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SUMMARY

Mechanisms and sources of spotted alfalfa aphid resistance in Ranger alfalfa and its five parental strains were investigated during 1957-1960.

Aphid-resistant plants were found in all parental strains, with the greatest number in strains A-111 and A-117 of Turkistan origin.

A-111 had the best overall performance for resistance in relation to high seedling survival, low aphid reproduction, and high frequency of resistant plants.

A-119 of Ladak origin survived as well but appeared somewhat more favorable for aphid reproduction and less favorable as a source of resistant plants than A-111 and A-117.

A-116 of Turkistan origin and A-110 of Cossack origin were the poorest sources of resistance and provided the smallest number of resistant plants.

A-110 was significantly more susceptible to killing than all other parental strains and Ranger.

Parent-progeny and selection studies were conducted on 96 randomly selected clones from Ranger.

A wide range in resistance levels was found. In two clones all introduced nymphs failed to survive. High aphid populations developed on most susceptible clones. Resistant classifications were assigned to 17 percent of the clones.

Wide-polycross progenies of the six clones most favorable for aphid reproduction and the six least favorable survived at an average rate of 5 and 37 percent, respectively, an indication that antibiosis resistance was inherited.

Nebraska Synthetic 20, produced by intercrossing the six most resistant clones in a cage containing honey bees, provided an experimental variety slightly less resistant than Lahontan and Moapa. High antibiosis in a clone was not always reflected by superior survival in its progeny, an indication of the importance of progeny testing in selecting for spotted alfalfa aphid resistance. These studies indicated the feasibility of developing an aphid-resistant variety from Ranger alfalfa.

Studies of the Mechanisms and Sources of Spotted Alfalfa Aphid Resistance in Ranger Alfalfa [1]

W. L. Howe, W. R. Kehr, M. E. McKnight, and G. R. Manglitz[2]

INTRODUCTION AND LITERATURE REVIEW

The spotted alfalfa aphid (*Therioaphis maculata*) was first observed in the United States in southeastern New Mexico in February 1954. Since then it has spread throughout much of southern and central United States. This aphid first appeared in Nebraska in August 1955, which indicates how quickly it can move over considerable distances. Since then it has appeared in Nebraska annually. Damage is largely dependent upon climatic conditions, which influence dispersion and population increase. Extended clear, warm, dry periods and favorable winds during the late summer and fall of 1956 appeared conducive to rapid dispersal and population increase. The loss to hay crops and fall plantings in Nebraska was estimated at more than $3 million. During the same year a loss of nearly $42 million was estimated for the entire United States (*2*).

Damaging infestations in the North Central States, such as Nebraska, where the parthenogenetic adults are not known to overwinter, have been attributed to summer dispersion of alate forms from states to the south. These have usually appeared first in southern or southwestern Nebraska between July 1 and August 1.

Recent observations indicated the presence of a spotted alfalfa aphid strain which produced sexual forms and eggs during the late fall. The aphids overwintered in the egg stage in two counties in east-central Nebraska during the winter of 1960-61 and may have also overwintered in this manner during the winter of 1959-60 (*16*).

During the fall of 1961, egg-laying forms of the aphid were found in 20 counties in central Nebraska and in 2 adjacent counties in south-

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[1] Contributions from the Entomology and Crops Research Divisions, Agric. Res. Serv., U.S.D.A., in cooperation with the Nebraska Agricultural Experiment Station. Contribution from the Departments of Entomology and Agronomy, University of Nebraska, Lincoln, Nebraska.

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central South Dakota. The overwintering of the species in this area, far removed from its normal overwintering habitat, provides a continuous source of infestation for outbreaks throughout the growing season when climatic conditions are suitable. Thus, the aphid may also become a serious threat to alfalfa in the northern portions of the United States. Ranger alfalfa is well adapted to and grown abundantly in many of these areas (Figure 1). Therefore, research on host-plant resistance and resistance sources in adapted types appropriately includes Ranger.

Breeding of alfalfa varieties resistant to the spotted alfalfa aphid plays a major role in research directed toward reducing damage by this destructive insect. The potential advantages of aphid resistance first became apparent in 1955 when the aphid invaded experimental alfalfa nurseries in southwestern United States (*10, 20*). Lahontan, a newly developed variety selected from Nemastan, remained green and productive, whereas the more susceptible varieties were almost completely killed. Subsequent study of resistance in Lahontan revealed that three of the five parental clones were unfavorable for aphid development and reproduction (*11*). The effectiveness of resistance in the field was again demonstrated in varietal plots (Figure 2) at Bakersfield, California (*12*).

Sources of spotted alfalfa aphid resistance were found in various alfalfa varieties susceptible to intermediate in reaction. Selection within intermediate African resulted in rapid development of Moapa adapted to lower desert regions of the Southwest (*12, 19*). Cody, a synthetic variety possessing effective resistance, was similarly developed by selection from susceptible Buffalo (*9*). Thus, aphid-resistant clones were present in susceptible varieties in a sufficiently high frequency to permit effective selection. Breeders found that even though resistant plants may be infrequent among adapted susceptible varieties, depending on the parental material involved, their utilization as parental material has been the most rapid method for developing aphid-resistant varieties.

