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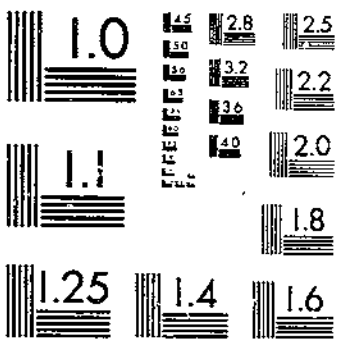

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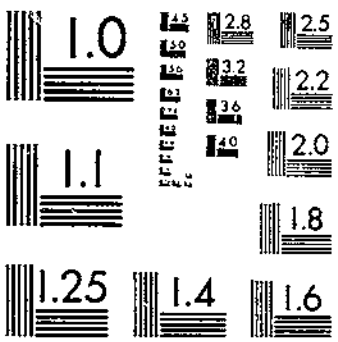

MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

TECHNICAL BULLETIN No. 704 NOVEMBER 1939

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

BACTERIAL WILT OF LESPEDEZA[1]

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CONTENTS

INTRODUCTION

In the summer of 1937 a bacterial parasite of annual lespedeza (*Lespedeza stipulacea* Maxim and *L. striata* (Thunb.) H. and A.) was first observed to be causing wilting and death of infected plants at the Arlington Experiment Farm, Arlington, Va.[3] Since then the causal organism has been isolated from diseased plants of annual lespedeza from Missouri, Kansas, Illinois, Tennessee, and New York. It would appear, therefore, that the disease is widely distributed throughout many of the areas in which the annual lespedezas have become an important forage crop, and that it must be considered a serious threat to the continued profitable use of these legumes for hay and pasture. For the past several years, reports have come from Missouri that the early strain of Korean lespedeza (F. C. No. 19604) was being attacked by a disease the cause of which was unknown. Since this strain of annual lespedeza has been shown by artificial inoculations and field observations at the Arlington Farm to be especially susceptible to bacterial wilt, it appears probable that this is the disease that has been active in lespedeza plantings in Missouri. This probability is strengthened by the ready isolation of the bacterium from typical diseased plants of No. 19604 sent to the authors from Missouri by C. A. Helm. This bulletin presents a description of the disease, the studies made of the characteristics of the causal organism, and the results of the artificial inoculations made to establish the pathogenicity

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[2] The authors are indebted to Florence Hedges of the Division of Fruit and Vegetable Crops and Diseases for supplying the cultures of *Bacterium phaseoli sojense* and *Bact. flaccumfaciens* used in the comparative studies.

[3] LEFEBVRE, C. L., AYERS, T. T., and JOHNSON, H. W. A BACTERIAL WILT OF LESPEDEZA. (Abstract) Phytopathology 29: 15. 1939.

and identity of the bacterium and to determine tentatively the relative susceptibility of various species and strains of lespedeza to bacterial wilt.

DESCRIPTION OF THE DISEASE

Dark, water-soaked spots on the leaflets appear to be the first visible symptom of infection. Infected leaves then soon become grayish brown, desiccated, and curled. A thin incrusting film of bacterial exudate is sometimes seen on the leaflets and upon sectioning an abundant bacterial extrusion occurs from the severed vascular bundles. The appearance of the infected leaflets of Early Korean lespedeza No. 19604 is shown in figure 1, *A*.

Systemic infection follows rather rapidly in the case of susceptible strains of annual lespedeza, and within a few weeks entire plants wilt and die as is shown in figure 1, *B*. Upon sectioning the stems of such plants an abundant bacterial extrusion occurs. Frequently the stems of systemically infected plants crack open and a yellowish bacterial exudate forms and hardens. Such drops of exudate are illustrated in figure 2, *A*-*C*. The ease with which the bacterial extrusion may be demonstrated provides a ready means of distinguishing this disease from a similar wilting and death of annual lespedeza plants caused by *Sclerotium rolfsii* Sacc. in the Southeastern States.

THE CAUSAL ORGANISM

ISOLATION

Bacteria are abundant in the leaves and stems of infected plants, and an identical organism has been isolated repeatedly. Isolation was preceded in all cases by surface rinsing of the tissues with alcohol and mercuric chloride solution. The surface-sterilized pieces of tissue were then transferred to a tube of nutrient broth and after standing for a time the tube was shaken and dilution plates were made from the bacterial suspension in the usual manner. In August 1937 five isolations were made from diseased plants of Early Korean lespedeza No. 19604 collected at the Arlington Farm. The pathogenicity of these isolants was proved by a preliminary set of inoculations and the bacterium was reisolated from the diseased plants. One reisolation was designated No. 303, and it has been used in the detailed cultural studies. Since then, the organism has been isolated from diseased plants of Korean lespedeza (*Lespedeza stipulacea*) collected at Columbia, Mo.; Manhattan, Kans.; Urbana, Ill.; Ithaca, N. Y., and Knoxville, Tenn. It has also been isolated from diseased plants of Tennessee 76 lespedeza (*L. striata*) collected at Knoxville. The isolations from Ithaca and Urbana were designated Nos. C-24 and C-34, respectively, and these have been used in comparative cultural studies and tests of pathogenicity with isolation No. 303 from the Arlington Farm. Isolations C-4 and C-6 from diseased plants of Tennessee 76 and Late Korean lespedezas collected at Knoxville have also been used in pathogenicity studies.

MORPHOLOGY

The pathogen is a small rod with rounded ends, usually occurring singly or in pairs but occasionally forming short chains. It stains readily with Ziehl's carbol fuchsin and carbol gentian violet (Nicolle)

FIGURE 1.—Early Korean lespedeza (No. 19604) growing at the Arlington Experiment Farm in August 1937: *A*, Leaflets showing the discoloration and necrosis caused by bacterial infection; *B*, dead plants caused by bacterial infection.

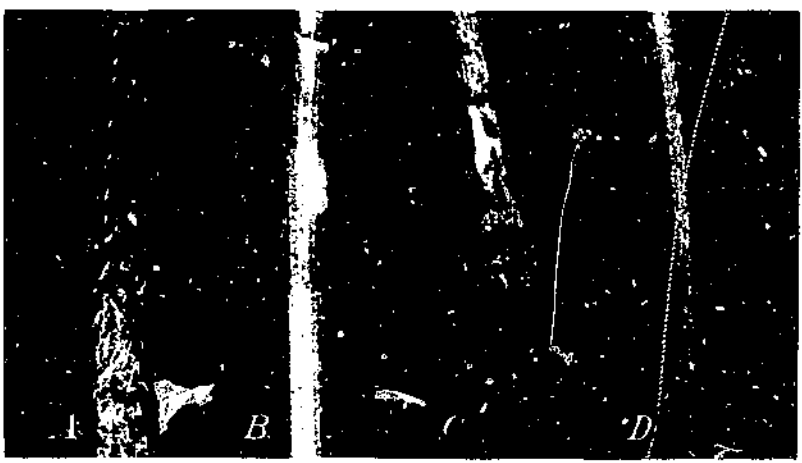

FIGURE 2.—Hardened drops of bacterial exudate from cracks in the stems of inoculated plants of: *A*, Kobe (F. C. No. 22456); *B*, Standard Korean (F. C. No. 22457); *C*, Early Korean (F. C. No. 19604) lespedezas. *D* is from a healthy, noninoculated check plant of Early Korean lespedeza. × 7.

but only lightly with Loeffler's alkaline methylene blue. When stained from 48-hour potato-dextrose-agar cultures with carbol gentian violet, the cells average 1.62μ by 0.56μ with extremes in length from 2.00μ to 0.90μ and in width from 0.72μ to 0.34μ.

