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SUMMARY

Sources of combined resistance to spotted alfalfa and pea aphids were found in Ranger alfalfa and its parental strains, foreign plant introductions, clones and their progenies.

Ranger alfalfa appeared to be a good source for selection of plants with resistance to both aphids.

Different selection sequences were found feasible. Aphid survival and reproduction were used to measure antibiosis in individual plants. Plant response to mass infestation was utilized for selection of resistant parental clones and evaluation of their progeny. Individual plants with high antibiosis to the spotted alfalfa aphid consistently produced progenies with high survival values when exposed to large spotted alfalfa aphid populations in the greenhouse. In contrast, plants with high antibiosis to the pea aphid produced progenies which varied in susceptibility and resistance.

The frequencies of pea aphid resistant plants among spotted alfalfa aphid susceptible or resistant plants and progenies did not vary significantly, which indicated that resistance to the two aphid species is controlled by different genes. Correlation analyses among large numbers of selections substantiated this conclusion.

Susceptible and resistant clones and progenies were subjected to resistance appraisals by using aliencolae (agamic females) originating from the Central Nebraska sexual egg-laying strain and the normal parthenogenetic strain. No differences were found in plant or aphid responses between strains of the spotted alfalfa aphid.

Correlations of pea aphid and spotted alfalfa aphid resistance with potato leafhopper damage, black stem, common leaf spot, and bacterial wilt disease reactions, and various agronomic characteristics were either nonsignificant or too low to be of predictive value.

Two synthetics with combined resistance were developed. In greenhouse and fiield cage tests, Nebraska Synthetic 27 of 8-clone parentage displayed spotted alfalfa aphid resistance equal to Lahontan and Cody and was far superior to named varieties in pea aphid resistance. Nebraska Synthetic 28, an 8-clone experimental of Ranger origin, was resistant to spotted alfalfa aphid but inferior to N.S. 27 in resistance to the pea aphid.

Appraisal for Combined Pea Aphid and Spotted Alfalfa Aphid Resistance in Alfalfa[1]

W. L. Howe,[2] W. R. Kehr,[3] and C. O. Calkins[4]

INTRODUCTION

Alfalfa in the United States has long been subject to damage and forage losses by the pea aphid, *Acyrothrosiphon pisum* (Harris). This damage was estimated at 4.1% of the nation's total crop or about 30 million dollars in a 1944 survey (2). Aphid loss potentials were further increased by the spotted alfalfa aphid, *Therioaphis maculata* (Buckton) after it appeared in the United States about 1953 and spread rapidly through alfalfa-growing areas in the southern two-thirds of the nation, particularly those areas west of the Mississippi River. Alfalfa forage and stand losses by the spotted alfalfa aphid in the Southwest and lower Midwest are presently estimated at several million dollars annually. The alarming destructive ability of the spotted aphid during its early spread prompted research on its biology, ecology, and control by several State Experiment Stations and the U.S. Department of Agriculture.

Observations of distinct differences in varietal injury encouraged and accelerated breeding programs to develop varieties resistant to the new destructive pest. Rapid progress and success in developing resistant varieties stimulated renewed studies of heritable pea aphid resistance with the goal of finding alfalfa resistant to both species. This Bulletin reports investigations of sources of resistance and development of materials with combined resistance to both aphids.

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REVIEW OF LITERATURE

Pea and spotted alfalfa aphids differ considerably in feeding habits, biology, and type of injury to alfalfa. For a clear understanding of the problem, we recommend a review of previous reesarch with special attention to alfalfa host-insect relationships, including appraisal of alfalfa resistance to each aphid and the factors involved in that resistance.

Appraisal of Alfalfa for Pea Aphid Resistance

Entomologists and plant breeders have studied alfalfa resistance to the pea aphid for many years. Despite their efforts, no variety that includes pea aphid resistance has been releaseed. This deficiency may be due partially to problems in appraisal caused by lack of distinct toxigenic feeding injury to foliage, difficulty in measuring the obscure stunting, and the ability of alfalfa to tolerate high populations and still produce acceptable growth.