During this study it was observed that the frequency of resistant plants was higher in alfalfa varieties of at least partial Turkistan origin than in those of non-Turkistan origin. Varieties of Turkistan origin were usually intermediate in aphid resistance (*12*) when Ranger, Orestan, Hardigan and others demonstrated either a reduction in aphid population or were resistant to killing or both. Nemastan, from which Lahontan was selected for resistance to the stem nematode, is also of Turkistan origin and has a degree of resistance only slightly lower than Lahontan (*8*). Thus, considerable resistance to the spotted alfalfa aphid would be expected in Lahontan. Antibiosis (*17*), such as found in the parental clones of Lahontan (*11*) and in other clones tracing to Turkistan, provided an effective tool for selection as well as aphid-resistant parental breeding stock. It was evident that resistance

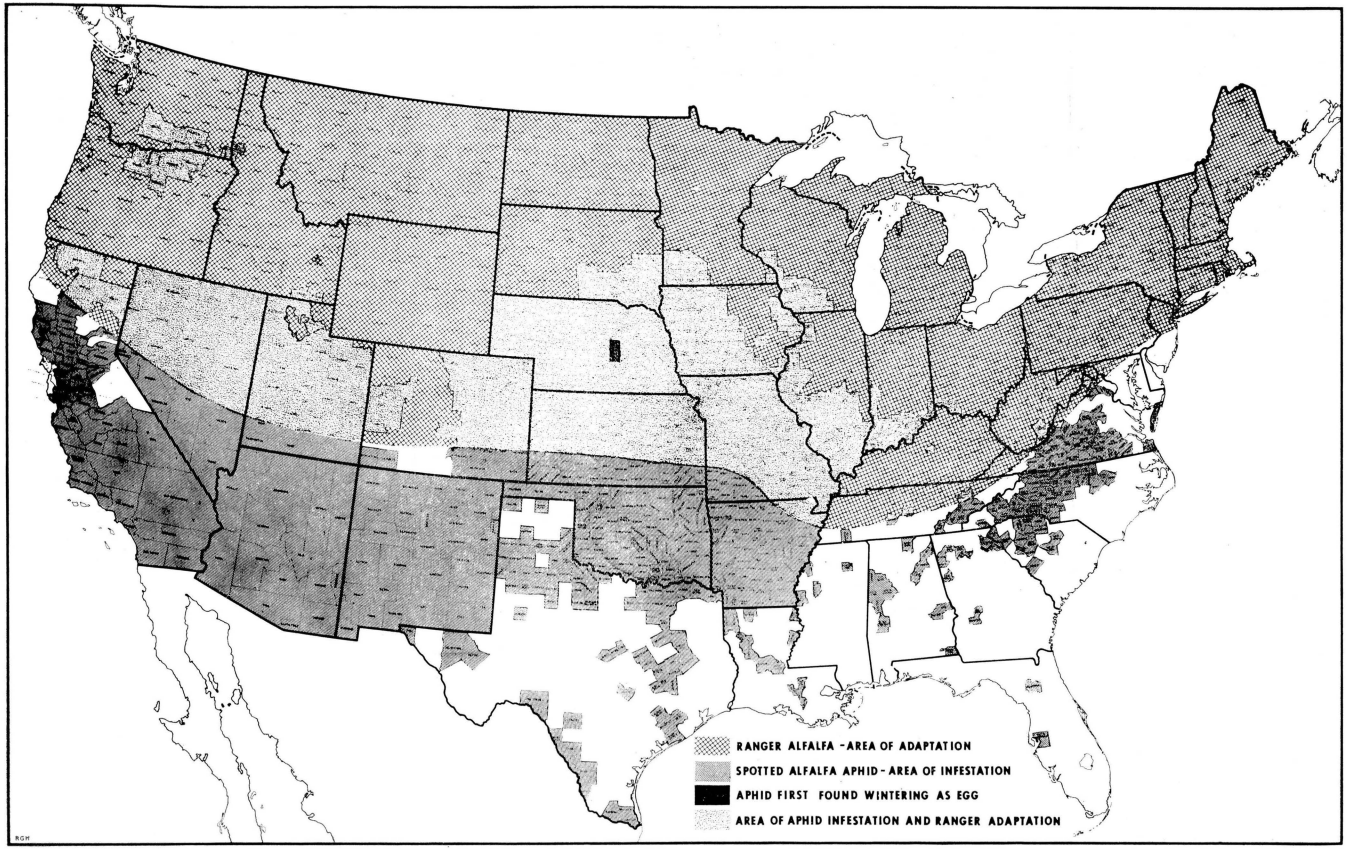

Figure 1. Distribution of the spotted alfalfa aphid in relation to the area of adaptation of Ranger alfalfa.

Figure 2. Differences in plant growth and injury in aphid-resistant and susceptible alfalfa varieties after infested in the field with spotted alfalfa aphid populations.

of the antibiosis type would be necessary to reduce the copious honeydew deposits associated with dense aphid populations.

Ranger alfalfa demonstrated some resistance to the spotted alfalfa aphid in both the seedling and mature plant stages of growth. It was developed through cooperative research by the U. S. Department of Agriculture and the Nebraska Agricultural Experiment Station (*21*). Information on its origin and performance was condensed and interpreted (*14*). It is a synthetic variety originated by compositing five basic or parental strains derived from three different varieties, namely Cossack, Turkistan, and Ladak. Parental strain A-110 originated from the variety Cossack. A-111 and A-117 originated from selections made in old Nebraska fields, presumably of Turkistan origin. The third strain of Turkistan origin, A-116, was selected from Foreign Plant Introduction No. 84401 introduced from Samarkand, Turkistan. A-119 was selected from the variety Ladak. Seed of A-110, A-111, A-116, A-117,

and A-119 was mixed so that their percentages in the Syn-0 composite, Ranger, were 45, 8, 13, 24, 10, respectively.

Ranger is grown over a larger area of the United States than any other variety. More than 9 million acres were grown for forage production and more than 29 million pounds of certified seed were produced in 1959 (*3*). Its area of adaptation in relation to the distribution of the spotted alfalfa aphid is shown in Figure 1.

