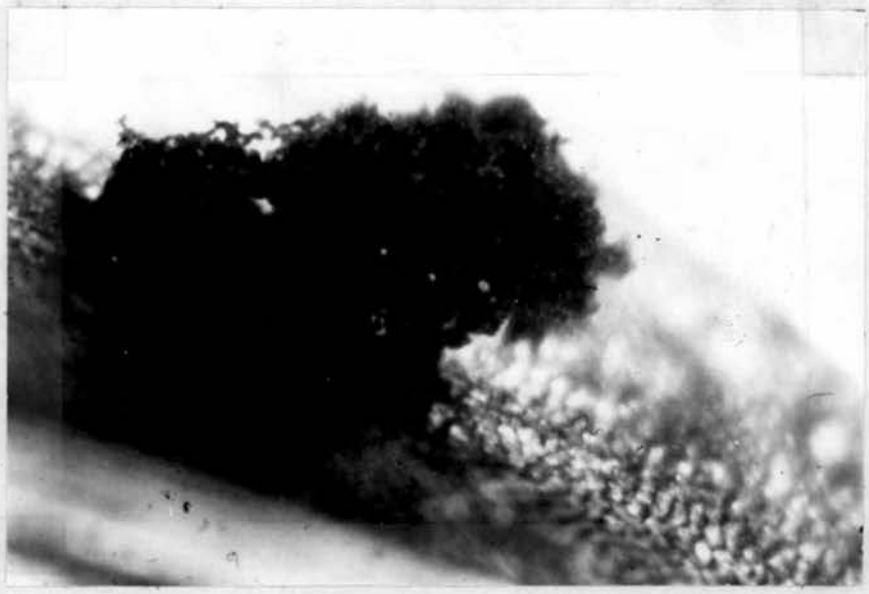


Figure 1. A mass of urediospores protruding from a sorus.

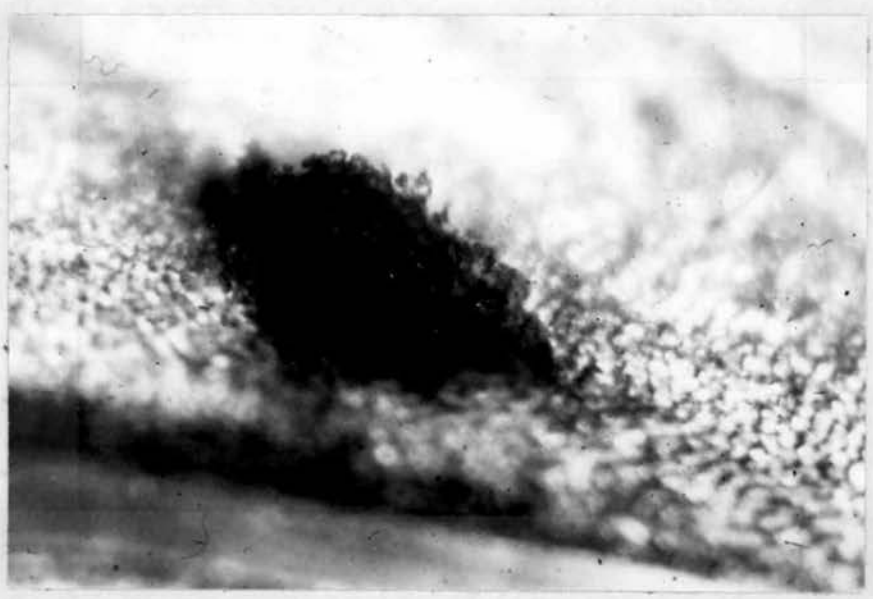


Figure 2. The same sorus after the mass of urediospores was removed.

Methods for Determining Reaction Grades with Pictures and Spot Cards

The measurement of pustules requires considerable time, especially when large numbers of plants are involved, and a more rapid yet accurate method of grading pustules would be useful. Two methods were developed and tested. In the first, natural-size pictures of infected leaves were used to compare with specimens to be graded. The method was satisfactory, yet unsuitable because several pictures must be available and each must be compared with each sample. The second method utilized photographs of black spots approximately 300 and 500 microns in diameter. The pictures were prepared by photographing Craf-Tone Design No. 81 at the magnifications required to obtain negatives with spots of the desired diameters. Printed cards were than prepared (Figure 3). The validity of the method was tested twice in replicated experiments. The experiment was composed of four replicates of each differential variety with a replicate made up of five plants in a number 10 can. Data were obtained by comparing the spot card with one primary leaf on each of five bean plants in each replicate.

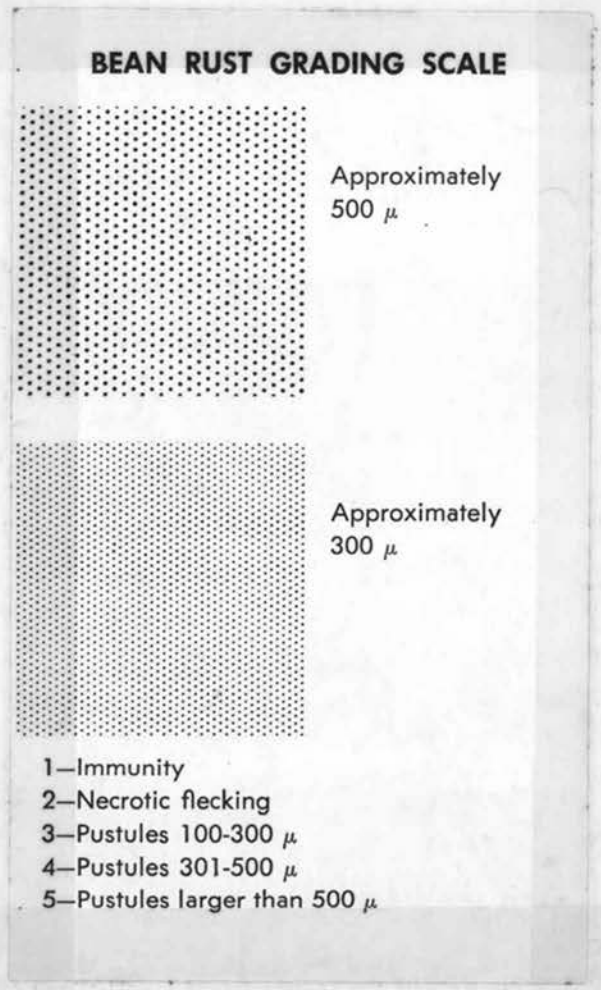


Figure 3. Bean rust grading scale used in determining appropriate grades of host-parasite reaction.

Results of Experiments to Determine Average Size of Pustules

Mean pustule diameter on each variety varied somewhat among replicates (Figure 4). The greatest variation appeared on variety No. 765 where the mean was 187 microns for one replicate and 316 microns for another. The smallest variation was on variety No. 643 where the mean for one replicate was 208 microns and 153 microns for another. Variations were great enough to extend over two to four grades in the Harter-Zaumeyer system of race identification (Figure 3, Table 4). Thus, if only a few plants and replicates are used, erroneous conclusion may be drawn.

Since Harter and Zaumeyer have not specified pustule dimensions for each of the grades in their system, attempts were made to determine by measurement the probable pustule dimensions intended using race 33 of U. phaseoli var. phaseoli. Hikida (14, p. 388) reported the grades of infection that race 33 produced on seven differential varieties (Table 3). The average pustule sizes on the differential varieties are presented in Table 1. The analysis of variance of the experiment is shown in Table 2.

Figure 4. Variation in pustule size and grades: U. phaseoli var. phaseoli, race 33.

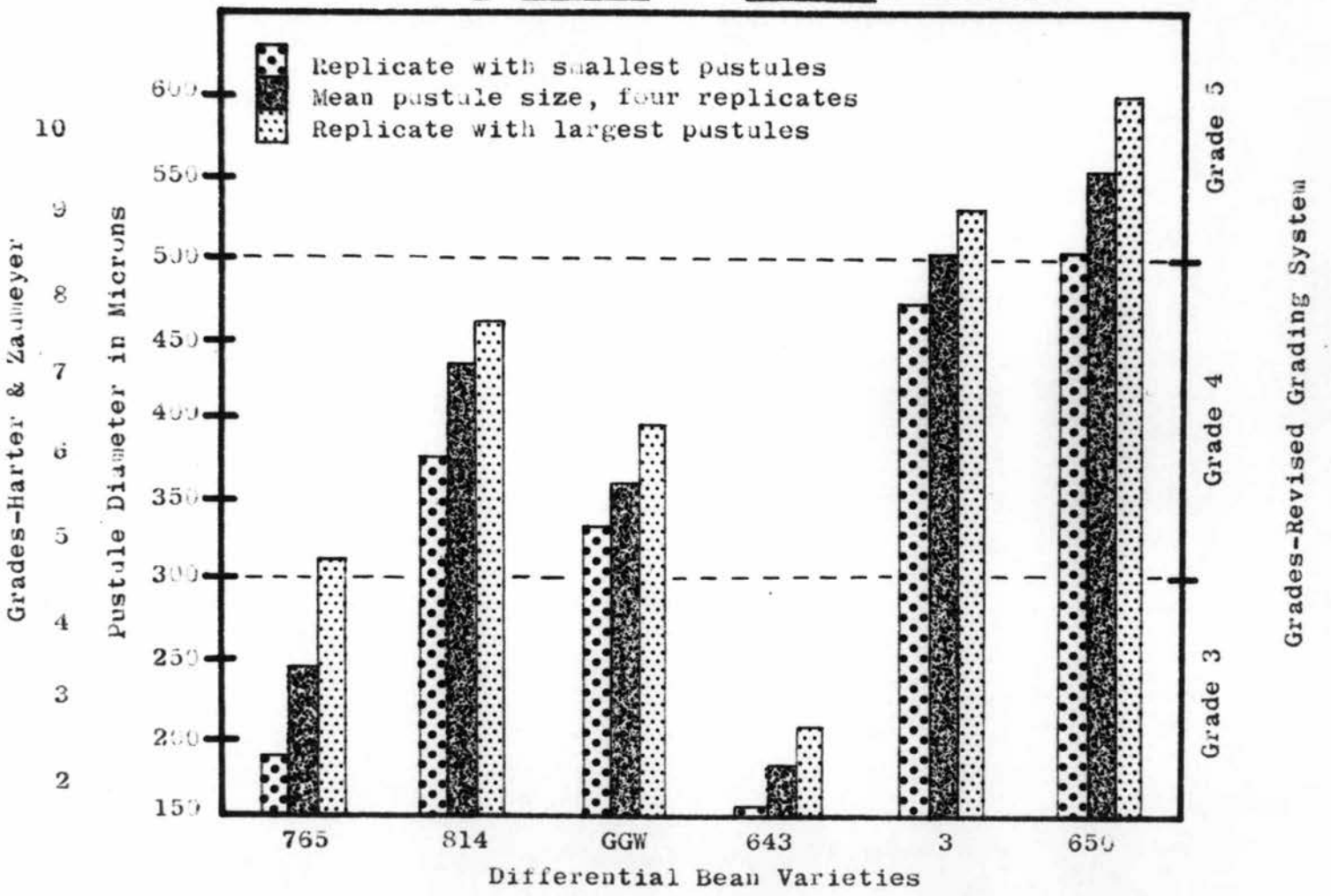


Table 1. Average size of pustules caused by race 33 of *U. phaseoli* var. *phaseoli* on differential varieties.

