

RACES OF BEAN RUST,  
UROMYCES PHASEOLI (PERS.) WINT. VAR. PHASEOLI,  
IN THE WILLAMETTE VALLEY

by

HIDEAKI ROBERT HIKIDA

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**APPROVED:** Redacted for privacy

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**Professor of Plant Pathology**

**In Charge of Major**

Redacted for privacy

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**Chairman of Department of Botany and Plant Pathology**

Redacted for privacy

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**Chairman of School Graduate Committee**

Redacted for privacy

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**Dean of Graduate School**

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RACES OF BEAN RUST, UROMYCES PHASEOLI (PERS.) WINT. VAR.  
PHASEOLI, IN THE WILLAMETTE VALLEY

INTRODUCTION

The snap bean, Phaseolus vulgaris L., is the most valuable vegetable grown in Oregon. During the 5 year period 1956-1960 an average of 83,500 tons, with a farm value of \$10,746,000, were harvested annually from 10,940 acres. Increases in acreage and in yield per acre over the years have accounted for this large production, most of which is used for canning and freezing. Oregon has been the leading producer of snap bean for processing since 1947 and during the period 1956-1960 accounted for 20 to 25 percent of the national production.

Snap bean acreages in Oregon are concentrated along the Columbia River near Portland and in the Willamette Valley as far south as Eugene. The Blue Lake pole type of bean, which requires trellis culture, is most widely grown, usually under overhead irrigation. This type is especially well adapted to the cool Willamette Valley where the environment favors the development of excellent color and appearance together with an unusually low fibre content in the bean. This combination accounts for high consumer appeal. The growing of this crop in Oregon is a high cost, concentrated industry in which disease problems may be economically critical.

Bean rust is one of the most widespread and consistent in occurrence among the diseases affecting beans in Oregon and in other areas. Although damage to the pods occurs only rarely, serious reductions in yield are not uncommon when the environmental conditions are favorable for the development of the disease, especially during the early stages of plant growth. Rust has been generally underrated as a limiting factor in bean production. Losses are indirect, resulting from decreased vigor due to foliage injury, a fact which tends to obscure the amount of reduction in yield except in very severe cases. While bean rust is commonly found throughout most of the bean growing areas in the United States, its presence and importance is complicated by the relatively large number of physiologic races involved.

In Oregon, during the years prior to 1941, there was an increase in severity of bean rust, followed by a decrease in its prevalence in the ensuing years. Again, prior to 1950, there was an increase in severity, followed by a decrease. These periods of decline in severity coincided with the introduction and adoption of new bean varieties. This suggested that introductions resistant to prevailing races of bean rust at time of adoption became susceptible at a later date. The variety F.M.1, which in the initial years after adoption was

resistant to the disease, has become increasingly affected and considerable loss of crop by some growers has been reported. Though the recommended program using sulfur dust provides excellent rust control, this practice cannot be carried beyond the time of pod-set.

The purposes of this study have been to identify the races of bean rust occurring in the Willamette Valley, to find sources of resistance, and to determine the mode of inheritance of resistance. Since one of the best means of controlling the disease is by breeding for resistance, such information should greatly facilitate the planning and execution of programs for breeding varieties resistant to bean rust.

## REVIEW OF LITERATURE

Nomenclature

Bean rust is caused by the fungus, Uromyces phaseoli (Pers.) Wint. var. phaseoli. For many years it appeared in literature as U. appendiculatus (Pers.) Fr. This name is invalid because it duplicates a name earlier applied to a different rust, U. appendiculatus Ung., on a distinctly different host (2, p. 296).

Persoon in 1795 gave the fungus the name, Uredo appendiculata phaseoli, which appeared also in 1801 in Persoon's Synopsis Methodica Fungorum, the starting point of nomenclature for Uredinales. In 1804, Reber raised it to species rank, Puccinia phaseoli, and in 1880 Winter transferred it to the genus Uromyces. In 1934 Arthur (2, p. 296) established the variety, typica, which under the International Code of Botanical Nomenclature (19, p. 45) becomes phaseoli. Therefore the fungus must be called Uromyces phaseoli (Pers.) Wint. var. phaseoli and the name Uromyces phaseoli (Pers.) Wint. var. typica Arth. must be relegated to synonymy.

Life Cycle

Bean rust is an autoecious obligate parasite. The life cycle is confined to a single host on which a complete

complement of spore forms is produced. Aecia are of rare occurrence in many places, but have been observed in the field by Eastman in British Columbia (8, p. 59), Milbrath in Oregon (24), Jones in New York (18), and Brien and Jacks in New Zealand (5, p. 282). Andrus (1, p. 560) induced production of sporidia and aecia on leaves of bean plants grown in a greenhouse. Uredia and telia, producing urediospores and teliospores respectively, are commonly found in the field during the summer and fall. In colder climates teliospores are always produced and serve to carry the fungus through the winter. In Florida (29, p. 25) and in California (15, p. 739), teliospores are rarely produced in the field. Apparently the fungus is able to perpetuate itself indefinitely by means of urediospores.

### Hosts

The principal host is Phaseolus vulgaris L. Arthur (2, p. 296) lists the following species of Phaseolus as susceptible to infection: P. lunatus L., P. multiflorus Willd., P. polystachyus (L.) B.S.P. (P. perennis Walt.), and P. sinuatus Nutt. Zaumeyer and Thomas (37, p. 39) obtained good infection on P. acutifolius var. latifolius and on some varieties of P. lunatus. They noted that lima beans are more resistant than the dry and snap beans,

though reactions to the several physiologic races of bean rust were similar. Fromme (12, p. 71) reported that U. phaseoli phaseoli can infect such other species as P. adenanthus G. Meyer, P. anisotrichus Schl., P. atropurpureus Moc. and Sease, P. coccineus Jacq., P. disophyllus Beth., P. obvallatus Schlecht, and P. retusus Benth.

### Symptoms

Bean rust attacks chiefly the leaves, where most of the injury results. It may also be found occasionally on the stems and pods. Small, reddish-brown spore masses or sori of rust, the uredia containing urediospores, appear on the leaves. In the field, they appear from late June to early September. The uredia, usually about the size of a pin head, are distributed over both surfaces of the leaves. There may be only a few, or so many that every available area of leaf surface is covered. The spores are carried by the wind and other mechanical means to neighboring plants where, under suitable conditions, they cause new infections. The disease spreads rapidly with favorable environmental conditions. Mature urediospores, capable of initiating new infections immediately, are produced 10 to 16 days after infection. If infection is severe, the leaf soon begins to yellow and eventually turns brown, shrivels, and falls to the

ground. Defoliation causes stunting of growth and consequent reduction in yield.

As each infected leaf on the plant matures, and the substrate becomes unsuitable for urediospore formation, the pustules change from a reddish-brown to a blackish-brown color. This change is brought about by the formation of another type of spores, the teliospores. These are black, thick-walled resting spores that carry the fungus through the winter season. Exposure to freezing temperature is required before the teliospores will germinate.

### Distribution

Bean rust was first reported from Germany in 1795 and since then has been found in almost every part of the world (37, p. 34). Rust infection is possible at any stage of plant growth in localities where relative humidity of 95 percent or higher is maintained for 8 to 10 hours or longer. It is rare at a lower relative humidity (15, p. 749).