The organism is motile by means of a single polar flagellum the presence of which has been demonstrated by the staining method of

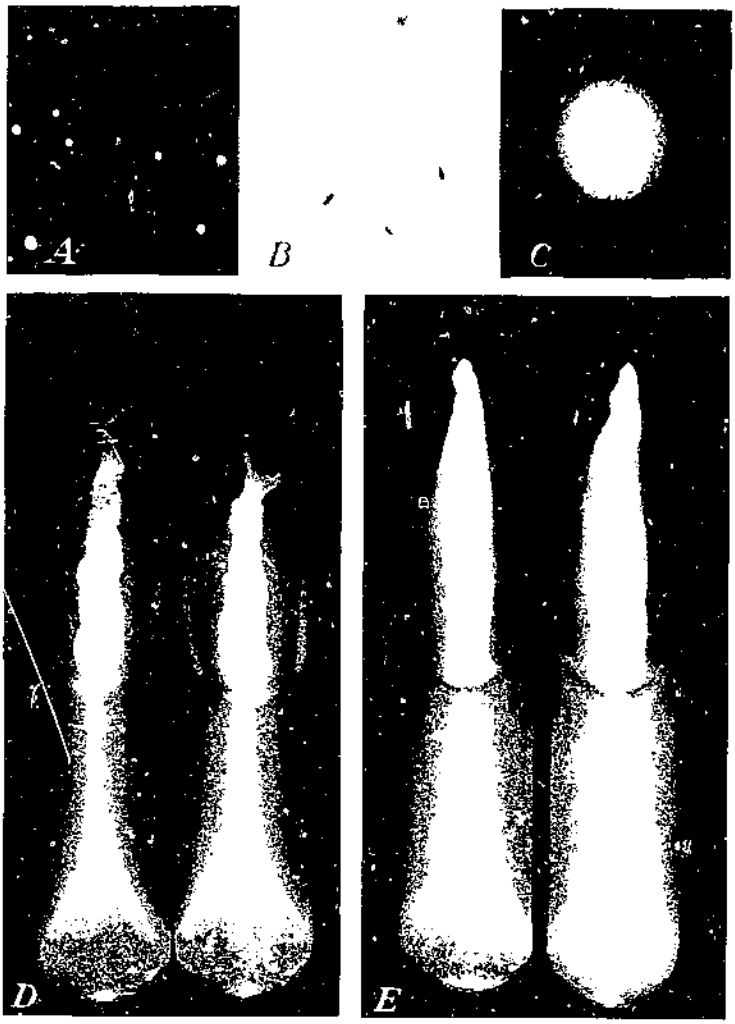

FIGURE 3.—Cultural and morphological characters of isolation No. 303 of *Phytomonas hyacinthi*: *A*, 3-day surface and embedded colonies on nutrient agar, × 1; *B*, 1-day culture on potato-dextrose agar, Casares-Gil's stain, × 1,700; *C*, 4-day surface colony on nutrient agar, × 7; *D*, 5-day nutrient-agar slants, × 1; *E*, 5-day potato-dextrose-agar slants, × 1.

Casares-Gil (fig. 3, *B*). Endospores and marked involution forms have not been observed. The organism is Gram-negative and has a thin, enveloping sheath, which has been demonstrated by Anthony's method of capsule staining.

CULTURAL CHARACTERS

The cultural characters as here described have been worked out with isolation No. 303 of the bacterium, which was reisolated from an artificially inoculated plant of Early Korean lespedeza (No. 19604) at the Arlington Farm. This is considered the type strain of the organism, and it has been used in all comparative cultural studies with other isolations of the bacterium. Dehydrated culture media were used in these studies and colony, stroke and nutrient broth characters were determined on cultures held in an incubator at 32° to 33° C. Gelatin-stab and plate characters were determined on cultures held in the refrigerator at 15° to 18°. The other cultures were kept on a table or open shelves at laboratory temperature. Color determinations follow the Ridgway color standards.[4]

Agar colonies.—On nutrient agar, colonies are evident within 48 hours and in 4 days have attained a diameter of 3 to 5 mm. (fig. 3, *C*). They are circular, with entire margins, raised, glistening, translucent, viscid, and primuline yellow in color. Submerged colonies remain small and are lenticular in shape (fig. 3, *A*).

On potato-dextrose agar growth is more abundant and the colonies become somewhat larger, but the characters are the same as on nutrient agar.

Agar strokes.—On nutrient-agar slants, growth is moderate after 24 hours and apparently little further growth occurs since it is still moderate after 7 days. It is filiform, appressed, glistening, viscid, and primuline yellow in color (fig. 3, *D*). The medium is unchanged and there is no odor.

On potato-dextrose-agar slants, growth is moderate after 24 hours and abundant after 3 days. It spreads across the base of the slant and is butyrous (fig. 3, *E*). The color is amber yellow. Other characters are the same as on nutrient agar.

Nutrient broth.—In nutrient broth (pH 6.7) there is slight clouding in 24 hours and moderate to heavy clouding in 48 hours. After 4 days there is flocculent surface growth and a thin pellicle has formed within 7 days. This breaks readily and falls in thin flakes. There is only a very slight odor and very little sediment is formed at the bottom of the tube.

Potato cylinders.—On autoclaved potato cylinders growth is moderate after 24 hours and abundant in 48. It spreads completely across the slant and collects abundantly at the butt of the cylinder. Growth is glistening and primuline yellow in color.

Gelatin plates.—On gelatin plates at 18° C. growth is slow, and after 6 days the colonies are less than 1 mm. in diameter. The beginning of liquefaction of the medium around the colonies is evident as cup-shaped depressions at this time.

Gelatin stabs.—Growth in gelatin stabs is best at top with slight filiform growth along line of puncture. Liquefaction is crateriform at first but soon becomes stratiform and after 18 days at room temperature the upper 12 mm. of gelatin in the tubes is liquefied. The controls remained solid during this time.

Skim milk.—In skim milk, complete peptonization occurred within 8 days; no curd was formed. The color of the medium changed from

[4] RIDGWAY, ROBERT. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.

an opaque cream buff to tawny. The pH reading changed from 6.4 at the start to 6.5 after 5 and 7.9 after 11 days.

