GENETICS OF RESISTANCE TO BACTERIAL WILT IN ALFALFA [1]

By R. A. BRINK, *professor of genetics, Wisconsin Agricultural Experiment Station;* F. R. JONES, *senior pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture;* and H. R. ALBRECHT, *assistant in genetics, Wisconsin Agricultural Experiment Station*

INTRODUCTION

In several diverse regions in the United States where bacterial wilt, caused by *Phytomonas insidiosa* (L. McC.) Bergey et al., has become a major factor in reducing the production of alfalfa, *Medicago sativa* L., there is urgent need of varieties which are resistant to the disease and, at the same time, suited in other respects to the areas involved. Various avenues which may lead to a satisfactory solution of the problems presented are being explored. Under the auspices of the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, a wide search is being conducted among American varieties and importations from Asia, Europe, and Africa for strains which will meet the new requirements, or which, at least, will provide material for the development of suitable resistant strains by more intensive methods. The possibilities offered by different systems of breeding which turn upon individual plant selections likewise are being studied. In the critical evaluation of material now in the trade and in the effective utilization of resistant individuals from various sources in developing varieties which will withstand the parasite, an understanding of the mode of inheritance of resistance would appear to be second in importance only to a sound knowledge of the disease itself.

The data on the genetic basis of resistance thus far obtained are not numerous. Nevertheless, significant evidence on certain aspects of the problem has been secured. Because of this and in view of the evident complexity of the problem and the likelihood that further progress in solving it will be slow, the results are presented now.

METHODS

The tests were all made in one season, 1933. Aside from foundation stocks, the seed used in the experiments was produced in greenhouses screened to exclude insects likely to transport pollen from plant to plant. The flowers were stripped by hand in selfing, 95-percent alcohol being used on the fingers after operating on each individual. In making crosses the ordinary precautions were observed to secure seed of known parentage. The tests were made on a fairly uniform plot of land to which water was applied as needed to maintain the plants in a thrifty condition. The seedlings were started in the greenhouse in December 1932, inoculated in the spring by scrap-

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ing the roots in a suspension of the wilt organism, and set in the field at once. The results from the check rows of Grimm and Hardistan alfalfa, treated in the same way as the test material and distributed throughout the plot, show that a uniform and relatively severe wilt infestation was secured. Among 754 Grimm check plants only 5, or 0.66 percent, were found to be uninfected on digging in the fall. The corresponding value for Hardistan, a strain known to possess considerable resistance, was 101 healthy plants in a total of 609, or 16.6 percent. The final counts are based on the number of plants living about 6 weeks after transplanting.

CLASSIFICATION OF PLANTS FOR RESISTANCE

The evaluation of individual plants for resistance at the termination of a test presents unusual difficulties. These difficulties, recently discussed by Jones,[2] appear from the evidence which he presents to arise from two circumstances: (1) Resistance seems to vary continuously among individuals, from slight to a condition approaching immunity, and (2) no single practicable test appears yet to have been devised that will distinguish precisely between individuals which may be supposed to differ only slightly in resistance. It is to be anticipated that among the environmental factors affecting the incidence of wilt in a test carried out in the field there will be a residuum of circumstances conditioning the reaction of the plant to the organism which will not have been identified and which were not under control. It may therefore be expected that more or less frequently two individuals of identical genotype and receiving similar treatment, so far as factors under control are concerned, will develop the disease in a significantly different degree. Moreover, it has been found in the practical work of classifying the plants that in some populations the character of the disease symptoms differs strikingly from that in other populations.

As a working plan, therefore, it is necessary to set up provisional classes each of which will embrace a particular segment of the presumably continuous array of reactions. Such a procedure, to be helpful, must satisfy at least two requirements: (1) The classes should be broad enough so that border-line cases, whose disposition must be more or less arbitrary, will constitute but a small part of the total; (2) each class should be predicated upon a "type" which is not only definable in terms of the results of the test applied but which also appears to have a validity beyond the conditions peculiar to the experiment. The validity sought here in classification is one which might reflect, in a general way, the probable fate of the plants under the most severe wilt conditions found in the field. It should be understood that the claim is not made that the behavior of the plants in the field would be expected necessarily to coincide with that on the wilt plot, but rather that the two would be parallel and in approximately the same order. The readings afforded by the wilt-plot test may prove to have been too high or too low as judged by the survival of plants grown in the ordinary way on wilt-infested soil; but it is believed that more drastic conditions for infection have been provided in the test than are likely to occur in the field.