Differential Variety No.	Spore Concentration (000)	Average Pustule Size Per Concent.	Average Pustule Size For Variety
765	20	246.92	
765	40	236.93	247.49
765	80	258.61	
814	20	440.30	
814	40	434.77	438.38
814	80	440.08	
GGW(1)	20	370.17	
GGW	40	368.90	365.07
GGW	80	356.15	
650	20	569.50	
650	40	535.71	557.03
650	80	565.88	
U.S. 3	20	501.50	
U.S. 3	40	510.63	502.84
U.S. 3	80	496.40	
643	20	162.14	
643	40	184.24	182.82
643	80	202.09	

(1) Golden Gate Wax LSD. 5% - 41.91u LSD. 5% - 24.19u
1% - 55.88u 1% - 32.26u

Table 2. Analysis of variance of experiment to determine average pustule size.

Source of Variation	D.F.	Sum of Squares	Mean Square	F	F Required
Replications	3	153.40	51.13	2.12	2.79 4.20
Varieties	5	35,367.45	7073.49	293.75	2.40 3.41
Concentration	2	21.55	10.78	0.45	3.18 5.06
Var. X Conc.	10	196.45	1.96	0.08	2.02 2.70
Error	51	1,227.83	24.08		
Leaves	288	1,950.72	6.77	0.28	1.48 1.76
Pustules	3240	10,292.30	3.18	0.13	1.44 1.68

Table 3. Grades of infection produced by race 33 on differential varieties.

Differential Variety	Grade
No. 643	2-3
U.S. No. 3	10
No. 650	10
No. 765	4
No. 814	8
No. 780	10
Golden Gate Wax	9

In the present studies, the range of pustule dimensions for race 33 on differential hosts varied from about 150 microns to a maximum of about 600 microns. The same results were obtained in several similar experiments. Table 4 illustrates the probable pustule dimensions for the Harter-Zaumeyer system as estimated on the basis of the known variations in pustule diameter for race 33.

Table 4. Probable pustule dimensions for Harter-Zaumeyer grading system.

Grade	Diameter of Pustules in Microns
0	Immunity
1	Necrotic flecking, no sori
2	150 to 199
3	200 to 249
4	250 to 299
5	300 to 349
6	350 to 399
7	400 to 449
8	450 to 499
9	500 to 549
10	550 up

The grades are based on 50 micron units and consistent separation of grades on the basis of such a small differences is very difficult. This was demonstrated when a group of Oregon State University plant pathologists and graduate students graded a sample set of beans infected by race 33. Little agreement among the individuals was found.

A revised system for race identification that reduced the number of grades from 11 to 5 and increased the range of pustule size in each grade proved very successful with regard to uniformity of results obtained by different workers and also to accuracy of race identification.

Grade 1 - totally immune, no necrosis or other evidence of infection.

Grade 2 - necrotic flecking without sori or spores. The necrotic spots may be of various sizes and shapes depending upon the host-parasite reaction.

Grade 3 - pustules with a mean diameter of less than 300 microns.

Grade 4 - pustules with a mean diameter of 301 to 500 microns.

Grade 5 - pustules with a mean diameter of 501 microns or larger.

Grades 1 and 2 do not differ from grades 0 and 1 of Harter and Zaumeyer (13, p. 721) except in designation (Figure 5, Table 5). These grades were raised numerically because in statistical analyses a zero (0) should not be used. Grade 3 encompasses grades 2, 3, and 4 of the

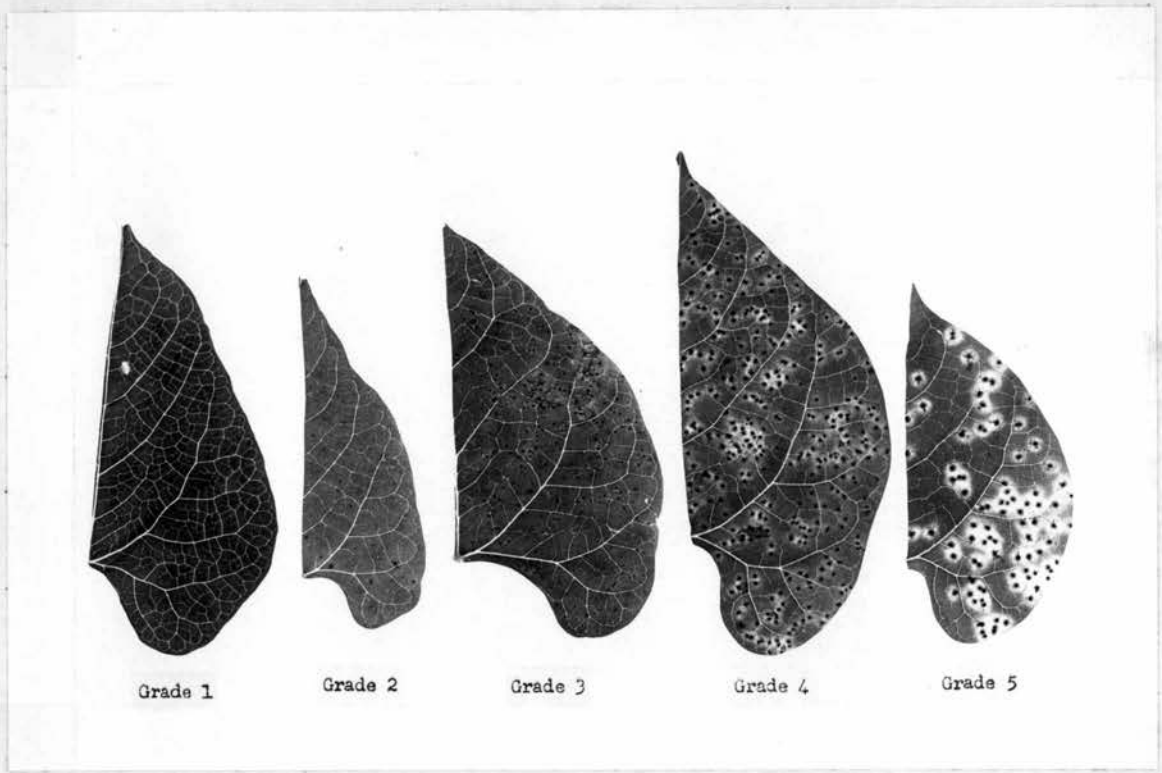


Figure 5. The five grades of the revised system for race identification.

Table 5. A comparison of the Harter-Zaumeyer system and revised system.

HARTER-ZAUMEYER SYSTEM 1941		REVISED SYSTEM 1962	
GRADE	DESCRIPTION	GRADE	DESCRIPTION
0	Immunity	1	Immunity
1	Necrotic Flecking	2	Necrotic Flecking
2	Small Sori, Few Spores		
3		3	Pustules less than 300 microns in diameter
4			
5			
6	Differentiated on basis of size of spore bearing pustules	4	Pustules 301 to 500 microns in diameter
7			
8			
9			
10		5	Pustules larger than 500 microns in diameter

Harter-Zaumeyer system while grade 4 includes the previous grades 5, 6, 7 and 8. Grade 5 combines grades 9 and 10.

When grades of the 34 described races are converted to the new values the integrity of each race is maintained (Table 6, 7). In no case was any race identical with another. The data in Table 6 are from references 6, 10, 13, 14, 19, and 33.

On occasion it may be desirable to grade rust specimens by actual measurement. Table 8 presents the standard error for various sample sizes as calculated from this investigation. As the number of pustules, leaves or replications are reduced the possible error increases. The sample sizes, therefore, depend on the degree of accuracy desired.

Table 6. Infection grades produced by various races of *U. phaseoli* var. *phaseoli* on differential bean varieties (Harter-Zaumeyer system).

	Differential Varieties										
Race	U.S. No. 3	No. 181	No. 643	No. 650	No. 765	No. 780	No. 814	(3) GGW	Z4	Bounti-ful	Pinto
1	2	8	9	10	1	1-2 (2)	1-2				
2	10	8	8	10	2	1	0				
3	9	8	2	10	2	1	9				
4	2	8	1	10	2	2	9				
5	10	8	9	10	2	1-2	10				
6	5-6 (1) / 3	8	9	10	2	2	9				
7	7 (1) / 3	8	9	10	2	4	9				
8	5 / 3	9	1	10	1	2	9				
9	10	9	9	10	5-6	2-3	10				
10	3	9	1	2	1	1	0				
11	10	8	9	10	6	6	10				
12	10	8	10	10	2	3	0				
13	10	8-9	9	10	9	10	9				
14	2	8	8	10	4-5	9	10				
15	10	8	5-6	10	2	2	9				
16	5	8	9	10	4 / 2	9	9				
17	10	7	1	10	5	1	0				
18	2	7	8	10	2	5	8				

Table 6 (Continued)

	Differential Varieties										
Race	U.S. No. 3	No. 181	No. 643	No. 650	No. 765	No. 780	No. 814	(3) GGW	Z4	Bounti-ful	Pinto
19	10	8	9	10	4	4/2	8				
20	9/7	8	9	10	5/2	9	9				
21	2	7	8	10	1	2	7				
22	2	6	8	10	4	6	1				
23	6	7	8	10	7	10	2				
24	3	6	3	10	4	2	7				
25	10	7	8	10	2	10	10				
26	2	4	5	10	2	10	10				
27	2		1	10	2	10	8	0	8		
28	0		0	0	1	2	0	0	10		
29	8		0	10	1	8	8	8	8		
30	2		9	10	3	9	8	0	7		
31	5-8		10	10	8	2	2	2			
32	1/3		0	-	0	1	0	3-4/10		10	0
33	10		2-3	10	4	10	8	9			
34	2		1	-	1	1	0	8		10	4-5
35	10		6	10	4	10	8	9			
36											

(1) Numerator designates grades occurring on upper leaf surface, denominator designates grade occurring on lower leaf surface.

(2) Indicates pustules of 2 sizes (2 grades) occurring on a leaf.

(3) GGW - Golden Gate Wax

Table 7. Infection grades produced by various races of U. phaseoli var. phaseoli on differential bean varieties (Revised system).