Seedling survival of Ranger under conditions of heavy infestation, though not exceptional, exceeded that of numerous susceptible varieties. In Kansas tests (*8*) 15- and 16-percent survival was observed; whereas most commonly grown susceptible varieties such as Buffalo, Ladak, Atlantic, Vernal, DuPuits, Narragansett, Grimm, Chilean, and others seldom showed more than 7-percent survival. Mature Ranger field plantings were considerably more resistant to killing by heavy populations than other varieties. Population buildup was much lower on Ranger than on most other varieties in California tests (*12*). A maximum population of about 150 aphids per stem developing on Ranger resulted in less than 5-percent plant mortality; whereas numerous susceptible varieties were 50- to 95-percent killed by populations of 300 or more aphids per stem. In these tests the rate of population buildup on Ranger was about half that of most varieties, with the exceptions of Orestan, Vernal, African, and Culver (A-600) and resistant varieties Lahontan and Bam. In Nebraska, further evidence of spotted alfalfa aphid resistance in Ranger was observed during field studies of aphid injury to randomly chosen clones from the variety and their S_1 and polycross progenies (*15*).

The degree of aphid resistance in Ranger, though effective in reducing plant mortality in mature stands, is far too low to protect the crop from severe forage losses resulting from high aphid populations. Effective resistance must utilize antibiosis to reduce aphid population buildup.

STUDIES OF RESISTANCE IN RANGER AND ITS PARENTAL STRAINS

These studies explored previous evidence of aphid resistance in Ranger alfalfa by making a detailed evaluation of sources and mechanisms of resistance in the Ranger variety and its parental strains. The information from these studies opens the way for logical planning for breeding an aphid-resistant variety of Ranger origin.

In the appraisal of resistance in Ranger and its parental strains, two general types of tests were utilized. The first appraised mass-plant response to large induced infestations; the second, termed aphid response, was concerned only with survival, growth, and reproduction of individual aphids when placed on caged plants. Interpretations of

results were based on the fundamental mechanisms of resistance—tolerance, preference, and antibiosis (*17*). Plant survival while under attack by high aphid populations was considered a function of all three basic mechanisms of resistance; whereas response of individual aphids on plants was interpreted on the basis of antibiosis—the adverse effects of the plant on aphid biology. The effectiveness of antibiosis in reducing aphid populations and subsequent injury makes it a most desirable characteristic in resistant selections.

Spotted alfalfa aphid cultures for all tests were reared in the greenhouse on susceptible or tolerant alfalfa varieties grown in flats. This asexual viviparous culture was started anew each fall from collections made in heavily infested fields in southwest Nebraska. Greenhouse tests were conducted with seedlings. Temperatures averaged 75°F ± 5° with a relative humidity ranging from 30 to 50 percent.

Materials and Methods

Plant-Response Tests

Ranger and its parental strains were subjected to heavy infestations primarily to determine differential levels of tolerance in the various parental strains.

In most mass-plant response tests seed was planted in rows 3/8 inch deep, covered with fine sand, and packed. Seed of the Ranger parental strains was obtained from the basic breeders stock held in cold storage at the University of Nebraska.

Test 1: A row of aphid-susceptible Buffalo alfalfa was seeded on each side of each randomly placed Ranger entry to assure adequate population buildup. Plants were infested in three stages of growth—cotyledonary, 1 to 2 trifoliolates, and 3 to 4 trifoliolates. Tests were conducted in a chronological sequence under uncontrolled conditions. Mortalities of seedling age groups were inconsistent and considered invalid. Hence, only mean mortalities of all groups are presented. These tests were terminated when the rows of Buffalo appeared to be over 80-percent killed. Stand counts before and after infestation were compared to determine plant mortality.

Test 2: An evaluation of the Ranger strains was repeated in 1959 to determine their relative susceptibility when infested naturally by alate females migrating from centrally placed aphid cultures. Tests were conducted in greenhouse benches rather than flats, as in the previous test. Each entry was replicated 10 times in rows 2 feet long and 2 inches apart on October 22, 1959. Most plants had emerged by October 27. Lahontan was included as a resistant check. Stand counts were made shortly after plants emerged. Records of injury were made on two dates—December 9, when plants were 43 days old, and on December 15, when plants were 49 days old.

Aphid - Response Tests

The first published reports in which aphid survival and reproductive rates were utilized as measures of antibiosis in alfalfa dealt with pea aphid resistance (*4*), and (*13*). This selection technique is especially valuable in identifying effective resistance in plants surviving heavy populations. A breeding program directed toward the selection of highly resistant plants of Ranger origin could use information on the presence and frequency of resistant plants in the parental strains.

Ranger, its five parental strains, Buffalo, and Lahontan were used. Individual seedlings were grown in cells in rectangular blocks cast from plaster-of-paris (Figure 3) similar to those described by Davis (*5*). The need for watering seedlings from above was eliminated by placing the blocks in water-filled galvanized pans. This technique satisfied the moisture requirements of the plant without disturbing the aphids. Each block 21 inches long contained a single row of 8 cells, 1 inch in diameter and 3 inches deep. Each seedling was caged in a plastic tube with the top (open end) covered with fine-mesh synthetic marquisette. Cages were firmly secured to the plaster-of-paris blocks by inserting tubing 1 inch in diameter through a hole bored in a plastic lid fitting the larger plastic-tube cage. Figure 4 shows the entire assembly partially removed from the plaster block. Aphid counts were easily made when the tube cage was removed from the plastic lid.

