PROGRESS IN THE DEVELOPMENT OF ALFALFA VARIETIES WITH RESISTANCE TO THE EGYPTIAN ALFALFA WEEVIL *Hypera brunneipennis* (Boh.)

W. F. Lehman and E. H. Stanford
Associate Agronomist and Agronomist
University of California, El Centro and Davis

The Egyptian alfalfa weevil (*H. brunneipennis*) is the most important insect pest of alfalfa in California. It is closely related to the Western alfalfa weevil (*H. postica*) which has been a serious problem only in local areas and the Eastern alfalfa weevil (*H. postica*) which is the most important insect pest in Eastern United States. Losses of more than 6 and 56 million dollars have been reported in California and Eastern United States, respectively.

The Egyptian alfalfa weevil was introduced into Yuma, Arizona, in 1939. It soon spread to most of the desert valley and coastal areas of Southern California. It was confined to Southern California until the 1960's when it started moving north into the Central Valley of California and soon became the most important insect pest on alfalfa in that area.

In Southern California the Egyptian alfalfa weevil is usually thought of as one of the lesser pests of alfalfa behind the spotted alfalfa aphid, the root rots, and scald. Its damage is generally limited to 1 or 1½ cuts in the early spring when pea aphid might also be a problem. Growers spray to control the weevil and the pea aphid and have learned to live with both of these insects. Predators were released and are working in the area, but peak activity of the predators usually comes too early to be of much value in weevil control.

The major control mechanism for the alfalfa weevil has been the use of insecticides. This method is expensive, can upset the biological balance in the field, and, if someone is careless, could leave chemical residues in the hay. Biological control through the use of predators and parasites can be effective, but to work, this method needs the presence of the insect pest. Biological control through the use of resistant varieties is the best means of control because the control mechanism is built into the plant. Nothing else is needed.

Breeding for Resistance in Eastern United States

Essentially no work was done on breeding for resistance to any of the alfalfa weevils until one of them became a serious problem in Eastern United States during the early 1960's. Good support for the plant breeding effort was received. A team of scientists primarily from the U. S. Department of Agriculture was assigned to this problem. After about 10 years of work a variety called Team was released. This variety contained a low level of resistance to the alfalfa weevil plus a combination of many other useful characters. In addition to variety development this work also produced good techniques and information useful in other programs.

The level of weevil resistance in Team was low, but was expected to be of value as part of an integrated control program which included good crop management and judicious use of insecticides. The variety was subsequently found to have less resistance than expected.

During the development of the variety Team many good procedures were developed or adapted. We were able to see this program and later adapt many of the techniques to our program. The main methods or techniques used in the East were: (1) A germplasm pool was established from diverse sources of germplasm. (2) The recurrent selection method of breeding was used where selections were made, intercrossed, and the progeny from these plants reselected and intercrossed, etc. Reselection and intercrossing was done to concentrate factors for resistance. For the variety Team this was continued for six generations. (3) Field selections based on low larval feeding were made in some generations. (4) Seedling selections were made by allowing adult weevil to feed on very young plants until a low number of plants remained. (5) Adult plants were tested by feeding leaf disks to adult weevil. (6) A second

adult plant test was the larval development test where the rate of growth of larvae fed on test plants was measured. (7) The egg laying stimulus test was an adult plant test which measured the effect of the test plant on the egg-laying ability of the weevil.

Breeding for Weevil Resistance in California

Work on breeding for resistance to the Egyptian alfalfa weevil in California was started in 1965 with selections made in San Diego County. This work was intensified in 1968 when the Egyptian alfalfa weevil started causing widespread damage in Central California. Techniques developed in the East were modified and applied. The germplasm used in the East could not be used directly because it was winter-dormant and susceptible to the spotted alfalfa aphid.

Weevil Collection

In California we were able to collect large numbers of adult weevil hibernating (aestivating) in trees, traps, and other hiding places. This is in contrast to the work in the East where active adult weevil are collected in the fields. The hibernating weevil were kept in a warm place (about 80°F) until December when they started to come out of hibernation. At this time they were placed in a refrigerator set at 40°F until used in tests. We found these weevil could be used in tests from late December until the end of May. Some weevil were kept in storage for more than two years, but there was little feeding and egg laying activity after one year.

Availability of large numbers of feeding adult weevil over long periods of time (more than five months) has been a major limiting factor in the work. Preliminary work has been done on extending this period, but this work was discontinued because research funds were very limited. One method investigated was the development and subsequent use of weevil populations which have no dormant period (non-aestivating populations) due to the special light and temperatures they require. A second method was the use of hormones which maintain adult weevil in an active stage or break the dormancy of hibernating populations.