	Differential Varieties										
Race	U.S. No. 3	No. 181	No. 643	No. 650	No. 675	No. 780	No. 814	(3) GGW	Z4	Bounti-ful	Pinto
1	3	4	5	5	2	2-3 (2)	2-3				
2	5	4	4	5	3	2	1				
3	5	4	3	5	3	2	5				
4	3	4	2	5	3	3	5				
5	5	4	5	5	3	2-3	5				
6	4 (1) 3	4	5	5	3	3	5				
7	4 3	4	5	5	3	3	5				
8	4 3	5	2	5	2	3	5				
9	5	5	5	5	4	3	5				
10	3	5	2	3	2	2	1				
11	5	4	5	5	4	4	5				
12	5	4	5	5	3	3	1				
13	5	4-5	5	5	5	5	5				
14	3	4	4	5	3-4	5	5				
15	5	4	4	5	3	3	5				
16	4	4	5	5	3	5	5				
17	5	4	2	5	4	2	1				
18	3	4	4	5	3	4	4				

Table 7. (Continued)

	Differential Varieties										
Race	U.S. No. 3	No. 181	No. 643	No. 650	No. 765	No. 780	No. 814	(3) GGW	Z4	Bounti-ful	Pinto
19	5	4	5	5	3	3	4				
20	5/4	4	5	5	4/3	5	5				
21	3	4	4	5	2	3	4				
22	3	4	4	5	3	4	2				
23	4	4	4	5	4	5	3				
24	3	4	3	5	3	3	4				
25	5	4	4	5	3	5	5				
26	3	3	4	5	3	5	5				
27	3		2	5	3	5	4	1	4		
28	1		1	1	2	3	1	1	5		
29	4		1	5	2	4	4	4	4		
30	3		5	5	3	5	4	1	4		
31	4		5	5	4	3	3	3			
32	2/3		1	-	1	2	1	3/5		5	1
33	5		3	5	3	5	4	5			
34	3		2	-	2	2	1	4		5	3-4
35	5		4	5	3	5	4	5			
36											

(1) Numerator designates grades occurring on upper leaf surface, denominator designates grade occurring on lower leaf surface.

(2) Indicates pustules of 2 sizes (2 grades) occurring on a leaf

(3) GGW - Golden Gate Wax

Table 8. Standard error for various sample sizes.

No. Pustules Measured/Leaf	No. Leaves	No. Reps.	Standard Error in Microns
10	5	4	14.8
7	5	4	15.2
5	5	4	15.7
4	5	4	16.2
2	5	4	18.3
5	2	4	19.6
5	1	4	24.7
5	5	2	22.3
3	3	3	22.3

Reactions of Primary and Trifoliate Leaves

Harter and Zaumeyer (13, p. 720) stated that they used primary leaves in their studies because these were ready for inoculation seven to ten days earlier than trifoliate leaves and gave the same results. Schein (20, p. 675) used trifoliate leaves in temperature experiments because primary leaf reactions were not typical of mature leaf reactions. He did not report how the reactions differed.

Because of Schein's report, a comparison of the reactions of race 33 on primary and trifoliate leaves appeared worthy of investigation. In addition, the revised grading system is based on the reaction of primary leaves to infection by U. phaseoli var. phaseoli and the reaction of trifoliate leaves should be determined.

Two sets of differential varieties were planted. Each set consisted of four replicates of each variety with each replicate consisting of five plants of a variety in a number 10 can. One set was planted three weeks before the other to obtain plants having trifoliate leaves when the other set had primary leaves. All plants were inoculated at the same time with a suspension containing about 20,000 spores per ml. The usual inoculation and incubation procedures were followed. After the moisture period, they were grown at the day-night temperatures of 80° - 65°F.

Data were taken two weeks after inoculation using the spot-card method.

Observed Reactions of Primary and Trifoliate Leaves

In general, on the varieties No. 650, U. S. No. 3 and No. 780, the rust pustules produced by race 33 on young trifoliate leaves were the same size as those produced on primary leaves (Figure 6, Table 9). However, on those trifoliate leaves which were fully expanded at the time of inoculation the resulting pustules were smaller. On the varieties Golden Gate Wax and No. 814, pustules produced on the primary and older trifoliate leaves were of the same size, while those on the expanding trifoliates were slightly larger (Figure 7, Table 9). No difference in size of pustule was observed on variety No. 643 although the chlorotic halo surrounding pustules on the trifoliate leaves was wider than on the primary leaves. On variety No. 765 there was more necrotic flecking on the trifoliate leaves than on primary leaves, but the pustules were the same size. The reaction of primary and trifoliate leaves of several bean hybrids tested for resistance to U. phaseoli var. phaseoli varied with individual plants indicating that genetic constitution also affects the host-parasite reaction (Table 10). In some cases the reactions observed were the same on primary and trifoliate leaves

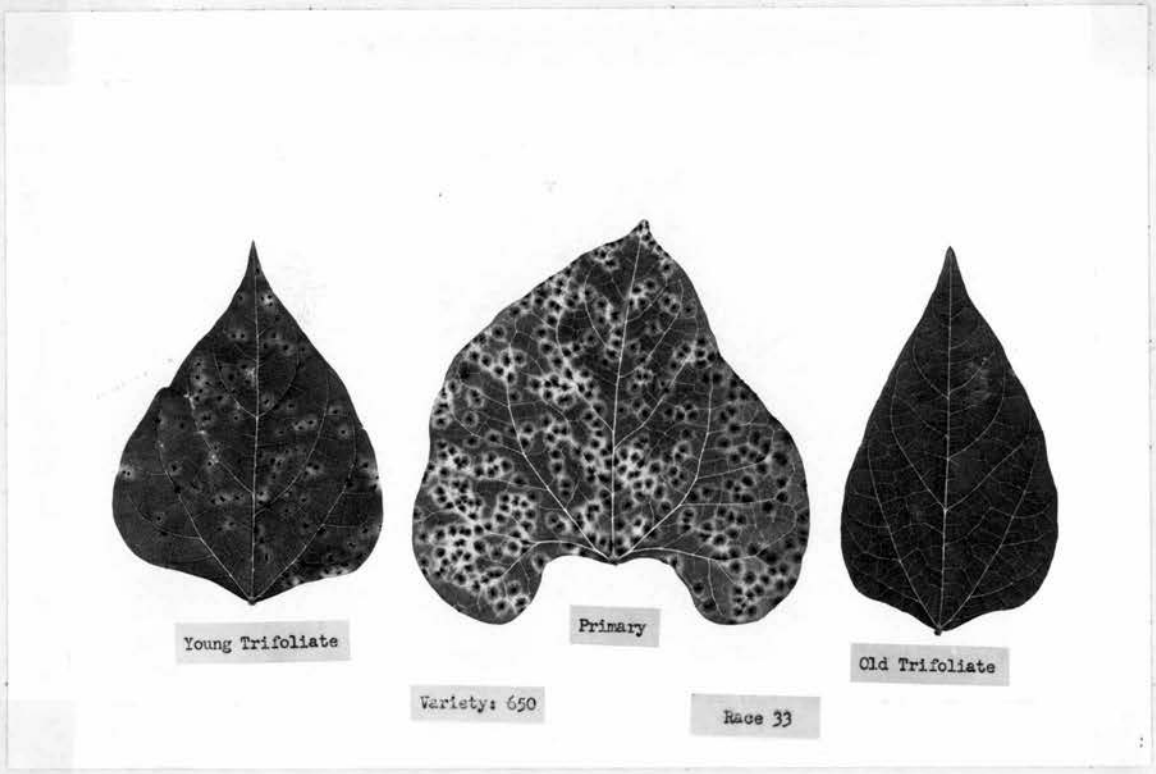


Figure 6. Effect of age of leaf on pustule size.

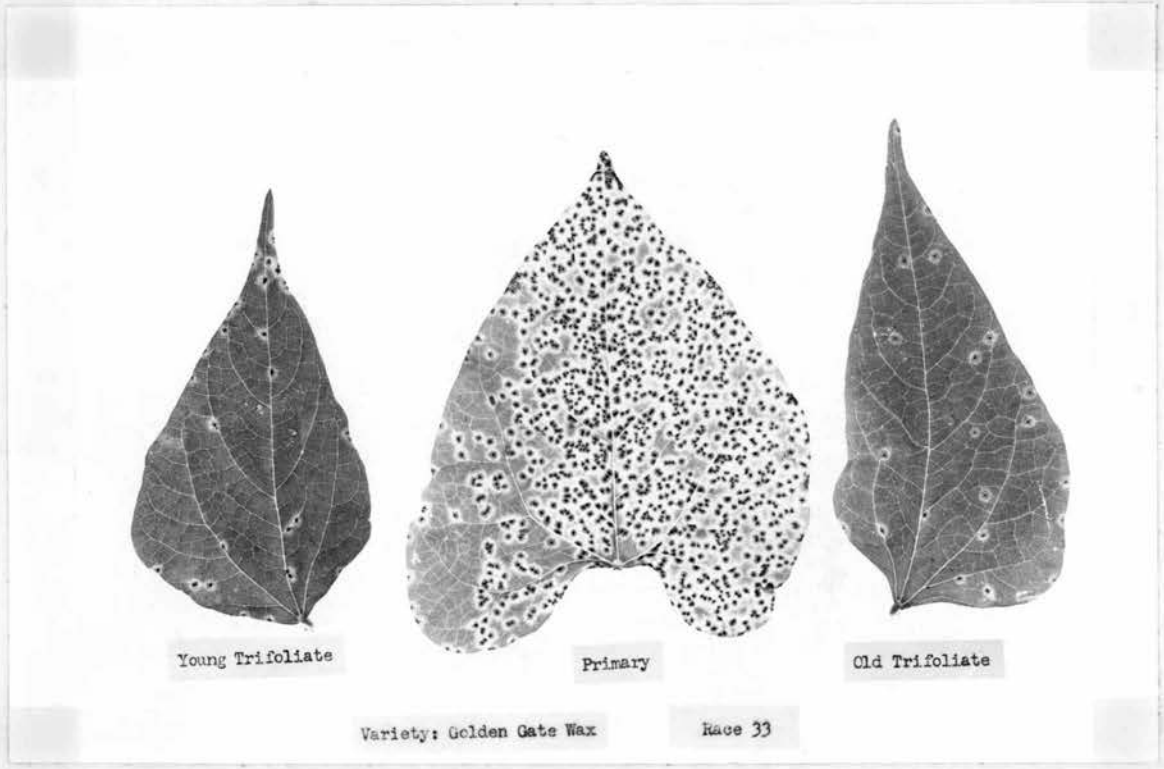


Figure 7. Effect of age of leaf on pustule size.

Table 9. Grades of pustules incited by race 33 of U. phaseoli var. phaseoli, on primary and trifoliate leaves.