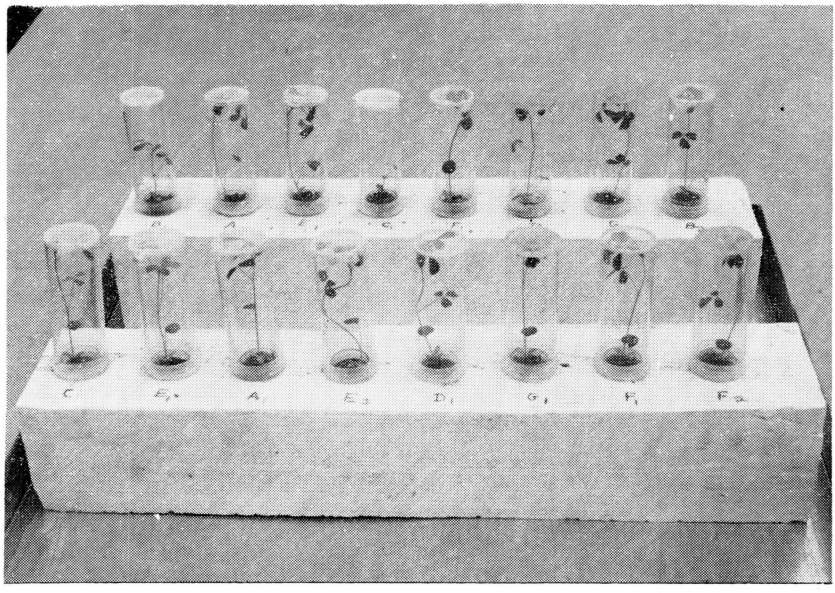

Figure 3. Cages used for determining differences in aphid development, reproduction, and mortality on random seedling populations from Ranger and its parental strains.

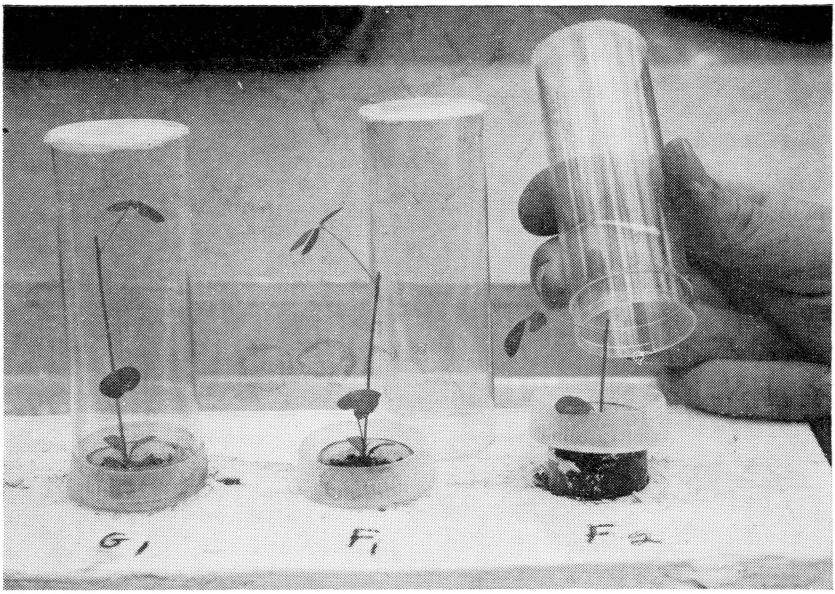

Figure 4. Seedling cage partially disassembled.

One month after planting, each seedling was infested with a single second- or third-instar nymph. The young were counted and removed from the plants, and missing or dead aphids were replaced every 2 days thereafter. The test was terminated after 13 days.

Each plant was assigned a resistance classification based on the number of young produced on it by spotted alfalfa aphid females. Plants which supported no aphid reproduction were classed highly resistant. Those on which 1 to 4 nymphs were born were intermediate; 5 to 10 nymphs, susceptible.

Results and Discussion

Plant-Response Tests

Test 1: The first expression of aphid injury on infested plants was vein chlorosis in the unifoliolates and trifoliolates among a portion of the seedlings of all entries. This injury, commonly observed in spotted alfalfa aphid-infested seedlings, is attributed to toxigenic effects of salivary secretions associated with aphid feeding (*18*). There appeared to be no correlation of this injury with greater or lesser susceptibility of seedling strains. Trends of injury, as the test progressed, were similar within each age group of the parental strains. All were more resistant than Buffalo. Seedlings infested in the cotyledonary stage were often injured to such a degree that no unifoliolate leaves were produced. Developing unifoliolates and trifoliolates were frequently stunted.

Table 1. Comparative seedling mortality rates of Ranger parental strains and two varieties in greenhouse flats infested with spotted alfalfa aphids.

Strain or variety	Average percent mortality[1]	Average mortality in percent of	
		Buffalo	Ranger
A-111	41[a]	46	66
A-119	46[a]	62	76
A-117	57[b]	65	94
Ranger	60[b]	68	100
A-116	64[b]	72	103
A-110	74[c]	83	122
Buffalo	88[d]	100	148

[1] Means having one or more letters in common are not different at the 5 percent level by Duncan's multiple-range test. Differences were significant at the 5 percent level according to Fisher's F test.

The average seedling mortalities (Table 1) indicated differential levels of average resistance in Ranger and its parental strains. Strains A-111 and A-119 showed significantly higher resistance than the other entries. Ranger occupied a median position, as might be expected. The greater survival of Ranger and its parental strains over that of Buffalo was strongly evident.

A series of the lesser injured seedlings was selected from this test to determine the proportion of plants with high antibiosis. These were potted and allowed to mature. Individual terminals were caged for observations of aphid reaction. Unfortunately, insufficient numbers of uninjured plants were available from each strain to make reliable comparisons of antibiosis frequencies among strains. Data accumulated on selections made from the five parental strains indicated that 50 percent possessed sufficient antibiosis to prevent maturation and reproduction of second- and third-instar nymphs. Thirty-eight percent of the Ranger selections were resistant. These data indicated that antibiosis was an important mechanism in seedling survival. Plants which allowed nymphal maturation and subsequent reproduction probably survived because of their tolerance or vigor.

Table 2. Response of Ranger parental strains and three varieties after exposure to injurious populations of the spotted alfalfa aphid. October-December, 1959.

