Progress in Development of New Germplasm with Multiple Pest Resistance

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The increase in alfalfa acreage and its concentration in certain regions during the past 3 to 4 decades has resulted in a rapidly increasing pest problem. Alfalfa stands have had a progressively shorter productive life during this period.

New research programs were begun and others were redirected in an effort to determine the cause of "alfalfa decline" and to develop methods of alleviating the problem. Most of these programs directed their efforts to individual causal organisms thought to be responsible for the problem. The first major breeding effort to develop disease resistance in alfalfa resulted in the bacterial wilt resistant variety Ranger in 1942 followed by Buffalo in 1943 and Vernal in 1953. Stem nematode resistance in addition to bacterial wilt resistance was concentrated in Lahontan in 1954. These varieties alleviated the decline problem in many areas where these diseases were severe. However, it was soon evident that resistance to these two disease organisms alone was not the final answer to "alfalfa decline".

During the past two decades plant breeders have developed resistance to a large number of insect and disease pests (table 1). Along with the development of pest resistance has come a greater appreciation of the economic importance of individual pests. Losses due to pests have been difficult to assess, however through the use of pesticides and variety comparisons during infestations or epidemics we now have fairly reliable data on losses caused by many of these pests.

Losses due to all diseases in 1965 were estimated at 24% of the potential production from the 30 million acres of alfalfa in the U.S. Research in Kansas indicated that control of foliar diseases alone by chemical means increased hay yield 18%, carotene content 21% and decreased defoliation 18%. Nematodes cause an estimated loss of 5% on the total U.S. alfalfa crop.

Insect losses of about $220 million occur annually on alfalfa in the U.S. Cost of insect control alone can be a serious economic problem in many alfalfa growing areas.

Recent research has indicated that alfalfa quality is affected by insects and diseases, especially those attacking leaves and stems. Beneficial components such as carotene may be reduced to an insignificant level while undesirable antimetabolites such as coumestrol will be increased to very high levels, seriously decreasing the nutritional value of the forage.

Pest resistance therefore is a major factor in stabilizing yield and quality of alfalfa forage. A diseased plant cannot express its full inherent productive potential because of its impared efficiency in the utilization of water, fertilizer and photosynthetic or metabolic capabilities.

I would like to discuss briefly the progress we are making in development of resistance to diseases, insects and nematodes, the breeding problems involved and finally a plan to conserve alfalfa germplasm.

Development of Resistance to Individual Pests

Ranger, Buffalo and Vernal were the first varieties of alfalfa developed specifically for resistance to bacterial wilt. These varieties still provide the major source of resistance in breeding programs throughout the country and have provided excellent protection from bacterial wilt. Although these varieties have been classified as highly resistant, recent selection programs indicate that much higher resistance is easily obtainable. Two cycles of selection in a relatively susceptible alfalfa population resulted in nearly twice the percentage of resistant plants found in Vernal (table 2). Selection in Vernal also resulted in significant improvement.

Moapa alfalfa was released in 1957 as the first variety developed specifically for resistance to the spotted alfalfa aphid. Moapa and the major resistant varieties developed since that time have reduced the damage caused by this aphid. Recently however more virulent biotypes of the spotted alfalfa aphid have been identified. Resistance in many of the earlier developed varieties is no longer effective in containing this pest (Fig. 1). Fortunately the resistance in such varieties as Washoe, Caliverde 65, Kanza, Dawson, Mesa Sirsa and Hayden is effective against these new biotypes. New resistant strains are being developed for the problem area of the Southwest.

Damage caused by the pea aphid on alfalfa was not fully appreciated until very recently. Only occasionally does this aphid cause a major loss in alfalfa, however, because of its almost universal distribution it is considered a major pest of alfalfa. The release of Washoe in 1966 marked the beginning of an accelerated nationwide breeding effort to develop pea aphid resistance in alfalfa. Resistance to pea aphid responds well to selection and high resistance is obtainable. Highly resistant varieties such as Washoe, Dawson, Kanza, Mesa Sirsa, Hayden, Team and Caliverde 65 represent almost every major alfalfa growing area in the United States.

