BREEDING ALFALFA FOR RESISTANCE TO THE SILVERLEAF WHITEFLY

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ABSTRACT

The silverleaf whitefly (*Bemesia argentifolii*) has been causing serious damage in California's Low Desert alfalfa (*Medicago sativa* L.)production since 1991. Imperial county Agricultural Commissioner's Reports suggest that through both direct and indirect impacts of the whitefly, average forage yields have decreased by 17 percent. Plant breeding procedures have recently been developed that are proving successful in developing genetic resistance to this insect. We expect to have adapted cultivars with Silverleaf whitefly resistance to growers by 2000.

Key words: alfalfa, lucerne, pest resistance, silverleaf whitefly, plant breeding, forage yield

INTRODUCTION

Silverleaf whitefly (*Bemisia argentifolii* Bellows and Perring) damage to crops nation-wide was estimated at $200 million and $500 million in 1991 and 1992, respectively. Formerly "strain B" of *Bemisia tabaci* (Gennadius), the silverleaf whitefly (SLWF) is present in both the Low Desert and the Central Valley and is a threat to California agriculture and horticulture statewide. The SLWF is a devastating agricultural pest in California's Low Desert alfalfa production region. From the fall of 1991 to April 1994, crop damage incurred from the SLWF totaled $336 million in California's Imperial Valley alone. In Imperial County, alfalfa ranks second in gross agricultural earnings and occupies approximately one third of all agricultural acreage. During this time alfalfa producers have suffered losses estimated to exceed $26 million per year.

Silverleaf whiteflies are more damaging and, unfortunately, more difficult to control than other whitefly species. Factors contributing to the severity of damage are: its higher reproductive rate than other whitefly species, a much wider host range, production of copious amounts of sticky honeydew exudate, and phytotoxic disorders in a number of plant species. Additionally (and importantly from the perspective of plant breeding), populations of this relatively new agricultural pest have demonstrated an astounding capacity to develop resistance to insecticides.

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Experiments that clearly delineate and quantify SLWF damage to either yield or reduction in forage quality of alfalfa are not available. This is in part due to the inability to create an uninfested control. However, grower records, Imperial County Agricultural Commissioner's annual reports, and UC forage yield trial records all strongly suggest that the SLWF may either directly or indirectly reduce alfalfa forage yield by ten to twenty-five percent. Imperial county agricultural commissioner's reports since 1990 show a 17 percent reduction in annual alfalfa hay yield. Lack of either resistant cultivars or chemical controls has prompted many growers to withhold all or part of the normal irrigation water for alfalfa during the late summer months. Commonly referred to as "dry-down", this practice often also results in serious stand loss. Studies we conducted cooperatively with Dr. Frank Robinson (UC Desert Research and Extension Center, retired) to determine the feasibility and influence on stand and yield of summer dry-down management are yet to result in management practices that eliminate the impact of the SLWF and avoid stand loss. Even if a dry-down management did exist, it would only avoid the problem of the SLWF by sacrificing hay production. In addition to yield reduction, there is concern that the SLWF may also reduce alfalfa forage quality. The insect's copious production of honeydew provides a suitable substrate for the growth of a sootymold fungus, *Capnodium spp*. Marketability of hay blackened by the growth of this sootymold is drastically reduced (Figure 1).