Litmus milk.—In litmus milk chemical changes were the same as in skim milk. Color change was from opaque light russet vinaceous to translucent liver brown. The pH reading changed from 6.4 to 7.0 after 5 and to 8.0 after 11 days.

Purple milk.—In purple milk complete peptonization occurred as in skim milk. Color change was from opaque deep olive buff to translucent garnet. The pH reading changed from 6.8 to 7.0 after 5 and to 7.9 after 11 days.

FIGURE 4.—Four-day culture of *Phytomonas lespedezae* on starch agar tested with iodine to show diastatic action.

Blood serum.—Blood serum stroke cultures showed moderate growth which slowly liquefied the medium and changed it from ivory yellow to cinnamon brown.

Egg albumin.—On egg albumin slow liquefaction occurred, and the color changed from white to mummy brown after 5 months.

Hydrolysis of starch.—The organism made abundant growth on starch agar and formed a streak about 8 mm. broad within 4 days. When tested with a saturated solution of iodine in 50-percent alcohol, a cleared zone 40 mm. broad was revealed (fig. 4). After 6 days, hydrolysis was complete in a zone 50 mm. wide. Tubes of nutrient broth to which had been added 0.2 percent of soluble starch were also

inoculated. After 4 days, tests with iodine solution showed no starch present, and tests with Fehling's solution revealed the presence of reducing sugars.

Indole production.—When cultivated in nutrient broth plus 0.1 percent of peptone containing tryptone and in a 0.1-percent aqueous solution of peptone containing tryptone, the organism produced indole after 11 days, as shown by the Ehrlich-Böhme and Gnezda techniques and the vanillin test.

Hydrogen sulfide.—Lead acetate strips were darkened in 7 days when suspended above cultures in nutrient broth. Lead acetate agar became dark brown along the margins of bacterial streaks.

Nitrate reduction.—Nitrates are not reduced. When the organism was grown on nitrate agar, no gas was produced and tests for nitrite with sulfanilic acid and alpha-napthylamine were negative. The presence of nitrates in these cultures after 6 days was confirmed.

Crystals.—Narrow, elongate colorless crystals were formed on the surface of cultures on nutrient agar and extended for about 5 mm. into the medium.

Fermentation tubes.—Cultures were grown in fermentation tubes containing phenol red broth with 0.5 percent of the following carbohydrates: Glucose, lactose, sucrose, maltose, soluble starch, and mannitol. No gas was formed. Clouding was moderate in the open arm and a ring was formed. There was no clouding in the closed arm. Tested with the glass electrode the inoculated tubes were always alkaline whichever carbon compound was used. From an original pH of 7.2 alkalinity gradually increased. Final pH ranged from 7.6 to 7.9 after 1 month.

Relation to oxygen.—The organism is an aerobe. Growth occurred only in the open arm of the fermentation tubes as recorded above. In shake cultures of potato-dextrose agar growth was at the surface only.

Temperature relations.—The bacterium was grown on potato-dextrose-agar slants and was incubated at temperatures ranging from 5° to 40° C. at 5° intervals. Growth was moderate at 30° and 35° after 24 hours. After 10 days there was scanty growth at 10° and abundant growth from 15° to 35°, inclusive. No growth was evident at 5° or 40°. The organism appears to grow well over a fairly wide range of temperature and apparently has an optimum near 35°, since more growth developed at that temperature than at 30°.

PATHOGENICITY

Preliminary tests of the various isolations made from diseased plants of Korean lespedeza No. 19604 at the Arlington Farm in 1937 demonstrated the ability of the bacterium to cause wilting and death of seedling plants of this strain of annual lespedeza within 2 weeks after inoculation under conditions prevailing in the greenhouse. The organism was reisolated readily from the inoculated plants, and one reisolation was designated No. 303. It has been used most extensively in the inoculation experiments made since to determine the relative susceptibility of various strains of lespedeza to this bacterial disease.

A typical inoculation experiment to show the extreme susceptibility of seedling plants of Early Korean lespedeza (No. 19604) to this isolation of the bacterium was begun on February 15, 1938. On this

date 15 lespedeza seedlings approximately 4 inches in height were inoculated by pricking 3 or 4 leaves with a needle dipped in a bacterial suspension while the leaves were supported from below on a pot label also dipped in the bacterial suspension. Five check plants were treated in the same manner except that sterile distilled water was substituted for the bacterial suspension. On February 28, 1938, 11 of the inoculated plants were dead and the other 4 showed definite symptoms of the disease. The presence of the bacteria in the vascular system of all 15 inoculated plants was demonstrated by sectioning the stems and observing the bacterial extrusion into water under the microscope. The 5 check plants were still healthy and approximately 5½ inches in height, whereas the inoculated plants appeared to have made no further growth during the 2 weeks after inoculation. The results of this experiment are illustrated in figure 5 where the stunting, leaf curling, wilting, and death of 3 of the inoculated seedlings is compared with the erect growth and healthy appearance of a check plant.

FIGURE 5.—Seedlings of Early Korean lespedeza (No. 19604) showing the stunting, leaf curling, wilting, and necrosis of the three inoculated seedlings (*A–C*) as compared with the healthy appearance of a noninoculated check seedling (*D*). The inoculations were made February 15, 1938, and the photograph was taken March 1.

On March 15, 1938, a preliminary experiment was made to determine the relative susceptibility of several strains of annual lespedeza to isolation 303 of the bacterium. On this date 5 seedlings each of 4 strains of annual lespedeza, Kobe (F. C. No. 22456), Common (F. P. I. No. 81742), Standard Korean (F. C. No. 22457), and Early Korean (F. C. No. 19604) were inoculated in the manner described above, and 3 check plants of each strain were pricked with a needle dipped in sterile distilled water. On April 2, 1938, all 20 inoculated plants showed definite symptoms of the wilt disease, and all 5 plants of Early Korean, 2 of Common, and 1 of Kobe were dead. By April 18 the remaining 3 plants of Common and 2 more of Kobe were dead, while the 5 inoculated plants of Standard Korean were still alive al-

though showing pronounced symptoms of disease. The check plants of each strain were still alive on this date and were from 1½ to 2 inches taller than the diseased plants.

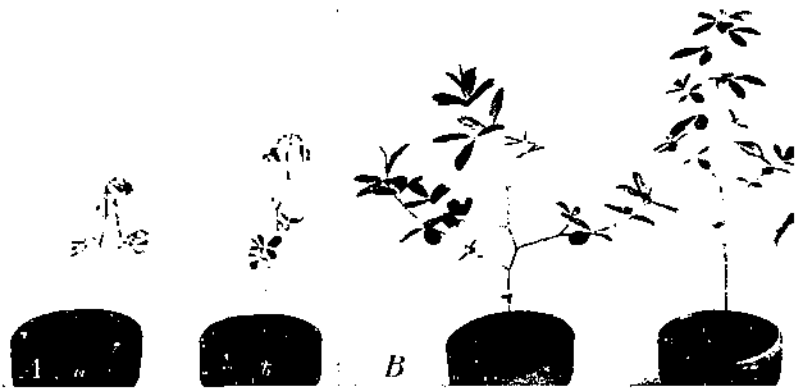

FIGURE 6.—*A*, Seedlings of Kobe (*a*) and Common (*b*) lespedeza showing the wilting and necrosis that resulted from bacterial inoculation. The inoculations were made March 15, 1938, and the photograph was taken April 4. *B*, Noninoculated seedlings of the same age.

