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INHERITANCE OF RESISTANCE TO THE PEA APHID IN ALFALFA HYBRIDS

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In this issue:

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THE DISCOVERY of a male-sterile barley and its use to facilitate floral infection with spores of *Helminthosporium gramineum* Rabh. (Suneson and Houston, 1942)[4] has provided a new method for study of the stripe disease in barley. The experiments reported in this paper were made possible by adaptations of this new technique for inducing infection.

To establish facts that will aid in breeding varieties resistant to stripe is an objective important for California; stripe was found in 12 of 53 fields surveyed in 1943 (Suneson and Santoni, 1943*b*) and in 49 of 99 fields surveyed in 1949 (Suneson, 1949). The continued presence of stripe in such proportions does not discredit recommended seed treatments for control but certainly does show that many farmers either do not treat seed at all or do not use seed treatments properly.

Distribution and Description of Stripe Disease. A general review of the literature (Dickson, 1939) shows that barley stripe is distributed throughout the humid and semihumid temperate regions of the world. Only rarely are more than 20 per cent of the plants in a field affected.

Diseased plants show yellow stripes soon after tillering. These ultimately darken. At heading time the entire plant darkens and becomes brittle, rarely producing heads or seed. At this period wind-borne spores initiate the floral infections, which are expressed in the succeeding crop.

Fundamental information regarding the life history of stripe and the role of environment in its development and expression is rather limited. Most investigators have depended upon natural infections, which at best produce only 10 to 60 per cent of diseased plants (Suneson and Santoni, 1943*a*). Seed treatment has reduced the severity of the disease.

Techniques for Inducing Infection. The technique followed in this paper (Suneson and Houston, 1942) provides positive deposition of spores within the hulls at the flowering period. An alternative controlled method of inoculation involves germinating seeds in direct contact with a mycelial mass of the organism grown on a culture medium. Shands and Arny (1944) using this method obtained satisfactory infections in only 5 out of 8 years. With it, a large number of culture plates is necessary. Moreover, the germinated seeds must have careful handling and timely planting. Recent California work has produced a variation in the method whereby the organism can be induced to sporulate in culture (Houston and Oswald, 1946). Furthermore, germination in contact with growing cultures apparently does not require

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[4] See "Literature Cited" for citations referred to in text by author and date.

highly specific time intervals and temperatures for infection, and seeds can be dried for 10 days following germination without harm to the organism (Houston and Oswald, 1948).

Floral or seedling infection does not assure full expression of the disease. A temperature of 15° C, or lower, during the period of emergence and a soil that is less than 40 per cent saturated have been reported as most conducive to stripe development (Leukel, Dickson, and Johnson, 1933). Vernalization for 38 days at 31° to 34° F resulted in a sharp increase in stripe over paired plantings of nonvernalized seed (Åberg, 1945).

Preliminary Work on Resistance to Stripe. Several workers have observed resistance to stripe. The preliminary California attempt to determine a genetic basis for resistance was based entirely on F_1 response (Suneson and Santoni, 1943*a*). In this work, the dominance of resistance in Hannchen, the intermediate reaction in Trebi, and the dominance of susceptibility in Club Mariout were indicated. A subsequent genetic analysis (Arny, 1945*a*) indicated a 3-factor difference in Oderbrucker × Brachytic. The resistance in Brachytic was different from that in Lion. Similarly, the susceptibility of Oderbrucker differed from that in Colsess IV.

Races of the Organism. Physiologic specialization of the stripe fungus has been noted by several workers. The basis for their differentiation has involved variances in spore development, in effect on growth, or in degree of infection. The greatest difference reported involves the variety Atlas. In tests conducted in Wisconsin for three years, Atlas developed no stripe (Shands and Arny, 1944), although it has long been known to be susceptible in California. This California susceptibility is not so complete, however, as for some other varieties (Suneson and Santoni, 1943*a*). A stripe culture capable of infecting Atlas has recently been reported from Wisconsin (Arny, 1945*b*).

ESTABLISHING AND EVALUATING INFECTIONS

Inoculation by the method reported in this paper differs from natural field infection only in controlling the time of deposition and the placement of the spores within the flower. Male sterility with its attendant open flowers is requisite. Fertilization and inoculation are consecutive or practically concurrent dusting operations (Suneson and Houston, 1942). The method is applicable to the F_1 generation from crosses with male-sterile, to backcrosses to male-sterile, or to segregates from crosses or backcrosses expressing male sterility.

Except for special race studies, a presumed single culture (no. 3) of stripe has been used throughout the experiments. It was propagated from season to season on male-sterile × Atlas. Other cultures for race studies have been similarly maintained. The only safeguard against contamination involved bagging after inoculation.