[2] JONES, F. R. TESTING ALFALFA FOR RESISTANCE TO BACTERIAL WILT. Jour. Agr. Research 48: 1085-1098. 1934.

Four classes have been established, as follows: (1) Healthy, (2) slightly infected, (3) badly diseased, and (4) dead. This classification is definitive for groups 1 and 4, but does not sharply delimit the other two groups. The criterion used in distinguishing between class 2 plants, slightly infected, and class 3 plants, badly diseased, was the extent of lesions in the roots. It is assumed that under field conditions plants in the former group, if infected at all, would show a relatively high resistance, whereas those of the latter group would succumb rather readily to the disease.

EXPERIMENTAL DATA AND DISCUSSION

In the intensive search which has been conducted during recent years for a wilt-resistant alfalfa among domestic plantings, two strains, aside from the Ladak variety, have been discovered which, as compared with the common and variegated forms, are outstanding in their ability to withstand the disease. These are Hardistan[3] and Kaw.[4] Both stocks appear to be of Turkistan origin. With the primary purpose of isolating some of the most wilt-resistant components of Hardistan and, secondarily, of obtaining light on the genetic behavior of resistance, 164 plants of this variety were selfed and the offspring of the 95 individuals yielding at least 80 seeds were tested for their reaction to the disease.[5] The 95 parent plants, represented on the wilt plot by as many families, were thus a random sample of the variety, except that the lowest seed producers were not included. The average number of plants per family 6 weeks after transplanting was 45. The results of this individual plant-progeny test are shown in figure 1. It will be noted that the progenies range continuously in proportion of resistant plants (classes 1 and 2) from 0 to 100 percent. The largest single subgroup of families, comprising 19 members, contains no resistant individuals. This fraction, combined with the progenies having some, but fewer than 10 percent, resistant segregates, makes up about one-third of the total. The remaining families are distributed in a rather uniform and unbroken series. In one of the two families falling in the highest class all the plants were resistant and in the other family all were resistant but one.

The facts here presented show the highly diverse character of families from individual plants in a stock known to carry, in the aggregate, a considerable amount of resistance. The fact that plants capable on self-pollination of giving at least some resistant offspring may vary continuously over a very wide range in the proportions actually thrown shows clearly that the genetic basis of resistance is far from simple. The cross section of the Hardistan variety afforded by this series of plant-progeny tests points to a complex of germinal elements making for resistance distributed very unevenly between different individuals in the stock and interacting in ways unknown at present to produce in each generation a certain, possibly fairly constant, proportion of resistant plants. It is a reasonably safe assumption also that by rather simple methods of selection the average resistance of the population could be shifted downward to zero, on the one hand, or,

[3] KIESSELBACH, T. A., ANDERSON, A., and PELTIER, G. L. A NEW VARIETY OF ALFALFA. Jour. Amer. Soc. Agron. 22: 189–190. 1930.

[4] SALMON, S. C. KAW—A NEW ALFALFA. Jour. Amer. Soc. Agron. 24: 352–353. 1932.

[5] The stock seed used was harvested from the original Hardistan field and came to the writers through the courtesy of L. F. Graber of the Wisconsin Station, who obtained it from the Nebraska Agricultural Experiment Station.

on the other, upward well above the present means to some unknown value, which would have to be determined empirically.

Since the ultimate solution of the wilt problem demands the production of varieties which will not only withstand the disease but will also be acceptable in other regards it is important to know whether resistance is compatible with other desirable qualities. A study was made of the relation between seed production and reaction to the disease in the above-mentioned Hardistan population. Hardistan, like other strains of Turkistan origin, is less productive of seed than the commonly grown American varieties. The 163 Hardistan plants from which the 95 families in the wilt test were derived produced an average of 207 seeds each on being selfed in the greenhouse. On an adjacent bench and under almost identical conditions of light, temperature, moisture, and handling, 380 unselected Grimm plants of the same age gave a mean yield of 358 seeds. The somewhat larger size of the Grimm plants gives this variety an advantage, but the difference, amounting to about 70 percent, is almost certainly not attributable to this factor alone. Adjacent and comparable plots of Grimm and Hardistan in the field in 1933 showed a much greater disparity in seed production than was observed in the greenhouse. Whether the low seed production and relatively high wilt resistance of Hardistan and of other Turkistan alfalfas is indicative of a general relationship between these two variables is a question of considerable importance.