Although bean rust occurs in all parts of the United States, losses seem to be especially severe in Virginia, West Virginia, Tennessee, Georgia, Florida, Alabama, Louisiana, Texas, Colorado and southern California. In the northern states, bean rust usually appears too late

in the growing season to cause much injury. In 1892, Beach (3, p. 332) reported that bean rust was abundant in New York, but the attack came late in the season and the foliage was damaged only slightly. This was one of the earliest reports of the disease in the United States. A few years later Halstead (14, p. 26) and Whetzel (32, p. 199) noted that the disease was widespread but not serious in New York. Fromme and Wingard (10, p. 3) found bean rust to be one of the most destructive of the several common diseases of beans in Virginia, sometimes causing complete destruction of the crop. Townsend (29, p. 24) reported that in the spring of 1936 rust was very destructive in southern Florida. Losses in certain counties at that time were estimated at 40 to 80 percent of the crop.

Campbell (6) and Campbell and Schwartze (7) reported that in Washington, bean rust was restricted primarily to the Whatcom county district where it caused heavy losses to the Blue Lake variety in 1936 and 1937.

In Oregon, bean rust was recorded as slight in 1921 (30, p. 344). A substantial increase in acreage of snap bean took place in the Willamette Valley in the late 1930's. In 1944, Milbrath (23, p. 1) reported bean rust to be increasing from year to year in certain bean-growing areas of Oregon to the extent that it was limiting

production. In the same year, from a survey of bean fields in the Willamette Valley, Boyle (4) found leaves of Early Blue Lake beans well covered with rust pustules. MacSwan and Raymer (22, p. 26) noted that the bean rust problem had almost disappeared when the susceptible variety F.M.65 was replaced during 1952 and 1953 by the highly resistant variety F.M.1. However, by 1958 the increased incidence of bean rust had caused considerable loss of crop to some growers. Loring (21, p. 10) reported a minor crop loss due to rust in Oregon in 1959.

#### Physiologic Races

In 1935, Harter, Andrus, and Zaumeyer (15, p. 753) demonstrated the presence of 2 physiologic races of the bean rust organism, U. phaseoli phaseoli. One was collected in southern California and the other in the vicinity of Washington, D.C. Later Harter (16) and Harter and Zaumeyer (17, p. 722-723) reported 18 additional races from collections made at various places in the United States and Hawaii. They demonstrated that certain varieties and strains of beans inoculated with different physiologic races showed varying degrees of infection, as measured by the size of the uredia. The following varieties of beans were employed in identifying the various physiologic races: (1) U.S. No. 3, a white-seeded

Kentucky Wonder type; (2) Bountiful (No. 181)\*, a common garden bean variety of the bush type; (3) a strain of California Small White (No. 643), a field type grown principally in California; (4) a strain of Pinto (No. 650), a speckled field bean grown extensively in the intermountain region of the west; (5) a selection (No. 765) from the Kentucky Wonder Wax variety; (6) a medium-late white-seeded Kentucky Wonder hybrid (No. 780); (7) a brown-seeded Kentucky Wonder hybrid (No. 814). On the basis of the differential reactions on these seven bean varieties, 20 different physiologic races were identified.

During the period 1941-1951, Fisher (9, p. 104) reported the reactions of 10 previously unrecorded races from Colorado, Maryland, Montana, Nebraska, Oregon, and Wyoming. He omitted variety No. 181 and added varieties Golden Gate Wax and Z4.

In 1952, Sappenfield (25) identified race 31 from New Mexico and in 1960 Zaumeyer (38, p. 459) identified race 32 from Maryland. Bean varieties used in identifying the latter two races were: U.S. No. 3, No. 643, No. 650, No. 765, No. 780, No. 814, and Golden Gate Wax.

\* Numbers in parenthesis following varietal designations are pure-line selections maintained in the seed files of Zaumeyer.

To date, 32 races of bean rust have been identified by their differential reactions to bean varieties.

#### Environmental Influence on Infection Reaction

Wei (31, p. 1103) studied the influence of several environmental factors on the type of reaction of the host to infection. Variations in temperature, in nutrient supply and age of host tissue did not affect significantly the type of reaction on varieties highly resistant or highly susceptible to infection. Aging of the host tissue, beyond a certain limit, reduced the amount of infection. Excess nitrogen increased and deficient nitrogen decreased the amount of infection per unit area. Potassium had the opposite effect. Reduction in light intensity prolonged the incubation period.

#### Infection Grades

The size of the mature rust pustule has been used as the criterion for determining the degree of susceptibility or resistance. Fromme and Wingard (11, p. 393) adopted a scheme of grading the variety Tennessee Green Pod as 100 percent susceptible. They then compared the number of infections on other varieties as a percentage of infections on Tennessee Green Pod. Wingard (33) separated varieties into 3 classes on the basis of their

reaction to rust: (1) immune, (2) severe flecking as a result of hypersensitivity to infection, and (3) susceptibility, with production of numerous pustules. Wei (31, p. 1103) graded infection into 5 major types (0 to 4). He considered 0, 1, and 2 as resistant, and 3 and 4 as susceptible.

Harter and Zaumeyer (17, p. 721) established an infection rating for determining the degree of susceptibility and resistance, based on development of the rust pustules 14 days after inoculation. Formation of secondary and tertiary rings of sori was not sufficiently constant for use in race identification. They described 10 grades of infection.

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 physiologic races of the organism; 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 in 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.

These grades were used to identify the 32 physiologic races of bean rust by Harter and Zaumeyer (17), Fisher (9), Sappenfield (25), and Zaumeyer (38).

#### Handling of Spores

Harter et al. (15, p. 741) found that urediospores of bean rust caused only scattered infections following storage for 26 weeks at 9°C with relative humidity at approximately 73 percent, and no infections following storage for 28 weeks. Teliospores stored under similar conditions germinated 50 to 60 percent at the end of 207 days, after which the test was discontinued. Wei (31, p. 1090) obtained satisfactory germination from urediospores stored up to 2 months at 8°C. Harter and Zaumeyer (17, p. 718) reported that spores in the ice compartment of a refrigerator remained viable for many months and good infections were obtained from urediospores stored for more than 2 years at -20°C.

Harter et al. (15, p. 744) found the optimum temperature for the germination of urediospores was approximately 14.5°C. The minimum was 1.8°C and the maximum about 34.5°C. They found optimum temperature for infection to be approximately 17°C and no infection was obtained at 27°C. Sempio (26) reported that the optimum temperature for development of rust was 19 to

to 20°C. Townsend (29, p. 27) found temperatures near 15.5°C to be most favorable for development of infection following artificial inoculation. Infection occurred at temperatures as low as 9°C and as high as 36.5°C.

Within the range of temperatures permitting spore germination, a relative humidity of 96 percent or higher was required for infection to take place readily (15, p. 744).

Maximum infection occurred on plants held in a high-humidity chamber for 12 to 18 hours. Only a few pustules were formed when plants were held in the high-humidity chamber for 8 hours and no infection took place when held for 2 to 6 hours. Decrease in percentage of infection occurred after 20 hours of confinement (15, p. 747). Reduced photosynthetic activities of inoculated plants held in subdued light was associated with this reduction in percentage of infection. However, Sempio (26) found that high relative humidity for 20 to 24 hours resulted in maximum infection.

According to Sempio (26) absence of light during incubation did not affect infection, but Wei (31, p. 1096) found that light was essential during the infection period for successful entrance of the fungus.