Plants were classified as diseased or healthy, depending on the presence or absence of spore-producing stripes on the leaves. In some seasons all diseased plants died before producing viable seeds, while in others a few plants produced viable seed on some tillers. Such seasonal variations were ignored in evaluating infections, however. Plants were treated as healthy or as totally diseased.

RESULTS

Effect of Humidity at Time of Flowering. Either a semihumid condition or wetting of spores is considered necessary for producing the stripe disease by floral infection (Dickson, 1939). In California and other parts of the arid Southwest, rains between flowering date and maturity are infrequent. Although the humidity may sometimes be high at night, it is generally very low from about noon until sunset. Nevertheless, natural infections may produce as much as 70 per cent stripe (Suneson, 1949).

TABLE 1

CHRONOLOGY ON THE DEVELOPMENT OF STRIPE FROM PAIRED HUMIDITY TREATMENTS IN THE CROSS *msms* × ROJO

Grown at Davis, California*

Pair No.	Dry spore inoculation and continuous low humidity after inoculation					Spores wet and high humidity maintained for 6 hours after inoculation				
	Number of plants established from spike	Number of plants with stripe on:†			Total stripe per cent	Number of plants established from spike	Number of plants with stripe on:†			Total stripe per cent
		April 10	April 18	May 11			April 10	April 18	May 11	
1	28	14	0	0	50	29	22	3	0	86
2	33	18	2	0	61	39	19	2	0	54
3	28	22	1	0	82	42	21	2	0	55
4	30	18	0	0	60	14	9	0	0	64
5	29	20	1	0	72	48	25	3	1	60
6	30	15	3	2	67	46	29	3	1	72
7	32	23	0	1	75	33	20	0	1	64
8	27	24	0	2	96	29	15	4	1	69
9	28	21	1	0	79	32	22	3	2	84
10	27	12	3	0	56	..	..	..	..	..

* Seeds were planted November 28 and plants emerged December 15, 1944. Plants were 10% headed on April 18, 1945.

† The plants were removed when classified as diseased.

The experiment reported in table 1 involved paired heads with contrasting treatments during the first 6 hours after pollination and inoculation. Subsequently, the natural closing of the hulls and the protective covering of the bag on each spike are believed to have cushioned the night–day variations in humidity. Pollination and inoculation were made at 11 a.m. The wet treatment included replenishment of water in the thick covering of absorbent paper around the spike each hour for 6 hours. Dry dusting of spores—the general procedure—produced essentially the same stripe infections as did the wetting of spores.

Certain data in table 1 are typical of all the experiments discussed. The number of plants established ranged from 14 to 48 per pollinated and inoculated spike. These plants had a range of from 50 to 96 per cent stripe infection. Maximum sporulation occurred just prior to flowering. The first stripe symptoms were observed on March 2; 90 per cent of the total ultimate stripe was evident by April 10. When the remaining nondiseased plants were 10 per cent headed on April 18, an additional 7 per cent of the total expressed stripe was

observable. On May 11, the seasonal stripe development was complete, the final 3 per cent occurring after the start of heading.

Effect of Stripe on Germination. Stripe infection of seeds apparently does not reduce germination. No significant differences in stand were noted during two seasons, in tests between paired uninoculated and inoculated seeds of several diverse stocks. In the first season only 63 and 64 per cent, respectively, of the seeds produced plants, whereas in the second, 93 per cent stands of both groups were obtained.

Uniform germination is a requisite in the malting of barley. A commercial maltster gave a "satisfactory" rating to three farm lots of 1948-crop barley, which produced 50–60 per cent stripe when sown at Davis without seed treatment. Thus, both field and malting tests show that the stripe fungus does not reduce germination.

TABLE 2

EFFECT OF STRIPE-INDUCED HULL DISCOLORATION ON PLANT ESTABLISHMENT AND STRIPE PERCENTAGE

Field tests at Davis, California, 1946

Cross	Dark-colored hulls			Bright hulls		
	Number of seeds planted	Per cent established	Per cent stripe	Number of seeds planted	Per cent established	Per cent stripe
msms × Atlas	194	94	54	48	96	36
msms × Club Mariout	180	97	51	67	97	24
msms × Vaughn	184	97	12	40	98	3
msms × Hannchen	113	93	11	31	87	0
All other crosses	307	93	19	106	96	7

Effect of Hull Discoloration on Stripe Development. The hulls on seeds infected with stripe at the flowering stage commonly exhibit moderate to severe darkening at maturity. Since the technique used insures deposition of spores inside all of the hulls at flowering time, it seemed desirable to compare the gross evidence of mycelial growth within and on the hulls (discoloration) with germination and ultimate stripe expression. These data (table 2) are based on common samples, separated according to hull color. They yield further evidence that the stripe organism does not affect germination. Equally interesting is the fact that conspicuous mycelial development is about equal on all varieties, including those with genetic resistance. Ultimate incidence of the disease was in all cases significantly less in seeds with no external evidence of stripe than in those of the same stock that were darkened by mycelium. On the other hand, mycelial development coincident with kernel development did not assure ultimate high levels of stripe expression, even in susceptible hybrids.