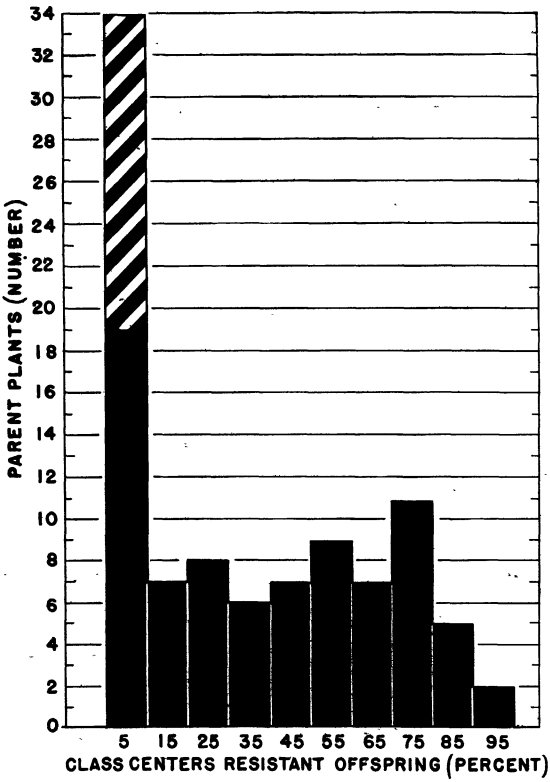

FIGURE 1.—Distribution of 95 families from once-selfed plants of the Hardistan variety, with reference to the proportion of resistant individuals in the progenies. The cross-hatched bar represents the families in which no resistant plants occurred.

The results obtained in the present study are depicted in figure 2. The 95 self-pollinated Hardistan individuals were grouped into classes according to the number of seeds produced, a uniform class interval of 200 seeds being used up to 800. The seven individuals lying above 800 and ranging up to 1,464 were lumped together at 1,100, the approx-

imate value of their mean. The average percentage of wilt-resistant offspring from the plants in each of these classes is indicated by the position of the circles with reference to the vertical scale. Reading from left to right the numbers of parent plants on which the 5 respective points are based are 42, 28, 9, 9, and 7. Since the frequency distributions for number of seeds and percentage of wilt resistance are both markedly asymmetrical there is no entirely satisfactory way of expressing in a single term the relationship between the two variables. Grouped in the way indicated, however, the data give a correlation ratio (η) of 0.29 for percentage wilt-resistant offspring on parent seed number. A finer grouping, as might be expected, leads to a somewhat higher value of η. Caution is necessary in judging the significance of this correlation ratio. There is no evident relationship between the two variables in the first four seed classes. The value of η is due, in large part, to the character of the array corresponding to the highest seed class. The seven plants producing more than 800 seeds each all proved to have very low percentages of wilt-resistant offspring. This is the most significant fact which the analysis brings to light.

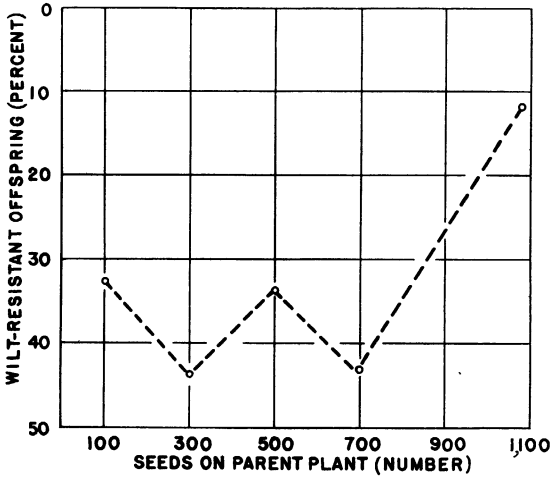

FIGURE 2.—Relationship between seed production in the parent plant and percentage wilt resistance in the offspring of 95 self-pollinated Hardistan plants.

The average percentage of wilt-resistant offspring from this group of once-selfed Hardistan plants is 14.8. This is to be compared with a value of 16.6 percent for the check rows of the Hardistan variety and 14.2 percent in a block of 1,006 Hardistan plants also included in the test. On genetic grounds it is to be expected that the average heterozygosity of the once-selfed stock would be about 50 percent less than that in the Hardistan variety. Since the large increase in homozygosity is not accompanied by a significant change in the average proportion of resistant plants it would appear that heterozygosity has little if any relation to wilt resistance. If this is true it appears probable that, other circumstances permitting, pure lines could be established by continuous inbreeding which would exhibit the various levels of wilt resistance found in the open-pollinated individuals of the parent variety.