### Varietal Susceptibility and Resistance

Bean varieties were first rated for resistance to rust by Fromme and Wingard (10, p. 15-18; 11). They found varieties of Phaseolus vulgaris to be fairly constant in their disease reaction and rated the varieties on a numerical scale based on the number and size of uredia, the incubation period, necrotic reaction of the host, and relative promptness of telia formation. They noted that of the varieties studied, those with indeterminate growth habit were more susceptible than those with determinate growth habit, green pod color more susceptible than wax pod color, and white seedcoat more than colored seedcoat. Harter et al. (15, p. 754) also recorded the relative resistance of many varieties of bean to 2 physiologic races of the fungus. Most varieties were highly resistant to one race and very susceptible to the other. Wei (31, p. 1093) demonstrated differences in rust reaction, ranging from highly resistant to highly susceptible, amongst several bean varieties. Zaumeyer (38, p. 459) reported that race 32 infected most of the bush snap bean varieties which are tolerant or resistant to the majority of the other races but was not able to infect most pole and dry bean varieties which are infected by the majority of the other

races.

For the varieties tested, Harter et al. (15, p. 751) found a close correlation between infection ratings obtained in the greenhouse and in the field.

#### Inheritance of Resistance

Wingard (33, 34) found that resistance to rust in certain bean varieties was controlled by a single dominant gene. He distinguished 3 types of reactions on the host that were characterized by no visible signs (immunity), severe flecking (hypersensitivity), and production of sori. Zaumeyer and Harter (36) reported that a single gene was involved in inheritance of resistance to physiologic races 1 and 2 while more than one factor governed inheritance to races 6, 11, 12, and 17. Factor for resistance was dominant in crosses inoculated with races 1, 2, 6, and 12, and incompletely dominant with races 11 and 17. They used the infection scale 0 to 10 based on the size of the sori. Plants graded 0 to 5 were considered resistant, and 6 or higher were regarded as susceptible.

## MATERIALS AND METHODS

### Sources of Bean Rust

Twenty-three collections of infected bean leaves taken in 1958, 1959, and 1960 from fields in the Willamette Valley, from Lane county in the south to Multnomah county in the north, were used for race identification. Each collection was identified by a serial number in which the first digit represented the year in which the collection was made (Table 1).

Except for one collection, 0120, taken from a selection with a bush habit, all were taken from varieties and selections with an indeterminate habit. Collections were made from late July when the urediospores became abundant and were continued until teliospores became abundant.

The infected leaves were dried between paper towels for two days at room temperature. Each collection was then packaged in a kraft paper bag of suitable size and stored in plastic containers at 1°C. This proved satisfactory when the urediospores were used within two months.

### Spore Collecting and Storage

Urediospores were collected from infected leaves by two methods. A scalpel with a rounded tip was used

Table 1. Collections of Uromyces phaseoli phaseoli from the Willamette Valley used in race identification.

Identity	Date	Host	County
8000	7-29-58	F.M.1	Yamhill
8010	7-29-58	F.M.1	Yamhill
8020	7-28-58	F.M.1	Multnomah
8030	7-31-58	F.M.1	Polk
8040	8- 7-58	F.M.1	Benton
8060	8-20-58	Pinto UI-72	Linn
8070	8-20-58	Pinto UI-111	Linn
8080	8-20-58	Asgrow Regular (197234)	Linn
8120	8-20-58	Red Mexican UI-34	Linn
8130	8-20-58	Great Northern UI-81	Linn
8140	8-20-58	Great Northern UI-123	Linn
9000	7-17-59	F.M.1	Lane
9010	8-25-59	F.M.1	Linn
9050	8-27-59	F.M.1	Lane
9070	8-29-59	F.M.1	Multnomah
0000	7-27-60	Pinto No. 650	Linn
0010	8- 5-60	F.M.1	Marion
0050	9- 2-60	F.M.1	Lane
0060	9- 2-60	F.M.1	Lane
0080	9- 8-60	F.M.1	Multnomah
0090	9- 8-60	F.M.1	Multnomah
0100	9-12-60	F.M.1	Multnomah
0120	9-12-60	Bush 1139	Linn

to scrape small quantities of spores into 1-dram shell vials. This method proved adequate for collecting spores from individual pustules and from pustules on a limited number of leaves.

A cyclone separation method was used to collect large quantities of spores (27, 28). For this purpose a 25 ml ehrlenmeyer flask was modified into a cyclone separator (Figure 1). The outlet of the separator was attached to

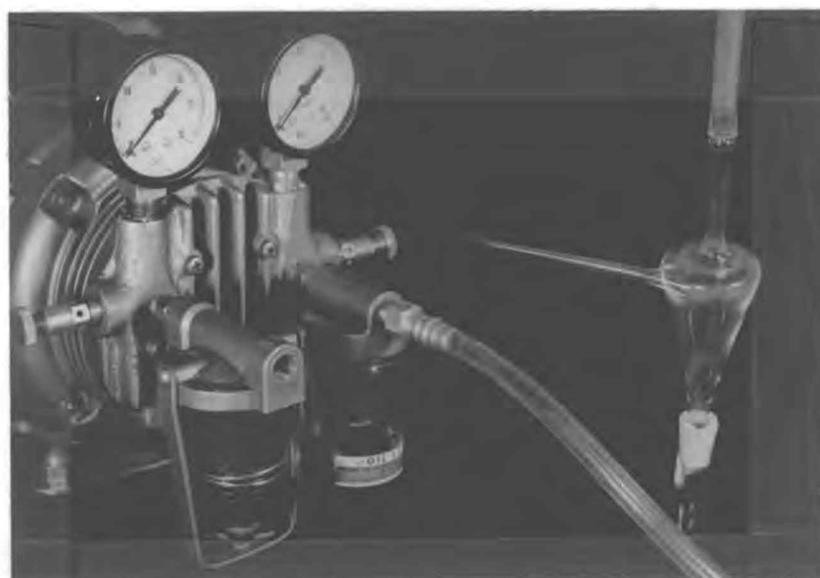


Figure 1. A cyclone separator made from a 25 ml ehrlenmeyer flask and an electrically driven portable vacuum pump.

an electrically driven portable vacuum pump with a length of polyethylene tubing. Spores were allowed to settle directly into a 1-dram shell vial. The cyclone separator provided a rapid means of collecting a large quantity of spores from infected leaves.

In order to prevent contamination among the different collections and isolates, scalpels and cyclone separators were washed in 70 percent alcohol and dried prior to use.

Urediospores for immediate use were kept in cork-stoppered 1-dram shell vials in a cold room at 1°C. Satisfactory germination of spores was retained up to 2 months. Spores for long term storage were sealed in gelatine capsules and stored in a freezer at -25°C.

### Inoculation Technique

A quantity of urediospores which could be picked up on the point of a penknife was wetted in a beaker with several drops of 0.01 percent Vatsol, a wetting agent, and a spore suspension prepared by adding 40 ml of 125 p.p.m. Ivory soap solution. Ivory bar soap was shaved or grated into thin sections, dried, and ground up finely with a mortar and pestle.

Inoculations were made by brushing the spore suspension with a No. 8 camel's-hair brush onto the upper and lower surfaces of the primary leaves when they were about one-half to two-thirds grown. When leaves up to two-thirds grown were inoculated, maximum infection reactions were expressed and when fully developed leaves were inoculated, pustules did not attain maximum size. Since identical infection grades were obtained on primary and trifoliate leaves, primary leaves, which were ready for inoculation about 10 days earlier than the trifoliate leaves, were preferred and used.

A chamber was equipped with several spray nozzles (.85 80A Delavan Des Moines) to supply a constant mist. It served as a high humidity environment favorable for the initiation of infection following inoculation. The

plants were inoculated during late afternoon, held under high humidity overnight (15 to 20 hours) and removed in the morning to a greenhouse bench. No attempts were made to alter light conditions or temperatures normally maintained in the greenhouse. Under these conditions, infections became evident about one week after inoculation. Records of the size of pustules were made about 14 days after inoculation or a few days later if it was suspected that development of rust was delayed because of low temperatures or for other causes.