Strain or variety	After 43 days' exposure			After 49 days' exposure		
	Percent killed	Percent injured	Percent healthy	Percent killed	Percent injured	Percent healthy
Lahontan	9.3	14.4	76.4	13.5	19.8	66.7
A-111	15.5	32.2	52.4	39.5	29.2	31.3
A-119	18.0	36.4	45.6	37.4	36.1	26.7
A-117	16.3	39.1	44.7	42.4	38.1	19.5
Ranger	18.4	42.2	39.5	48.2	35.8	16.0
A-116	28.6	39.4	32.0	58.5	29.7	11.9
A-110	21.5	50.4	28.1	58.4	34.0	7.6
Buffalo	30.4	50.8	18.8	79.4	18.4	2.2
LSD 5 percent	15.5			12.8		
1 percent	21.1			16.7		

Test 2: Initially, the infestation of alates from the open cultures to the bench-planted seedlings was slow and the typical venation injury did not appear until most plants were in the 2- to 3-trifoliolate stage. Aphid populations increased rapidly thereafter, and severe injury appeared on December 9 when first observations were recorded. Plant mortality increased considerably from December 9 to 15 (Table 2). As in Test 1, varied degrees of plant injury and mortality were observed among the parental strains. The identical ranking of strains in both tests was considered to contribute to the validity of their relative position in resistance to the aphid and indicated that no further testing of this type would be necessary.

Aphid-Response Tests

Observations indicated that mean level of antibiosis and the frequencies of resistant plants varied in the parental strains. Table 3 records differences in mortality of introduced nymphs, fecundity, and number of reproductive days. Number of reproductive days was considered that period between the production of the first nymph by a mature female and its death or the end of the test. A-117 and A-111 were not significantly less desirable hosts than Ranger, but were less favorable than A-119, A-110, and A-116. The frequency distribution of the plants classified in this test is presented in Table 4. More resistant plants were identified in Ranger, A-111, and A-117 than on A-110, A-116, and A-119. The percentages of Ranger plants resistant to the aphid were approximately the same as that from A-111. The reason for this is unknown.

A comparative ranking of all observations made on plant injury and subsequent evaluation of antibiosis in the parental strains is summarized in Table 5. The parental strains contributed to aphid resistance in varying degrees. A-111, A-117, and A-119 were injured to a lesser degree than other strains. A-111 and A-117 demonstrated a generally higher level of antibiosis than other strains. A-111 and A-117

Table 3. Mean spotted alfalfa aphid maturity and reproductive rates on Ranger parental strains and three varieties.

Strain or variety	Percent nymphs which matured	Number of reproductive days	Number of young per reproductive day[1]
Lahontan	35.4	3.2	2.0[a]
A-117	66.8	5.9	2.6[b]
A-111	69.4	5.3	2.9[b]
Ranger	63.6	6.0	2.9[b]
A-119	74.5	7.2	3.3[c]
A-110	76.8	6.7	3.4[c]
A-116	77.6	7.4	3.4[c]
Buffalo	79.1	7.9	3.7[d]

[1] Means having one or more letters in common are not different at the 5 percent level by Duncan's multiple-range test. Differences were significant at 5 percent level according to Fisher's F test.

Table 4. Distribution of a random population of plants of Ranger parental strains and three varieties into resistant, intermediate, and susceptible classes.

Strain or variety	Plant classification			
	Percent highly resistant	Percent resistant	Percent intermediate	Percent susceptible
Lahontan	21	39	18	21
Ranger	11	21	7	61
A-111	14	11	21	54
A-117	7	11	21	61
A-119	4	4	7	85
A-110	4	4	14	78
A-116	4	0	14	82
Buffalo	0	4	7	89

Table 5. Summary of ranking of Ranger alfalfa and its parental strains for factors used to measure their resistance to the spotted alfalfa aphid (1 = most resistant).

Strain or variety	Plant mortality test		Mean aphid reproduction	Number resistant plants	Overall rank
	1	2			
A-111	1	1	2	1	1
A-117	3	2	1	3	2
Ranger	4	4	2	2	3
A-119	2	3	4	4	4
A-110	6	5	5	4	5
A-116	5	6	6	6	6

strains appeared to be the best potential source of resistance. The third Turkistan strain, A-116, was susceptible to killing and favorable for aphid reproduction. Only 1 plant from a random population of 28 plants of A-116 appeared resistant; whereas A-111 and A-117 had 7 and 5 resistant plants, respectively. Strain A-119 had a moderately low rate of killing under heavy infestation, supported a rather high fecundity rate, and had a low frequency of plants classed as resistant. Therefore, A-119 was considered the most tolerant strain. Based on high plant killing when heavily infested, support of a rather high reproductive rate, and a low frequency of plants with antibiosis, A-110 was considered to be the most susceptible of the strains. A-116, also susceptible, was injured to a lesser degree than A-110.

STUDIES OF RESISTANCE IN CLONES OF RANGER ORIGIN AND THEIR PROGENIES

This work was conducted to obtain a preliminary evaluation of parent-progeny relationship for resistance in Ranger clones and to evaluate this source of resistance for the development of aphid-resistant varieties. Work was divided into three phases:

1. **Selection and evaluation of clones:** Determine variability of spotted alfalfa aphid resistance in a random population of Ranger clones.

2. **Progeny evaluation:** Evaluate aphid reaction of progenies from selected resistant and susceptible clones.

3. **Performance of a synthetic variety of Ranger origin:** Evaluate resistance of a synthetic variety derived from the most resistant clones.

This investigation was closely integrated with a study of genetic variability in Ranger alfalfa conducted cooperatively by the U. S. Department of Agriculture, Crops Research Division, and the Nebraska Agricultural Experiment Station. It was initiated in 1953 to determine the nature and extent of genetic variability in forage yield, to estimate forage yield heritability, and to discuss the findings relative to systems of alfalfa breeding.