In table 1 the results are shown of progeny tests on 34 plants selected for their resistance to wilt out of large populations of alfalfa of widely different types.[6] The nonhardy class is represented by the Spanish-Moroccan group, the widely grown American varieties by selections out of Kansas, Montana, South Dakota, and Utah Common,

[6] The authors are indebted to L. F. Graber for the initial seed stocks of a number of these lots.

and the hardier types by Grimm, Ladak, Hardistan, and the Turkistans. These plants were selfed and their offspring inoculated with the wilt organism. There are three progenies containing no resistant individuals, a result, probably, of the severity of the test. The average proportion of wilt-resistant offspring in the entire group is only about 35 percent. This result is not due to the behavior of the strains from any particular variety or class of varieties. Rather, the explanation is the occurrence of families throughout the whole series which contain very few resistant plants, together with the variable composition of the remaining progenies. If the 14 families containing one-fourth or fewer resistant segregates are eliminated the average resistance of the rest is found to be nearly 60 percent. Four plants in the group gave over 75 percent resistant offspring. These results are of considerable significance for the light they throw on the methods most likely to lead to success in breeding wilt-resistant varieties. In making selections for wilt resistance, individuals should be sought which will not only withstand the disease themselves but which will also be capable of transmitting resistance to a large proportion of their offspring. It is evident from the performance of these 34 families that selected resistant individuals differ greatly in their ability to transmit resistance, and that this ability can be determined only by a progeny test.

TABLE 1.—*Progeny tests with self-pollinated resistant selections from alfalfas of diverse origin*

Pedigree	Stock designation	Source	Plants	Resistant plants		
				Class 1	Class 2	Classes 1 and 2
			Number	*Percent*	*Percent*	*Percent*
1-33-101	F.P.I. 89904	Portugal	44	9.1	15.9	25.0
1-33-102	F.P.I. 89630	Spain	62	11.3	16.1	27.4
1-33-103	F.P.I. 89631	do	51	.0	.0	.0
1-33-104	F.P.I. 89631	do	61	.0	14.7	14.7
1-33-105	F.P.I. 89022	Chile	46	17.4	26.1	43.5
1-33-106	F.P.I. 89043	Manchuria	59	42.4	27.1	69.5
1-33-107	F.P.I. 89854	Spain	37	24.3	16.2	40.5
1-33-108	F.P.I. 89897	do	57	21.0	38.6	59.6
1-33-109	F.P.I. 011701	Morocco	64	.0	.0	.0
1-33-110	F.P.I. 011706	do	49	2.0	6.1	8.1
1-33-111	F.P.I. 84371	Turkistan	35	20.0	37.1	57.1
1-33-112	F.P.I. 84371	do	55	.0	1.8	1.8
1-33-113	F.P.I. 84371	do	46	41.3	34.8	76.1
1-33-114	F.P.I. 84371	do	59	1.7	10.2	11.9
1-33-115	F.P.I. 84371	do	70	21.4	38.6	60.0
1-33-116	F.P.I. 84371	do	54	44.4	33.3	77.7
1-33-117	1356	do	45	2.2	.0	2.2
1-33-118	1356	do	63	.0	1.6	1.6
1-33-119	2	do	71	46.5	25.3	71.8
1-33-120	F.P.I. 84443	do	52	.0	1.9	1.9
1-33-121	Hardistan	Nebraska	40	7.5	12.5	20.0
1-33-122	989	Turkistan	61	.0	16.4	16.4
1-33-123	989	do	55	9.1	36.4	45.5
1-33-124	Hardistan	Nebraska	58	6.9	15.5	22.4
1-33-125	989	Turkistan	59	15.2	44.1	59.3
1-33-127	1355	Grimm	46	.0	.0	.0
1-33-128	1356	Turkistan	68	19.1	30.9	50.0
1-33-129	875	South Dakota	23	13.0	30.4	43.4
1-33-130	1173	do	64	65.6	15.6	81.2
1-33-131	1334	Ladak	37	48.6	32.4	81.0
1-33-132	1300	Montana	45	51.1	17.8	68.9
1-33-133	1300	do	56	12.5	39.3	51.8
1-33-134	Engle	Kansas	53	43.4	30.2	73.6
1-33-135	1158	Utah	38	13.2	28.9	42.1

A series of hybrids studied involved as one parent a highly resistant plant (J3–13–2) of Turkistan origin which on self-pollination gave 21 healthy, 8 slightly diseased, and no badly diseased or dead offspring. This individual was crossed with two unrelated Grimm plants both of which on self-pollination gave entirely susceptible progenies. The F_1 hybrids were not tested for their reaction to wilt. The F_2 family from one of them, however, contained 2 class 1, 9 class 2, 95 class 3, and 53 class 4 plants. In other words, only about 7 percent of the F_2 segregates showed well-marked resistance. The other F_2 family showed 1 class 1, 11 class 2, 100 class 3, and 35 class 4 plants. Again, over 90 percent of the plants were more or less highly susceptible to the disease. There were, however, a few wilt-resistant segregates.