The brushes used for inoculations were suspended in 70 percent alcohol in a large test tube and were rinsed in tap water before use. Hands were thoroughly washed between inoculations to prevent carry-over of spores and to maintain purity of the isolates.

#### Greenhouse Culture of Bean Plants

Bean plants used in these studies were grown in No. 10 cans on greenhouse benches. The soil consisted of about 6 parts sandy loam, 2 parts builder's sand, and 2 parts pulverized peat moss. A complete commercial fertilizer was mixed into the soil at the rate of five grams per No. 10 can. The mixture was used without sterilization.

To insure uniform germination the soil surface was firmed and dibbled. Seeds treated with Spergon were dropped into the holes and covered to a depth of one inch. The soil surface was then smoothly packed and watered. Seedlings were thinned to five plants in each can for each variety tested.

Temperature in the greenhouse was maintained between 60 and 75°F from late fall to spring. During the summer months, afternoon temperatures in the greenhouses frequently rose to 90-100°F despite manipulation of ventilators and doors.

Light intensities are not adequate for optimum plant growth during the cloudy winter weather in western Oregon. However, the bean seedlings responded consistently to rust inoculations under conditions of available light duration and intensity in the greenhouse.

#### Varieties of Beans used to Identify Races of Rust

The varieties of beans used in identifying the physiologic races were: (1) U.S. No. 3, (2) No. 643, (3) No. 650, (4) No. 765, (5) No. 780, (6) No. 814, and (7) Golden Gate Wax. Physiologic races of bean rust were identified on the basis of the differential infection reactions on these bean varieties.

Only a small quantity of seed of each of these

pure-line selections was available from Dr. W. J. Zaunmeyer, United States Department of Agriculture at Beltsville. Therefore each variety was increased during the summer of 1959 and again in 1960 on the farm of the Vegetable Crops section, Department of Horticulture, Oregon State University. Identification of the isolates, therefore, could not be started until the fall of 1959.

#### Single-spore Isolation

Rust was pure-lined by starting with urediospores taken from a single sorus. Primary leaves of bean variety F.M.1 were inoculated with light spore suspensions so that only few well-isolated pustules developed on a leaf. Urediospores from each sorus were carefully scraped with a scalpel into a vial serially labelled for identification. The spores collected from a single sorus were then inoculated onto F.M.1 plants. The urediospores from the resulting infections were then collected and stored in individual vials to retain their purity. Three to 40 single-spore isolates were made from each selected collection.

From isolate 8140-1, 30 single-spore sub-isolates were made to determine the purity after 6 generations and 14 months culture in the greenhouse.

A total of 292 single-spore isolates were made from the 23 selected collections and 30 sub-isolates were made from the single-spore isolate, 8140-1, the total being: 83 single-spore isolates from 11 collections made in 1958, 28 single-spore isolates from 4 collections made in 1959, 181 single-spore isolates from 8 collections made in 1960, and 30 sub-isolates from isolate 8140-1.

### Infection Grades

Infection grades of 0 to 10, based on the development of rust pustules 14 days after inoculation, were used. These grades on selected varieties of beans had been used in the identification of the 32 races of bean rust by previous workers (17, 9, 25, 38). Grade 0 indicated no lesions or other evidence of infection. Grade 1 indicated necrotic flecks of variable shapes and sizes but without any spore development. Grade 2 indicated development of small pustules with some spores. Grades 3 to 10 were differentiated on the basis of the spore-bearing pustules. Plants graded 0 to 5 were considered resistant, and 6 or higher were regarded as susceptible.

To determine uniformity of infection reactions, 10 primary leaves of the 5 plants in each can were inoculated with the same isolate. Repeated tests indicated that reaction grades obtained from 5 plants of

each variety were dependable.

Uniformity of infection grades under variable environments in the greenhouse was tested by inoculating with the same isolate over a period of 6 months or more. Infection grades on the varieties were consistent in spite of the environmental variability existing in the greenhouse during the test period.

### Sources of Resistance

Tests to find bean varieties, strains, and selections carrying sources of resistance to races of bean rust isolated from collections made in the Willamette Valley were begun in the winter of 1959-1960. Since the results of the reactions of the isolates up to this time suggested the presence of only one race, resistance to this race alone was sought at first. With the finding of another race in February, 1961, source of resistance to this race was also sought. Lack of greenhouse space and supply of bean varieties were limiting factors for an intensive as well as an extensive search for sources of resistance.

The culture of bean seedlings, the inoculation of primary leaves with urediospores, and the recording of infection reactions were carried out according to the prescribed methods.

Seeds of bean varieties (Table 3) used for this test were received from: (1) Associated Seed Growers, Inc., (2) Ferry-Morse Seed Co., (3) Northrup, King & Co., (4) Oregon State University, Horticulture Department, (5) Oregon Trail Farms, Inc., (6) Rogers Bros. Seed Co. Inc., (7) Canada Department of Agriculture Experiment Station, Morden, Manitoba, and (8) Dr. W. J. Zaunmeyer, United States Department of Agriculture, Beltsville.

#### Hybridization and Seed Production

Hybridization between bean varieties was accomplished by means of hand emasculation and pollination. A plump bud, which was about one day prior to anthesis, was selected for emasculation and held gently between the thumb and forefinger of the left hand. The suture which closed the banner around the other flower parts was opened with sharp, slightly flattened, pointed forceps. Extreme care was taken that the margins were not torn. The left side of the banner was everted and held down with the thumb of the left hand. The left wing and the upper side of the distal portion of the coiled keel was removed. The distal end of the pistil was then lifted up carefully until it was free and all of the stamens could be counted and removed. A freshly-opened flower in which the anthers had already dehisced

was selected from the staminate parent. By holding the banner and arching the wings ventrally, the pistil was forced out of the coiled keel. Pollination was accomplished by removing the distal portion of the pollen-laden pistil with a pair of clean forceps and rubbing the pollen onto the stigma of the emasculated bud. After pollination, the left side of the banner was returned to its normal position and the other slightly disarranged parts were carefully tucked underneath so that the bud was almost completely closed. The suture was then sealed by folding a one-inch length of cellophane tape over the dorsal side and sandwiching the bud. The tape dropped off or was carried along at the apical end as the young pod developed. Sealing the bud with cellophane tape made possible the maintenance of internal humidity comparable to that at anthesis in normally selfed unopened buds.

Crosses between resistant and susceptible varieties were made in an effort to determine the nature of inheritance of resistance to the predominant single race of bean rust. All crosses were made on plants grown in No. 10 cans.

The selection of the parents for the crosses were based on the results of inoculation tests (Table 3). F.M.1, a commercially grown variety, was selected as a

susceptible parent. Selection No. 643 and Florigreen were chosen as resistant parents. In the spring of 1960, the following crosses were made:

No. 643 x F.M.1

F.M.1 x Florigreen

During the summer and fall of 1960, the  $F_2$  generation and the  $F_1$ 's backcrossed to resistant and susceptible parents were produced from these two crosses. The  $F_1$ ,  $F_2$ , and the backcross populations were tested during the fall and winter of 1960. The  $F_2$  family identity was maintained during the testing program.

Hard seedcoat in the  $F_2$  seeds, especially in the cross No. 643 x F.M.1, necessitated scarifying each seed prior to planting. The resulting poor germination and weak seedlings were attributed to this treatment. The hard seedcoat factor, causing uneven germination, appeared to be a genetic factor in the F.M.1 variety.