One hundred twenty randomly selected plants produced from certified Ranger seed were initially used. Cuttings were made in the greenhouse and, subsequently, transplanted to a wide-polycross seed-production nursery in April, 1953. Failure to obtain adequate quantities of polycross seed from some clones and difficulty with propagation of others reduced the number of clones for further study to 96. Agronomic data obtained on the 96 clones and their polycross progenies from 1953-57 indicated they varied greatly in every characteristic (*15*). The clones also differed considerably in field injury to a heavy natural infestation of the spotted alfalfa aphid in the field plots in mid-October 1956. This random population of clones provided a useful and appropriate source of germ-plasm for continued study of Ranger variability for spotted alfalfa aphid resistance.

Materials and Methods

Selection and Evaluation of Clones

This phase was conducted to evaluate the range of resistance in a random population of clones, and to select resistant and susceptible plants for subsequent inheritance and progeny studies. As in previous appraisals, two types of tests were used—a cage antibiosis test measured aphid reaction on each plant and an excised-stem test was used to appraise clonal reaction to large aphid populations. Lamp chimney cages used for antibiosis tests are shown in Figure 5.

Aphid development, reproduction, and mortality were determined by previously described methods of caging stems with only slight variations. After introduction of three nymphs, observations of aphid reproduction and survival were made twice weekly. Subsequent introductions of aphids were made if no survival occurred. The test was ended in 2½ weeks after counts were made of the aphid population on each caged stem.

Appraisal of clonal reaction to high aphid populations was obtained by a simplified technique in which a single excised stem was used to represent each clone. Individual healthy stems from the nursery

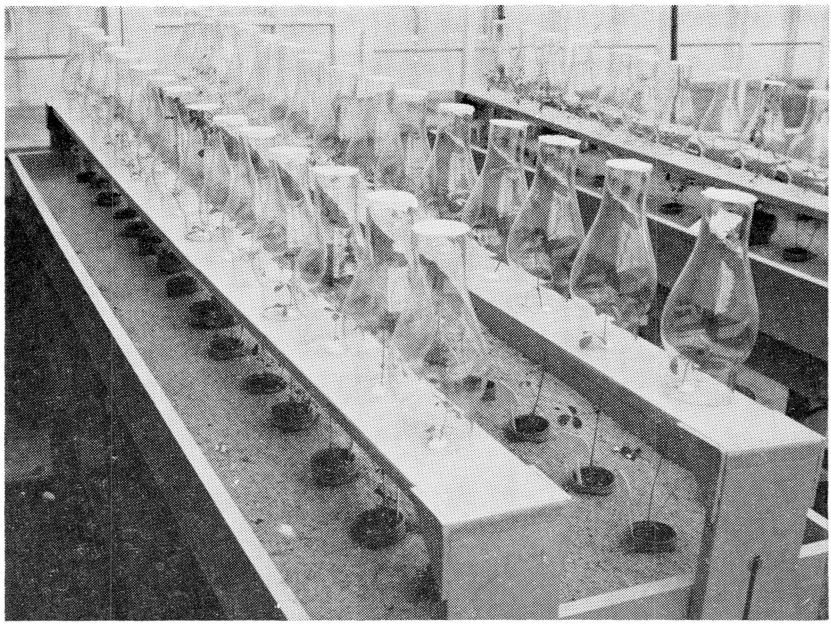

Figure 5. Adjusted racks with lamp chimney cages for evaluating antibiosis in potted plants.

were placed in individual vials which contained distilled water. The test consisted of a block assembly containing 196 closely bored holes. A vial was placed in each hole and covered tightly with a sheet of heavy aluminum foil. A centered puncture above each vial permitted entry of the excised stem. The aluminum foil provided a good surface which allowed aphid access to the stems and retarded evaporation of water from the vials. After stems were in place, they were heavily infested with aphids in all stages of development. Injury ratings based on degree of yellowing were assigned to each stem after one week. Plants with no yellowing were rated as highly resistant; those with slight yellowing, resistant; partial yellowing, intermediate; and complete yellowing, susceptible.

Progeny Evaluation

Parent-progeny relationships of spotted alfalfa aphid resistance and susceptibility evaluated the efficiency of the previously described clonal selection techniques. Resistance of a transitory or unstable type was observed for pea aphid in alfalfa (*1, 6*). Spotted alfalfa aphid resistance is known to vary with temperature (*8, 11*). The progeny test provided a valuable tool for eliminating potential sources of classification error.

The wide-polycross progenies of the six most resistant and six most susceptible clones from the previous clonal test were selected for this appraisal of inheritance. Seed was obtained from a wide-polycross nursery which contained all 120 of the Ranger clones; therefore, it is assumed that this seed resulted largely from intercrossing between resistant and susceptible clones. Plantings of polycross seed from the 12 clones were made in metal greenhouse flats on July 17, 1958. Each entry was interplanted with Buffalo. Other entries included Ranger, A-111, Lahontan, Moapa, African, and Kansas B-1 (now Cody). A randomized-block design of four replications was used for the 18 entries. Plants were infested in the 2- to 3-trifoliolate stage of growth. Observations of plant survival were made on August 22 and September 22.

Since most of the 120 clones selected at random from Ranger were susceptible to the aphid, it seemed desirable to compare the reaction of wide-polycross versus S_1 progeny of the six most resistant clones. Unfortunately, sufficient S_1 seed of clone 62 was not available; therefore, progenies of only five clones were compared. Seed was planted on January 9, 1959, and seedlings were infested in the first-trifoliolate stage. Seedlings were rated for injury on February 12, 1959. A scale of 1 to 10 was used to appraise injury—1 being no injury and 10 indicating complete killing.

