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Improving Procedures Used to Select for *Lygus* Bug Resistance in Alfalfa

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Introduction

Since the 1960's, attempts have been made to reduce alfalfa flower damage from *Lygus* bug feeding by developing resistant alfalfa varieties. Much of this work was conducted at New Mexico State University by Dr. B. A. Melton and his graduate students (Vering 1968, Knipe 1969, Arledge 1972, Auld 1973). In order to identify plants that could withstand damage from *Lygus*. bug feeding these workers developed techniques for testing flower buds in the field and seedlings in a lath house. Unfortunately, after one or two cycles of selection, evaluations of germplasm developed by these methods showed that progress had been limited. No germplasm releases or new cultivars resulted from this breeding program, but seed stocks remain from several germplasm selections. Dr. M. Nielson with the USDA at Tucson used a modified lath house technique to screen a number of alfalfa varieties, which he rated for their potential resistance to *Lygus* (Nielson 1974).

In the mid-1980's Dr. Bill Lehman at the UC Desert Research and Extension Center in Holtville proposed modifying the techniques developed in New Mexico and comparing the results derived the from the two. Dr. Lehman passed away in 1989 after he and L. Gibbs had completed two years of selections using only the flower bud technique. Dr. L. R. Teuber and L. Gibbs continued with this program, using both flower bud and seedling techniques.

Reports from all previous investigators had expressed concern about the degree of confidence that should be placed in their results because of the extreme variability among trials and the difficulty they had in showing statistical differences among germplasm tested. We were also concerned that the excessive variability might obscure differences that among alfalfa germplasm we tested. A successful selection program is dependant upon a reliable screening technique. Therefore, we initiated a series of studies in 1989 that were designed to reduce the variability to levels that would allow us to be confident that we could detect real differences.

We felt that exerting greater control over environmental conditions and the bugs used for testing would result in substantial reductions in variability. Plant damage results from bug feeding activity, which is influenced by the hunger, health, age, sex, and aggregation behavior of the *Lygus* bugs. The temperature and lighting conditions during the test also influence both bug feeding activity and the growth and physiological condition of the plants. Cold weather slows bugs down and reduces feeding activity, resulting in lower damage than would occur on a warm day. During the 1991 experiments it became evident that bug mortality was a serious problem which probably contributed greatly to the variability in results. In 1992 an insect biologist (Dr. Gordon) was hired to supervise the project in order to improve control over the bug populations within and among experiments. To refine the techniques, we focused on 1) improving the survival and age structure of bugs, 2) determining the appropriate bug densities and feeding periods which would allow families to be adequately separated based on damage, and 3) controlling environmental conditions in the seedling technique by performing it in a greenhouse.

Materials and Methods

Lygus hesperus adults, all of the same age, were purchased from a commercial insectary. Bugs were raised under conditions of ideal temperature and humidity and were allowed to feed continuously on an artificial diet until the time they were placed in plastic snap-top vials for inoculation into cages. Actively feeding and egg-laying adult females (23-25 days old) were used for experiments. Experiments were carried out during the summer of 1992.

Flower Bud Technique: Two experiments were performed in which the bugs were inoculated into cages in the morning (7:00 AM) or evening (7:00 PM) to determine if bug survival was improved by allowing them to adapt to field conditions overnight. Both experiments tested the same combinations of numbers of bugs (two bugs and four bugs), and length of the challenge period (twelve hours and four hours) and were repeated three times at one week intervals. Bug dose and exposure periods were tested in cages containing one mature, pre-bloom flower bud. Each experiment was conducted on a different set of half-sib families. The same plants were used during each repetition unless there were not enough flower buds available, in which case another plant in the same family was used.

Seedling Technique: These studies were conducted in a greenhouse on the UC. Davis campus. Temperature and lighting were controlled by whitewashing the greenhouse, heating or cooling as necessary (maximum 38^{o}C, minimum 27^{o}C), and providing supplemental lighting to maintain a 16:8 hour light: dark cycle of uniform intensity. Three alfalfa cultivars, Moapa 69, CUF101, and Rincon were grown in alternating rows radiating within circular cages. When seedlings reached the unifoliolate leaf stage they were thinned to 8 plants per row and bugs were inoculated. Two factors were tested to determine the appropriate conditions to yield an adequate separation of damage between the three cultivars: 1) Bug dose (0, 0.25, 0.5, 0.75, 1.0 bugs per seedling), and 2) length of exposure period to bugs (36, 48, 60, 84 hours).

Results and Discussion

Flower Bud Technique: We achieved a tremendous improvement in bug survivorship during the 1992 field experiments (Table 1 and Fig. 1), and increased both the efficiency and reliability of the technique. Bug survival averaged 88 % when inoculation occurred at 7:00 AM and 87% when inoculation occurred at 7:00 PM (Table 2). With this increase in survival (3.5 times that in 1991), damage scores that required four days of bug exposure in 1991 were achieved in twelve to twenty-four hours in 1992 (Tables 1 and 2).