The appearance of an inoculated plant of Kobe and Common lespedeza on April 4, 1938, is compared in figure 6 with the appearance of a noninoculated check plant of each strain. The more rapid

FIGURE 7.—*A*, Seedlings of Standard Korean (*a*) and Early Korean (*b*) lespedeza showing the wilting and necrosis that resulted from bacterial inoculation. The inoculations were made March 15, 1938, and the photograph was taken April 4. The more rapid killing of the Early Korean seedling indicates the extreme susceptibility of this strain to bacterial wilt. *B*, Noninoculated seedlings of the same age.

killing of the Early Korean plants in this experiment is evident in figure 7, as is the greater ability of the Standard Korean to withstand the disease and make some growth even after the bacteria are introduced into it by wounding. The stunting that resulted from bacterial

inoculation is obvious by comparing the diseased plants (*A*) with the noninoculated plants (*B*) in figure 7.

Three more extensive inoculation experiments to determine the relative susceptibility of various strains of annual lespedeza to bacterial wilt were made in the greenhouse at the Arlington Farm at later dates in 1938. The plants were inoculated in the manner described on page 8 with isolation 303 of the causal organism, and in each experiment the control plants were treated the same as the inoculated plants, except that sterile distilled water was substituted for the bacterial suspension. The results of these three experiments are summarized in table 1. All of the strains of annual lespedeza tested proved to be susceptible in some degree to bacterial wilt. Practically 100-percent infection was obtained with all strains, but the severity of infection varied from light in the cases of Standard Korean (No. 22457) and Common (F. C. No. 22590) to severe in the cases of Early Korean (both F. C. No. 19604 and F. P. I. No. 59379), Harbin (F. P. I. No. 65280), and Tennessee 76 (F. C. No. 30611). In the cases of severe infection many or all of the inoculated plants wilted and died within a few weeks after inoculation, but in the cases of light infection few or none of the inoculated plants died and the symptoms of disease appeared to be more or less restricted to the upper portions of the plants where the inoculum had been introduced. Of the 101 control plants that were a part of these 3 inoculation experiments, 16 developed the disease while the other 85 remained healthy. Since the control plants were kept on the greenhouse bench in close proximity to the inoculated plants, it is possible that bacteria were spread to some of the checks during watering.

TABLE 1.—*Relative susceptibility of various strains of annual lespedeza to bacterial wilt as determined by greenhouse inoculations made at Arlington Experiment Farm, Arlington, Va., in 1938, with isolation No. 303 of the causal organism*

Species	Common name	F. C. or F. P. I. No.	Plants inoculated	Plants infected	Severity of infection [1]
			Number	*Number*	
Lespedeza stipulacea	Early Korean	19604	35	35	4
	do	59379	50	50	4
	Harbin	65280	18	18	4
	Standard Korean	22457	73	73	2
	Late Korean	19601	74	74	3
Lespedeza striata	Common	81742	72	72	3+
	do	22590	73	72	2
	Kobe	22456	75	75	3
	Tennessee 76	30611	24	24	4

[1] In the scale used to denote severity of infection, 0 indicates no infection; 1, very light; 2, light; 3, moderate; and 4, severe infection.

Inoculation experiments similar to those just described were made also to test the pathogenicity of other isolations of the bacterium. On October 10, 1938, 25 seedlings of Early Korean (No. 19604) were inoculated with isolant C–34 of the bacterium, which had been isolated from a diseased plant of Korean lespedeza collected at Urbana, Ill. Two weeks later, 23 of the inoculated plants were in various stages of wilting while the 5 control plants appeared healthy. On October 11, 21 seedlings of this strain of lespedeza were inoculated with isolant C–24 of the bacterium which had been isolated from a diseased plant

of Early Korean lespedeza collected in the Soil Conservation Nursery at Ithaca, N. Y. On October 28 all of the 21 inoculated plants showed symptoms of infection with 19 definitely wilting. The 5 check plants were all still healthy. On October 18, 20 seedlings of Tennessee 76 lespedeza were inoculated with isolant C–4 of the bacterium, and the same number were inoculated with isolant C–6. These isolations had been made from diseased plants of Tennessee 76 and Late Korean lespedezas collected at Knoxville, Tenn. On November 2, 1938, 12 of the 20 plants inoculated with isolation C–4 were entirely dead, while the remaining 8 were partially killed by the disease. Of the 20 plants inoculated with isolation C–6, 17 were entirely dead by this date and the remaining 3 were partially dead. The severity of infection in these cases indicates the high susceptibility of the Tennessee 76 strain of lespedeza to these isolations of the causal organism. Its susceptibility to isolation 303 of the bacterium has been mentioned on page 10 in discussing the data presented in table 1.

Although this bacterial disease has not yet been observed to affect perennial species of lespedeza in the field, artificial inoculations in the greenhouse have shown that several perennial species are susceptible to some extent. In one experiment a total of 74 seedlings of *Lespedeza sericea* (Thunb.) Benth. (F. C. No. 04730) and 75 seedlings of *Lespedeza* sp. (F. P. I. No. 82098), a species closely related to *L. sericea* and *L. juncea* Wall., were inoculated by pricking the leaves with a needle dipped in a bacterial suspension of isolation 303, as described on page 8. On *L. sericea* the inoculated leaves soon turned gray, became desiccated and curled, and eventually dropped from the plants. However, growth of the plants was not stopped, and the newly formed tip leaves appeared free from symptoms of the disease (fig. 8). Although there were no external symptoms indicating that the bacteria had invaded the vascular system of these plants, microscopic examination of sections cut from the stem near the ground line showed bacteria streaming out from the conducting strands.

The symptoms on *Lespedeza* sp. No. 82098 are quite similar to those on *L. sericea*, but in this species growth appears to cease upon inoculation and the entire tips of the plants die (fig. 9, *A*). In this species also, the presence of bacteria in the conducting system of the stem near the crown of the plants can be readily demonstrated by microscopic examination. It would appear, therefore, that both of these perennial species of lespedeza are susceptible to bacterial wilt but that the severity of infection is different for the two species. On the basis of the inoculations made to date, infection on *L. sericea* may be classified as very light to light, whereas infection on *Lespedeza* sp. No. 82098 should probably be considered as moderate. The greater susceptibility of the latter species, at least to isolation 303 of the bacterium, is shown further by the fact that 74 of the 75 inoculated plants developed symptoms of the disease, whereas in the case of *L. sericea*, only 42 out of 74 inoculated plants became diseased.