The data from the inheritance study were compared statistically with theoretical genetic ratio by means of the chi-square test for goodness of fit. Calculations were made using the formula:

$$\chi^2 = \sum \frac{D^2}{E}$$

where D equals the difference between the observed and expected (theoretical) values, and E equals the expected

value. Since the ratio included only two classes, when the expected frequencies were small, Yates' correction for continuity was applied, using the formula:

$$\chi^2 = \sum \frac{(D - 0.5)^2}{E}$$

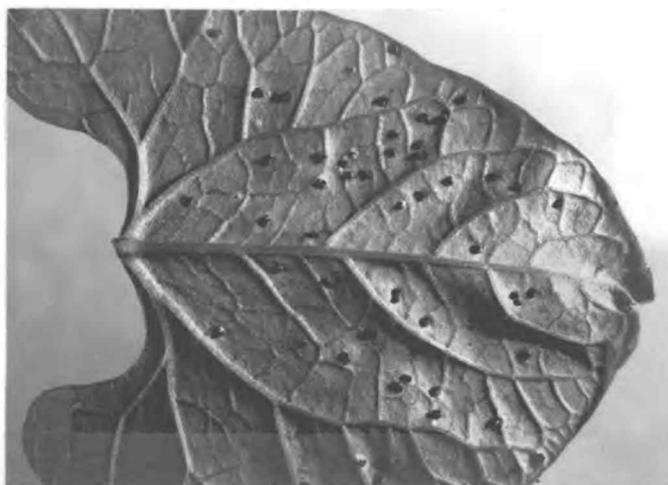
## EXPERIMENTAL RESULTS

Identification of the Single-spore Isolates

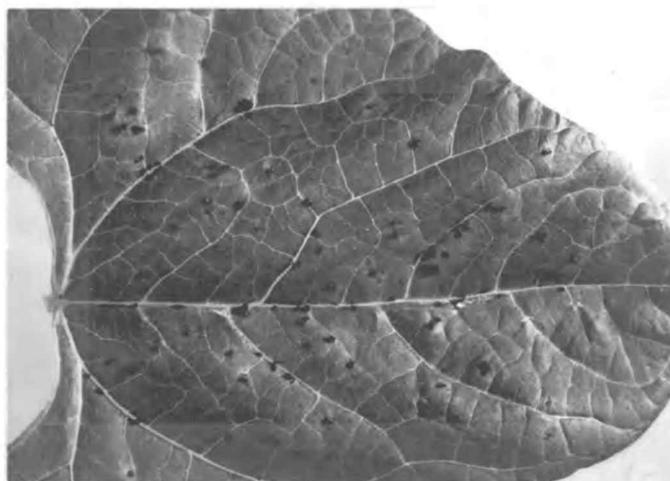
The grades of infection reactions of the 83 single-spore isolates made in 1958 were uniform, indicating that a single race was present. Grade 10 reaction was recorded on differential varieties U.S. No. 3, No. 650, and No. 780; grade 9 on Golden Gate Wax; grade 8 on No. 814; grade 4 on No. 765; and grade 2-3 on No. 643. Typical grades of infection reactions are illustrated in Figures 2, 3, 4, 5, and 6. The infection grades of this race on the differential varieties differed from those recorded for the 32 previously described races. On this basis it was called race 33. The infection grades of race 33 on these varieties are compared with several previously recorded races in Table 2. These races were selected for comparison on the basis of the grades 0 to 6 recorded on No. 643.

The grades of infection reactions of the 28 single-spore isolates made in 1959 were uniform and similar to those of race 33.

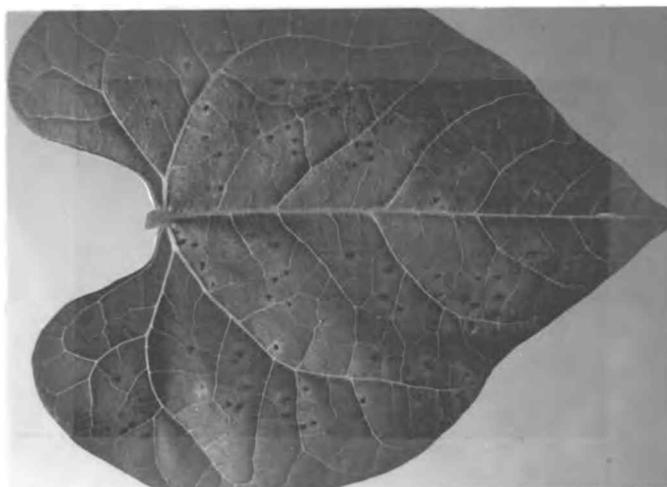
All but 2 of the 181 single-spore isolates made in 1960 also were race 33. The 2 isolates, 0100-13 and 0100-15, differed from race 33 in that infection grade



**Figure 2. Grade 10 infection reaction of race 33 on variety No. 650.**



**Figure 3. Grade 9 infection reaction of race 33 on variety Golden Gate Wax.**



**Figure 4. Grade 8 infection reaction of race 33 on variety No. 814.**



**Figure 5. Grade 4 infection reaction of race 33 on variety No. 765.**

Table 2.

Infection reactions of bean varieties to several selected races of bean rust

Differential variety	Infection grade <sup>a</sup> produced by physiologic race									
	3	4	8	15	17	24	27	29	33	34
No. 643	2	1	1	5-6	1	3	1	0	2-3	6
U.S. No. 3	9	2	5/3 <sup>b</sup>	10	10	3	2	8	10	10
No. 650	10	10	10	10	10	10	10	10	10	10
No. 765	2	2	1	2	5	4	2	1	4	4
No. 814	9	9	9	9	0	7	8	8	8	8
No. 780	1	2	2	2	1	2	10	8	10	10
Golden Gate Wax	-	-	-	-	-	-	0	8	9	9
No. 181	8	8	9	8	7	6	-	-	-	-
Z4	-	-	-	-	-	-	8	8	-	-

<sup>a</sup> Infection grades ranged from 0 for immunity to 10 for the highest degree of susceptibility.

<sup>b</sup> Numerator indicates size of pustule on upper leaf surface and denominator on lower surface.

6 instead of 2-3 was recorded on variety No. 643 (Figure 7). Grades recorded on the other varieties were identical to those recorded for race 33. These 2 isolates are therefore designated race 34. The infection grades of race 34 on the varieties are shown in Table 2.

In 1961, 30 single-spore sub-isolates were made from the 6th generation of isolate 8140-1. Infection reactions on the varieties indicated that a pure-line culture, identical to race 33, had been maintained during 14 months' culture in the greenhouse.

#### Sources of Resistance

Of 49 varieties, strains, and selections of beans inoculated with race 33 of bean rust, 5 appeared resistant. A grade 4 infection reaction was recorded for Florigreen and No. 765, grade 3 for Extender, grade 2-3 for No. 643, and grade 1 for N203 (Table 3). When inoculated with race 34, only 2 appeared resistant. A grade 4 infection reaction was recorded for No. 765 and grade 1 for N203 (Table 3).

Resistance in N203 was a hypersensitive reaction with necrotic flecks of varying size appearing at points of infection (Figure 8). Resistance in No. 643, Extender, No. 765, and Florigreen caused production of small sori (Figures 5, 6, 9).

Table 3. Infection grades of bean varieties inoculated with races 33 and 34 of Uromyces phaseoli phaseoli.