Effect of Seeding Date on Stripe. Surveys in California in 1943 and 1944 indicated that stripe was much more prevalent and severe in fields sown early in the fall than in those sown later. Frequently, early- and late-sown fields were observed on the same farm. The same lot of seed had apparently been used in both plantings, since the same characteristic mixtures were present

in both fields. A consistent low incidence or a total absence of stripe was evident in the late-sown fields, irrespective of the stripe infection in the early-sown ones. Since stripe in other areas often occurs in spring seedlings, it seemed desirable to investigate the relation of seeding date to stripe infection.

The data (table 3) show progressive declines in stripe disease in three plantings from November to March. Elsewhere in America, where barley is sown in the spring, stripe develops despite late seeding. In the series emerging March 17, 1946 (table 3), sporulation was poor on the leaves of tillers with symptoms of stripe disease. Though a considerable number of these plants failed to head, they never exhibited either chlorotic stripes or fruiting structures. In this case, it seems probable that stripe infection retarded the development of the host plants to such an extent that the parasite was unable to complete its life cycle.

TABLE 3

EFFECT OF SEEDING DATE ON STRIPE EXPRESSION

Davis, California

Cross	Per cent stripe, 1944–45			Per cent stripe, 1945–46			Per cent of abnormal plants* 3/17/46
	Emergence date and interval			Emergence date and interval			
	12/8/44, 15 days	2/11/45, 16 days	3/15/45, 10 days	12/25/45, 20 days	1/24/46, 19 days	3/17/46, 16 days	
msms × Atlas	19	0	7	53	50	0	20
msms × Rojo	36	14	0	61	25	4	4
msms × Trebi	13	0	0	..	..	..	..
msms × Club Mariout	..	..	..	58	50	5	5
msms × Winter Tennessee	..	..	..	46	46	2	14

* Plants were dwarfed and nonheading but gave no evidence of spore development. The malformations may have resulted from stripe infection within the plant. Restricted to 3/17/46 emergence group.

Temperatures above 20° C have an inhibiting effect upon stripe development (Leukel, Dickson, and Johnson, 1933). Although soil temperature records were not available in this experiment, emergence intervals were noted carefully. The tests in 1944–45 required 10 to 16 days from seeding to emergence; those in 1945–46, from 16 to 20 days. Soil temperatures in relation to emergence interval have been reported (Leukel, Dickson, and Johnson, 1933)—a temperature of 10° C produces emergence in about 18 days, and one of 15° C in about 10 days. Thus, all seedings (table 3) appear to have germinated at temperatures well below the reported inhibiting temperature of 20° C. It seems, therefore, that factors other than soil temperature or soil moisture were operative in producing the progressive reductions in stripe. To confound the situation further, seeding date and prevailing temperature had little effect upon percentage of disease in recent laboratory inoculation experiments (Houston and Oswald, 1948).

Physiologic Specialization of the Stripe Organism. The technique for inoculation and testing of F_1 hybrids is not well suited for either testing or perpetuating cultures that may react differently on a series of host testers. Occasional natural crosses can be detected; the dispersal of spores in a contiguous area is known to be far more voluminous than that of the pollen.

Information on physiologic specialization of the stripe organism in California seemed very necessary to the breeding of resistant varieties.

A total of eight cultures of *Helminthosporium gramineum* were tested in two or more seasons, from 1944 to 1947. Originally one collection was obtained from Club Mariout (no. 1) and seven from Atlas. The latter were from widely separated fields of this variety in California.

Contamination in field cultures prevented clear-cut race identification on the host testers, which were F_1 from hybrids between *msms* and Atlas, Club Mariout, Trebi, Hannchen, or Vaughn. There was evidence, nevertheless, for two distinct races. The one, common to four of the eight cultures, was characterized by poor spore production on the hosts and relatively low levels of infection. The other involved differences in pathogenicity on the hosts. Thus, culture 6 produced a mean of only 28 per cent stripe on *msms* × Club Mariout for four seasons—an obviously low value for this cross, which regularly produces in excess of 50 per cent stripe with culture 3. With the cross *msms* × Vaughn, culture 3 had a mean of 8 per cent stripe for four seasons, but culture 8 produced 58 per cent stripe in one season. On *msms* × Trebi and *msms* × Hannchen, substantially the same resistance reactions were obtained with all cultures in all seasons.

Since the physiological specialization of the stripe organism encountered in California produced widely different infections, it seemed advisable to restrict the present genetic investigations to a single pure culture of the stripe organism.