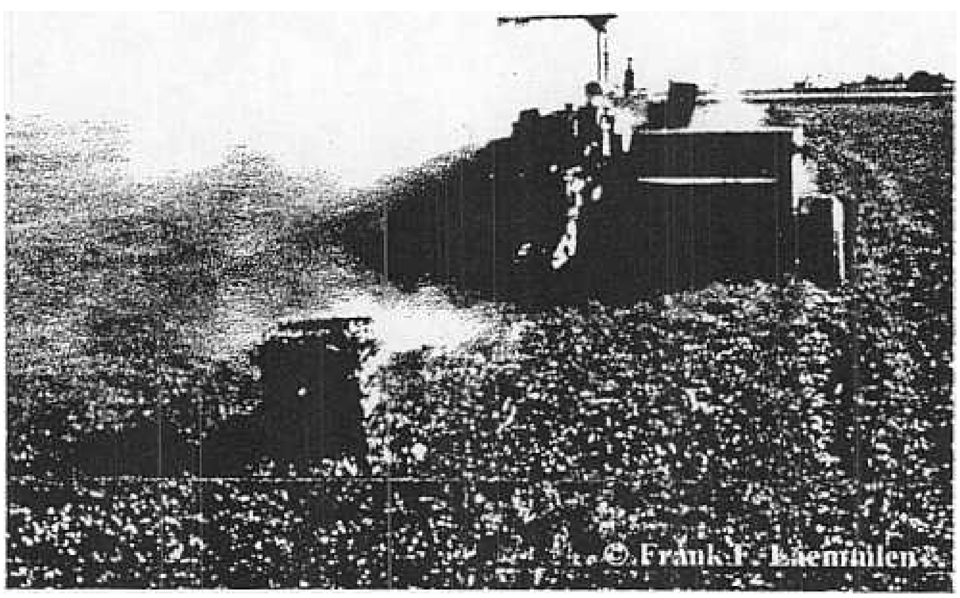

Figure 1. Honeydew secreted by the silverleaf whitefly interferes with harvesting and the sooty mold that grows on the honeydew significantly reduces marketability of the hay.

Also, sticky alfalfa foliage increases the energy demand for swathing which increases harvest costs.

Although alfalfa does not appear to be a primary host of the SLWF, alfalfa occupies a very high percentage of agricultural land year round in the Low Desert. This alone provides the potential to serve as a habitat and reservoir for large SLWF populations. This reservoir likely serves as a source for infestation of other crops. The importance of alfalfa (and the Low Desert) to the agricultural economy of California, its role in harboring whitefly populations, and the current lack of effective and economical pesticides or alternative cultural controls for this insect in alfalfa necessitates the development of alfalfa germplasm/cultivars with resistance to the SLWF. Historically, breeding for insect and disease resistance has been particularly successful in alfalfa. More than 250 cultivars are currently registered in the United States which possess stable long term economic field resistance to several agricultural pests and diseases. Our program to develop SLWF resistance has 4 primary objectives: 1) To devise a visual method of quantifying the level of whitefly infestation that could be used to assess differences among genetic materials. 2) To develop baseline information on the development of SLWF populations in alfalfa planted at different densities. 3) To quickly assess the potential for developing genetic resistance and the time this would take. 4) To rapidly incorporate resistance into commercially viable cultivars.

PROCEDURES

In October of 1992, 73 alfalfa plants exhibiting apparent resistance to the SLWF were identified in the field at the University of California Desert Agriculture Research and Extension Center (DREC) in El Centro, California. These plants were taken from a study containing more than 10,000 plants that had been planted in replicated half-sib families (a group of plants that have the same female parent) composing the germplasm UC-356. Consistent identification of potentially resistant plants in half-sib families with reduced levels of infestation provided us with encouragement that alfalfa cultivars could be developed with resistance to the SLWF.

Plant regrowth stage and spacing

Prior to our work, plant breeders had not studied the SLWF as a pest of alfalfa. This made it necessary to conduct research that would determine conditions to be used in further experimentation. Our principal concern was to identify the stage of regrowth (time after cutting) that would provide the greatest information about differences in whitefly damage. Furthermore, alfalfa breeding programs are commonly based on the evaluation and/or selection of individual plants. This made it necessary to determine the relationship between whitefly infestations in densely planted stands (similar to a hay production field) and stands with the plants spaced a foot or more from one another. Replicated dense- and space-planted plots were established at the University of California Desert Research and Extension Center. Whitefly infestation parameters (Figure 2; Table 1) were measured on a weekly basis between June and September. Individual plots were scored for a period of seven weeks. While this is well beyond the normal period for hay production, it provided us with important information regarding both SLWF population development and the time when differences in plant response to the SLWF could be most accurately assessed.

Infestation parameters

Measurements of SLWF immature numbers (Figure 2a), honeydew stickiness level (Figure 2b), and sooty mold quantities on foliage are taken randomly from each plot. Each parameter is scored according to a five-category scale (Table 1).

