POSSIBILITIES FOR DEVELOPING ALFALFA VARIETIES FOR RESISTANCE TO THE EGYPTIAN ALFALFA WEEVIL (Hypera brunneipennis)*

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The Egyptian alfalfa weevil was introduced into the United States near Yuma, Arizona in 1939 and soon spread across southern California to the coastal areas. It was confined to these southern California areas until the 1960s when it started moving north into the Central Valley of California. After the Egyptian alfalfa weevil arrived in the United States, the pest was identified, information was obtained about its biology, and studies were made on the control of this pest through the use of insecticides, but no breeding work was started at that time.

An embryo program designed to develop varieties resistant to the Egyptian alfalfa weevil was started in California in 1965. This work was expanded in the early 1970s because funds designated to do this work were obtained. These funds have recently been reduced to a low level in California.

Resistance to Alfalfa Weevil in Eastern United States

When the alfalfa weevil (Hypera postica) invaded the eastern part of the United States in 1952, damage was severe and strong programs in every area of research including breeding for resistance were mounted.

Work in the alfalfa weevil programs in the East resulted in the release of the variety Team in 1969 and also contributed to the release of the variety Arc in 1973. Team, as the name implies, was developed through the cooperative efforts of the U. S. Department of Agriculture and several agricultural experiment stations. There is a small acreage of both of these varieties being grown for seed production in California. Neither of these varieties are being grown for hay production in California because they are winter dormant and may be lower in hay production.

Team is reported to have a moderate level of resistance to the alfalfa weevil. Weevil can be expected to be found on Team, but damage and the number of insecticide treatments can be expected to be lower than for other varieties. In variety tests Team may occasionally be equal to or slightly poorer than other varieties in a particular trial, but it is usually among or better than the highest rated varieties in the test. However, reports have been obtained through personal communications about trials where Team has exhibited little or no resistance to the alfalfa weevil.

Arc derives its resistance to the alfalfa weevil from Team. The Arc germplasm received one additional cycle of selection for weevil resistance beyond Team, and it is reported to be slightly more tolerant to alfalfa weevil larval feeding than Team.

Breeding for Resistance in California

Work on breeding for resistance to the Egyptian alfalfa weevil was started in California because of the need for additional methods of biological control to reduce the repeated applications of insecticides. In addition, and perhaps of greater importance to the entire biological control program, this work will also serve as a pilot program in the development of resistance to the number of other "worms" attacking alfalfa in California. Some of these are the alfalfa caterpillar, beet armyworm, leaf roller, and

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varigated cutworm. Prior to now, we have only obtained resistance in alfalfa to sucking insects such as the spotted alfalfa aphid and pea aphid which are much more discriminating in their tastes. The "worms" are foraging insects which eat like cattle and are much less discriminating in what they eat. If we can develop good methods and resistance levels for the Egyptian alfalfa weevil, we will have helped the immediate problem of controlling the Egyptian alfalfa weevil and we will have gone a long way in developing resistance to the other "worms" that can be very destructive to our California alfalfa fields in the late summer.

Weevil Collection

Many of the techniques or methods used in the University of California Egyptian alfalfa weevil program were developed or adapted from methods developed in Eastern United States because it was thought breeding behavior and, perhaps, genes for resistance would be the same for the two weevil. However, collection of weevils to be used in resistance tests in California is different. This is primarily because the period of activity, hibernation (aestivation), and climate are different for the Egyptian alfalfa weevil. Adult weevil used in the California tests are collected from the bark of eucalyptus trees, stacked lumber, cardboard, etc. during the late spring and summer while the adults are in hibernation. They are stored in the laboratory at a temperature of about 26° C until about mid-November. Storage is then changed to a chamber set at about 4° C where they remain until used in tests. Other dates for starting cold storage were tried, but mid-November appeared to be the best time. Earlier introduction (spring or mid summer) into cold storage resulted in poor feeding and later storage resulted in high mortality. The adults have been fed a sugar water solution during their entire storage period. Recently we have been trying to place only potatoes in the storage containers because sugar water would occasionally wet the cage or paper and kill many weevil. Testing is started about the first of the year when it appears the adults are feeding actively after being placed on alfalfa.

Methods of Selection

Three methods of selection have been used: (1) Greenhouse flat tests are used for seedlings. (2) Leaf disk tests are used on adult plants. (3) Selections are made in infested fields. The greenhouse flat test is conducted by growing seedlings in greenhouse flats, infesting them with adult weevil when the plants have one or two leaves, removing the weevil when most plants have been damaged, and then saving the plants which survive in good condition. These plants are then tested and further eliminated by using the leaf disk test. This seedling method is being largely discontinued because it is difficult to control feeding, adult weevil are used rapidly, feeding or killing of alfalfa plants seemed random, and the number of escapes appeared too great.