In later experiments 12 other perennial species of *Lespedeza* were inoculated by pricking the leaves with a needle dipped in a bacterial suspension prepared from cultures of isolation 303 of the parasite. The results of these experiments are summarized in table 2. These perennial species also vary in their susceptibility to the disease under conditions prevailing in the greenhouse. *L. daurica* Schindl. (No.

82435), *L. frutescens* (L.) Britton, *L. inschanica* Schindl., *L. procumbens* Michx., and *L. virginica* (L.) Britton appear to be highly susceptible, as shown by the high percentage of plants infected and by the severity of infection. The stunting, leaf curling, and wilting that

FIGURE 8.—Seedlings of *Lespedeza sericea* (No. 04730), a perennial species showing leaf curling and partial defoliation as a result of bacterial inoculation. Growth was not stopped in this species, however, and the newly formed tip leaves appeared free from symptoms of the disease as is shown by comparing the noninoculated plant (*A*) with the two inoculated plants (*B–C*). The seedlings were inoculated September 13, 1938, and photographed October 17.

resulted from artificial inoculation of three of these species is shown in figure 9, *B* and in figure 10, *A* and *B*. *L. capitata* Michx. appears to be moderately susceptible as far as severity of infection is concerned, although all 25 of the plants inoculated became diseased. On the other hand, *L. bicolor* Turcz., *L. cyrtobotrya* Miq., *L. daurica* (prostrate

form, No. 90355), *L. formosa* (Vogel) Koehne, *L. hirta* (L.) Hornem., *L. latissima* Nakai, and *L. thunbergii* Nakai appear to be highly

FIGURE 9.—Seedlings of *Lespedeza* sp. (No. 82098) (*A*) and of *L. inschanica* (*B*) showing stunting and wilting as a result of bacterial inoculation. Noninoculated plants at *a*. The inoculations of *Lespedeza* sp. 82098 were made September 13, 1938, and the photograph was taken October 17. The inoculations of *L. inschanica* were made January 6, 1939, and the photograph was taken February 20.

resistant to this parasite, as they show no external symptoms of the disease. In these species, extrusion of the bacteria could be demonstrated only from sections of the petioles of the leaflets that had been

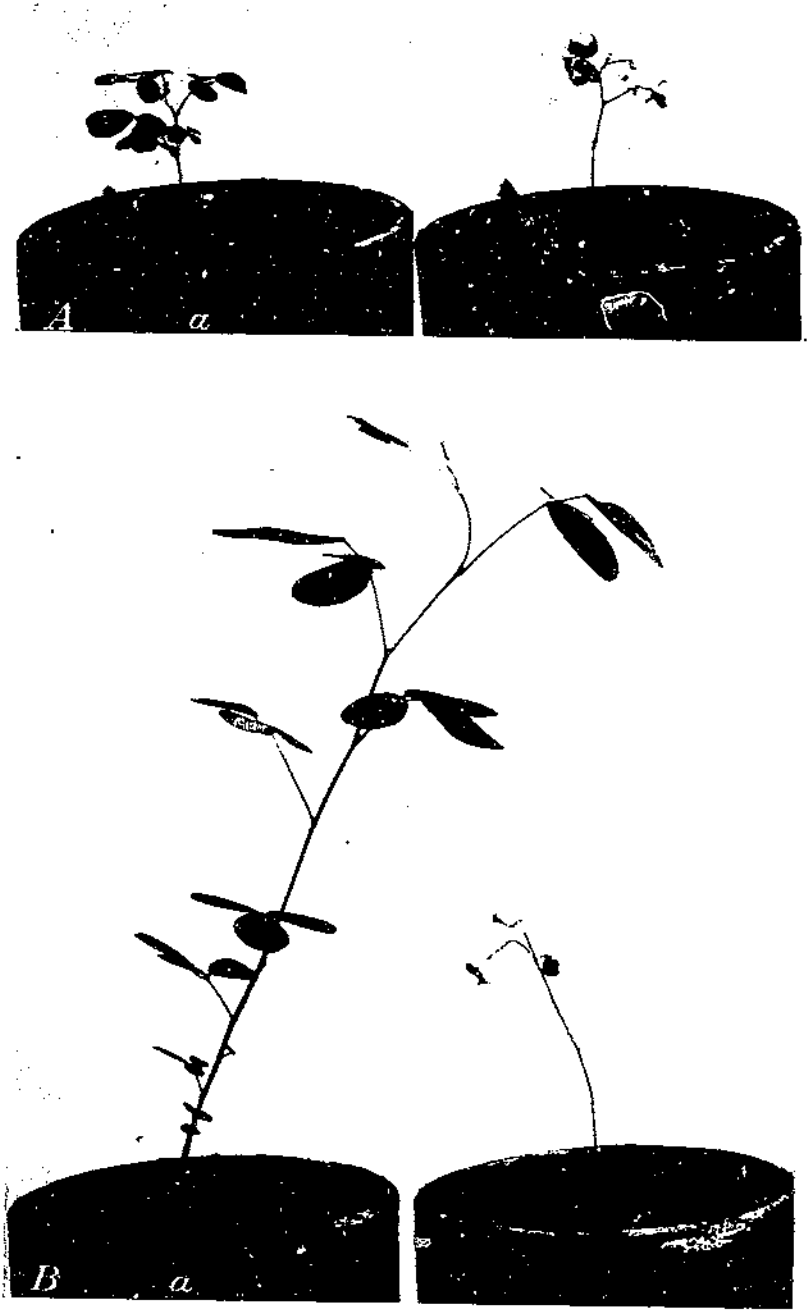

FIGURE 10.—Seedlings of *Lespedeza procumbens* (*A*) and *L. frutescens* (*B*) showing stunting and wilting as a result of bacterial inoculation. Noninoculated plants at *a*. Inoculations were made January 7, 1939, and photographs were taken February 20.

inoculated, and it would appear that for some reason the parasite is unable to progress in the vascular system of these plants.

TABLE 2.—*Relative susceptibility of different perennial species of* Lespedeza *to bacterial wilt as determined by greenhouse inoculations made at Arlington Experiment Farm, Arlington, Va., during the winter of 1938–39, with isolation 303 of the bacterium*

Species	P. C. or P. P. I. No.	Plants inoculated	Plants infected	Severity of infection[1]	Species	P. C. or P. P. I. No.	Plants inoculated	Plants infected	Severity of infection[1]
		Number	*Number*				*Number*	*Number*	
L. bicolor	62861	35	0	0	*L. hirta*	21069	17	0	0
L. capitata	------	25	25	2	*L. inexhanica*	21886	25	25	3
L. cyrtobotrya	62863	57	0	0	*L. latissima*	19285	95	0	0
L. daurica	82435	25	23	4	*L. procumbens*	05228	34	32	3
Do	90355	69	0	0	*L. thunbergii*	25009	13	0	0
L. formosa	82481	30	0	0	*L. virginica*	02333	25	25	4
L. frutescens	21054	25	24	4					

[1] See table 1, footnote 1 for scale of severity.