Differential Variety	Primary Leaves				Trifoliate Leaves				
	Replicate				Replicate				
	I	II	III	IV	I	II	III	IV	Exp. No.
U. S. No. 3	5	5	5	5	4-5 [a]	4-5	4-5	4-5	I
	5	5	5	5	4-5	4-5	4-5	4-5	II
No. 765	3	3	3	3	3-3	3-3	3-3	3-3	I
	3	3	3	3	3-3	3-3	3-3	3-3	II
No. 814	4	4	4	4	4-5	4-5	4-5	4-5	I
	4	4	4	4	4-4	4-5	4-4	4-5	II
No. 650	5	5	5	5	4-5	4-5	4-5	4-5	I
	5	5	5	5	5-5	5-5	4-5	5-5	II
No. 643	3	3	3	3	3-3	3-3	3-3	3-3	I
	3	3	3	3	3-3	3-3	3-3	3-3	II
No. 780	5	5	5	5	4-5	4-5	4-5	4-5	I
	5	5	5	5	5-5	5-5	5-5	5-5	II
Golden Gate Wax	4	4	4	4	4-5	4-5	4-5	4-5	I
	4	4	4	4	4-5	4-5	4-5	4-5	II

[a] The left number in the pair is the grade assigned to pustules on mature trifoliate leaves, the right number is the grade on young trifoliate leaves at the time of inoculation.

Table 10. Reaction grades of primary and trifoliate leaves of bean hybrids inoculated with race 33 of U. phaseoli var. phaseoli.(1)

Hybrid No.	Plant Number 1		2		3		4		5		6		7	
	P*	T**	P	T	P	T	P	T	P	T	P	T	P	T
FM-1	5	5	5	5	5	5	5	5	-	-	-	-	-	-
2835	5	5	5	5	5	5	5	5	-	-	-	-	-	-
3103	5	5	2	2	3	3	3	5	5	5	3	5	3	3
3305	5	-	3	5	3	3	4	5	4	5	4	5	4	5
3078	5	-	4	5	5	-	4	5	3	5	5	5	5	-
3109	4	5	5	5	3	5	3	3	3	4	3	3	3	3
G-50	3	4	3	4	3	4	3	4	-	-	-	-	-	-
3079	4	5	4	5	3	3	4	4	5	-	3	4	3	3
3081	4	5	3	-	-	5	3	3	3	3	3	4	3	4
3079	3	5	3	5	3	4	3	4	-	-	-	-	-	-

* Rating on primary leaves.
** Rating on trifoliate leaves.
(1) Hybrids are breeding material used by Dr. W. A. Frazier in developing new bean varieties.

but on others the reactions were different.

Because of the variation in reaction between trifoliate and primary leaves it is felt that in testing hybrids for resistance or susceptibility to U. phaseoli var. phaseoli primary and trifoliate leaves should be used. Reliable results will be obtained if only primary leaves are used in rust race determinations.

Effects of Inoculum Concentration

Inoculum suspensions containing approximately 10,000, 20,000, 40,000, 80,000 and 160,000 urediospores of race 33 per ml were used at the rate of ten ml per five plants to inoculate differential bean varieties. Five sets of five plants of each differential variety were inoculated with each of the inoculum concentrations. Following inoculation the plants were held in a moisture chamber for 16 hours, then returned to the greenhouse bench. Fourteen days after inoculation the number of pustules per cm^2 was determined by counting the number of pustules in four cm^2 on each of five leaves from each can of plants and calculating the average. Average size of the pustules on each variety was determined by measuring with an ocular micrometer ten individual pustules on each of four leaves from each inoculum level. The germination percentage of the urediospores was determined by spraying five ml per plate of each inoculum suspension onto the surface of PDA in petri dishes and after 24 hours incubation at 20°C, counting the number of germinating spores per 100 spores observed. Two hundred spores were counted in each of the four plates. Only those spores having a germ-tube length of twice the diameter of the spore were considered to have germinated.

Results of Inoculum Concentration Studies

The average number of pustules per cm^2 increased progressively on plants of all varieties inoculated with suspensions containing 10,000, 20,000 or 40,000 spores per ml. The highest pustule density occurred on plants sprayed with the suspension containing 40,000 spores per ml; the density decreased progressively on plants inoculated with suspensions containing 80,000 or 160,000 spores per ml (Table 12, Figures 8 through 14). Pustules on all varieties of plants inoculated with the 40,000 spores per ml suspension were smaller than those on plants inoculated with the other spore suspensions (Table 13, Figures 8 through 14). The grades, which are based on pustule size, were affected in several cases. In all other experiments, race 33 of U. phaseoli var. phaseoli has produced grade 5 infections on varieties U. S. No. 3, No. 650 and No. 780 but produced grade 4 pustules on plants inoculated with 40,000 spores per ml (Table 13).

The germination percentage of the urediospores decreased with increasing numbers of spores in the inoculum (Table 11). The urediospores used to prepare the inoculum had been stored at -20°C for about 6 months prior to use which may account for the generally low germination.

Table 11. Germination percentage of urediospores used as inoculum.

No. of Spores per ml	Percent Germination
10,000	7.25
20,000	4.00
40,000	4.12
80,000	0.75
160,000	0.25

Figure 12. Number of pustules per cm^2 on differential varieties inoculated with various concentrations of inoculum.

Approx. no. spores per ml	Average no. of pustules per cm^2						
	U. S. No. 3	No. 765	No. 814	No. 650	No. 780	No. 643	GGW(1)
10,000	5.00	0.37	5.75	4.37	1.12	1.50	--
20,000	6.87	6.37	11.75	8.87	12.87	4.37	8.00
40,000	23.87	11.50	19.87	25.12	30.37	8.37	15.50
80,000	8.25	5.62	17.25	6.62	18.12	1.63	6.50
160,000	6.50	4.62	13.00	9.37	1.50	3.12	1.50

(1) Golden Gate Wax

Table 13. Pustule size and grade produced by various concentrations of inoculum.

Approx. no. spores per ml	Average pustule size on differential varieties inoculated with various concentrations of inoculum.						
	Differential Varieties						
	U. S. No. 3	No. 765	No. 814	No. 650	No. 780	No. 643	GGW(3)
10,000	584(1) 5	276 3	451 4	612 5	571 5	212 3	425 4
20,000	593 5	213 3	457 4	597 5	624 5	199 3	405 4
40,000	457 4	157 3	368 4	419 4	360 4	195 3	384 4
80,000	561 5	206 3	444 4	650 5	585 5	199 3	366 4
160,000	461 4	238 3	450 4	582 5	611 5	204 3	412 4
Ave. pustule size (2)	502 5	247 3	438 4	557 5	--- -	182 3	365 4

(1) Upper number is the size of the pustules in microns, lower number is reaction grade.

(2) Average pustule size as determined in other experiments.

(3) Golden Gate Wax

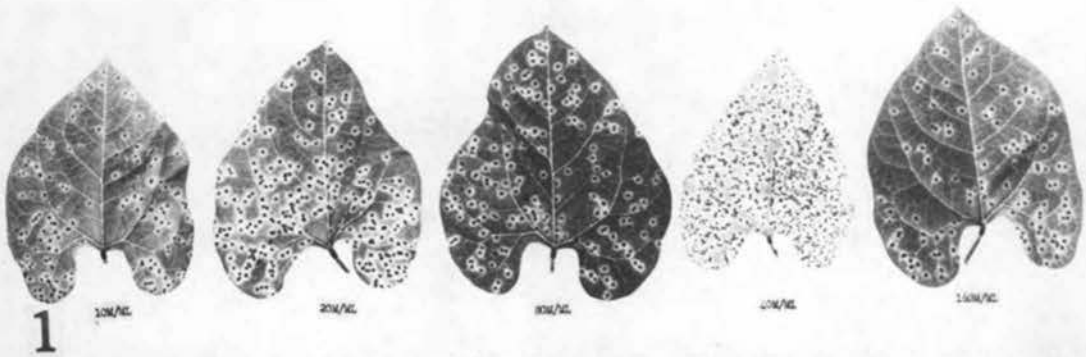


Figure 8. Effects of inoculum density on number of pustules per cm^2 and on size of pustules on variety U. S. No. 3.

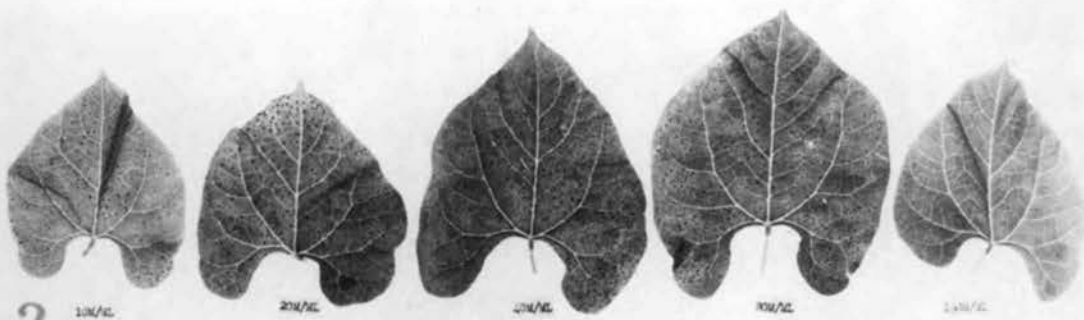


Figure 9. Effects of inoculum density on number of pustules per cm^2 and on size of pustules on variety No. 765.

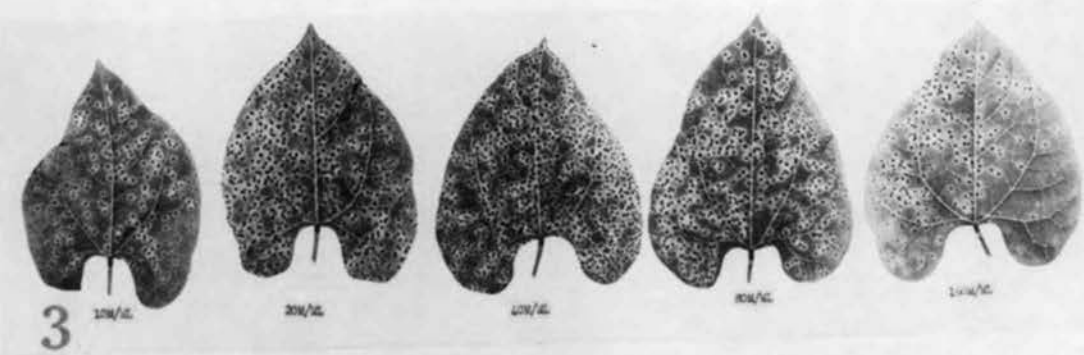


Figure 10. Effects of inoculum density on number of pustules per cm^2 and on size of pustules on variety No. 814.