The leaf disk test is conducted by collecting eight leaves from the upper three nodes of eight different stems, if possible. The detached leaves are then placed on a pad of moistened blotter paper. One leaf disk about one-fourth inch in size is cut from each trifoliate. The leaf disks are weighed after being allowed to come to an equilibrium for about two hours. After the leaf disks are weighed, eight random, actively feeding adult Egyptian alfalfa weevils are placed in each petri dish containing the leaf disks and moistened paper. These disks are placed in a dark chamber set at 86° F and allowed to remain for 18 to 24 hours. Weevil are removed from the petri dishes when an average of about 50% of the leaf material has been eatened or they have been feeding for 24 hours. After the weevil are removed, the remaining plant material is weighed and percent feeding loss is calculated.

Field selections for weevil resistance are made in fields severely damaged by a prolonged attack from the Egyptian alfalfa weevil. Plants are selected which have low feeding damage by the Egyptian alfalfa weevil and which also have the ability to produce regrowth under similar conditions.

Variety Types Being Used

Nondormant and semidormant alfalfa varieties and germplasm adapted to Southwestern United States are being used in this work. This is in contrast to the dormant varieties such as Team used in Eastern United States. Use of nondormant and semidormant varieties is very important in California, but this has tended to confound the work on resistance

to the Egyptian alfalfa weevil because the more dormant plants tend to appear more resistant. There are exceptions to this such as the variety Lahontan. In addition, the weevil is most active during the winter and early spring when dormancy differences are greatest.

The number of alfalfa varieties and sources of germplasm being used in the alfalfa weevil breeding program is being kept large because we are not sure which varieties will have the best source of resistance. Genes for resistance may have to be obtained from many sources and then concentrated through the recurrent selection procedure of breeding in order to get a higher level of resistance.

One recent source of germplasm added to the program is the variety Monsefu from Peru (PI 247,790). It is related to Hairy Peruvian and has been reported by workers in Missouri to have some resistance to the alfalfa weevil.

Results of Leaf Disk Tests

Leaf disk tests have been one of the main tests used to make resistant selections because this appears to be the best test we have for antibiosis (killing or reducing growth and reproduction of the feeding insect). By using this test it has been possible to obtain a numerical score and easily classify each plant. The test is also adapted to using adult weevil. Over 1,000 leaf disk tests have been conducted each year for the past few years. In an effort to determine the progress being made using these tests, we selected and compared some of our best and poorest plants (Table 1). An average percent leaf disk eaten (by weight) was calculated using six replications. Individual differences in leaf disk feeding activity between resistant and susceptible clones were as high as 38%. The average difference between the two groups in Table 1 was 22%.

New varieties have been made on the basis of the results from leaf disk tests. To date five experimental varieties have been made using this method of selection. Only two of these varieties (UC 63 and UC 68) have been tested. UC 63 appears to have a slight tolerance to the Egyptian alfalfa weevil, and UC 68 appears to have no tolerance. Since field testing for Egyptian alfalfa weevil resistance is very slow, three of the newer varieties have had essentially no testing. Field testing of varieties and selected plants has been a serious limitation in this work. Field populations of the weevil have been erratic. In some years, like last year, weevil numbers are not high enough to obtain an adequate test.

Results from Field Selections

To date, experimental varieties made from field selections appear to be showing most promise. On the basis of personal communication from other workers, this appears to be the case in other programs also. We have been working with two varieties, UC 73 and UC 77, which were made from field selections made in NK Blend 9-19. Both varieties are from a second cycle of selection for weevil resistance. The parent plants of these varieties were selected from an area in the Western part of San Diego County where no pesticides are used. Prior to the time the selections were made, the plants were subjected to severe prolonged damage from the Egyptian alfalfa weevil. In tests made at several locations of California these varieties were generally among the best varieties for weevil resistance in the test (Table 2). However, they appear to do best in areas most similar to the area in which the parent plants were selected and poorer in areas where they were less well adapted like Davis.

UC 73 and UC 77 appear as though they might have a useable level of resistance in some areas of California. They also seem to have some other unknown advantages. However, the level of tolerance to the Egyptian alfalfa weevil does not appear high enough to justify release as a variety. In addition, their level of resistance to the spotted alfalfa aphid may be too low.

At present we plan to use UC 73 and UC 77 primarily as a source of parents for new varieties. A large area in San Diego County will be planted with UC 73 this winter. Selections will be made in this area during one of the severe weevil attacks. In addition, we will decide this winter if UC 73 should be released as germplasm so that other plant breeders will have access to its good characteristics.

National Weevil Test

A national weevil test has been designed to compare alfalfa varieties selected for Egyptian alfalfa weevil and alfalfa weevil in areas of the United States where the weevil is a problem. Two of our best weevil varieties have been entered in this test. The test was planted in the spring of 1975 in nine locations. First results from this test should be available in 1976.