A bacterium resembling isolation 303 in culture was reisolated from inoculated plants of *L. sericea* and *L. cyrtobotrya*, and its pathogenicity on Early Korean lespedeza (*L. stipulacea*, F. C. No. 19604), was proved by successful inoculations.

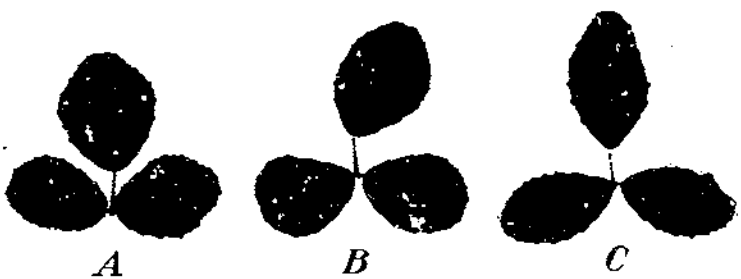

FIGURE 1.—Two inoculated leaves (*A*, *B*) and a noninoculated leaf (*C*) of white sweetclover (*Melilotus alba*), showing the localized discoloration that developed around some of the inoculation wounds on this plant.

Several isolations of the lespedeza bacterium were used also to inoculate a few plants of each of the following species and varieties of legumes: Alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), white sweetclover (*Melilotus alba* L.), ladino clover (*Trifolium repens* var. *latum*), broadbean (*Vicia faba* L.), rattlebox (*Crotalaria spectabilis* Roth and *C. striata* DC.), Refugee bean (*Phaseolus vulgaris* L.), kudzu (*Pueraria thunbergiana* (Sieb. and Zucc.) Benth.), lotus (*Lotus corniculatus* L.), and Hahto, Hollybrook, Ito San, Laredo, Mammoth Yellow, Midwest, Tokyo, and Yellow Biloxi varieties of soybean (*Soja max* (L.) Piper). Some of these plants were entirely unaffected by the introduction of this bacterium into the leaves by needle pricks whereas in others a small discolored area developed around some of the inoculation wounds. Such a host reaction is illustrated in figure 11, which shows two inoculated leaves and a check

leaf of white sweetclover. In no case was any wilting observed, and it would appear from these preliminary inoculations that these species and varieties of legumes are not susceptible to the bacterial wilt of lespedeza.

RELATION TO THE HOST

The relation of the parasite to the host—the routes by which the bacteria gain entrance, the manner of their spread through the tissues, the conditions under which the disease progresses to cause the death of the plant, and the bearing which age may have on the response of plants to infection—has not been fully investigated. It seems desirable, nevertheless, to present here a statement of the more obvious features that have been observed and examined.

METHOD OF INFECTION

Direct inoculations of lespedeza plants with pure cultures of the parasite have been made successfully by three methods, which involved wounding of the host. The one used most commonly in this investigation consisted of pricking the leaves with a needle dipped in bacterial suspension, as was described under Pathogenicity (p. 8). Infection was obtained also by cutting the roots of seedlings while they were immersed in bacterial suspension and by cutting across young leaflets followed by immediate dipping of the cut surfaces into bacterial suspension. Usually the plants were placed in a moist atmosphere for 24 hours after inoculation. It was found, however, that this treatment was not necessary to secure infection. A high percentage of wilt developed when seedling plants inoculated by the needle-prick method were placed at once on a greenhouse bench in direct sunlight at a time when the greenhouse temperature was approximately 94° F.

Early attempts to secure infection by atomizing or swabbing the leaves of older plants with bacterial suspension proved unsuccessful. Later it was found that young seedlings with only the primary leaves exposed become infected when atomized with bacterial suspension. Numerous, small, scattered, water-soaked spots developed on the primary leaves, especially on their lower surfaces, which would suggest that the bacteria had gained entrance to the leaves through the stomata. The presence of the bacteria in the stems of these young seedlings was later demonstrated by sectioning and microscopic examination. From this it appears that the parasite can make its way through parenchyma from wounds or from stomata to the vascular tissue.

DISTRIBUTION WITHIN THE HOST

Whatever the method by which the parasite gains entrance into the vascular system of infected plants, it apparently travels rather rapidly in that system, passing from leaves to the base of the stem or from roots through the crown to the stem without appreciable difficulty. Seedling plants of the susceptible early strain of Korean lespedeza are often wilted and dead within 3 weeks after inoculation, and abundant bacterial extrusion occurs from segments cut from any point in the stems of these plants. A similar extrusion of masses of bacteria from the vascular system of the stem was observed in the case of all the species and strains of annual lespedeza inoculated, and in the case of several perennial species, although in some of the latter vascular in-

vasion did not appear to cause a general wilting and necrosis of the infected plants.

To demonstrate more fully the presence of the bacteria in the water-conducting vessels of the host, pieces of the stem of infected plants of Early Korean lespedeza were killed and fixed in formalin acetic alcohol. These were then embedded in paraffin, sectioned, and stained with crystal violet and orange G. A longitudinal section showing vessels in the stem filled with bacteria is presented as figure 12. Whether the material clogging the vessels is entirely of bacterial origin or is deposited in part by the plant is not known.

SEED INVASION

Since the annual lespedezas bear their seed in the leaf axils along the stems and bacteria are found in leaves and stems close to the seed,

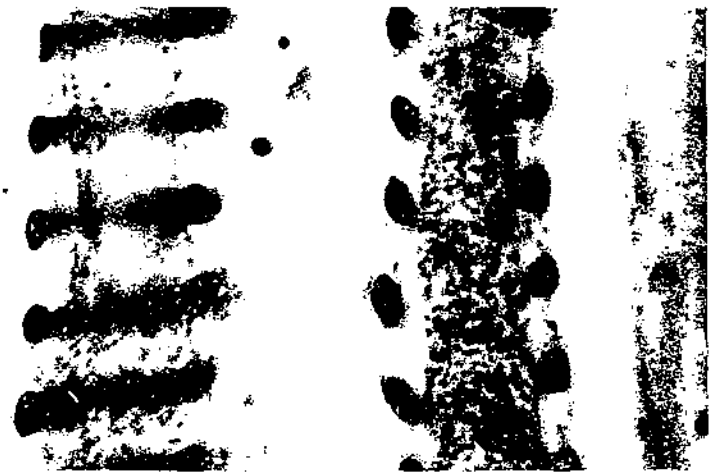

FIGURE 12.—Longitudinal section of the stem of an inoculated plant of Early Korean lespedeza showing two water-conducting vessels filled with bacteria. The leaves of the plant were inoculated by the needle-prick method on March 8, 1939, and the stem segment was killed and fixed on April 5. × 1,900.

it seemed that the parasite might at times enter the seed itself. Attempts were made, therefore, to isolate the organism from seed of Early Korean lespedeza (No. 19604) harvested at the Arlington Farm in 1938 from a row of plants in which many were diseased. Cultures of a bacterium that resembled the parasite causing wilt were obtained from hulled seed with apparently intact seed coats and from seed with the seed coats removed. The pathogenicity of the culture isolated from seed with intact seed coats was proved by inoculation of healthy seedlings.