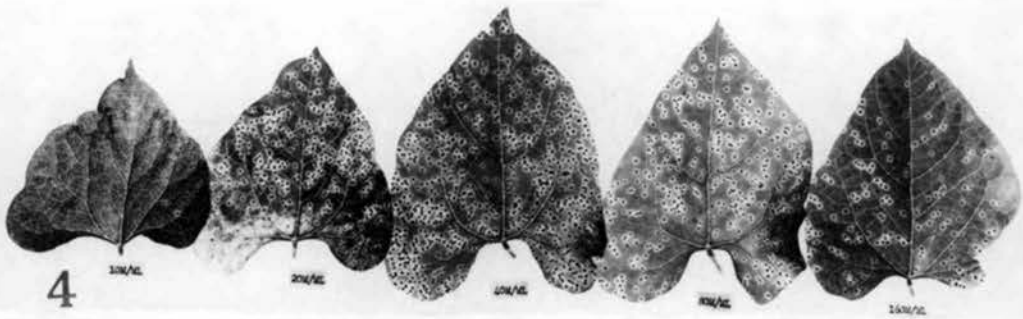


Figure 11. Effects of inoculum density on number of pustules per cm^2 and on size of pustules on variety Golden Gate Wax.

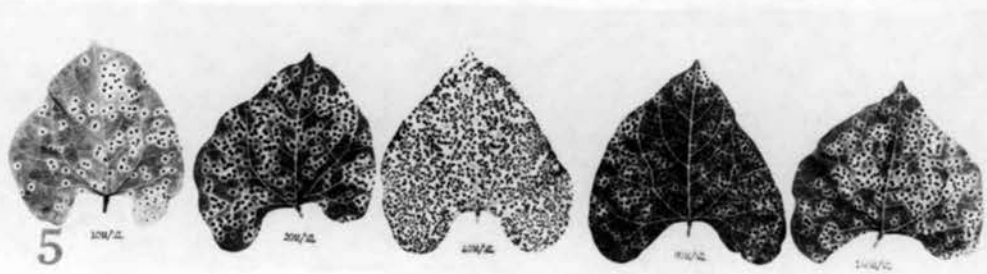


Figure 12. Effects of inoculum density on number of pustules per cm^2 and on size of pustules on variety No. 650.

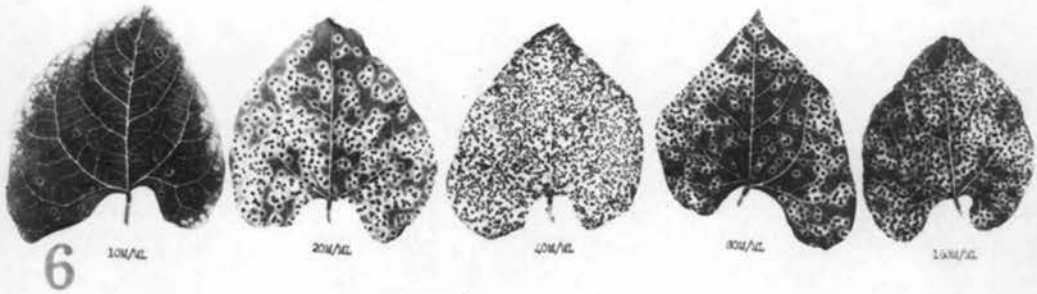


Figure 13. Effects of inoculum density on number of pustules per cm^2 and on size of pustules on variety No. 780.

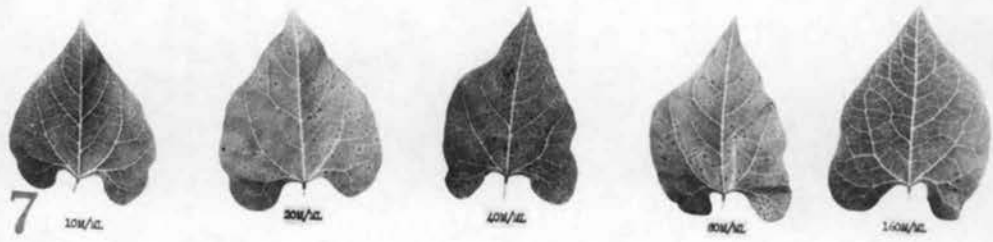


Figure 14. Effects of inoculum density on number of pustules per cm^2 and on size of pustules on variety No. 643.

The fact that the concentration of spores in the inoculum affects the size of the sori and the number of pustules that develop is of significance because identification of races of U. phaseoli var. phaseoli is based on the size of the sori produced on differential bean varieties. Under the conditions of the experiment erroneous grades would have been assigned to the reaction of U. S. No. 3, No. 650 and No. 780 to race 33 if only one level of inoculum containing a concentration of 40,000 spores per ml had been used.

Effects of Temperature Treatment After Inoculation

Schein (20, p. 675) reported that when infections between 72 and 96 hours old were subjected to a constant temperature of 90°F the host-parasite interaction was adversely affected in proportion to the time it endured the condition. A 4-hour exposure delayed attainment of full sporulation by about 1 day; 8 and 15 hours exposure caused a 2-day delay; and 24 hours, a 3-day delay. He also reported that infections 96 and 120 hours old developed a local necrotic response while those 72 hours old were killed when exposed to a temperature of 90°F for twelve days. Infections older than 120 hours became static without further development. Schein also reported that infected plants grown at 90° - 80°F temperatures showed no

symptoms after ten days but normal progression of symptoms and sporulation occurred when the plants were transferred to a 80° - 70°F environment. There was some reduction in the number of lesions.

In the present studies two replicates of five plants each of the seven differential varieties were grown in the greenhouse at 80° - 65°F prior to inoculation with race 33, after which they were placed in a moisture chamber for 16 hours. They were then divided into groups and transferred to chambers maintaining temperatures of 50°, 60°, 70° and 90°F. After 72 hours they were returned to the greenhouse where day-night temperatures were 70° and 55°F. Data were taken 14 days after inoculation, using the spot-card method.

Results of Temperature Treatments

There were striking interactions between differential variety, race and temperature (Table 14). A reduction in number of pustules per leaf occurred on all varieties at 90°F (Figures 15 through 21). In several cases no pustules had developed 18 days after inoculation in one of the two replicates. Varieties thus affected were No. 814, No. 650 and Golden Gate Wax. The few pustules in the other replicate were the same size as recorded in other experiments (Figures 15, 16, and 17). Variety No. 643

Table 14. Effects of post-inoculation temperature on infection grades.

Temperature[1]	Differential Varieties							Replicate
	814	650	765	643	780	GGW	3	
50	4.0[2]	4.0	3.8	3.0	5.0	4.8	4.8	I
	4.0	4.6	3.8	3.0	4.4	5.0	5.0	II
60	4.0	4.0	4.0	3.0	4.6	5.0	4.0	I
	4.2	4.8	4.0	3.0	5.0	5.0	4.0	II
70	4.0	4.6	4.0	3.0	5.0	4.8	5.0	I
	4.0	4.6	4.0	3.0	5.0	5.0	5.0	II
90	4.0	5.0	3.0	1.0**	5.0	5.0	5.0	I
	1.0**	1.0**	3.0	1.0**	5.0	1.0**	5.0	II

L.S.D. 5% - 1.40*
1% - 1.90**

(1) Plants held for 72 hours in constant temperature chambers of 50°, 60°, 70° and 90°F and then returned to the greenhouse.

(2) Each value is the average of five individual leaf ratings per replicate.

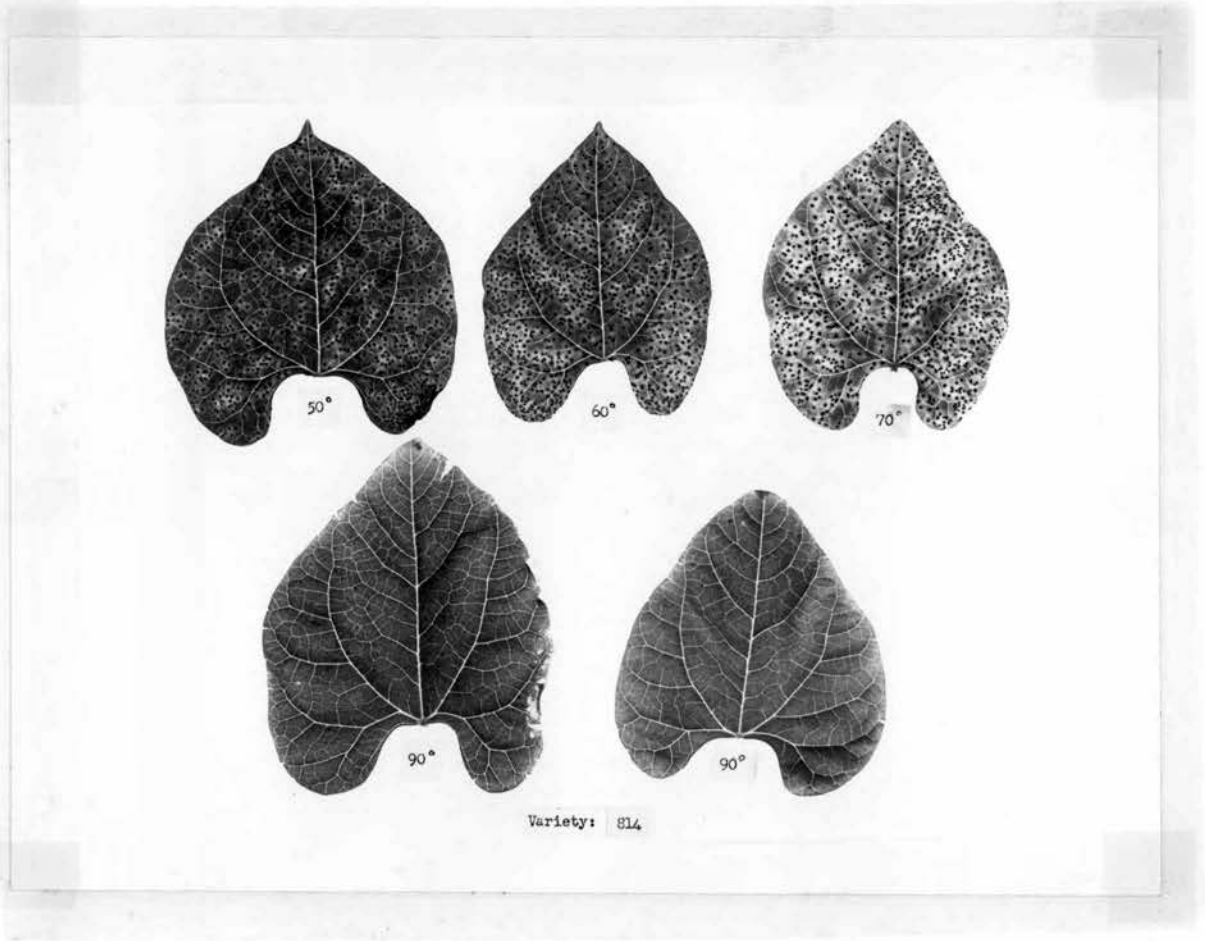


Figure 15. Effect of temperature on pustule development. A temperature of 90°F for 72 hours caused a marked reduction in the number of pustules.