Performance of a Synthetic Variety of Ranger Origin

Spotted alfalfa aphid resistance in six Ranger clones evaluated in the previous studies appeared great enough to observe their performance when intercrossed to produce a synthetic variety. This test seemed further justified since the yield of progenies of all six clones was superior to Ranger in tests conducted during 1955-57 (*15*). Seed from the individual clones, designated narrow-polycross progeny, and their composite, designated Nebraska Synthetic 20, Syn-1, was produced in a cage in California during 1959. Honeybees were used for pollination. This seed was planted in a greenhouse bench during late 1959 for evaluation of aphid resistance. Rows were 2 feet long, 3 inches apart, and replicated three times. Infestations of alate females from open-culture flats were made when plants were primarily in the cotyledonary stage of growth.

Results and Discussion

Selection and Evaluation of Clones

All degrees of antibiosis were represented in the series of 96 Ranger clones summaried in Table 6. Aphid numbers on individual caged terminals ranged from 0 to 153 at the end of the test. In two clones no aphid survived from one observation to the next, an indication that no aphid establishment occurred after a total of 15 nymphs were intro-

Table 6. Classification groupings of 96 randomly selected Ranger clones based on aphid reaction and plant injury.

Clonal classification final rating	No. clones	Antibiosis		Tolerance		
		Number of aphids per plant		Percent plant injury		
		Introduced	Final population	None to light	Medium	Heavy
Highly Resistant	7	9.0	2.6	100	0	0
Resistant	9	8.7	5.6	0	67	33
Tolerant	3	3.0	31.0	100	0	0
Intermediate	4	5.3	15.8	0	100	0
Susceptible	20	4.4	50.5	0	55	45
Highly Susceptible	53	3.2	93.3	0	0	100

duced. The original three introduced nymphs were responsible for the final population observed on clones demonstrating little or no antibiosis reaction. It was clearly evident that most of these clones (76 percent) of Ranger origin were favorable for aphid reproduction. Classifications of individual clones for antibiosis and tolerance are presented in Table 10.

Results of the excised-stem test, recorded in Table 6 as tolerance, portrayed distinct differences in injury. The possibility existed that lack of vigor in a portion of the cut stems could have hastened yellowing. This test probably served satisfactorily as a preliminary screening measure for large volumes of plant material under greenhouse conditions. Excised-stem tests should be followed by the more critical antibiosis and progeny tests. High antibiosis in clones was later reflected by reduced injury to aphid infested stems.

Classification of clones into resistance categories shown in Table 6 was based on results of both tests. As in previous tests, plants favorable for aphid reproduction that had comparatively low injury from heavy infestations were classified as tolerant. Susceptibility in the antibiosis test and low injury in the excised-stem test was interpreted to indicate tolerance.

Progeny Evaluation

Parental selection based on high antibiosis was successful for resistance, as reflected by superior seedling-progeny survival in mass-infestation tests (Table 7). After 16 days of infestation the mean percent survival from the six most resistant clones was 57, whereas that from the six most susceptible was 23. The survival rate of seedling progenies of high antibiosis clones approached the 63- and 62-percent levels observed in Lahontan and Moapa, respectively. The progeny resistance level was considerably below that of the resistant varieties after 46 days of infestation. Mortality of the wide-polycross progenies of resistant Ranger clones dropped to 37 percent, whereas that of the resistant varieties did not fall below 50 percent. This difference would be ex-

Table 7. A comparison of spotted alfalfa aphid population increase on six resistant and six susceptible Ranger clones and the survival of their seedling wide-polycross progenies in a greenhouse infestation, 1958.

Ranger clone number	Clonal tests		Progeny tests	
	Aphid population		Percent plant survival after:	
	No. introduced	No. final	16 days	46 days
Resistant				
12	3	5	57	23
47	11	0	51	25
57	15	4	67	59
62	5	0	50	26
99	15	0	69	53
109	12	4	47	36
Average	10	2.2	57	37
Susceptible				
53	3	131	10	2
64	3	126	27	9
68	3	104	37	9
84	3	137	33	6
101	3	135	19	4
113	3	101	12	1
Average	3	122	23	5
Check Varieties				
Resistant				
Lahontan	...	...	63	55
Cody	...	...	69	53
Moapa	...	...	62	50
Susceptible				
African	...	...	20	7
Ranger	...	...	28	10

pected since the wide-polycross seed resulted from intercrossing among clones that were predominantly susceptible. Survival rates of Ranger were distinctly lower than those of the polycross progeny of resistant clones—28 percent after 16 days and 10 percent after 46 days of infestation. Polycross progenies from Ranger clones 57 and 99 were about equal in resistance to the resistant varieties, Lahontan, Moapa, and Cody. The prolonged intensive infestation was very injurious to progenies of the resistant selections and to the susceptible varieties. The alternate rows of Buffalo were completely killed after the 46-day exposure period.

As shown in Table 8, the S_1 progeny of resistant clones appeared to exhibit a different frequency distribution for injury than the wide-polycross progeny of the same clone. Mean injury ratings for S_1 progeny were lower than for the wide-polycross progeny of the same clone. S_1 progeny ratings closely approached those found for the resistant checks Lahontan, Cody, and Moapa. The percentage of resistant individuals from S_1 progeny was higher for each clone in each case than in the comparable wide-polycross progeny. These tests further verified the inheritance of resistance identified in the initial selection tests. These

Table 8. Comparative spotted alfalfa aphid reaction of self-pollinated (S_1) and wide-polycross progenies of aphid-resistant Ranger clones in comparison with check varieties.