The differences in damage that resulted from the morning and evening inoculation experiments suggests that the bugs feed mainly during the daytime (Fig. 1). When inoculated in the morning, the damage after twelve hours would have been caused during the daytime. Since, within bug dose categories, the damage after twenty four hours was not significantly different than the damage after twelve hours, it appears that very little feeding occurred during the night. The same effect can be seen in the evening inoculation experiment when the first twelve hours of bug exposure occurred at night, and the following twelve hours were daylight. During the night, there was no difference in damage between cages with two or four bugs. Increasing the exposure period from twelve to twenty four hours (adding daylight hours) did result in increased damage. Comparing between the two-bug doses, an additional twelve hours of daylight exposure resulted in significantly more damage. Comparing the four-bug doses also reveals significantly more damage resulting from the daylight exposure.

Seedling Technique: There was no significant difference in damage based on the position of seedling rows within cages. The uninfested control (dose-l) showed no damage across all exposure periods, but damage increased significantly with increases in bug density (Fig. 2). Statistically significant (but small) differences in damage among cultivars were detected at several exposure periods and doses. Too high a dose (reflected by damage levels

greater than category g) reduced the ability to detect differences among cultivars. Inadequate separation of cultivar damage also resulted from too low a dose (less than category e). Differences in germplasm sources were maximized by using a dose of $^1/_2$ bug per plant and scoring after 48 hours (Fig. 3). Within this dose and exposure combination, significant differences were detected between Moapa 69 and Rincon. Moapa 69 and CUF101 are nondormant cultivars with no prior selection history for resistance to *Lygus.* Rincon is a semidormant cultivar with part of its parentage derived from the New Mexico *Lygus* selection program.

Conclusions

With these improvements, both the flower bud and seedling techniques now provide reliable results that we can base our selection program on. While we can get very similar results when we repeatedly test the same germplasm sources with the seedling technique, the flower bud technique produces more variable results. This is partly because of the environmental variability inherent in field tests. While we would prefer that the flower bud technique provided greater agreement in ranking families by damage when the same families are tested repeatedly, it is encouraging that significant differences can be detected between some families (Table 3). We believe we can live with this variability by conducting multiple evaluations and reducing selection intensity.

The differences in damage that can be detected among cultivars are small. The encouraging thing is that our tests repeatedly produce the same results. What remains to be determined is if these differences are heritable. The fact that Rincon was partially developed from germplasm selected by the seedling technique is very encouraging. Moapa 69 and Rincon can now be used as susceptible and resistant checks for screening seedlings.

We are now in a position to begin a selection program and feel there is a greater chance of success than any time in the past. In 1993 we plan to complete screening of half-sib families, make selections from these families, and establish a breeding program that will utilize both flower bud and seedling screening techniques. We expect that it will take several complete cycles of selection to determine if significant progress can be made. We will monitor progress in each selection cycle so that we will be able to identify trends at each step along the way.

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Table 1. Survival rates of bugs and damage induced during experiments using the flower bud technique 1991, before it was improved. One caged flower bud was exposed to combinations of three *Lygus* bug doses for a period of either four or eight days. Dead bugs were replaced daily with live bugs to maintain the bug dose throughout the exposure period. Bugs used in the experiment were collected from the alfalfa field.

	Total Number Bugs Replaced [1]		Percent of Bugs Surviving [2]		Flower Bud Damage [3]	
Bug Dose	4 Days	8 Days	4 Days	8 Days	4 Days	8 Days
2	5.17	11.58	35	28	1.67	4.58
4	11.50	25.67	28	20	1.92	4.75
6	16.83	38.33	30	20	2.50	4.92
				$LSD_{.05}$	0.09	0.09
				CV %	27.10	27.10

1. Average number of bugs placed in cages by the end of exposure period to maintain bug dose level.
2. Proportion of bugs surviving each day during the exposure period.
3. Average Severity Index: 1 = no damage, 5 = completely blasted.

Table 2. Survivorship of bugs during experiments with improved technique in 1992. One caged flower bud was exposed to two bug doses for twelve or twenty four hours.

Time of Inoculation [1]	Bug Dose	Percent of Bugs Surviving 12 Hrs	Percent of Bugs Surviving 24 Hrs
Morning			
	2	92	89
	4	88	83
Evening			
	2	89	86
	4	93	81

1 Bugs inoculated into cages at 7:00 AM or 7:00 PM.

Table 3. Differences in flower bud damage among alfalfa half-sib families resulting from *Lygus* bug feeding.