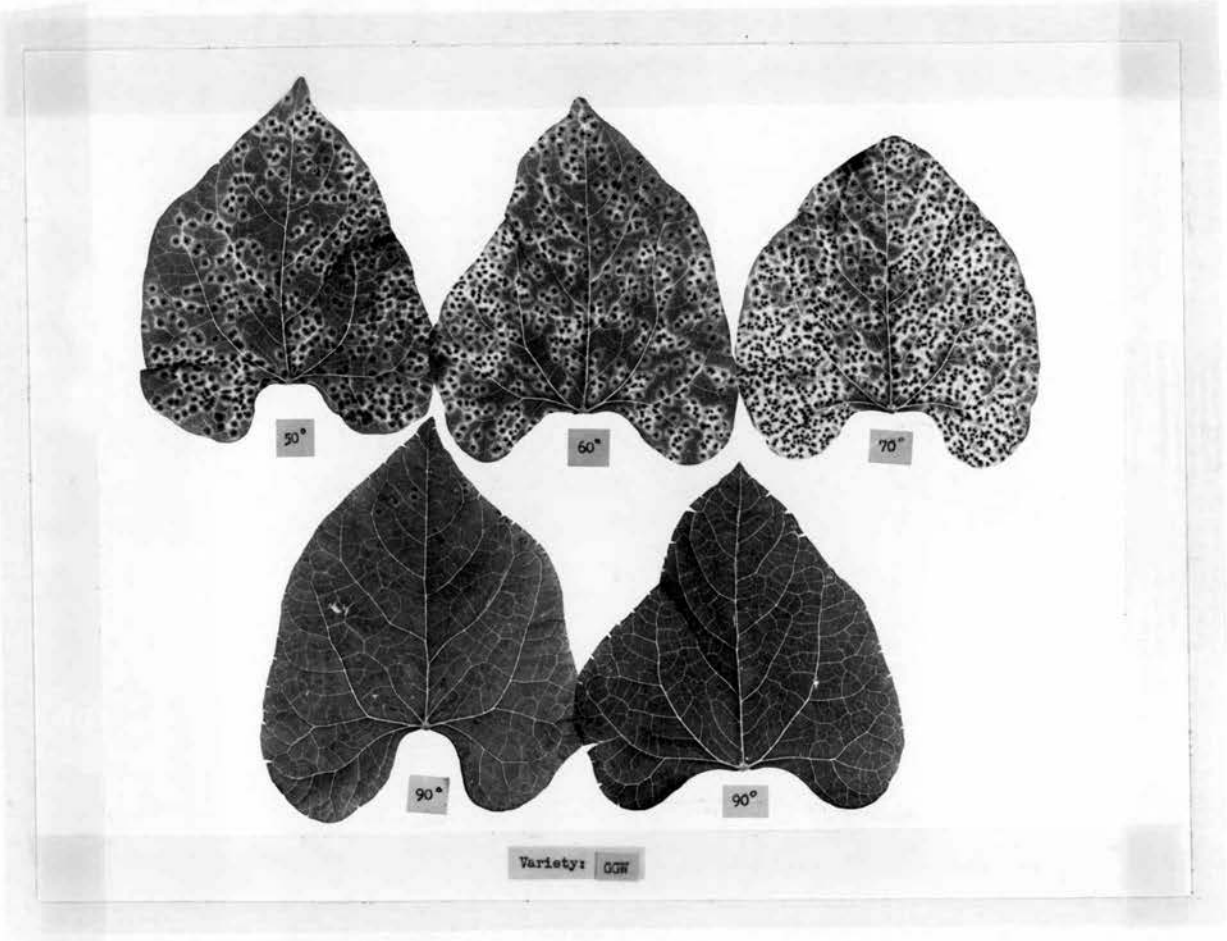


Figure 16. Effect of temperature on pustule development. A temperature of 90°F for 72 hours caused a marked reduction in the number of pustules.

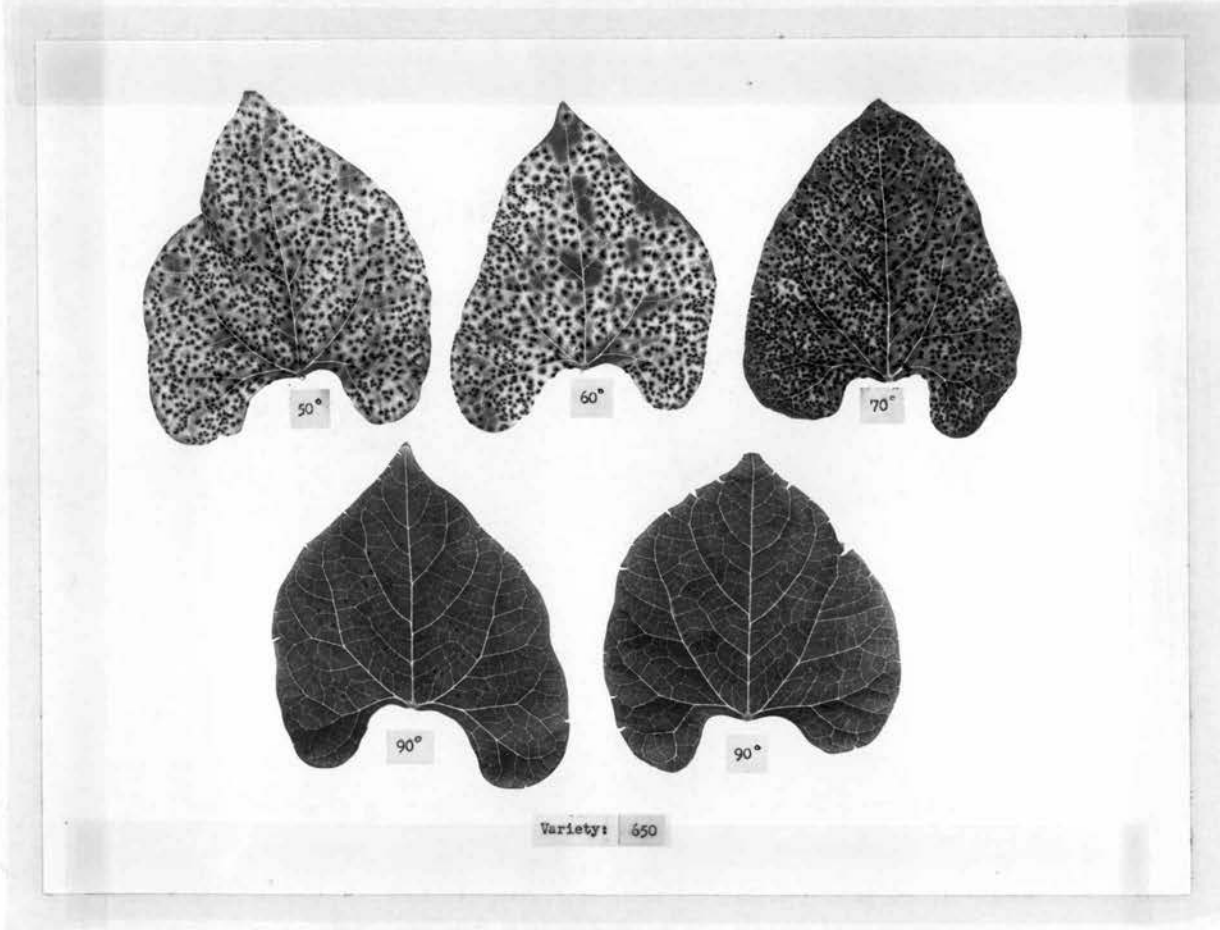


Figure 17. Effect of temperature on pustule development. A temperature of 90°F for 72 hours caused a marked reduction in the number of pustules.

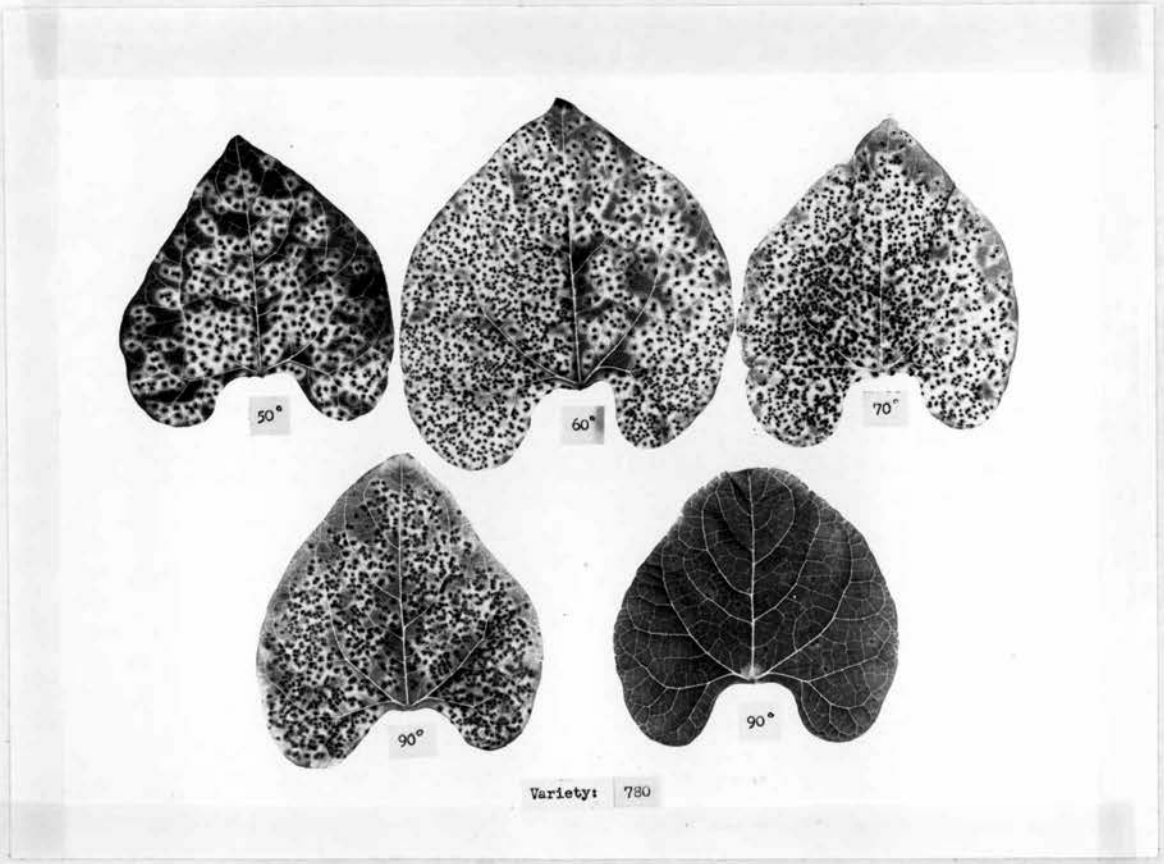


Figure 18. Effect of temperature on pustule development. A temperature of 90°F for 72 hours caused a reduction in the number of pustules in one replicate but not in another.