In Nebraska greenhouse tests, Du Puits and Lahontan were classified as intermediate in pea aphid reaction (26). Plants with apparent resistance in the seedling stage were further tested for antibiosis resistance. Open-pollinated progeny of clones with antibiosis resistance were able to make near-normal growth under severe infestation.

In an evaluation of seedling survival of 27 entries in a Kansas greenhouse, a resistant selection, P-42, significantly exceeded all other entries (13). Such Flemish types as Socheville, Alfa, Du Puits, and Tourneur 501 survived relatively well, but varieties with Turkistan parentage generally showed only intermediate or good resistance. In the field Du Puits ranked high, with smaller aphid populations present and less injury than other entries. Ladak and varieties of Turkistan origin demonstrated some degree of resistance in Kansas (31).

In nursery cage tests the relative rank of varieties indicated that Moapa and Lahontan were injured to a lesser degree than Cody, Culver, Caliverde, and Ranger in one test (5). In another test a synthetic developed for resistance was injured much less than named varieties. In this test Lahontan and Du Puits were similar in resistance.

The presence of intermediate resistance to the pea aphid in certain alfalfa varieties of Turkistan origin—like Ranger—which also provide good sources of spotted alfalfa aphid resistance (17), would appear to favor the possibility of developing resistance to both aphids.

Detailed studies of resistance within lines, varieties, and individual plants have been made intermittently for many years (29). Observations showed that antibiosis could be measured by differences in aphid reproductive rates (4, 6). This mechanism of resistance was utilized in studies of the inheritance of alfalfa resistance to the pea aphid (23).

Pea aphid-resistance appraisal in alfalfa was apparently complicated by phenotypic variations which resulted from certain environmental conditions. For example, a condition described as "instability" of resistance and a similar condition designated as "temporary immunity" were reported (1, 8). Further complication in appraisal of alfalfa resistance may have been caused by pea aphid biotypes (14) or by seasonal forms (9). These reports indicated that identification of genetically inherited resistance was not simple and that improved procedures would be necessary.

During 1953 and 1954, testing techniques involved pea aphid and plant response observations in the USDA pea aphid nursery in Orange County, California (Howe, unpublished). Nursery plantings were subjected to heavy infestation under plastic screen cages which excluded primary predators. Resistance in individual lines was measured by rate and volume of regrowth after cutting, under conditions of high aphid population stress. Some lines were killed, but others were tolerant to a high population. The most promising lines produced abundant growth and appeared to be less desirable to the aphid since they supported only comparatively low populations. Further appraisal was needed to eliminate the preference factor and to verify the presence of antibiosis, described as an adverse effect of the plant on the biology of the aphid (30). Aphid development and reproduction were measured on vigorous plants growing *in situ* in the nursery. Stem terminals of spaced nursery plants were isolated and infested with aphids in height-adjustable cages (21). Repeated measurements of aphid reproduction on new regrowth were made after periodic cuttings of growth on individual clones. Other tests were made on replicated clones and on separate stems of the same plant. These tests eliminated much error involved in abnormal overaged pot-grown plants and other atypical phenotypes. Antibiosis identified in these repeated tests was correlated with low populations observed under the previously described mass infestation tests. High plant survival after exposure to large aphid populations was consistently correlated with low aphid reproduction rates on caged isolated stems.

Further evidence on the nature and appraisal of resistance of alfalfa to the pea aphid was obtained in tests on field-selected plants which continued to grow under extremely high field infestation. Excellent field sources of resistance were found in certified Ranger seed fields at high elevations in southern California (Howe, unpublished). Occasionally pea aphid infestations developed in sufficient intensity to cause almost complete killing of top growth throughout entire fields. Small portions of plants which retained growth were removed to the nursery and vegetatively propagated for further study. Replicated tests conducted on 8 clones from field selections revealed an average reproduction rate of 1.0 nymphs per day compared with

4.7 per day on clones derived from plants with completely necrotic foliage. This finding indicated that antibiosis to the pea aphid in normal, mature, deep-rooted plants was reasonably stable under varied conditions of cutting, plant age, and climate. Antibiosis was apparent in asexually propagated clones.