The alfalfa stem nematode is becoming more widely recognized as a problem in alfalfa, especially in irrigated areas. Wherever it occurs stand decline is rapid. In the Columbia Basin of Washington a three year stand life is normal (Fig. 2). In some areas of Nevada the stem nematode limits the life of the stand to 5 years (Fig. 3, 4).

Since the release of Lahontan in 1954 as the first variety highly resistant to the stem nematode, interest in developing resistant varieties has not been great. The frequency of stem nematode resistance genes is low in most of our alfalfa germplasm, however, high levels of resistance have been incorporated by either selecting within an adapted variety or transferring resistance from Lahontan or other Turkestan materials. In addition to Lahontan the varieties Washoe, Caliverde 65, and Apalachee all have high levels of resistance.

We have only recently begun to recognize the effect of root-knot nematodes on alfalfa. Root knot infestations reduce seedling vigor and are often responsible for establishment failures. We have been accumulating much research evidence indicating that nematodes are responsible for increasing the incidence or severity of other diseases. We have shown that root knot can increase the incidence of bacterial wilt in alfalfa.

Most of the non-hardy alfalfa varieties of African or Indian origin are highly resistant to the Southern root knot and Javanese root knot nematodes. Resistance to Northern root knot nematode has been found in low frequency only in Vernal or Vernal derived varieties. We have successfully transferred this resistance into Nemastan and African germplasm. Experimental varieties of hardy and non-hardy alfalfa have been developed at Nevada with complete resistance to the Northern root knot nematode while carrying resistance to both the Southern and Javanese root knot nematodes. We think there is a great need for an alfalfa with this type of resistance to be used as a rotation crop to reduce nematode populations for susceptible crops to follow. The use of root knot resistance for this purpose is being tested in Nevada and Washington.

Resistance to the alfalfa weevil has been difficult to achieve. An accelerated breeding program by USDA and several cooperating states to develop resistance to the Eastern alfalfa weevil resulted in the release of Team. The resistance found in Team is not sufficiently high to be used alone as a control measure but it does an excellent job in supplementing other control measures. Efforts to up-grade resistance in widely adapted germplasm are being continued.

The California Agricultural Experiment Station has made excellent progress in developing resistance to the Egyptian weevil. Selection response, like that for resistance to the Eastern weevil is slow but results indicate usable resistance will be developed (Table 3).

Leaf and stem diseases as a group cause large losses both in yield and quality of hay. Much like pea aphid damage, these pests are often very unspectacular and go virtually unnoticed. Too often however, these diseases cause heavy losses in certain periods of the growing season. Some of the more important of these diseases are leaf rust, common leafspot, downy mildew and Stemphylium leafspot. Selection response to these diseases has been excellent in a wide range of alfalfa types. Some of the recent germplasm releases by USDA at Beltsville combine high resistance to rust, common leafspot and Stemphylium leaf spot. Many of the new non-hardy varieties are resistant to downy mildew. Most private breeding programs have placed considerable emphasis on this group of diseases. We presently have two programs at Nevada to develop resistance to the foliar diseases in widely adapted populations along with resistance to pea aphid, spotted alfalfa aphid, stem nematode and bacterial wilt.

Only within the past five years have we really begun to appreciate the effect of anthracnose and phytophthora on yield and stand depletion in alfalfa. A true appreciation for anthracnose as a destructive disease did not occur until breeding produced resistant varieties for comparison. In the Eastern states resistance to anthracnose is now considered one of the major causes of yield and stand decline.

High levels of anthracnose resistance have been bred into several widely adapted alfalfa populations at Beltsville, Maryland. Selection response has been excellent as indicated by the high levels of resistance obtained in 2 to 3 cycles of recurrent selection (Fig. 5). In addition to the hardy populations developed at Beltsville, high levels of resistance have been incorporated in the varieties Vernal, Saranac, Narragansett, and Glacier. We have completed 3 cycles of selection for resistance to anthracnose in Moapa 69 and in a composite population of elite non-hardy breeding materials from Arizona, California and Nevada.