Density of immature SLWF is determined by randomly removing stems from a plant and looking at the undersides of mature leaves (Table 1; Figure 2a). A score of "1" signifies no discernible immature whiteflies. A score of "2" is given if several immatures are found on occasional leaves. A "3" is assigned if several immatures occurred on nearly every leaf or they are dense on occasional leaves. A score of "4" is given if more than several immatures occurred on each mature leaf or are dense on many leaves. A "5" is used if most mature leaves have at least 30 to 50% of the underside covered with immature SLWF's.

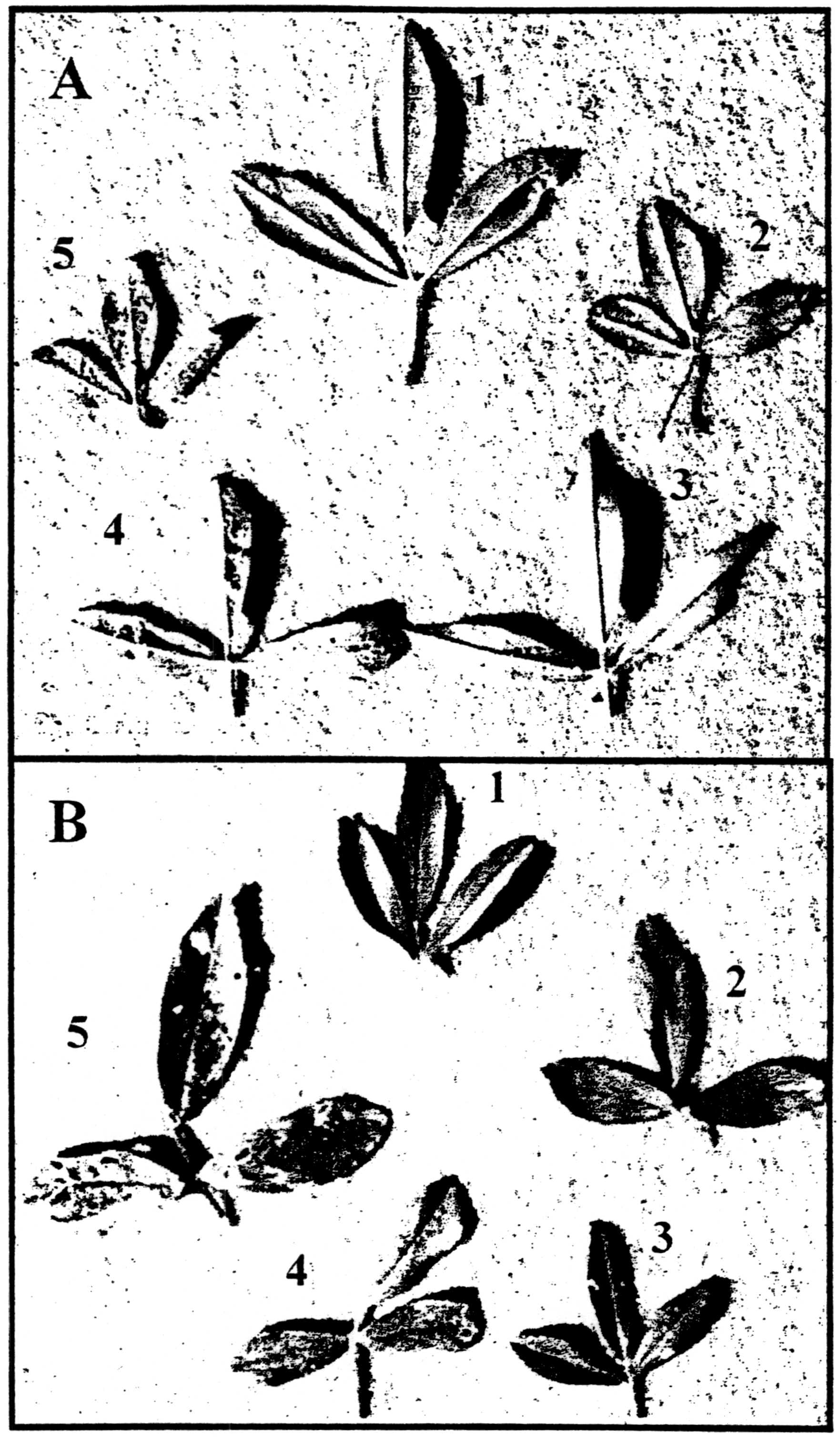

Figure 2. Scoring Classes for number of immature whiteflies (A) and the amount of stickiness (B).