Family ID	ASI	Grouping [1]
35612723	2.54	a
35634605	2.38	a b
35612724	2.25	a b c
35612742	2.12	a b c
35612740	2.00	a b c
35612738	2.00	a b c
35612715	2.00	a b c
35612725	1.25	b c
35634601	1.00	c

1. Means with the same letter are not significantly different. (protected LSD, $P \leq .05$).

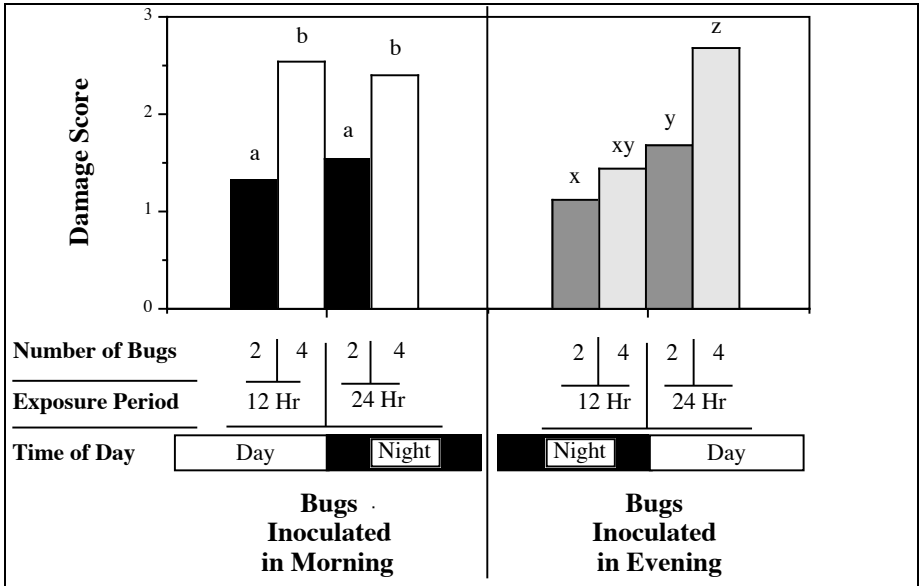

Figure 1. Damage to alfalfa flower buds resulting from different numbers of *Lygus* bugs and exposure periods. One flower bud was exposed to two or four adult female bugs for a period of twelve or twenty four hours. Bugs were inoculated at 7:00 AM or 7:00 PM in two different tests. Means indicated by the same letter are not significantly different (Protected LSD, $P \leq 0.05$).

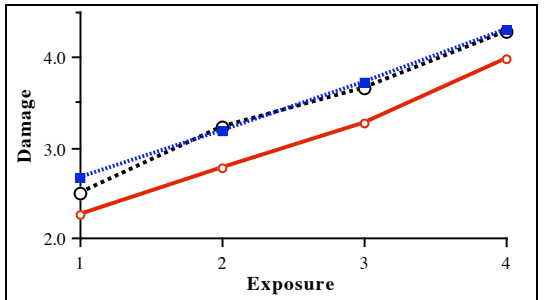

Figure 3. Differences in *Lygus* bug damage to three alfalfa cultivars at dose-3 (0.5 bugs per plant). Differences are maximized at exposure-2 (48 hours). Exposure 1= 36, 2= 48, 3= 60, 4 = 84 hours.

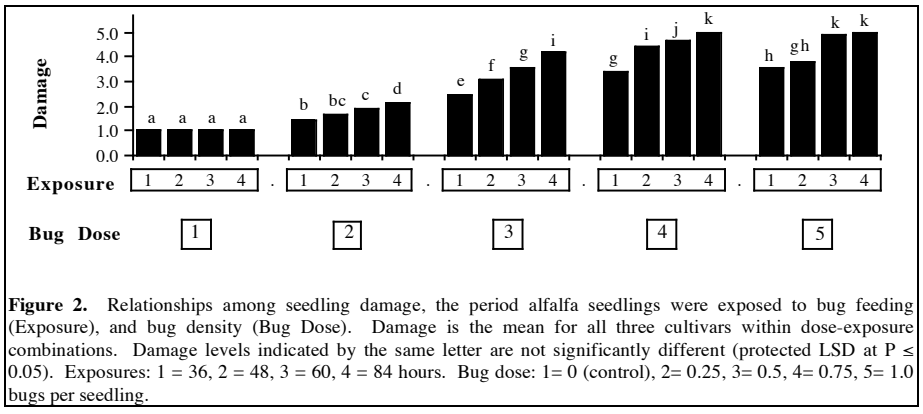

Figure 2. Relationships among seedling damage, the period alfalfa seedlings were exposed to bug feeding (Exposure), and bug density (Bug Dose). Damage is the mean for all three cultivars within dose-exposure combinations. Damage levels indicated by the same letter are not significantly different (protected LSD at $P \leq 0.05$). Exposures: 1 = 36, 2 = 48, 3 = 60, 4 = 84 hours. Bug dose: 1= 0 (control), 2= 0.25, 3= 0.5, 4= 0.75, 5= 1.0 bugs per seedling.