Phytophthora root rot has long been suspected of contributing to stand decline in the non-hardy alfalfa region of the Southwest as well as in some areas of the midwest. Very little progress was made in selection for resistance to this specific disease until recently. Selection under laboratory conditions at California and Minnesota has resulted in levels of resistance higher than were expected (Table 4).

Lahontan has been known to have better persistence than most varieties. We now know that this could be attributed to a rather high level of resistance to phytophthora. Even Lahontan responds well to selection for phytophthora resistance (Fig. 6).

We now have high levels of phytophthora resistance in Lahontan and African germplasm. Selection within very hardy materials has been in progress in Minnesota (Fig. 7). Field tests of the Lahontan and African type selections were established in 1971 at Logandale and Pahrump, Nevada and at El Centro, California. Selection in California for resistance to this disease has shown very marked improvement in stand maintenance.

Development of Multiple Pest Resistance

We have made a meager beginning in incorporating resistance to all of the major pests together into alfalfa. Only 6 years ago Washoe was released as the first variety with resistance to four pests. We have since added resistance to Northern root-knot nematode. Germplasm of this type however is of limited use because of its narrow range of adaptation. Our present program at Nevada includes development of multiple pest resistance in four populations and two modified varieties. Our plans are for the development of resistance to bacterial wilt, stem nematode, pea aphid, spotted alfalfa aphid, common leafspot, root-knot nematode, Phytophthora, anthracnose, downy mildew, leaf rust and leafhopper yellowing. Resistance to the first five of these pests has already been accomplished in two hardy populations. Two non-hardy populations with moderate resistance to pea aphid, spotted alfalfa aphid, and downy mildew have undergone three cycles of selection for anthracnose and one for Phytophthora resistance. Public release of this germplasm will be made at appropriate intervals while the improvement program continues.

The development of pest resistance in wide genetic base germplasm pools by USDA at Beltsville illustrates our most concentrated effort in this regard (Fig. 8). This is the first program aimed at improvement of multiple pest resistance in alfalfa germplasm with wide adaptation. We should see many such programs in the near future. An accelerated program of this type among public breeders throughout the country, coupled with release of improved germplasm, should simplify the synthesis of new and improved alfalfa varieties.

Breeding Problems

Although we have shown that development of pest resistance into alfalfa varieties is relatively simple we do not infer that it is without technical problems and considerations. We referred earlier to the problem caused by biotypes of the spotted alfalfa aphid. This problem can often be minimized by using a wide genetic base in the plant material being screened for resistance as well as a wide collection of the pest organisms. Studies in the past year at Nevada and Oregon indicate that resistance to Northern root knot nematode is stable with a wide collection of nematodes. Throughout our program to develop root-knot resistance we used nematodes collected in three states. Maintaining cultures of the pest under artifical conditions for many generations will very often alter its virulence. Nat ural field collections should be used at least at intervals in the program.

We often find that certain sources of plant pest resistance are sensitive to environmental conditions such as temperature. Use of this type of resistance exclusively in a breeding program could induce problems under some field conditions. The type of stem nematode resistance in Nemastan alfalfa is sensitive to temperature and could conceivably breakdown under certain conditions. We are presently collecting and integrating as many sources of resistance as possible for use in future screening programs. We know that certain of these sources are not temperature sensitive. On the other hand, most pests parasitize and reproduce under certain optimum conditions. These conditions should be known and maintained in a screening program for resistance.

Screening for resistance to any pest can be faster, easier and less costly if resistance can be determined in the seedling stage. Seedling screening techniques should always be tested against mature plant reaction. After several years of screening for stem nematode resistance in the seedling stage we found that seedling reaction does not necessarily reflect resistance or susceptibility in the mature plant.