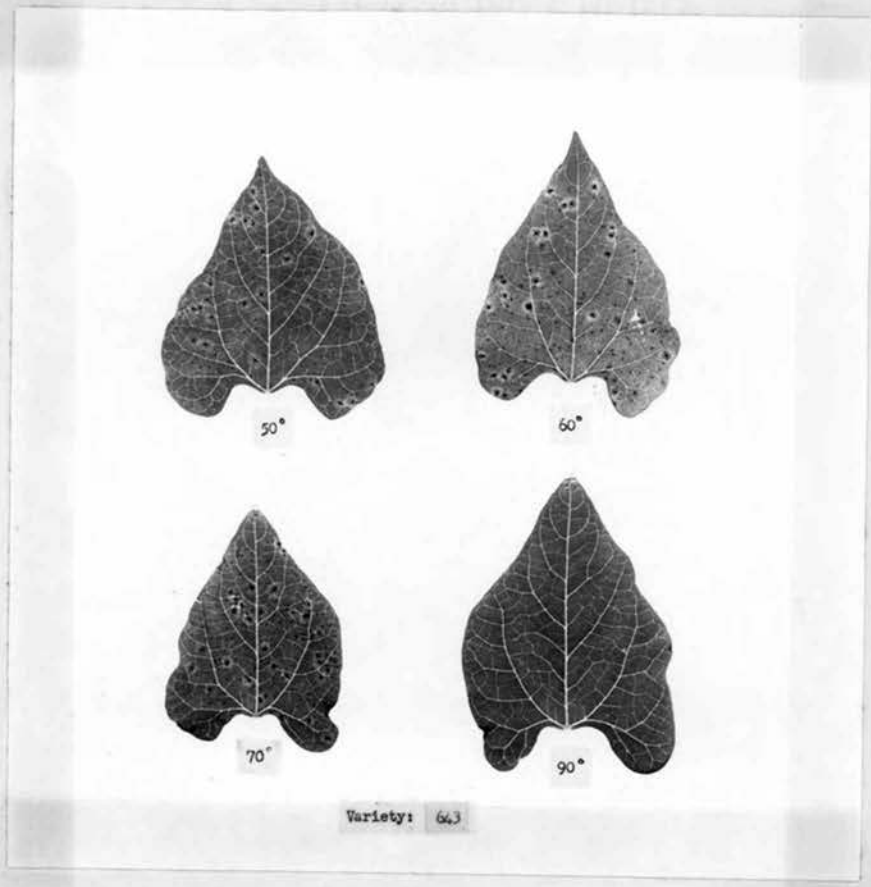


Figure 19. Effect of temperature on pustule development. A temperature of 90°F for 72 hours prevented pustule development on variety No. 643.

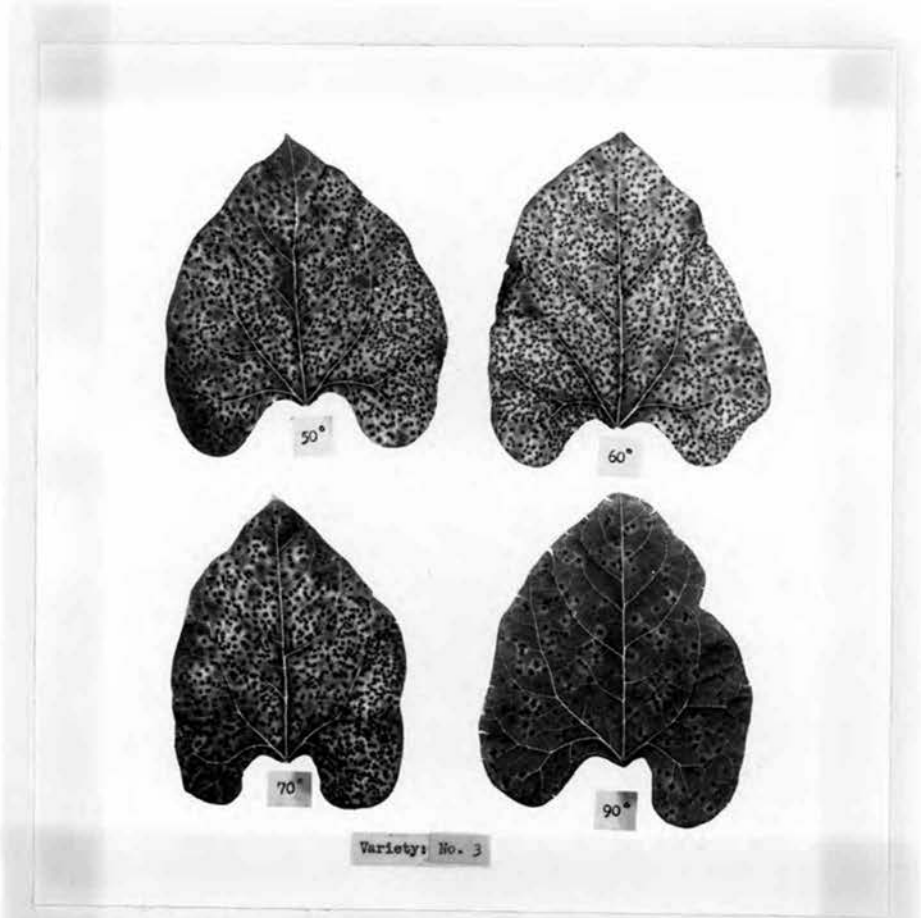


Figure 20. Effect of temperature on pustule development. A temperature of 90°F for 72 hours caused a slight reduction in the number of pustules.

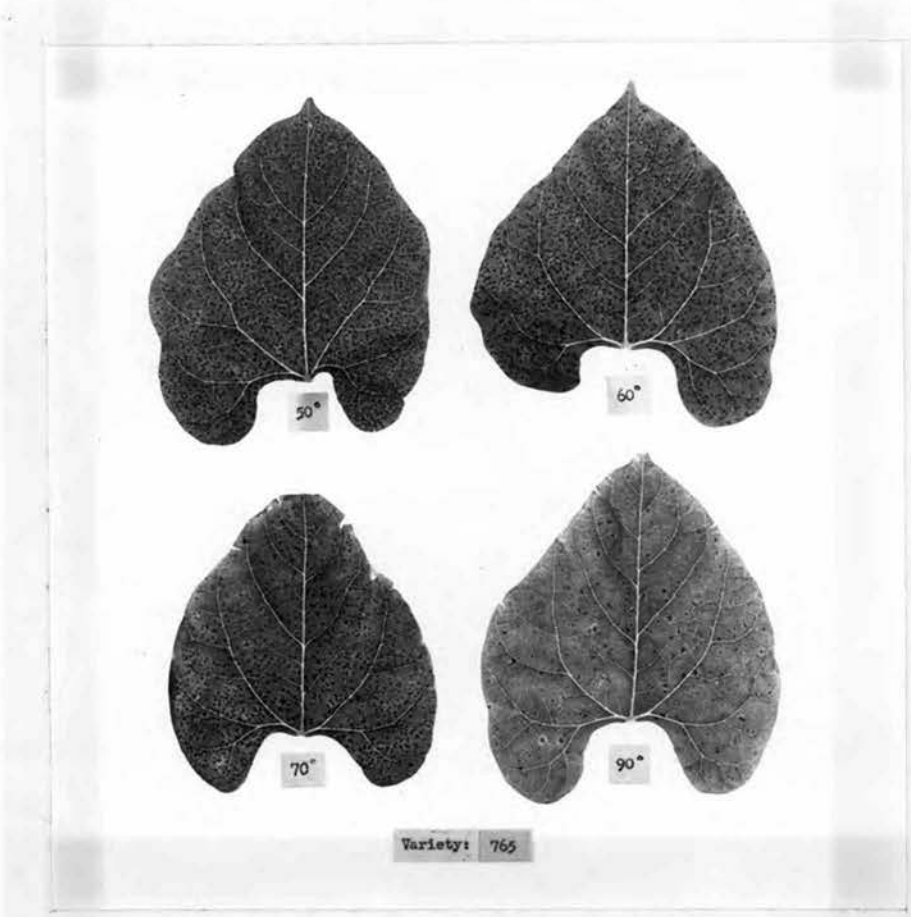


Figure 21. Effect of temperature on pustule development. A temperature of 90°F for 72 hours caused a slight reduction in the number of pustules.

gave an immune reaction on both replicates at 90°F (Figure 19). Temperatures of 50°, 60° and 70°F delayed the development of symptoms about two days.

Schein (20, p. 678) reported that plants exposed to 90°F for 48 hours immediately after the moisture period and then placed at 80° - 70°F showed a marked time lapse in symptom expression and a slower development rate but that full sporulation eventually occurred. No mention was made of a reduction in the number of pustules per leaf. The present experiments confirm his report that symptom expression is slower but also show that there was a reduction in number of pustules on all varieties (Figures 15 through 21). Sempio (22, p. 48) reported that temperatures of 90° - 93°F for four days or 93° - 97°F for two days "sterilized" rust infections. The present studies indicate that such "sterilization" is dependent on host variety, length of temperature treatment and probably the race of U. phaseoli var. phaseoli (Table 14).

The reaction grade on varieties No. 765 and Golden Gate Wax did not agree with the pustule size as determined in other experiments. In both cases the pustules were rated one grade higher. Variety No. 765, when grown at day-night temperatures of 80° - 65°F, has in previous experiments produced a grade 3 reaction while Golden Gate Wax has produced a grade 4 reaction. Wei (25, p. 1093-1095)

reported that on certain mesothetic varieties, temperatures of 16°C (60.8°F) and 28°C (82.4°F) caused larger pustules to be formed. After the temperature treatments the plants were grown at a mean daily temperature of about 16° to 17°C. This could account for the higher grades on varieties No. 765 and Golden Gate Wax which are mesothetic hosts for race 33. These results point up the need for detailed studies of the effects of environment on pustule development.

Effects of Temperature Treatment Prior to Inoculation

Schein (21, p. 486) reported the induction and appearance of a necrotic response in Pinto 111 after seven days at a temperature of 32°C following inoculation. In the present investigations all plants, prior to temperature treatment, were grown at day-night temperatures of 80° - 65°F for eight days. At that time, five plants of each variety were placed in temperature chambers maintained at 50°, 60°, 70° and 90°F for 72 hours. The plants received constant light from an incandescent 200-watt lamp hung 32 inches above the cans. After 72 hours the plants were inoculated and placed in a moisture chamber for 16 hours, then returned to the greenhouse where day-night temperatures of 80° - 65°F were maintained. The experiment was repeated using the same techniques. Data were

taken 14 days after inoculation, using the spot-card method.

Results of Pre-Inoculation Temperature Treatments

Under the conditions of the experiment no differences in pustule size or numbers of pustules per cm^2 were observed when plants were held at constant temperatures of 50°, 60°, 70° and 90°F for 72 hours immediately prior to inoculation.