Stickiness level is subjectively ascertained by touching plants (Table 1; Figure 2b). A score of

"1" signifies no discernible stickiness. Plants with barely discernible stickiness on any surface are scored a "2". Light but readily discernible stickiness on lower plant or very low levels on much of plant is scored "3". Copious stickiness on lower plant or moderate stickiness on entire plant receive "4", and saturated or gooey build-up on most of the plant is scored "5".

Table 1. Description of scoring classes for evaluating silverleaf whitefly infestation parameters on alfalfa .

	Trait		
Scoring Class [1]	Immature numbers	Stickiness level	Index
	no./ cm^2 ---	--- score ---	--- score
1	0	None	None
2	< 1	Barely discernible	Some
3	< 50	Readily discernible	Many
4	< 100	Copious	Numerous
5	> 100	Saturated	Continuous

[1] Scores are based on the average infestation on a randomly chosen stem from each plant scored.
Sootymold growth is measured visually by looking at shaded foliage where mold might grow. A score of "1" signifies no discernible mold. If only several barely discernible mold colonies are present; a plant is scored "2". If more than several leaves of a plant exhibit several colonies, the plant is scored "3". If many leaves on plant showed numerous black mold colonies it receives a "4". If the mold colonies are so dense as to appear continuous on the lower or shaded third or more of a plant, it receives a "5".

RESULTS

Whitefly infestation development

Both SLWF immature numbers and stickiness level increase at a very rapid rate for the first 3 to 4 weeks after cutting (Figure 3). Thereafter, both continue to increase at a slower rate. Less than three weeks after cutting, both SLWF immature numbers and stickiness level on the plants exceeded what we believe to be the economic threshold (Score between 2 and 3). Sootymold appearance and growth lags behind the increase in number of immature SLWF's and stickiness of the foliage. Spaced plantings exhibit much less sootymold than dense plantings. This is probably due to lower humidity in the canopy when plant density is less. Number if immature whiteflies and stickiness in dense- and spaced-planted plots were in close agreement. This agreement permits us to evaluate individual plants for immature density and stickiness level with confidence that it is representative of what would occur in a hay production field. Evaluations are made when regrowth is three weeks of age. Sootymold growth is not considered a useful measure for plant breeding purposes. This is because sootymold growth is apparently dependent on higher humidity than would normally be present in spaced plantings.