Although early seedling tests for pest resistance have many advantages we must emphasize the many bonus effects from recurrent selection (selection followed by recombination and reselection) both in the field and after long periods of growth under less than optimum conditions in the greenhouse. Plants growing under such conditions are subjected to the total complex of pest actions and interactions. Selected plants are healthier in ways beyond that imparted by resistance to the pest for which selection was practiced. Lahontan was bred for resistance to stem nematode and bacterial wilt however it has moderately high resistance to phytophthora and spotted alfalfa aphid. Populations A and B developed by USDA had resistance to anthracnose imparted by field selection for other traits. Much of the weevil resistant material developed at Beltsville is resistant to ozone imparted by naturally high ozone levels in greenhouse atmosphere.

After many generations of selection for alfalfa weevil resistance under greenhouse conditions without use of pesticides, Team alfalfa was found to be as resistant to pea aphids as Dawson or Kanza whose resistance was the result of intensive selection programs. We can cite many such examples of bonus improvement resulting from recurrent selection under field or other conditions which subject selected plants to unintentional stresses or pest problems.

We briefly mentioned that our studies showed that root-knot nematodes increased the incidence of bacterial wilt in alfalfa. Similar results were reported from Iowa. Research in Canada showed a similar relationship between stem nematode and bacterial wilt. The Canadian studies also showed evidence that resistance to bacterial wilt could be broken down by stem nematodes. The predisposition of otherwise resistant plants to disease by nematodes has been reported for a large number of crop plants.

When working with many traits in a plant the plant breeder must be aware of certain genetic pitfalls which might produce undesirable results. Intensive selection in a population low in the frequency of the trait desired, could result in selection and subsequent interbreeding of related clones (inbreeding). Negative correlation between two desired traits is always a problem for plant breeders since selection for one results in loss of the other. If these are caused by genetic linkages they may be overcome, sometimes with difficulty. In most of our efforts to breed for resistance to several pests, provisions are made to test for these undesirable effects at intervals in the program.

Conserving Alfalfa Germplasm

Germplasm resources in alfalfa, not unlike those of other crop plants, are being diminished by encroachment of agriculture on their native habitat. Much of the germplasm collected over the years has been exploited and lost through close breeding programs. We have suggested plans to conserve the genetic diversity still available in alfalfa and at the same time exploit this germplasm by developing combined resistance to diseases and insects*. The plan involves worldwide collection, genetic recombination in regional germplasm pools and mild selection to improve this germplasm for use in breeding regionally adapted varieties.

*Hanson et al. 1972. Jour. Environmental Quality 1:106-111.

Table 1. Principal pests of alfalfa to which resistance is being developed.

Common name of disease or insect	Scientific name
*Bacterial wilt	*Corynebacterium insidiosum* (McCull) H.L. Jens
*Stem nematode	*Ditylenchus dipsaci* (Kuhn) Filip.
*Northern root-knot	*Meloidogyne hapla* Chitwood
*Phytophthora	*Phytophthora megasperma*
*Anthracnose	*Colletotrichum trifolii* Bain and Essary
*Rust	*Uromyces striatus* Schroet. var. *Medicaginis*
*Common leafspot	*Pseudopeziza medicaginis* (Lib.)
Eastern alfalfa weevil	*Hypera postica*
Egyptian alfalfa weevil	*Hypera bruneipennis* (Boh.)
*Spotted alfalfa aphid	*Therioaphis maculata* (Buckton)
*Pea aphid	*Acyrthosiphon pisum* (Harris)
*Potato leafhopper	*Empoasca fabae* (Harris)

*High levels of resistance developed

Table 2 Response of alfalfa populations A-C4 and B-C4 to mass selection for resistance to bacterial wilt as determined by frequency distribution of plants in wilt reaction classes and severity index, and three variety checks.