New Testing Procedures

New methods of selection are being investigated which might be useful in extending the selection period, in screening plants, and in determining the nature of resistance. At present, workers depending on field populations of Egyptian alfalfa weevil have only one chance each year to classify plants or varieties. If no heavy weevil population develops, the entire year is lost. The leaf disk test can be used over three to four months, but tests are needed which will extend the testing period longer and produce more reliable results.

Cooperative work on the nature of resistance and plant selection procedures was started with Dr. Weiss, USDA Utilization Laboratory, Albany, California. Some of the resistant and susceptible plants shown in Table 1 have been supplied to Dr. Weiss. The approach to the problem he plans to use, simply stated, will be to use biological assay (weevil feeding) and forms of chromatography to determine the chemical factors, if any, responsible for weevil resistance in alfalfa. This appears to be a very interesting and encouraging part of the weevil resistance work. Success seems possible even with small differences. If successful, this could lead to simplified tests for the factors responsible for weevil resistance. It could also open the way toward developing resistance to other leaf-feeding insects.

Experiments were conducted on the relationship between general plant color and resistance because observations made on various types of material seemed to indicate plants with a general dark green color also had less weevil feeding. If this were true, it might mean that elimination of a portion of susceptible plants found in a population could be made by selecting for a general dark green plant color. This idea was tested by rating plants and varieties for color and correlating this with tests made for weevil feeding. The following results were obtained:

Damage rating of plants x plant color (N=82) r = 0.12
Damage rating of plants x leaf disk tests (N=82) r = 0.41
Damage rating of varieties by stems x variety color (N=25) r = 0.18
Damage rating of varieties by plots x variety color (N=25) r = 0.47
Damage rating of varieties by plots x variety color (N=11) r = 0.44
Damage rating of varieties by plots x variety color r = 0.63

In general, the correlation coefficients (r values) were low to moderate and dependent on the type of weevil test used. It appears dark color might be used to screen plants for resistance, but, if so, it will have to be used with caution. Refinement of the test by using better color indexing, a different time of year, and replications might be helpful.

One color component found in plants is anthocyanin. This gives the stem a red color, and it is fairly easy to identify in cooler seasons of the year. Two cycles of selection were made for high and low anthocynin content in an effort to see if one population might have more resistance to the weevil. Seed has been produced, but, thus far, experimental varieties have not been tested for resistance to the Egyptian alfalfa weevil. However, correlations between red stem and weevil feeding were made on one set of plants rated for red stem and weevil feeding. A r value of 0.33 was obtained. This value was fairly low, but if greater differences among the plants for red stem (anthocynin) were obtained, this correlation might change.

Another area of weevil resistance work we tried was to determine if there might be any physical difference among the leaves of plants selected from cultivated alfalfa (*Medicago sativa*) and species of Medicago previously determined to have some resistance to the alfalfa weevil. Methods were a problem, and more work will be necessary to find a good test method if this work is to be continued. In general, we tried to clear and dissolve part of the leaf tissue. We then rated the leaves for number of veins and density

of the individual veins. The veins were stained in some of the tests to help provide a better reading. The results of six preliminary tests were combined in the following table:

Identification	Previous classification of the material	Rating for number of veins*	Rating for density of veins*
W 315 (*M. sativa*)	Susceptible weevil plant	2.40	2.75
Monsefu (*M. sativa*)	Susceptible weevil variety	2.50	2.50
M. rugosa	Resistant species	1.00	1.00
M. suffruticosa	Resistant species	2.15	2.00
M. arborea	Resistant species	2.40	2.00
W 45 (*M. sativa*)	"Resistant" weevil plant	2.30	2.25

*Plants were rated on a scale of one to five. A rating of one was a large number or dense rating. A rating of five was the opposite.

M. rugosa appeared definitely better than the other materials. The lines classified as susceptible had slightly poorer scores than the rest. This procedure has promise of being useable as a selection method through the year. Techniques need to be found which will do a better job of identifying routinely the number and/or density of the veins. Correlations between veining and resistance will be needed for specific clones.

A last selection method being tried is to use weevil larvae in the screening of plants of various ages. In this method, female weevil are allowed to lay eggs in stems. The egg-filled stems are then placed in a refrigerator until larvae are needed. When larvae are needed, the stems are brought into a warm room and remain there until the eggs hatch. After larvae start hatching from a number of stems differing slightly in age, it is possible to obtain enough larvae for most tests in one night's hatch. Only day old larvae are used.

In tests using old plants five young larvae are placed on one terminal for one to three weeks or until the larvae are past the first two instars but before the adult stage. At this time the stems are cut and placed in a vial of alcohol for reading at a later date. No results of this test are available as yet.