Effects of Inoculum Source

The possibility that urediospores from grade 3 pustules might be more virulent than those from grade 5 pustules of the same race was investigated. Mass spore collections were made from the differential varieties No. 765 (grade 3) and No. 780 (grade 5) which had been inoculated with race 33. These were stored in individual glass vials at 36°F until used to prepare an inoculum suspension containing about 20,000 urediospores per ml. Ten ml of the inoculum suspension was sprayed on each replicate consisting of five plants per number 10 can. The experiment was replicated twice and included six differential bean varieties.

Fourteen days after inoculation, the average number of pustules per cm^2 was determined by counting the number of pustules in 20 individual cm^2 on five primary leaves

of each variety.

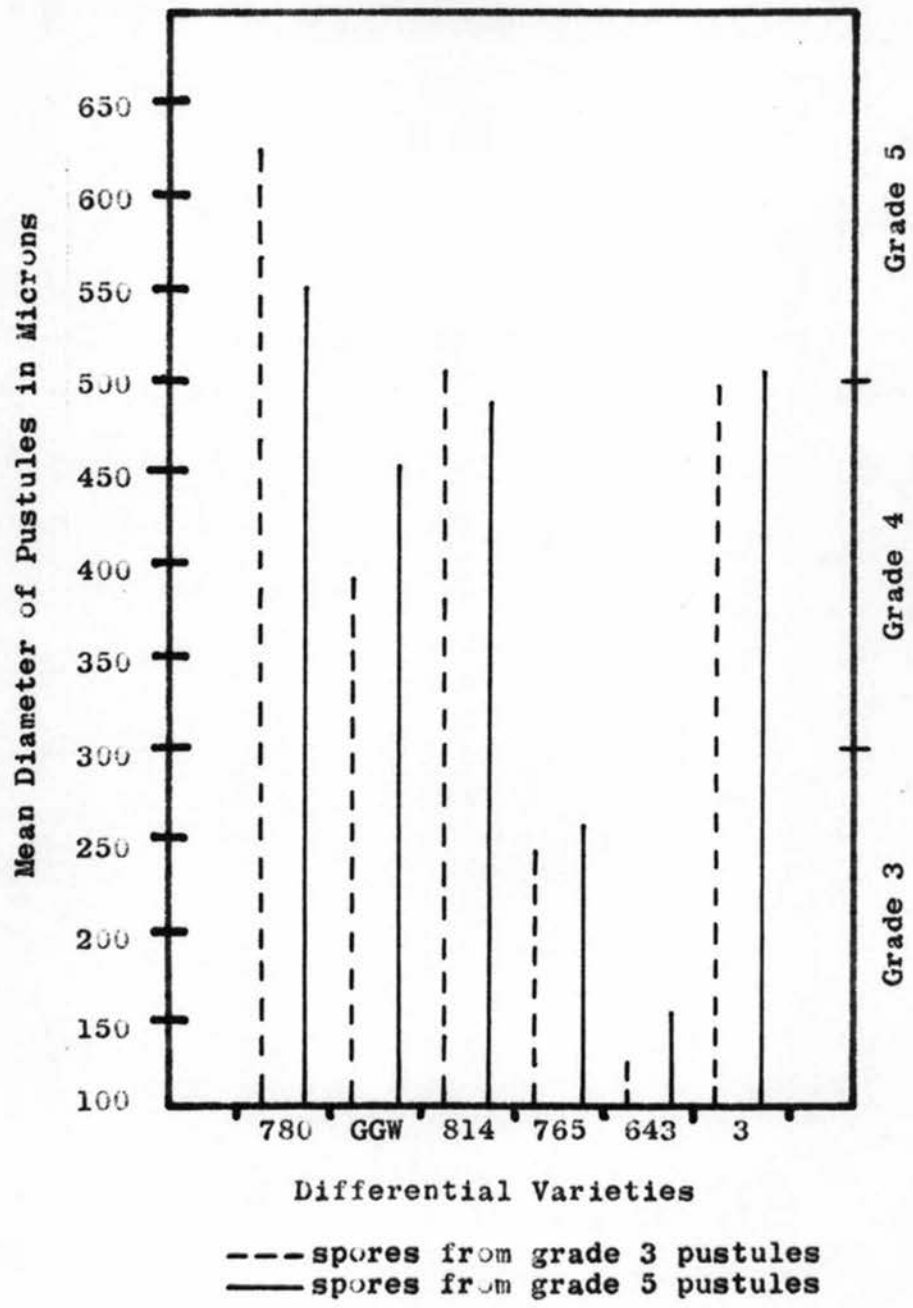
One primary leaf from each plant in each replicate was collected and stored in a plastic freezer bag at 36°F. Pustule sizes were determined by measuring ten pustules per leaf on the five leaves from each replicate. The two replicates were then averaged to find the mean pustule size produced by each source of inoculum on each differential variety.

Percentage germination of spores from each inoculum source was determined by spraying five ml of the spore suspension on the surface of potato dextrose agar in petri dishes. The plates were kept at room temperature (72° to 75°F) for 24 hours at which time 100 urediospores were counted on each plate and the percentage germinating noted. Only those having germ tube lengths at least twice the diameter of the spore were considered to have germinated.

Results of Inoculum Source Study

No differences in the virulence of urediospores collected from grade 3 and grade 5 pustules was detected (Figure 22). On the differential varieties No. 780, Golden Gate Wax, No. 765 and No. 643 the mean pustule sizes from each inoculum source are approximately the same and both fall within the same grade. In the case of variety U. S. No. 3, one of the mean sizes falls in grade 5,

Figure 22. Comparison of rust pustule sizes with source of inoculum.



the other in grade 4. However, it should be noted that on variety U. S. No. 3 race 33 produces pustules having a mean diameter of approximately 500 microns.

About 21 percent of the urediospores from each inoculum source germinated. Therefore, it seems evident that spores from grade 3 are not more viable than those from grade 5 pustules. On this basis the relatively small number of spores produced by grade 3 pustules are not as important in the spread of bean rust throughout a field as are grade 5 pustules which produce many more spores.

DISCUSSION AND CONCLUSIONS

The identification of races of U. phaseoli var. phaseoli is essential in the development of resistant varieties. Increased emphasis on the development of bush-type beans, having the quality of F. M. 1, makes resistance to bean rust more important because the destruction of the limited foliage could have severe effects on yield. In 1941, Harter and Zaumeyer (13) proposed a system of race identification which utilized a 0 through 10 grading scale. Grade 0 denoted immunity and grade 1 a necrotic flecking reaction. Grades 2 through 10 were differentiated on the relative size of the spore-bearing pustules. Certain difficulties were encountered when the system was used to identify races present in the Willamette Valley of Oregon. Distinct size of pustules for each grade had not been defined and a collection of all identified races had not been maintained for comparative purposes.

To avoid these difficulties and to make race identification easier and more reliable a revised system is proposed. The number of grades has been reduced from eleven to five with each grade clearly defined. Grade 1 denoted immunity, grade 2 indicated necrotic flecking, grade 3 includes pustules less than 300 microns in diameter. Grade 4 encompasses pustules having a diameter of 301 to 500

microns while pustules larger than 500 microns in diameter are assigned to grade 5. When the reaction grades of all described races are converted to the new grades, all races retain their identity. This indicates that only five grades are necessary. Use of the printed Bean Rust Grading Scale facilitates rapid determination of the grades.

Harter and Zaumeyer (13, p. 720) reported that pustules occurring on primary and trifoliate leaves were comparable in size. Present studies do not agree with their findings. In some cases the pustules produced on primary and trifoliate leaves are not the same size. In most cases the grade is not affected but occasionally there will be a difference. Therefore, only primary leaves should be used in race identification studies, but in testing bean hybrids for resistance both primary and trifoliate leaves should be used.

Results of one inoculum concentration study indicated a close correlation between the inoculum concentration and the size and number of resulting pustules, however, in later studies little correlation was observed. Wilson (27, p. 595) has determined by quantitative chromotography that aspartic and glutamic acids are present in the urediospores and in the tissues surrounding the pustules in quantities sufficient to account for inhibition of urediospore germination. Wilson concluded that the temperature

and humidity at which the urediospores are produced apparently affect the phenomenon and that in some cases the spores contain little if any of the inhibitors. The urediospores used as inoculum in the present studies were collected at various times and were stored at -20°C until used. Low temperature may have had an effect on the inhibitors. The variation among tests on the effect of inoculum density on pustule number and size does not decrease the importance of the results. It was clearly demonstrated that in some cases inoculum density can influence the germination percentage, the number of pustules and consequently, the grade assigned to a particular host-parasite reaction. Additional work is needed to clarify this phenomenon.

Various investigators have demonstrated that post-inoculation temperature influences total number of urediospores produced, and may in some cases kill young infections. In both cases, however, only one host variety was used and the race of U. phaseoli var. phaseoli was not specified. The results of the present investigations indicate that various bean varieties react differently to temperatures following inoculation. A reduction in number of pustules per leaf occurred on all varieties at 90°F but a difference in the size of the few remaining pustules was not recorded. Infections were stopped in their development

only on variety No. 643, although pustule development was slowed somewhat on all varieties. Results indicate that the effects of post-inoculation temperature are influenced by the variety of bean, the length of exposure to various temperatures and probably by the race of U. phaseoli var. phaseoli. Detailed studies of the effect of environment should be made under carefully controlled conditions.

The possibility that the size of pustules, from which inoculum was taken, might influence the reaction grade was investigated. It was concluded that the source of urediospores of a given race of U. phaseoli var. phaseoli has little influence on the infection grades or on the identification of that race.

The present investigations clearly indicate that more emphasis should be placed on the study of U. phaseoli var. phaseoli and of the environmental factors influencing the host-parasite reactions on various varieties of Phaseolus vulgaris and other species of Phaseolus.

SUMMARY

1. A revised system for identification of races of U. phaseoli var. phaseoli is proposed which utilizes five reaction grades in place of the eleven grades of the Harter-Zaumeyer system.
2. Reaction grades were found to differ in some cases between primary and trifoliate leaves. Therefore, only primary leaves should be used in race identification studies, but both primary and trifoliate leaves should be used in testing bean hybrids for resistance to the fungus.
3. Temperature influences the number of pustules produced and may have an effect on the size of the pustules.
4. Inoculum density can affect the number and size of pustules and consequently, the reaction grade.
5. The size of pustules from which inoculum was collected was not found to influence the infection grades or the identification of a given race of the fungus.
6. It was demonstrated that one or more factors may affect race identification even when the revised grading system is used. The same factors undoubtly cause even greater errors when the Harter-Zaumeyer system is used.
7. These investigations clearly indicate that more emphasis should be placed on the study of U. phaseoli var.

phaseoli and of the environmental factors influencing the host-parasite reactions.

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