Selection Methods

Three main methods of selection have been used in the California work. They are field selection, seedling non-preference tests, and the leaf disk test. Field selections have been made in severely damaged, old fields in San Diego where the dairymen fear spraying of alfalfa with insecticides because insecticide residues might be found in the milk. In this method we try to find a severely damaged field where individual plants are easy to identify. Plants which have had a good chance of being attacked but are relatively free from damage are saved.

The seedling non-preference test is conducted by growing seedlings in greenhouse flats, infesting them with adult weevil when the plants have one or two leaves, and then saving the plants which survive in good condition. These plants are then tested and further eliminated by using the leaf disk test. In this test a leaf disk about 1/4 inch in size is cut from leaves of test plants. These disks are weighed, fed to adult weevil, and weighed again. The plants with the lowest feeding values were saved.

No selection has been done on the basis of larval feeding or egg laying because these tests are more expensive and because special studies have shown adult tests to be equally effective in selecting for resistance to larval growth and egg laying.

Germplasm Source

The germplasm source (alfalfa varieties) used in the California weevil breeding program has been kept very large because we are not sure which varieties have the best potential or source of resistance. Genes for resistance may have to be obtained from many sources and then concentrated through the recurrent selection procedure in order to get a high level of resistance. The germplasm being used is largely from varieties adapted to California because other work indicates it should be possible to select for weevil resistance in most varieties. By using adapted varieties a minimum of work will be needed on other characters. The varieties being used in this

work are African, Lahontan, Caliverde, California Common, Indian, Arizona 21-5, introductions from foreign countries, and elite materials from the breeding program. The selections made in San Diego County were primarily from the blend 9-19.

Experimental Varieties

Through the past few years we have produced eight experimental varieties which are expected to have some resistance to the Egyptian alfalfa weevil. Four of these were made from field selections, three from leaf disk tests, and one from seedling selection. We are planting these varieties in as many locations as possible throughout California in an effort to get good field evaluations. Since local weevil infestations, growers spraying practices, timing of the last cutting, weather, and many other factors affect the weevil infestations, many tests are needed to obtain a few good weevil readings. Last spring we had about 10 tests planted and were unable to get any good evaluations. However, we do have some evaluations.

One is a test in Tulare County (Table 1). The synthetics selected for weevil resistance had fewer larvae and better appearance than the check variety. Of particular interest is UCW3 because it should have a better agronomic type than the other two. UCW3 was also observed to be better than the check variety, Sonora, in San Diego County during the spring of 1970. A second cycle of selection for weevil resistance has been made in this variety.

In a variety test near El Centro a small, but significant, difference was obtained between the weevil synthetic UC63 and the named varieties, Table 2. No consistent differences between weevil synthetics and named varieties were obtained in 1972. As will be shown later, this difference may be related to cool winter temperatures in 1972. No tests were evaluated in 1971 because field populations were low.

Resistance in Experimental Varieties

Test results obtained to date seem to show the varieties tested have a slight advantage over non-selected varieties but not enough advantage to resist heavy populations. The level of resistance seems to be similar to the results given for Team. However, no direct comparisons have been made with Team because it is winter dormant and susceptible to the spotted alfalfa aphid. Like Team, these new experimental varieties, when combined with good cultural practices and biological control, may be effective in helping to control the alfalfa weevil. However, we feel higher levels of resistance than the tests have shown to date are possible and should be obtained before any varieties are released.

Continuous Evaluation

Because breeding for foraging (leaf eating) insects is relatively new in alfalfa breeding, we must continually evaluate the program and look for new and better sources of resistance and methods.

The methods we are using to select for weevil resistance appear reliable, but they are slow and expensive. If any method is going to be truly successful, it must be inexpensive, easy to use, and useable on large populations. Work on bacterial wilt, spotted alfalfa aphid, and Phytophthora root rot has shown this. One of the major objectives of this program is to find an easy and effective method to use in selecting for weevil also.

New Areas of Research

In the process of working with the weevil and evaluating the program, we have observed several points that seem important and should be investigated as time and money permits. One factor which is quite striking as we view our weevil synthetics through the state is that they are always darker in color and have more purple color or anthocyanin in the stems. This also seems to be the case of the better plants found in 9-19 in San Diego County. A correlation coefficient (statistical test which shows relationship) calculated between weevil damage scores and plant color resulted in an r value of 0.59. This indicates a fairly close relationship between the two characters. We wonder if there is a chemical or some other factor associated with

the dark color which is objectionable to the weevil. If so, we wonder if this chemical is anthocyanin, a color compound, or tannin, a chemical affecting plant color. In preliminary testing for tannin on about 100 clones we have found distinct differences between clones. We plan to conduct more of these tests to select for high concentrations of these chemicals and then try to correlate this with weevil resistance. Unfortunately, these chemicals have been shown to fluctuate with weather factors in other crop plants. We expect the same problem in alfalfa.