In the seedling screening method, space-planted seedlings were infested with one first instar weevil larva. Damaged seedlings were discarded as they were found. Undamaged seedlings were reinfested two or three times before they were saved. The retained seedlings will be tested later with the leaf disk or cage test. It is too early to know if this test will be effective.

Summary

The weevil breeding program in Eastern United States on the alfalfa weevil has resulted in the release of the varieties Team and Arc. These varieties have tolerance to the alfalfa weevil but can be expected to suffer feeding damage. Their major value is in an integrated program using insecticides and other forms of biological control. When used in this type program, they are expected to be able to eliminate the need for insecticide applications under light populations and delay the need for insecticides under heavy populations.

The Egyptian alfalfa weevil breeding program in the University of California has produced seven Egyptian alfalfa weevil experimental varieties. Only the first four have been tested. Two of these appear to have a useable level of tolerance, especially in areas where they are best adapted. Both of these better looking experimental varieties were from field selections made in Western San Diego County. Testing of all materials has been slow because the Egyptian alfalfa weevil is active only during the spring of the year and in some years populations are very low.

Testing nondormant varieties with semidormant and dormant varieties has been difficult because dormant plants tend to appear more resistant. Some of our better experimental

varieties are now being compared in a national weevil test planted in nine locations in the United States.

Resistant plants are being selected by using seedling screening, leaf disk tests, and field selections. Adult Egyptian alfalfa weevil have been used in most testing in the past, but tests are being started using larvae.

The variety types being used are primarily nondormant since they are the types being used in the areas most damaged by the Egyptian alfalfa weevil.

New selection techniques are constantly being tried which will help make more effective selections and also extend the selection period. The new techniques or methods used have been: biological assay plus chromatography, relation of resistance to general plant color, importance of leaf structure, and use of day-old larvae on adult and seedling plants.

Table 1. Amount of feeding by adult Egyptian alfalfa weevil on leaf disks obtained from plants classified as resistant and susceptible by the leaf disk test.

Clone	% leaf disk eaten	Clone No.	% leaf disk eaten
Resistant clones		Susceptible clones	
W 45	54.9	W 376	78.3
W 274	60.6	W 929	79.3
W 370	66.0	W 1286	79.1
W 845	44.0	W 1339	82.4
W 851	60.7		
W 920	66.0		
W 1137	51.3		
W 1178	63.0		
W 1386	54.0		
Average	57.8	Average	79.8

Table 2. Reaction of alfalfa varieties to the Egyptian alfalfa weevil (EAW) in six trials grown in California.

	Weevil damage score[1/]					
	Test 6-7E[2/] El Centro 3-5-73	Test A60[2/] El Centro 1972	KFS[2/] Parlier 1973	San Pasqual[3/] 1975	UC Davis[3/] 1974	Manteca[3/] 3-24-74
UC 73 (EAW)	-	3.9	1.8	7.0	-	6.0
UC 77 (EAW)	2.7	-	-	6.8	5.8	-
UC 63 (EAW)	2.8	-	1.9	-	-	-
UC 68 (EAW)	3.6	-	2.3	-	-	-
UC Cargo	3.1	4.6	1.9	9.0	6.9	6.3
UC Salton	2.3	4.7	1.9	9.0	7.5	-
Moapa	-	-	2.4	-	-	4.0
Moapa 69	4.3	5.1	-	-	8.9	6.0
Sonora	3.1	4.9	-	-	-	8.5
Hayden	3.0	4.3	-	-	-	9.5
El Unico			-			
Mesa Sirsa	4.	4.3	-		-	10.0
Lahontan	-	-	1.5		10.0	9.0
Team (AW)	2.8		.2			
Weevlchek (AW)	1.8		.3			

1/ 1 = no damage, 10 = very severe damage.
2/ Visual damage rating of individual stems.
3/ Visual damage rating of the plot.

Selected References

Barnes, D. K., C. H. Hanson, R. H. Ratcliffe, T. H. Busbice, J. A. Schillinger, G. R. Buss, W. V. Campbell, R. W. Hemken, and C. C. Blickenstaff. 1970. The development and performance of Team alfalfa. U. S. Dept. Agr., Agr. Res. Serv. ARS 34-115, 41 pp.

Barnes, D. K. and R. H. Ratcliffe. 1969. Evaluation of annual species of Medicago as sources of alfalfa weevil resistance. Crop Science 9: 640-2.

Lehman, W F. and E. H. Stanford. 1971. Egyptian alfalfa weevil, breeding resistant alfalfa. California Agriculture 25(5): 7-8.

Lehman, W. F. and E. H. Stanford. 1972. Progress in the development of alfalfa varieties with resistance to the Egyptian alfalfa weevil Hypera brunneipennis (Boh.). Proceedings 1972 California Alfalfa Symposium. pp. 23-27.