In another experiment, selected hulled seeds with apparently intact seed coats were surface sterilized in alcohol and mercuric chloride, then washed in five changes of sterile distilled water. In the final washing the seed coats were slipped off, and the cotyledons were dropped into a tube of sterile nutrient broth. After 10 minutes soaking, dilution plates were poured with nutrient agar. Bacterial

colonies that resembled those of the parasite causing wilt of lespedeza developed in these plates, and the organism was isolated in pure culture.

In another experiment, seed of this strain of lespedeza was soaked in sterile distilled water for 3 hours after which the wash water was used as inoculum. Within 3 weeks after inoculation, the seven plants that had the wash water pricked into the leaves were completely wilted and dead. On several occasions seeds of early Korean lespedeza were placed on moist filter paper in Petri dishes to observe germination. Several of these seeds extruded yellow masses of bacteria, and the seedlings from such seed usually became infected and died soon after germination had started.

It would appear from these experiments and observations that the bacterium causing wilt of lespedeza may occur either in or on the seed. It is possible that in this way the organism is carried over winter in established fields and is carried into new fields where the disease has not occurred previously.

OVERWINTERING

Since in the usual farm practice the annual lespedezas are permitted to reseed themselves year after year in the same fields, infected seed and the trash from diseased plants would be suspected as harbors for the parasite during the winter months. A 1938 nursery row of Early Korean lespedeza (F. C. No. 19604), in which there were many diseased plants, was allowed to stand throughout the winter of 1938–39 and plant stems were collected from this row on January 12, February 13, March 17, and April 14. Examination under the microscope revealed the presence of bacteria in some of the stems collected on each date, and the organism was isolated from these stems in pure culture. The pathogenicity of these isolations was proved by inoculation of healthy seedlings of Early Korean, Standard Korean, Common, and Tennessee 76 annual lespedezas, with the production of typical wilt symptoms similar to those occurring in nature. These experiments showed that the bacterium could persist from summer to the following spring in diseased plant trash that overwintered out of doors at the Arlington Farm.

Although no experimental evidence is yet available to prove that the bacterium overwinters in the seed the fact that seed invasion has been shown to occur would suggest this as another likely method of overwintering.

COMPARISON WITH OTHER BACTERIAL SPECIES PARASITIC ON LEGUMES

No bacterial plant pathogen, so far as the authors could determine, has been described as causing a wilt of any species in the genus *Lespedeza*. However, several bacterial species parasitic on legumes are yellow in culture and appear to differ only slightly from the lespedeza wilt organism in morphology, cultural characters, and biochemical properties. It appeared wise, therefore, to make comparative studies with these species, insofar as possible, with particular attention being paid to cross inoculations. Since lespedeza wilt was found originally in a nursery adjoining soybean varietal test plots where bacterial pustule (*Bacterium phaseoli sojense* Hedges) was quite prevalent, a comparison with this organism seemed in order. Cultures were made, therefore, from infected soybeans at

the Arlington Farm, and other cultures, previously demonstrated to be pathogenic on soybean, were obtained from Florence Hedges, of the Division of Fruit and Vegetable Crops and Diseases. It was found that *Bact. phaseoli sojense* differs in culture from the organism causing wilt of lespedeza in the consistency of its cultures (butyrous as compared with viscid) and in the internal markings that characterize the colonies of the former.

When inoculations were made by spraying the leaves of seedlings, the soybean organism infected the Tokyo and Hollybrook varieties of soybean, but when pricked into the leaves of annual and perennial lespedezas it failed to cause any symptoms of disease. The lespedeza organism, on the other hand, readily infected the species of lespedeza inoculated but failed to produce symptoms of disease on the Hahto, Hollybrook, Ito San, Laredo, Mammoth Yellow, Midwest, Tokyo, and Yellow Biloxi varieties of soybean. Hedges [5] found that all of these varieties were attacked by *Bacterium phaseoli sojense* except Yellow Biloxi.

More marked cultural differences exist between the lespedeza wilt organism and *Bacterium flaccumfaciens* Hedges, but since the latter causes a dwarfing and wilting of beans comparable in many ways to lespedeza wilt, cross inoculations were made. In these tests, the bean wilt organism did not attack the Standard Korean variety of annual lespedeza, and the lespedeza wilt organism did not attack plants of the Refugee variety of bean or the Ito San variety of soybean, which was reported by Hedges [6] to be susceptible to *Bact. flaccu. faciens.*

The lespedeza wilt organism appears similar also in leaf symptoms produced and in many cultural characters to *Phytomonas alfalfae* Riker, Jones, and Davis,[7] which causes a bacterial leafspot of alfalfa. However, when seedlings of several strains of annual lespedeza were inoculated by spraying or by pricking *P. alfalfae* into the leaves, no symptoms of disease developed. Similarly the lespedeza wilt organism failed to produce disease on alfalfa seedlings. In view of these facts, the lespedeza wilt organism is considered to be an undescribed species of bacterium and the following name is proposed: *Phytomonas lespedezae*, n. sp.

TECHNICAL DESCRIPTION

Phytomonas lespedezae, n. sp.

Organism is a rod with rounded ends, occurring singly, in pairs, or occasionally in short chains. The average size is 1.62μ by 0.56μ. It is motile by one polar flagellum and is Gram-negative. The organism is an aerobe and makes most rapid growth between 30° and 35° C. Capsules are present but no spores.

On nutrient agar plates, colonies are circular, raised, glistening, translucent, and viscid. They have entire margins, are yellow, and after 4 days at room temperature isolated colonies are 3 to 5 mm. in diameter. Submerged colonies remain small and are lenticular in shape. On nutrient-agar slants, growth is moderate, filiform, appressed, glistening, viscid, and yellow. More abundant growth occurs on potato-dextrose agar and on potato cylinders. Nutrient broth is clouded in 48 hours. The organism liquefies gelatin, egg albumin, and blood serum. Milk becomes alkaline and is peptonized. Starch is hydrolyzed readily, indol is produced, as is also hydrogen sulfide. Nitrates are not reduced. Neither acid nor gas are formed from glucose, lactose, sucrose, maltose, soluble starch, or mannitol.

[5] HEDGES, FLORENCE. A STUDY OF BACTERIAL PUSTULE OF SOYBEAN, AND A COMPARISON OF BACT. PHASEOLI SOJENSE HEDGES WITH BACT. PHASEOLI EFS. Jour. Agr. Research 29: 229-251, illus. 1924.