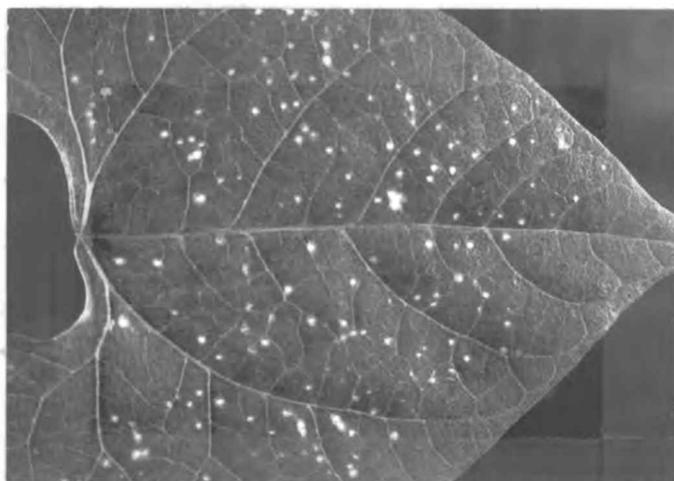
Variety	Infection grades	
	Race 33	Race 34
Bountiful 76229 (1) <sup>a</sup>	8	8
Brown Seeded Pole No. 192 (2)	9	9
Cherokee 2/T15 (3)	7	8
Contender 9/T52 (3)	8	7
Contender 03188 (6)	8	8
Earligreen 03236 (6)	8	8
Earliwax 03294 (6)	7	7
Extender 3/T1 (3)	4	7
Extender 00015 (6)	3	7
Florigreen 10020-10476 (2)	4	7
F.M.1 (4)	9	10
F.M.65 (4)	10	10
Golden Gate Wax (8)	9	9
Golden Gate Wax PC-521868 (1)	9	9
Golden Lake (5)	9	-
Harvester 46252 (1)	8	8
Higrade 60185 (6)	9	9
Improved Landreth Stringless 03041 (6)	9	8
Improved Supergreen 03057 (6)	9	9
Improved Top Notch Wax 03331 (6)	8	8
Kinghorn Wax 03322 (6)	8	7
Morse's 191 12020-10488 (2)	9	9
N203 (4)	1	1
OSC - 284 (4)	9	-
OSC - 1827 (4)	9	-
Pearlgreen 38/T94 (3)	7	8
Pencil Pod Wax 03252 (6)	8	8
Processor 12920-10434 (2)	9	9
Puregold Wax 93083 (6)	8	8
Resistant Tendergreen 46482 (1)	8	8
Richmond Wonder (7)	8	8

Table 3. - continued.

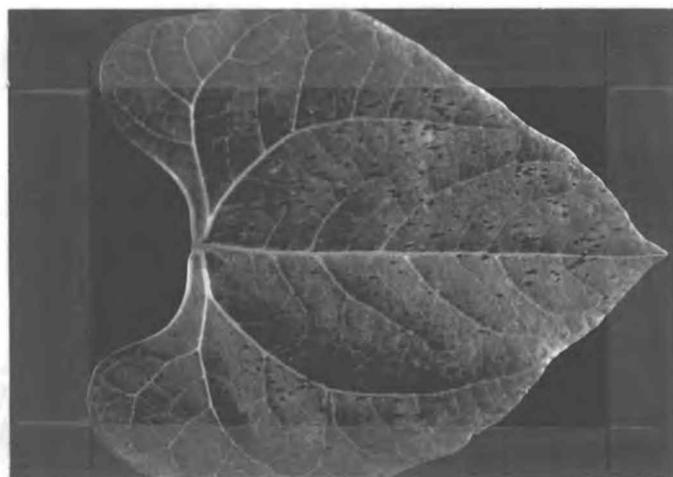
Variety	Infection grades	
	Race 33	Race 34
Seminole 93172 (6)	8	8
Slendergreen 93092 (6)	8	9
Slenderwhite 93102 (6)	7	8
Tendercrop 03134 (6)	7	8
Tendergreen 32304 (6)	8	8
Tenderlong 15 (1)	7	7
Tenderwhite 93037 (6)	8	8
Topcrop 46526 (1)	9	9
U.S. No. 3 (8)	10	10
Wade 46631 (1)	7	8
Wadex 03226 (6)	8	8
White Seeded Slendergreen 60173 (6)	8	8
White Seeded Tendercrop (4)	7	7
No. 643 (8)	2-3	6
No. 650 (8)	10	10
No. 765 (8)	4	4
No. 780 (8)	10	10
No. 814 (8)	8	8

<sup>a</sup> Figures in parenthesis refer to seed source.

- (1) Associated Seed Growers, Inc.
- (2) Ferry-Morse Seed Co.
- (3) Northrup, King & Co.
- (4) Oregon State University, Horticulture Department
- (5) Oregon Trail Farms, Inc.
- (6) Rogers Bros. Seed Co. Inc.
- (7) Canada Department of Agriculture Experiment Station, Morden, Manitoba.
- (8) Dr. W. J. Zaumeyer, U. S. Department of Agriculture, Beltsville.



**Figure 8. Grade 1 infection reaction of race 33 on variety N203.**



**Figure 9. Grade 4 infection reaction of race 33 on variety Florigreen.**

F.M.1, a variety grown commercially in the Willamette Valley, was highly susceptible. A grade 9 infection reaction occurred when inoculated with race 33 (Figure 10) and grade 10 with race 34 (Table 3).

#### Inheritance of Resistance

In the  $F_1$  generation of the cross No. 643 x F.M.1 inoculated with bean rust race 33, there were 32 plants with infection grades 2-3 classed as resistant (Figure 11) and 1 plant with infection grade 7 classed as susceptible. Control inoculations of No. 643 resulted in infection grade 2-3, resistant, and F.M.1 in grade 9, susceptible.

In the  $F_1$  generation of the cross F.M.1 x Flori-green, there were 44 plants with infection grades 3-4

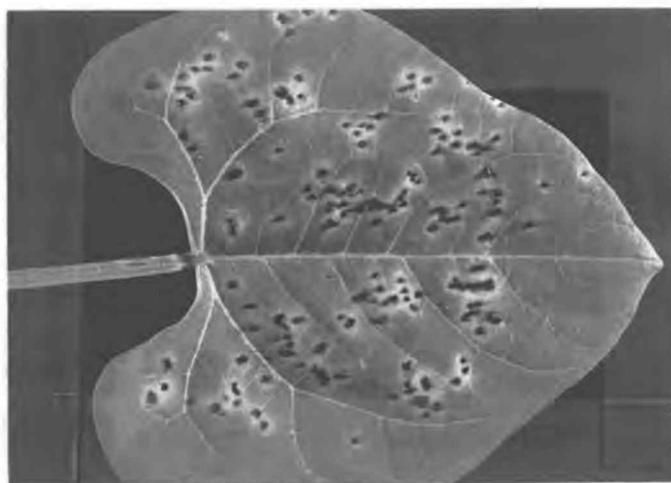


Figure 10. Grade 9 infection reaction of race 33 on variety F.M.1.