Entry	Number of plants in resistance category indicated						Mean[a] injury rating	Percent res.	Percent int.	Percent susc.	Percent killed
	No. resistant		No. intermediate	No. susceptible		Total					
	1–2	3–4	5–7	8–9	10						
Selfed progeny											
Ranger 12	6	4	2	4	0	16	4.3	63	13	25	0
Ranger 47	7	1	6	13	2	29	6.2	28	21	52	7
Ranger 57	12	6	8	8	1	35	4.7	51	23	26	3
Ranger 99	6	7	3	2	1	19	4.1	68	16	16	5
Ranger 109	10	7	11	5	1	34	4.6	50	32	18	3
Wide-polycross progeny											
Ranger 12	2	2	6	4	6	20	7.0	20	30	50	30
Ranger 47	0	1	6	8	2	17	7.5	6	35	59	12
Ranger 57	3	1	5	2	1	12	5.4	33	42	25	8
Ranger 99	0	0	2	0	5	7	8.9	0	29	71	71
Ranger 109	2	2	3	10	2	19	7.0	21	16	63	11
Check varieties											
Lahontan	5	6	8	1	1	21	4.5	52	38	10	5
Ranger	0	0	1	4	11	16	9.4	0	6	94	69
Kansas B-1 (Cody)	4	2	7	7	0	20	5.7	30	35	35	0
Vernal	0	0	2	1	6	9	8.9	0	22	78	67
Buffalo	0	0	0	1	4	5	9.7	0	0	100	80
Moapa	3	4	3	4	0	14	5.0	50	21	29	0

[a] 1 = no injury; 10 = completely killed.

data emphasize that pollination control in seed production is vital to progeny evaluation.

Performance of a Synthetic Variety of Ranger Origin

The Syn-1 generation of Nebraska Synthetic 20 as 14-day-old seedlings demonstrated effective resistance but to a lesser degree than the resistant checks—Lahontan, Cody, and Moapa (Table 9). Narrow-polycross progenies composited to form Synthetic 20 performed about the same as the synthetic, as would be expected. Subjection of the synthetic and progenies to a steadily increasing population of aphids for approximately 14 days resulted in a rather high mortality—39 percent for the synthetic and 36 percent average for the progenies, compared to 81 percent mortality in Ranger. This represents about a 100-percent increase in resistance of the synthetic over the parent variety Ranger and clearly demonstrates that an intensive selection program in Ranger or its parental strains could provide a variety highly resistant to the spotted alfalfa aphid.

This exploration and appraisal of spotted alfalfa aphid resistance in Ranger and its parental clones provides strong evidence that sources of resistance in the variety are abundant in its broad genetic base. Through proper selection, an aphid-resistant synthetic could be developed and still maintain the agronomic and disease-resistant attributes of Ranger.

Table 9. Comparative survival of intercrossed (narrow-polycross) seedling progenies of six selected aphid-resistant Ranger clones, the Syn-1 synthetic produced from these six clones, and resistant and susceptible check varieties.

Entry	Progeny reaction			
	Percent dead	Percent injured	Percent healthy	Percent survival
Narrow-Polycross[a]				
12	26	19	55	74
47	44	29	27	56
57	25	15	60	75
62	42	36	22	58
99	38	18	44	62
109	44	16	40	56
Average	36	22	41	63
Nebraska Synthetic 20 (Syn-1)	39	8	53	61
Resistant Checks				
Lahontan	23	21	57	78
Cody	10	19	71	90
Moapa	21	17	62	79
Susceptible Checks				
Ranger	81	17	2	19
Buffalo	92	5	3	8
LSD: 5 percent	13			
1 percent	18			

[a] Ranger clone numbers.

APPENDIX

Table 10. Classification for spotted alfalfa aphid resistance in 96 alfalfa clones from Ranger.

Clone number	Test results		Classification
	Antibiosis (aphid reaction)	Tolerance (plant reaction)	
12	R	R	HIGHLY RESISTANT
47	HR	R	
54	R	R	High antibiosis
57	R	R	High tolerance
62	R	R	
99	HR	R	7 plants
109	R	R	7.3 percent
4	R	I	RESISTANT
32	R	I	
36	R	S	High antibiosis
41	R	I	Moderate to low
70	R	I	tolerance
79	R	I	
82	HR	I	9 plants
95	R	S	9.4 percent
117	R	S	
29	S	R	TOLERANT
32	I	R	Low to moderate
83	S	R	antibiosis
			High tolerance
			3 plants
			3.1 percent
11	I	I	INTERMEDIATE
27	I	I	
69	I	I	Moderate antibiosis
104	I	I	Moderate tolerance
			4 plants
			4.2 percent
1	S	I	SUSCEPTIBLE
5	S	I	
21	S	I	Low antibiosis
23	S	I	Moderate tolerance
30	S	I	
39	S	I	10 plants
43	S	I	10.4 percent
49	S	I	
85	S	I	
119	S	I	
9	I	S	SUSCEPTIBLE
31	I	S	
60	I	S	Moderate antibiosis
76	I	S	Low tolerance
85	I	S	
93	I	S	10 plants
94	I	S	10.4 percent
96	I	S	
102	I	S	
112	I	S	

Table 10 (Concluded)

Clone number	Test results		Classification
	Antibiosis (aphid reaction)	Tolerance (plant reaction)	
3	S	S	HIGHLY SUSCEPTIBLE
6	S	S	
8	S	S	Low antibiosis
10	S	S	Low tolerance
14	S	S	
15	S	S	53 plants
17	S	S	55.2 percent
18	S	S	
19	S	S	
22	S	S	
24	S	S	
25	S	S	
26	S	S	
28	S	S	
34	S	S	
35	S	S	
37	S	S	
40	S	S	
42	S	S	
44	S	S	
45	S	S	
46	S	S	
50	S	S	
52	S	S	
53	S	S	
55	S	S	
58	S	S	
59	S	S	
64	S	S	
65	S	S	
66	S	S	
67	S	S	
68	S	S	
71	S	S	
72	S	S	
73	S	S	
74	S	S	
75	S	S	
77	S	S	
78	S	S	
84	S	S	
87	S	S	
88	S	S	
89	S	S	
92	S	S	
101	S	S	
103	S	S	
106	S	S	
108	S	S	
110	S	S	
113	S	S	
114	S	S	
115	S	S	

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