Genetic Studies. The general nature of the resistance in the four varieties used in this experiment has already been reported (Suneson and Santoni, 1943*a*). Data presented in table 4, using culture 3, however, are conclusive in showing the nearly complete dominance of resistance in Hannchen, the partial dominance of resistance in Trebi, and the dominance of susceptibility in Club Mariout. It is further shown that when male-sterile is sibbed (*msms* × *Msms*), the progeny is not so susceptible as *msms* × Club Mariout. The male-sterile stock, therefore, possesses a weak resistance, which makes genetic evaluation of crosses with it more difficult. It should be noted also that the reaction groups—resistant, intermediate, and susceptible—in table 4 are not identical entities but are merely a device for indicating modal classes observed in each group of crosses.

The cross *msms* × Club Mariout required two backcrosses to Club Mariout to produce homozygous resistant progeny. Recombinations produced by backcrossing revealed an intermediate reaction class. This result suggests multiple gene action. In any case, resistance in Club Mariout seems to be conditioned by at least two recessive genes.

The results from the cross *msms* × Trebi point to a single gene difference, with dominance incomplete. The evidence was more positive when backcrossing the F_1 plants to susceptible male-sterile than when allowing segregation to occur in the F_2 generation and then backcrossing to Trebi. Resistance was stabilized after two backcrosses to Trebi, however.

Backcrossing male-sterile × Hannchen to the susceptible parent resulted in recovery of both intermediate and susceptible classes. Since this intermediate resistance does not show in the first generation, where dominance is complete,

it must represent a type of gene action with incomplete dominance. Preliminary test crosses with Trebi have not been conclusive, but they suggest a genetic difference. The Hannchen resistance has therefore not been fully determined, but it probably involves two or more genes.

The resistance genes herein reported have not been related to those found in Brachytic and Lion (Arny, 1945*a*).

TABLE 4

STRIPE-REACTION SEGREGATIONS IN CROSSES OF MALE-STERILE WITH THREE VARIETIES IN SEVERAL TEST GENERATIONS

Cross	Generation tested	Number of test years	Total plants grown	Type of observed reaction to stripe					
				Resistant		Intermediate		Susceptible	
				Number of lines	Stripe per cent	Number of lines	Stripe per cent	Number of lines	Stripe per cent
msms × *Msms*.....	F_1	4	756	0	..	0	..	24	55
msms × Club Mariout.....	F_1	4	296	0	..	0	..	4	69
F_2 sel. × Club Mariout.....	BC F_1	1	334	0	..	2	35	13	55
BC F_2 sel. × Club Mariout.....	BC^2F_1	1	167	1	0	4	28	0	..
BC^2F_2 sel. × F_1.....	$BC^2F_2 \times F_1$	2	446	5	5	10	28	0	..
msms × Trebi.....	F_1	3	187	0	..	3	20	0	..
msms × F_1.....	BC F_1	3	649	0	..	7	22	7	68
(*msms* × F_1) × F_1...	BC $F_1 \times F_1$	2	818	0	..	14	33	10	55
(*msms* × F_1) × *msms*	BC^2F_1	1	639	0	..	3	32	16	60
F_2 sel. × Trebi.....	BC F_1	1	169	0	..	3	15	3	45
BC F_2 sel. × Trebi	BC^2F_1	1	276	8	2	0	..	0	..
msms × Hannchen	F_1	6	204	6	4	0	..	0	..
msms × F_1.....	BC F_1	3	1,535	12	3	24	27	6	57
(*msms* × F_1) × F_1...	BC $F_1 \times F_1$	2	401	6	6	12	24	3	57

CONCLUSIONS

Complete floral inoculation of male-sterile barley with spores of the stripe-disease organism is possible. Wetting of spores and maintenance of high humidity for 6 hours after inoculation had no effect on subsequent stripe development. Under the conditions of these tests, stripe did not reduce germination.

Seeds darkened by mycelial growth coincident with development produced higher levels of stripe than did bright seeds that evidenced no spore growth prior to seed germination.

The seasonal decline from fall to spring noted in stripe expression was due to delayed seeding and was apparently independent of soil temperature or soil moisture.

At least two distinct physiologic races of the stripe organism exist in California.

Four sources of genetic resistance to stripe were recognized. These were

derived, respectively, from the varieties Hannchen, Trebi, Club Mariout, and male-sterile. Respectively, these show: dominance of resistance, partial dominance of resistance, dominance of susceptibility, and weak resistance. Collectively, at least six different genes appear to be involved.

From these and other genetic studies (Arny, 1945*a*), it is evident that resistance to the stripe disease is conditioned by a rather large number of genes. A similar broad dispersal of genes covering mildew resistance has been reported (Briggs, 1945). Further contributions on the genetics of stripe resistance, using the male-sterile technique, seem to require combining each of the several genes with the gene for male sterility. When this transfer has been accomplished, further gene identification and differentiation will be possible with relatively small populations.

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