In our 1972 disk tests we noticed that four check or control plants which were tested over long periods of time gave poor results part of the time and good results at other times. We suspected weather to be a factor because we had a long, cool period followed by warmer weather. In multiple correlations calculated between weevil resistance in the check clones and the combined effect of temperature and relative humidity r values between 0.23 and 0.50 were obtained. The low correlation was from a clone with some dormancy. These values indicate there is a tendency for resistance to improve as temperatures increased and humidity decreased. We are not certain how important this relationship might be, but research has shown clearly that resistance to the spotted alfalfa aphid is increased as temperature is increased. If this is true for the weevil, we might expect the resistant varieties to show good resistance during warm springs and then have the resistance break down during a cool spring. If so, this might explain the inconsistent results obtained on the varieties near El Centro during 1972. More work is needed on this.

Work by the USDA in the East and by us in California has shown that certain species of Medicago (close relatives of cultivated alfalfa) are resistant to the weevil. In species we have observed such as Medicago arborea and M. suffruticosa the weevil only skins the surface of the leaf like it would the stem on cultivated alfalfa. We wonder if there might be a hard layer of cells which the weevil does not like or some other barrier. Since hybridization of these particular species with cultivated alfalfa is not possible, we are unable to use this resistance. However, we should try to see if these resistant species contain chemicals or structures causing this resistance. If so, we must then try to find this same thing in cultivated alfalfa. In addition, we should check for weevil resistance in many collections of species that will cross with cultivated alfalfa.

Priorities for Future Work

Through this report I have discussed areas of research, where work is being conducted, and others which look promising. A good question to ask now is, "Where do we go from here?" (1) Briefly, we plan to continue the recurrent selection program using seedling and leaf disk selection methods with adult weevil. (2) Obtain reliable test results on experimental synthetics. (3) Develop materials with high tannin and anthocyanin and test this material for weevil resistance. (4) Make detailed studies on factors such as temperature and humidity which seem to have an effect on resistance. (5) Test species closely related to Medicago and, if resistant, cross them to the cultivated alfalfa. (6) Develop non-hibernating populations of Egyptian alfalfa weevil and test hormone treatments to break dormancy. (7) Cross weevil resistant lines from California with Team. During the seasons when we are not actively testing we are collecting weevil and will also do preliminary work on other foraging insects such as the alfalfa caterpillar. Perhaps the major end result of this weevil resistance work will be the development of research methods and, ultimately, varieties resistant to the weevil as well as other foraging or chewing insects.

Selected References

Barnes, D. K., and R. H. Ratcliffe. 1967. Leaf Disk Method of Testing Alfalfa Plants for Resistance to Feeding by Adult Alfalfa Weevils. Journal of Economic Entomology 60: 1561-1565.

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.

Busbice, T. H., D. K. Barnes, C. H. Hanson, R. R. Hill, Jr., W. V. Campbell, C. C. Blickenstaff, and R. C. Newton. 1967. Field Evaluation of Alfalfa Introductions for

Resistance to the Alfalfa Weevil Hypera postica (Gyllenhal). U. S. Dept. Agr. Agr. Res. Serv. ARS 34-94, 13 pp.

Byrne, H. D., C. C. Blickenstaff, J. L. Huggans, A. L. Steinhauer, and R. S. VanDenburgh. 1967. Laboratory Studies of Factors Determining Host Plant Selection by the Alfalfa Weevil Hypera postica (Gyllenhal). Maryland Agr. Expt. Sta. Bull. A-147, 27 pp.

Koehler, P. G. 1971. An Association Between the Alfalfa Weevil's Larval Growth Response and Adult Feeding Response to Its Host Plant. Ann. Entomol. Soc. 64: 1230-1233.

Lehman, W. F., and E. H. Stanford. 1971. Egyptian Alfalfa Weevil, Breeding Resistant Alfalfa. California Agriculture 25(5): 7-8.

Norwood, B. L., R. S. VanDenburgh, C. H. Hanson, and C. C. Blickenstaff. 1967. Factors Affecting Resistance of Field-Planted Alfalfa Clones to the Alfalfa Weevil Crop Science 7: 96-99.

Norwood, B. L., D. K. Barnes, R. S. VanDenburgh, C. H. Hanson, and C. C. Blickenstaff 1967. Influence of Stem Diameter on Oviposition Preference of the Alfalfa Weevil and Its Importance in Breeding for Resistance. Crop Science 7: 428-430.

Table 1. 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
Outside 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

Table 2. Egyptian alfalfa weevil damage ratings and larvae counts made on 4 varieties grown on the University of California Imperial Valley Field Station on 3-6-70 and 3-9-70.

Variety	Average damage rating per plot 1/2/	Number of larvae per plot 1/
Moapa	6.6*	11.0 NS
Sonora	6.7	11.5
El Unico	6.5	12.5
UC 63	5.5	14.0

1/ 40 stems were taken from each plot.
2/ Damage of 1 = none, 5 = moderate, 9 = severe.
* Significant at the 5% level.