[6] ——— BACTERIAL WILT OF BEANS (BACTERIUM FLACCUMFACIENS HEDGES), INCLUDING COMPARISONS WITH BACTERIUM PHASEOLI. Phytopathology 16: 1-22, illus. 1926.

[7] RIKER, A. J., JONES, F. R., and DAVIS, MARGUERITE C. BACTERIAL LEAF SPOT OF ALFALFA. Jour. Agr. Research 51: 177-182, illus. 1935.

The organism is pathogenic on annual and perennial species of *Lespedeza*, filling the vascular system and causing death of the plants.

CONTROL MEASURES

Since the bacterium is seed borne, at least in the case of Early Korean lespedeza (No. 19604), prevention of the disease would require primarily the use of disease-free seed. The importance of securing seed from healthy plants was demonstrated incidental to the attempts to secure healthy seedlings of this strain of lespedeza for the artificial inoculations in this study. Poor stands were always obtained when Arlington-grown seed was planted, and, as was pointed out above, when this seed was placed on moist filter paper in Petri dishes, extrusion of yellow masses of bacteria occurred not infrequently. Seed of Early Korean was obtained from Missouri, therefore, and when placed

FIGURE 13.—*A*, Complete lack of stand as a result of planting Arlington-grown seed of this strain in sterilized soil; *B*, thick stand of healthy seedlings that developed from Missouri-grown seed of Early Korean lespedeza (No. 19604) sown in sterilized soil.

in Petri dishes no bacterial extrusion was observed and healthy-appearing, green seedlings developed.

For further comparison, pots of sterilized soil were planted with seed from the two sources and were placed in the greenhouse side by side so that all pots were subjected to essentially the same environmental conditions. A thick stand of healthy green seedlings developed in the pots planted with Missouri-grown seed of Early Korean, whereas only a few seedlings emerged from the soil in the pots planted with Arlington-grown seed, and these seedlings soon wilted and died (fig. 13). In this test the Arlington-grown seed was known to have come from a 1938 nursery row in which there were many diseased plants. The exact origin of the Missouri-grown seed is not known, but judging from its freedom from seed-borne infection it would appear that it had been harvested from a planting in which the disease has not yet appeared.

Once the disease appears in a field of annual lespedeza, there seem to be no practical control measures that will check its spread. Seed probably should not be harvested from such fields, since it is quite

possible that in this way the disease will be disseminated to new fields. How long a time must elapse before Early Korean lespedeza should be replanted in a field known to have been diseased can be determined only by future experience.

It would appear from the artificial inoculations reported in this bulletin that Standard Korean and some strains of Common lespedeza may be more tolerant of the disease than is the Early Korean. In the greenhouse, at least, the disease progresses more slowly in these strains than in Early Korean. Because of later maturity, however, these strains are not adapted to the more northern latitudes where Early Korean is being grown intensively. It would appear, therefore, that the development of an early strain of annual lespedeza resistant to bacterial wilt, either by selection or hybridization, might eventually prove necessary for the control of this disease in the northern portion of the area in which the annual lespedezas have become an important forage crop.

SUMMARY

In the summer of 1937 an apparently hitherto unreported bacterial wilt of annual lespedeza (*Lespedeza stipulacea* and *L. striata*) was observed at Arlington Experiment Farm, Arlington, Va. Since then the causal organism has been isolated from diseased plants of annual lespedeza from Columbia, Mo., Manhattan, Kans., Urbana, Ill., Knoxville, Tenn., and Ithaca, N. Y.

Dark water-soaked spots on the leaflets are the first visible symptom of infection. Infected leaves then soon become grayish brown, desiccated, and curled. A thin incrusting film of bacterial exudate is sometimes seen on the leaflets. Systemic infection follows rather rapidly in the case of susceptible strains of annual lespedeza, and eventually entire plants wilt and die. Frequently, the stems of systemically infected plants crack open, and a yellowish drop of bacterial exudate forms and hardens.

The causal bacterium is a rod-shaped, Gram-negative, monotrichous organism that digests starch vigorously and grows especially well on starchy media. Colonies on agar are primuline yellow and those on nutrient agar are appressed and extremely viscid. The organism liquefies gelatin, egg albumin, and blood serum and forms both hydrogen sulfide and indol. Nitrates are not reduced and neither acid nor gas are formed from dextrose, lactose, maltose, saccharose, starch, and mannitol. Milk becomes alkaline and cleared. Optimum temperature for growth of the organism in culture is near 35° C.

Greenhouse inoculations have shown that the following strains of annual lespedeza are susceptible to bacterial wilt: Early Korean (both F. C. No. 19604 and F. P. I. No. 59379), Harbin (F. P. I. No. 65280), Standard Korean (F. C. No. 22457), Late Korean (F. C. No. 19601), Common (F. P. I. No. 81742), Common (F. C. No. 22590), Kobe (F. C. No. 22456), and Tennessee 76 (F. C. No. 30611.) Infection varied from light in the cases of Standard Korean and one strain of Common (F. C. No. 22590) to severe in the cases of Early Korean (both strains), Harbin, and Tennessee 76.

The disease has not yet been observed to affect perennial species of lespedeza in the field, but greenhouse inoculations have shown the following species and strains to be susceptible: *Lespedeza capitata*

L. daurica (S2435), *L. frutescens*, *L. inschanica*, *L. procumbens*, *L. sericea*, *L.* sp. (S2098, closely related to *L. sericea* and *L. juncea*), and *L. virginica*. On the other hand, *L. bicolor*, *L. cyrtobotrya*, *L. daurica* (90355, a prostrate form), *L. formosa*, *L. hirta*, *L. latissima*, and *L. thunbergii* appear to be highly resistant to this parasite, since upon inoculation they show no external symptoms of the disease.

Only very localized infection and no necrosis was obtained when species of the Leguminosae belonging to genera other than *Lespedeza* were inoculated with the bacterium.

Infection of annual lespedezas was obtained by three methods of inoculation which involved wounding of the host. Atomizing with bacterial suspension resulted in infection only in the case of young seedlings with just the primary leaves exposed. In the latter case it appeared that the bacteria had gained entrance to the leaves through the stomata.

Once within a susceptible host, the parasite soon gains entrance to the vascular system and apparently travels rather rapidly throughout the plant, filling the water-conducting vessels and causing wilting and death.

It would appear from experiments and observations that the bacterium causing wilt of lespedeza may occur either in or on the seed. In this way the organism may be carried into new fields where the disease has not occurred previously.

Experiments have shown that the bacterium can persist from summer to the following spring in diseased plant trash overwintered out of doors at Arlington Experiment Farm.

Since the bacterium is seed-borne, at least in the case of Early Korean lespedeza, prevention of the disease would require primarily the use of disease-free seed. There seem to be no practical control measures that will check the spread of the disease once it appears in a field of annual lespedeza.

The lespedeza wilt organism is considered to be an undescribed species of bacterium and the name *Phytomonas lespedezae*, n. sp., is proposed. An extensive description is given of the pathogen.

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