Population or variety	Percentage of plants in wilt reaction classes*						Severity index	No. plants tested
	0	1	2	3	4	5		
A-C4†	3	6	3	12	57	19	3.72	217
A-CW2	13	19	11	15	35	7	2.53	213
A-CW3	37	29	12	8	10	4	1.38	194
B-C4†	0	0	1	6	60	33	4.25	228
B-CW2	1	0	3	14	64	18	3.94	242
B-CW3	0	1	8	21	65	6	3.67	255
B-CW4	1	7	16	35	39	2	3.13	204
B-CW5	4	13	26	35	21	2	2.63	245
Vernal	15	22	17	24	21	1	2.17	229
Ranger	6	10	13	26	36	8	3.04	219
Narragansett	0	1	0	4	45	51	4.46	195
LSD (.05)							.42	

*Scored 0 - 5: 0 = healthy, 5 = dead. †Unselected for bacterial wilt resistance
From Barnes et al. 1971. Crop Sci. 11:545-546.

Table 3. Egyptian alfalfa weevil counts made by W. Sallee in Tulare, County, California, April 8, 1969.

Variety	Ave. larvae for 3 sweeps	Damage
UC W1	17	Little
Ouside of plot	50	Severe
UC W2	24	Much less than outside
Outside of plot	44	Severe
UC W3	35	Much less than outside but poorer than UC W2
Outside of plot	78	Severe

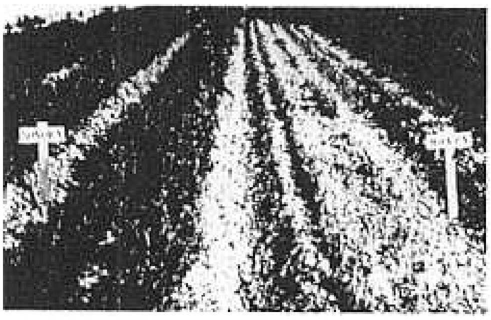
By courtesy of W. H. Lehman, UC, El Centro.

Table 4. Reaction of UC Salton and Moapa to *Phytophthora megasperma* at Davis, California*

Variety	Surviving plants by infection classes: Slight	Moderate	Severe	Total
UC Salton	116	104	166	386
Moapa	29	99	134	262

*Equal amounts of seed were planted in two replications. The two varieties were located adjacent to each other in the test. No record of dead plants is available but better survival of plants in UC Salton is another indication of tolerance.

By courtesy of W. H. Lehman, UC, El Centro.



Moapa alfalfa, seriously damaged by a new biotype of the spotted alfalfa aphid, shows little of its former resistance.

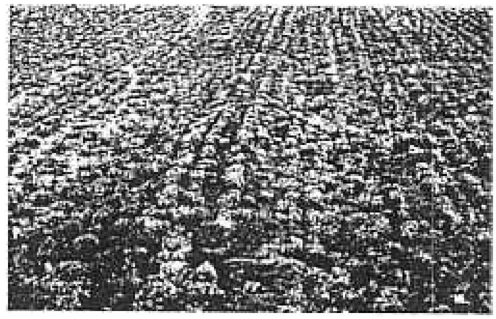


Fig. 2. A relatively young stand of alfalfa with noticable stand decline caused by stem nematode.

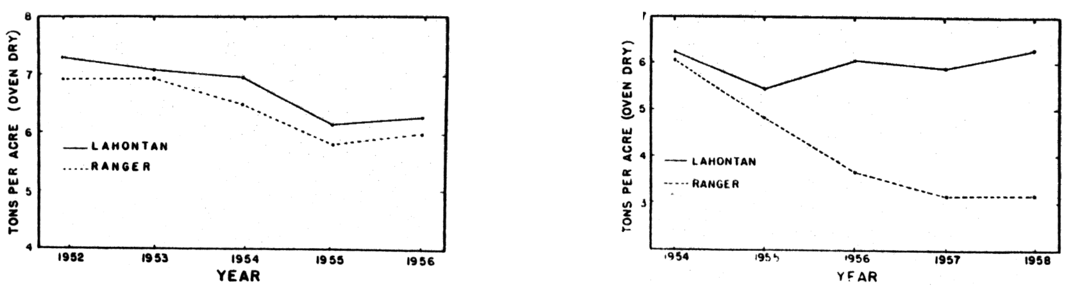
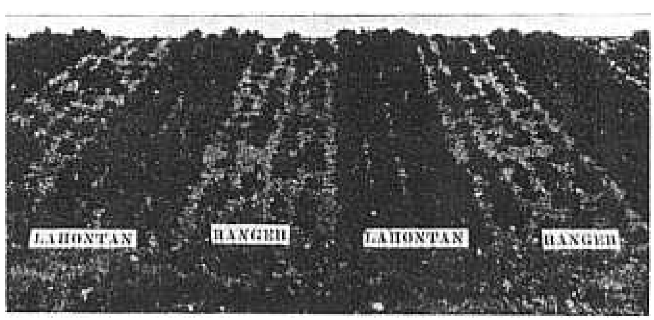