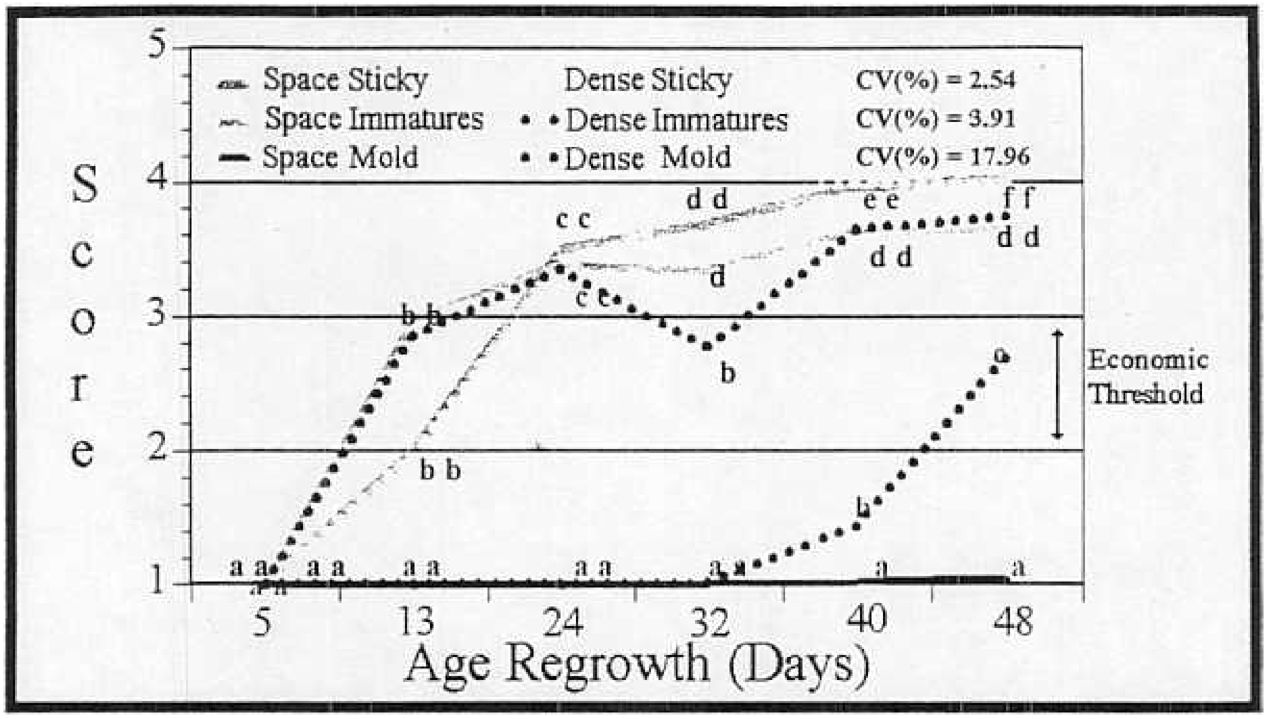

Figure 3. Comparison of silverleaf whitefly infestation parameters measured in dense and spaced alfalfa during July and August at the Desert Research and Extension Center. Regrowth ages on pairs of lines (e.g. stickiness) associated with the same letter are not significantly different (P < 0.05, Fisher's Protected LSD)

Studying the development of SLWF populations in alfalfa also provided valuable information regarding cultural management. Early cutting has been proposed as a means of controlling SLWF damage. As proposed, this practice would break the lifecycle of the SLWF and would also remove the foliage (hay) prior to the time that it becomes too sticky. Normal monthly harvests do break the life cycle of SLWF on alfalfa. However, honeydew stickiness levels reach an economic threshold as early as two weeks after cutting (Figure 3). Therefore, timely or even early harvest is unlikely to substantially reduce economic damage to alfalfa hay production. Early cutting would also produce low yields and would over time seriously reduce the stand.

Selection progress:

Developing host plant resistance to the SLWF was our highest priority, but we had virtually no information to guide our decisions about breeding methodology. We needed a reasonable understanding of the potential for developing resistance. Fortunately, the initial 73 selected plants had already set seed when they were identified. That seed was harvested from each individual plant creating half-sib families. Those families were then used to establish a study that would provide us with estimates of the genetic variability present among the selected plants. We then used those estimates to develop predictions of the rate that we could increase resistance to the SLWF. Estimates of the rate of selection progress were made assuming selection based on immature numbers, stickiness level, and an index score. Index score is the average of the stickiness and immature scores.

Heritability (percentage of the parent characteristics for a trait that is passed to its offspring) estimates for immature numbers, stickiness level, and the index were all moderate (Table 2). Both heritability (56.8 %) and the rate at which resistance can be developed are slightly higher when selection is based on the index. This also fits well with our desire to reduce both the size of the

Table 2. Heritability estimates for Silverleaf whitefly evaluation parameters measured either once or twice during the evaluation period at the University of California Desert Research and Extension Center.

Evaluation frequency	Trait: Immature numbers	Stickiness level	Index
	%	%	%
Once	35.4	37.0	39.6
Twice	50.7	54.0	56.8