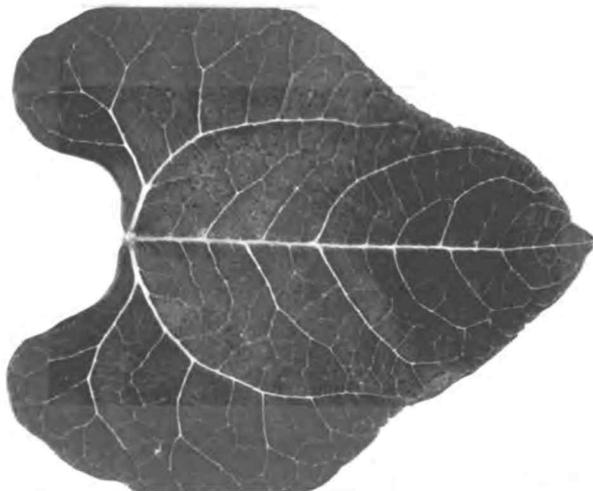


Figure 11. Grade 2-3 infection reaction of race 33 on  $F_1$  plants of cross No. 643 x F.M.1.



Figure 12. Grade 3-4 infection reaction of race 33 on  $F_1$  plants of cross F.M.1 x Florigreen.

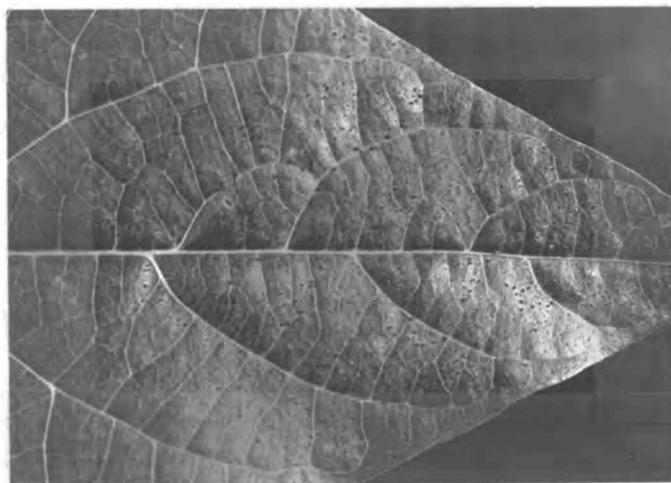
classed as resistant (Figure 12) and 3 plants with infection grade 9 classed as susceptible. Control inoculations of Florigreen resulted in infection grade 4, resistant, and F.M.1 grade 10, susceptible.

The 1 susceptible plant in the cross No. 643 x F.M.1 could not be readily explained. The 3 susceptible plants in the cross F.M.1 x Florigreen came from the same seed pod and undoubtedly resulted from a selfed flower.

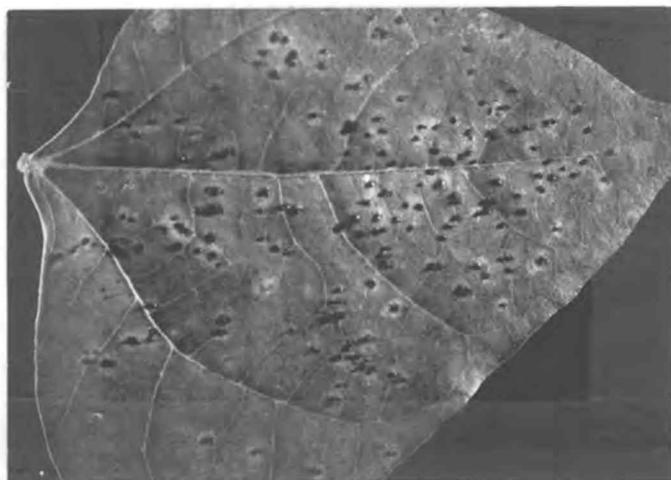
Dominance of resistance in the  $F_1$  generation was clearly shown.

In the  $F_2$  generation of the cross No. 643 x F.M.1, 396 plants from 16 single cross progenies each with 5 to 74 plants were inoculated with bean rust race 33. There were 300 plants classed as resistant and 96 plants classed as susceptible. The classes were discrete and were readily identified (Figures 13, 14). Control inoculations showed No. 643 was resistant and F.M.1 susceptible. A chi-square value of .121 with a probability range of .70 to .80 indicates that the ratio fits satisfactorily a theoretical 3:1 inheritance ratio.

In the  $F_2$  generation of the cross F.M.1 x Florigreen, 358 plants from 25 single cross progenies each with 4 to 41 plants were inoculated with bean rust race 33. There were 267 plants classed as resistant and 91 plants classed as susceptible. As with the other cross, the



**Figure 13. Grade 2-3 infection reaction of race 33 occurring in resistant class of  $F_2$  population of cross No. 643 x F.M.1.**



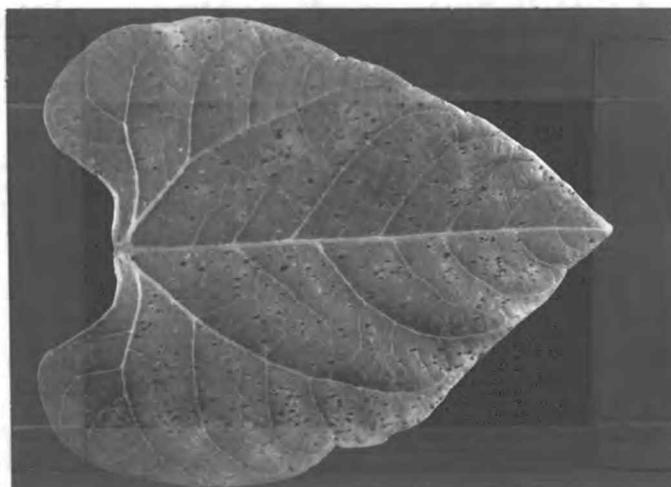
**Figure 14. Grade 9 infection reaction of race 33 occurring in susceptible class of  $F_2$  population of cross No. 643 x F.M.1.**

classes were discrete and readily identified (Figures 15, 16). Control inoculations showed Florigreen was resistant and F.M.1 susceptible. A chi-square value of .033 with a probability range of .80 to .90 indicates that the data fits satisfactorily a theoretical 3:1 inheritance ratio. In this cross, 4 resistant plants and 21 susceptible plants appeared in 2 families. This deviation was attributed to selfing having taken place in the original cross.

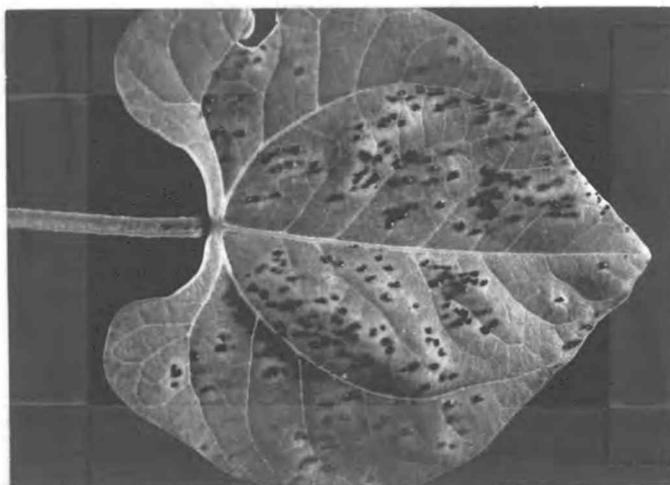
In the No. 643 x F.M.1 cross, when the  $F_1$  was backcrossed to No. 643, 12 resistant and 0 susceptible plants were recorded. When the  $F_1$  was backcrossed to F.M.1, 14 resistant and 12 susceptible plants were recorded.

In the F.M.1 x Florigreen cross, when the  $F_1$  was backcrossed to Florigreen, 16 resistant and 0 susceptible plants were recorded. When the  $F_1$  was backcrossed to F.M.1, 12 resistant and 10 susceptible plants were recorded.

The data from these tests indicate that resistance to race 33 occurring in No. 643 and Florigreen was due to a single dominant gene.