Fig. 3. Effect of stem nematode on yield of a susceptible variety of alfalfa (Ranger) and a resistant variety (Lahontan). Left: Grown under nematode free conditions. Right: Grown under nematode infestation.



4. Effect of stem nematode on a susceptible variety (Ranger) and a resistant variety (Lahontan) three years after seeding.

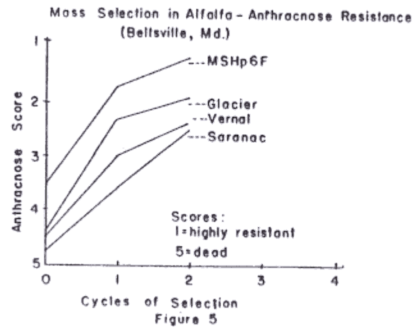


Fig. 5. Chart showing selection response for anthracnose resistance in alfalfa.

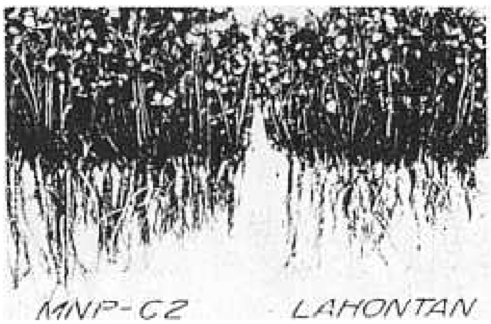


Fig. 6. Healthier top and root development of Phytophthora resistant selection MnP-C2 compared to parent variety Lahontan.

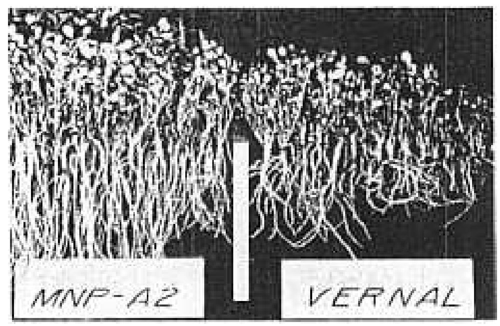


Fig. 7. Healthier top and root development of Phytophthora resistant selection MnP-A2 compared to parent variety Vernal.

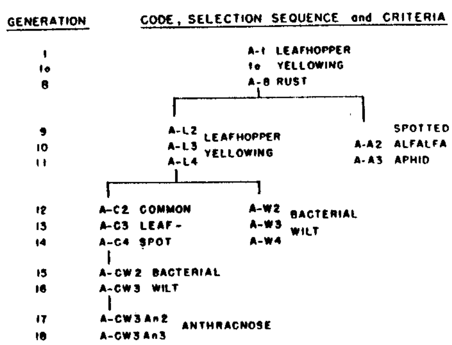


Fig. 8 - Plan of recurrent phenotypic selection in germplasm pool A from 1950 to 1970. Comparable plan was used for pool B except that four cycles of selection for bacterial wilt resistance followed A-C4 in pool B instead of two as in pool A

From Hanson, et al. 1972. J. Environ. Quality 1:106-111