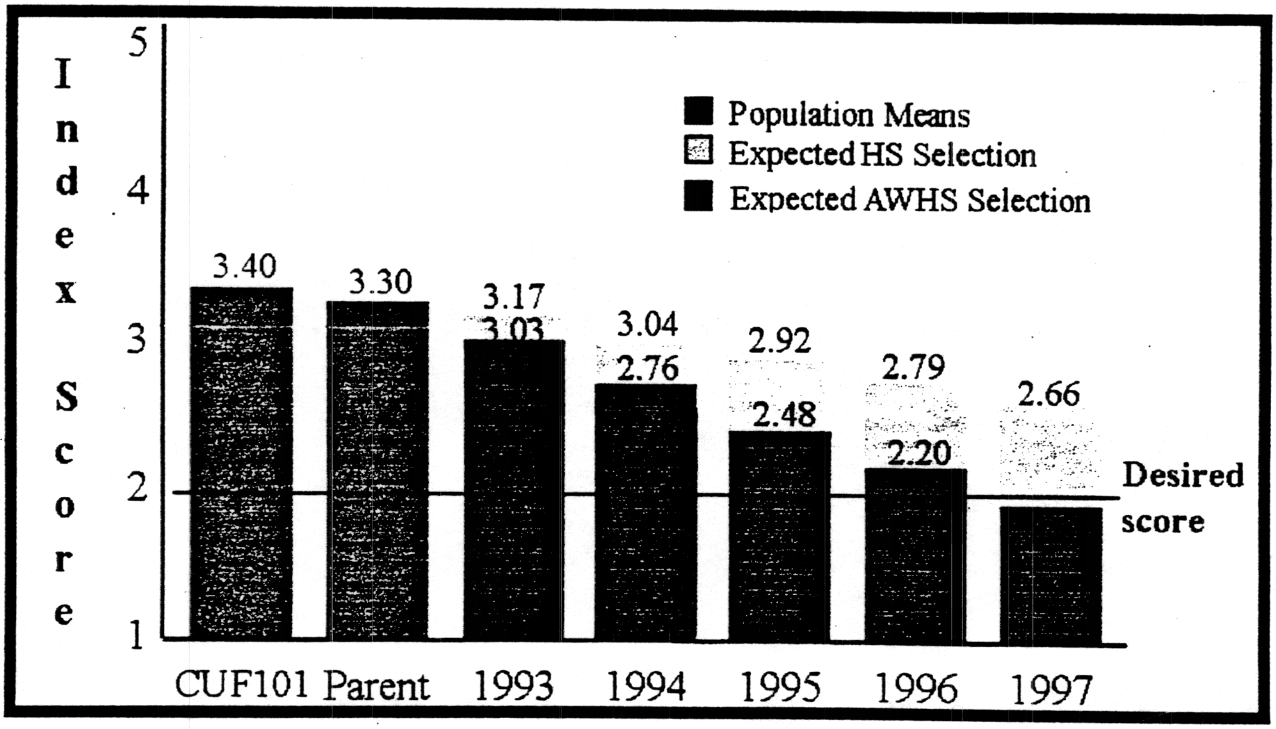

Figure4. Initial prediction of selection progress for silverleaf whitefly resistance using half-sib family selection (HS) and among-and-within half-sib family selection (AWAS), based on open-pollinated seed derived from UC-356.

whitefly population that develops in alfalfa (reservoir for infestation of other crops) and the stickiness of the foliage. Environment (*eg.* location in the field, time of day) have a large influence on individual scores. This convinced us that we should devise a breeding program based on family selection rather than on selection among individual plants. Using the above heritability values and the corresponding estimates of genetic variability, predictions of selection progress were made for both half-sib family and among and within half-sib family selection (selection for the best families and then the best individuals from the best families). Selection progress was predicted to be most rapid with among and within half-sib family selection. Considering observations we made in the regrowth/spacing study, we set a goal of developing populations with a mean index of two or less. Predictions of selection gain were then extrapolated for several years to determine how soon we might expect to identify economic levels of resistance. Approximately five cycles of selection will be required to reach our goal of a population with a mean SLWF damage index of less than two (Figure 4).

For the past 4 years we have established selection nurseries in March containing ten- to fifteen-thousand individual plants in replicated half-sib families. Selection is based on the average of two observations on each plant, during August and September, for immature numbers and stickiness level. Two- to three-hundred individuals are selected from the best 20 percent of the families based on the SLWF resistance index and agronomic type. These plants are dug in late September and transported under special permit to Chile (Figure 5). Seed is produced under field conditions in "winter" nurseries in Chile that are harvested in March. Seed production on these plants during the summer in Chile permits us to produce as much as 500 times more seed than we could produce in a greenhouse during the winter in California. Consequently we also obtain more rapid evaluation for forage yield and resistance to other economically important insects and diseases. Overall, this permits us to concentrate our efforts on germplasm pools that have the greatest yield potential and to quickly improve, if necessary, other insect and disease resistance levels.