**Figure 15.** Grade 3-4 infection reaction of race 33 occurring in resistant class of  $F_2$  population of cross F.M.1 x Florigreen.



**Figure 16.** Grade 10 infection reaction of race 33 occurring in susceptible class of  $F_2$  population of cross F.M.1 x Florigreen.

## DISCUSSION AND CONCLUSIONS

Bean rust is caused by the parasitism of the fungus Uromyces phaseoli (Pers.) Wint. var. phaseoli of which 32 physiologic races have been reported (17, 9, 25, 38). The occurrence of so many races presents many difficulties in the development of rust resistant snap bean varieties acceptable to the growers and processors. This study was undertaken to identify the races of bean rust prevalent in the Willamette Valley, to find some sources of resistance, and to determine the mode of inheritance of resistance. Such information is necessary for the development of resistant varieties.

Single-spore isolates from collections made in the Willamette Valley in 1958 and 1959 indicated the presence of a single race. Isolates from collections made in 1960 indicated the presence of a dominant race identical to that of the two previous years and a minor race representing 1 percent of the isolates. The infection reactions of these two races on differential bean varieties were different from those of the 32 races previously reported. Based on these reactions, the isolates were described as race 33 and race 34. Races of bean rust recorded earlier from Oregon were 18 and 22 in 1941 and 29 in 1951 (9). These 5 represent the

races identified since bean rust was recorded in Oregon in 1921.

Harter and Zaunmeyer (17, p. 719) noted that a single field collection of bean rust often consisted of two or three distinct physiologic races and in practically every case one form greatly predominated. They (17, p. 727) and Fisher (9, p. 105) presented evidence that races of rust established in a locality in any year may be followed by a different race or groups of races in the following year. Races of rust in Oregon appear to follow this pattern.

Hybridization in fungi has been demonstrated and the origin of new races and morphologic characters by mutation is generally accepted. The different races of bean rust undoubtedly originated by one or the other of these methods, if not by both. Bean rust is autoecious and aecia occur frequently in Oregon under field conditions. Hence races may readily hybridize under natural conditions. Although no one has demonstrated that mutations occur in bean rust, it is probably safe to assume that new races originate in this manner. If these methods of origin are accepted, one is justified in concluding that new races could originate with increasing frequency in the future.

The sudden and dramatic changes in the rust race

population can be attributed to the production of large numbers of spores. Yarwood (35, p. 22) estimated that about 1 million urediospores per  $\text{cm}^2$  are produced from infections of 2 to 100 pustules per  $\text{cm}^2$ . Therefore in the presence of a susceptible host and favorable environment, bean rust, caused by the appearance of new races by hybridization and mutations, may approach epidemic proportions.

Grades of infection reactions on a scale ranging from 0 to 10 were used. Grade 0 represented immunity with no evidence of infection. Grade 1 represented a hypersensitive reaction of the host tissue to infection resulting in necrotic flecks. Grades 2 to 10 represented degrees of resistance or susceptibility based on the size of the spore-bearing pustules. Plants graded 0 and 1 were highly resistant, those graded 2 to 5 were commercially resistant, and those graded 6 to 10 were susceptible.

In a search for sources of resistance, 49 varieties, selections, and strains were inoculated with bean rust races 33 and 34. When inoculated with race 33, a grade 4 infection reaction was recorded on Florigreen and No. 765, grade 3 on Extender, grade 2-3 on No. 643, and grade 1 on N203. When inoculated with race 34, a grade 7 infection reaction was recorded on Florigreen

and Extender, grade 6 on No. 643, grade 4 on No. 765, and grade 1 on N203. Other varieties were rated from 7 to 10 with only slight difference between infection reactions of races 33 and 34. These results indicate sources of resistance to races 33 and 34. Furthermore, they suggest that these races have different nutritional requirements.

Rust resistance in beans is not the result of any morphological character of the plant but is caused by something inherent in the protoplasm of the plant tissues. Nutrients available in the host tissue appear to affect the ability of the rust to infect, survive and produce spores.

Harter and Zaumeyer (17, p. 727) noted that differential host varieties often gave slight differences in the degree of rust infections when inoculated with the same physiologic race at different times. Zaumeyer and Harter (36, p. 619-620) attributed the shifting downward of an infection grade to unfavorable environment, and concluded that certain uncontrollable differences in environment, even though slight, definitely affected the results and played a more important role with some races than with others.

In this study the infection grades on the varieties inoculated with race 33 during a period of 6 months or more were consistent.

It is possible to control bean rust by the use of resistant varieties produced by hybridization. Zaumeyer and Harter (36, p. 621) reported that a single gene was involved in inheritance of resistance to races 1 and 2 and more than one factor governed inheritance to races 6, 11, 12, and 17. Factor for resistance was dominant in crosses inoculated with races 1, 2, 6, and 12 and incompletely dominant with races 11 and 17.

This inheritance study of 2 crosses, No. 643 x F.M.1 and F.M.1 x Florigreen, indicated that resistance to race 33 was controlled by a single dominant gene. Since disease resistance behaves genetically in a manner similar to and independent of morphological characters, except where linkages occur, it is possible to combine disease resistance with any group of morphological characters possessed by the parents.

The results presented here dealt with resistance in the seedling stages of growth under greenhouse conditions. Goulden, Neatby, and Welsh (13, p. 656) indicated that resistance to Puccinia graminis tritici in the maturing plants was inherited independently of seedling resistance and that the two types of resistance were distinct. Levine and Smith (20, p. 727) reported a close agreement in the reactions of seedling and maturing plants to races of oat rust, Puccinia graminis avenae. Though this phase

of the bean rust problem was not studied, observations indicated that bean varieties susceptible in the maturing stages in the field are equally susceptible in the seedling stage in the greenhouse.

With the large number of known races of rust, the chances of developing resistant varieties decreases. A knowledge of the nutritional requirements of the races becomes important in an extensive program of breeding for rust resistance. The susceptibility or resistance of many bean varieties has been determined for some races. A grade 0 or 1 reaction for all races would be the ultimate goal. The best source of resistance now occurs in the variety No. 765, a selection from Kentucky Wonder Wax. It is resistant to all races except races 13, 23, and 31.

## SUMMARY

Differential infection reactions of single-spore isolates from collections of bean rust, Uromyces phaseoli (Pers.) Wint. var. phaseoli made in 1958 and 1959 in the Willamette Valley, indicated the presence of a single race. Results from collections made in 1960 indicated the presence of the same predominant race and a minor race representing 1 percent of the isolates. Each differed in reaction from the 32 previously described races. They were considered to be unrecorded physiologic races of bean rust and were designated as race 33 and race 34. The grades of infection reactions for these two races on the standard differential bean varieties are described.

Most of the 49 varieties, strains and selections of bean (Phaseolus vulgaris L.) inoculated with race 33 and race 34 were susceptible.

Resistance to race 33 was found in bean varieties N203, California Small White selection No. 643, Kentucky Wonder Wax selection No. 765, Florigreen, and Extender. Resistance to race 34 was found in N203 and No. 765.

Resistance in N203 caused a hypersensitive reaction of the host to infection resulting in necrotic flecks of varying sizes and shapes.

Resistance in No. 643, No. 765, Florigreen, and

Extender caused formation of small sori and production of relatively few spores.

Inheritance studies involving crosses No. 643 x F.M.1 and F.M.1 x Florigreen showed that resistance to race 33 in No. 643 and Florigreen was controlled by a single dominant gene. The resistant and susceptible classes were discrete and readily identified.

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