Another cross between an untested Grimm plant and (J3–13–2) gave no wilt-resistant F_2 plants in a population of 53.

The behavior of an F_2 population from a hybrid between the same resistant Turkistan parent (J3–13–2) and a plant of the Hairy Peruvian variety stands in marked contrast with these foregoing results. The reaction to wilt of the Hairy Peruvian individual used is not certainly known as it was not inoculated and no selfed seed was obtained. Judging, however, from the frequency with which resistant plants are found in this variety, the chances are roughly 1,000 to 1 that it was susceptible. Fifty-eight percent of the F_2 plants from this cross were resistant. The 171 plants in the family were classified as follows: 33 class 1, 66 class 2, 60 class 3, and 12 class 4. The progeny contained many large and vigorous plants which, by their habit of growth and pubescence, exhibited clear evidence of Hairy Peruvian ancestry. Among 117 F_2 plants from a similar but distinct cross involving these same varieties 18 percent resistant individuals were found. A cross between the resistant Turkistan plant, J3–13–2, and an untested, but probably susceptible, *Medicago falcata* plant gave 54 percent wilt-resistant segregates in F_2. The distribution was 13 class 1, 25 class 2, 17 class 3, and 15 class 4 plants. In this family the plant type, in general, was very different from that in the Hairy Peruvian cross, the individuals being small and semiprostrate in their habit.

The wide divergence in size and form of the plants in these two progenies from crosses of a common resistant Turkistan plant with a Hairy Peruvian individual, on the one hand, and with a *Medicago falcata* individual on the other, associated as it is with nearly equal percentages of resistant segregates, suggests that there is little if any relationship between the gross morphology of the alfalfa plant and its reaction to the wilt organism. The evidence from these two latter crosses does not support the view expressed by Peltier and Schroeder [7] that differences in resistance and susceptibility are due mainly to variations in the morphology of the plant. Attention should be called to the fact that Hairy Peruvian is a nonhardy alfalfa, whereas *Medicago falcata*, in general, can endure considerable cold. It is to be expected, consequently, that the two hybrid families would differ considerably in this respect. If they do, wilt resistance is probably not necessarily associated with winter hardiness.

[7] PELTIER, G. L., and SCHROEDER, F. R. THE NATURE OF RESISTANCE IN ALFALFA TO WILT (APLANOBACTER INSIDIOSUM L. MC.). Nebr. Agr. Expt. Sta. Research Bull. 63, 28 pp., illus. 1932.

SUMMARY AND CONCLUSIONS

A preliminary study was made of the inheritance of resistance to bacterial wilt in alfalfa. The main conclusions to which the results seem to point are as follows:

Resistance to bacterial wilt in alfalfa behaves in inheritance as an intergrading character and probably rests upon a complex genetic basis. A factorial interpretation is at present impossible.

Wilt-resistant plants may differ markedly in composition with respect to the genes governing resistance and susceptibility, as shown by the widely divergent proportions of resistant offspring obtained on selfing such individuals. The same appears to be true of wilt-susceptible plants.

The Hardistan variety comprises a diverse array of plants which on self-pollination yield families varying in number of resistant plants from 0 to 100 percent under the conditions of the test.

A study of the behavior of 95 once-selfed Hardistan families suggests that there may be a small inverse correlation between seed production and wilt resistance. The apparent relationship, however, is complex and the data are by no means conclusive.

Wilt resistance appears to bear no relationship to the external form of the plant or to winter hardiness. If this conclusion is confirmed there would seem to be no insuperable obstacle to the incorporation of wilt resistance into any of the diverse regional types of alfalfa now in use.

Insofar as it may be judged from the small sample of plants tested, Grimm alfalfa appears to be of a genetic composition, with respect to the factors conditioning resistance and susceptibility, which permits relatively few wilt-resistant segregates in crosses with highly resistant Turkistans. On the other hand, two similar matings of the Turkistans with Hairy Peruvian gave in one instance 58 percent and in the other 18 percent resistant offspring. A single cross of a resistant Turkistan with a *Medicago falcata* plant gave 54 percent resistant segregates.