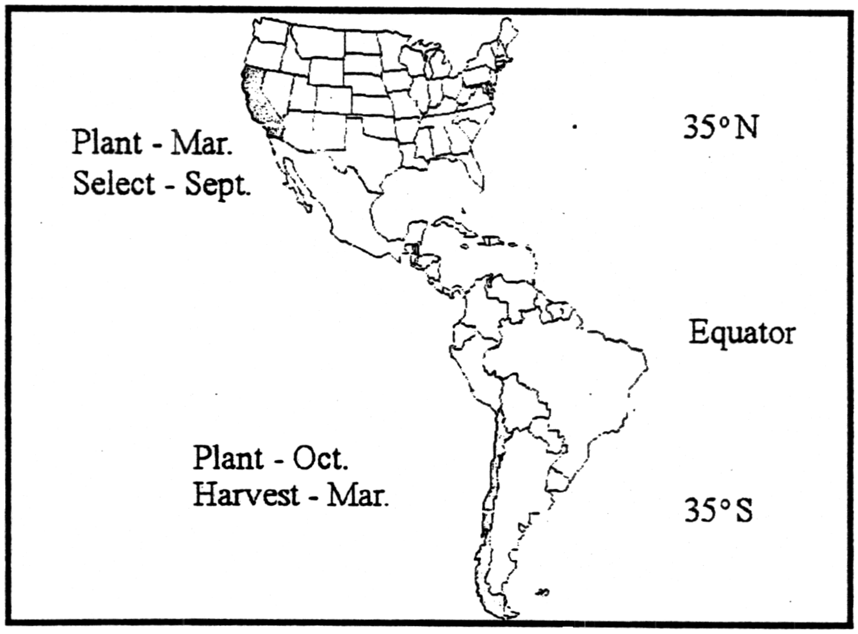

Figure 5 Selection for resistance occurs in September at the Desert Research and Extension Center in California, and seed is harvester from those selections in March in a "winter" nursery south of Santiago, Chile.

We have now completed five cycles of selection and have completed evaluation of the first three and have partial data on the fourth cycle. Seed is currently being produced in Chile on the cycle-5 selections. Significant improvement has been made in resistance to the SLWF (Table 3). The rate at which we are making improvement is in

Table 3. Silverleaf whitefly resistance and forage yield of check materials and best populations selected for resistance to the silverleaf whitefly.

Germplasm		Trait				
		Whitefly damage		Forage Yield		
					-- Rank[4] --	
Identification	Characteristic	- Index[1] -		- Tons/A[3] -	1996	1997
UC-2239	Cycle-3	2.3	2	8.57	8	5
UC-2230	Cycle-3	2.3	1	8.48	1	7
UC-2241	Cycle-3	2.4	5	8.13	2	15
UC-356	Parental source	2.6	12	--	--	--
UC-Cibola	Check	--	--	8.38	10	8
CUF 101	Check	2.7	17	7.83	3	18
Test mean		2.7		8.32		
$LSD_{0.05}$		0.2		1.25		
CV (%)		12.0		11.6		

[1] Index = [(stickiness level score) + (immature numbers score)]/2
[2] Rank among 21 entries in the trial
[3] Average yield per cutting on a 100% dry weight basis
[4] Rank among 18 entries in the trial

almost exact agreement with the predictions we developed in 1993. One of the most encouraging facts is that three of the populations exhibiting the greatest resistance to the SLWF were also high yielding, based on two years of yield data. Furthermore, although we are not able to detect statistically significant differences in forage yield, lines selected for resistance to the silverleaf whitefly are producing 15 to 30 percent more forage than CUF 101 between August and October when the whitefly is most prevalent (Figure 6). This is exciting because whitefly populations were very high during this period in 1997. We will continue to select for SLWF resistance and improved forage yield. We expect to observe significant increases in yield with later generations of selection when we have increased resistance (reduced score) to levels below the assumed economic threshold level of 2.0.

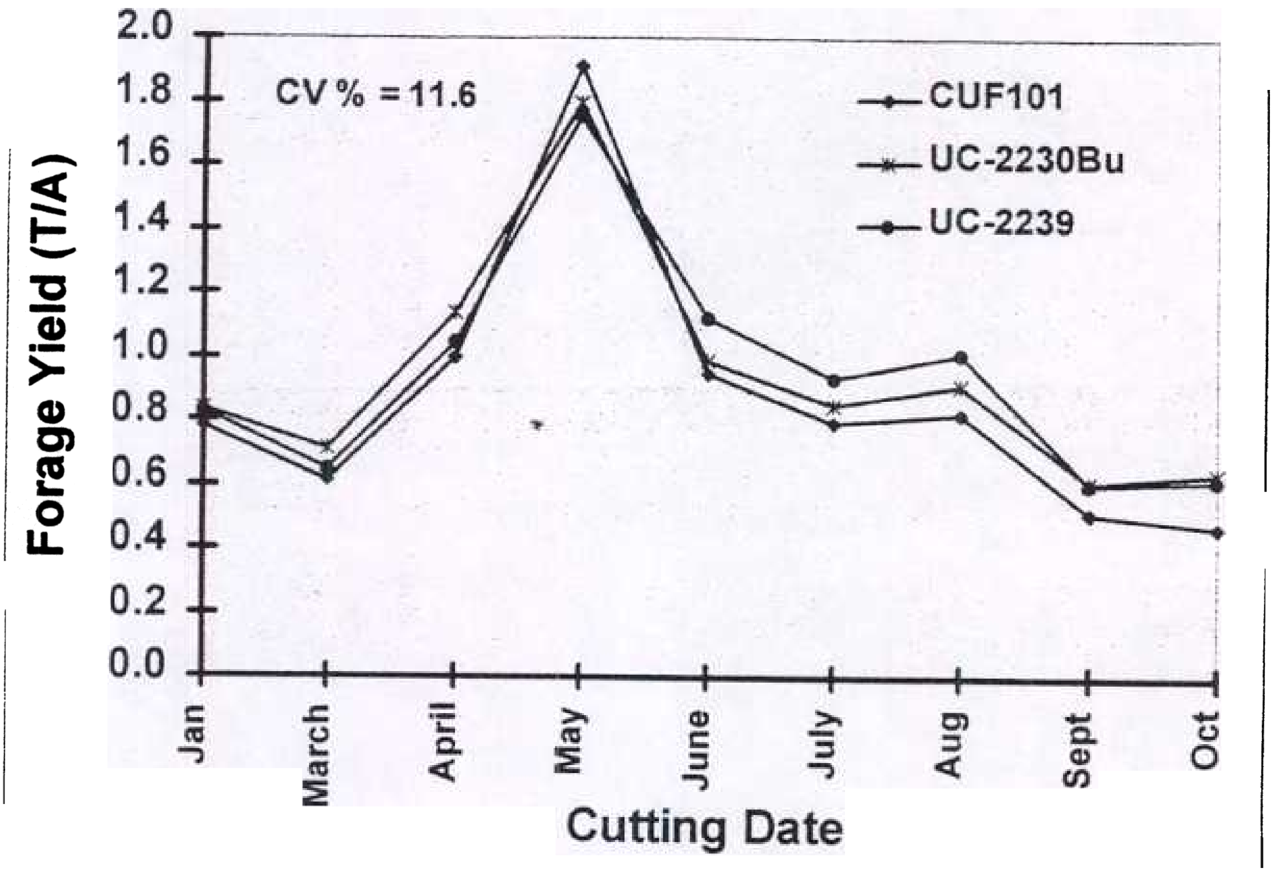

Figure 6. **Seasonal forage yield at the Desert Research and Extension Center of populations selected three cycles for resistance to the silverleaf whitefly compared to CUF 101**

CONCLUSION

Cultural management of the SLWF in alfalfa either by chemical control or by cutting management is not feasible. We have developed plant breeding methodology to successfully select for genetic resistance to the SLWF. Screening is conducted under field conditions in the Imperial Valley during August and September. Seed is produced on the selected plants between September and March in a "winter" nursery in Chile. This permits two generations per year and complete pest and yield evaluation at more than one location starting in the spring following the year of selection. Taking into account both our early predictions and our current progress, we expect that populations with economic resistance to the SLWF will be selected this fall. We are working to provide California growers with resistant cultivars as rapidly as possible.

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