More recently, accelerated research on pea aphid resistance was conducted by several investigators after the initial pressure for spotted alfalfa aphid resistance studies subsided. Successful greenhouse and field selection techniques were employed in Kansas for identifying heritable pea aphid resistance (29). Selection of seedlings on the basis of vigor after their exposure to large aphid populations in the early seedling stage was considered a positive and rapid method for selecting resistant plants. A high correlation was found between antibiosis in caged leaf tests of plants and seedling survival of their progeny.

Appraisal of Alfalfa for Spotted Alfalfa Aphid Resistance

The methodology described for selection and evaluation of pea aphid resistance was generally adaptable for the spotted alfalfa aphid. Greenhouse methods which must be used under Midwest climatic conditions appear standardized with a few variations in technique. Greater accuracy seemed possible in appraisal for spotted alfalfa aphid resistance than for pea aphid resistance because injury symptoms were more definitive and phenotypic variation in alfalfa appeared to have less effect on the expression of resistance.

Numerous reports recorded differences in spotted alfalfa aphid resistance among varieties, breeding lines, clones, etc., toxigenic effects of feeding, environmental factors affecting expression of resistance, selection of appraisal methods, and the actual breeding and release of resistant varieties, differential behavior of aphids on resistant and susceptible plants, evidence of aphid biotypes, the appearance of an aphid sexual strain, and the influence of plant age on expression of resistance. It is evident that despite the comparatively short period of spotted alfalfa aphid occurrence in the United States intensive research has provided greater knowledge leading to improved control through varietal resistance for the spotted alfalfa aphid than for the pea aphid.

Varietal differences in field and greenhouse response to injury were reported (12, 17, 18, 20, 21, 24, 35). Comparative varietal rankings have appeared surprisingly consistent despite differences in climate, soil, moisture, temperature, and individual appraisal methods. Lahontan was invariably classed as resistant. Varieties of Turkistan or partial Turkistan origin, such as Ranger, Orestan, and Nemastan, certain foreign varieties, and others, were usually considered intermediate in resistance with tolerance being an important contributing mechanism. African generally appeared partially resistant, with considerable survival under infestation conditions which nearly destroyed susceptible varieties. Susceptible types included the so-called Common

Alfalfas, Chilean, Indian, Atlantic, Narragansett, Du Puits, and many others.

Other significant research concerned with alfalfa host relationships and the aphid was valuable in providing a clearer understanding of plant resistance to the spotted alfalfa aphid. The toxic effects of feeding were observed and their nature was described (7, 28, 32). This insect-induced toxemia, characterized by yellow vein banding, causes an overall weakening and stunting and sometimes death of the plant. It is probable that tolerant and resistant plants can resist the injurious effects of the toxin.

Environmental effects on the expression of resistance were also partially explored. Most significant were the observations that lower temperatures were more favorable for aphid reproduction and survival on resistant plants (11, 21,22). Humidity had less influence on aphid biology than temperature. High humidity had less influence on decreased survival of adults and number of nymphs produced (10, 22).

The development and release of varieties resistant to the spotted alfalfa aphid was exceptionally rapid and undoubtedly had a significant effect in alleviating losses in some areas. Moapa, the first resistant variety developed for farm plantings, was released in 1957 (34). It is a 9-clone synthetic of African origin (20, 34). Observations of aphid population and injury in field plantings indicated greatly reduced populations and less injury than on susceptible varieties (3). Sources of resistance were also found in the susceptible variety Buffalo (15). They were utilized in the development of another resistant variety, Cody, a 22-clone synthetic developed at the Kansas Agricultural Experiment Station. Field populations of spotted alfalfa aphid were about four to ten times greater on the parent variety Buffalo than on Cody (16). Zia, another spotted alfalfa aphid resistant variety, is considered resistant in tests conducted within its normal range of adaptation in New Mexico (37).

Sources of resistance were found also in the parental strains of Ranger and in an experimental resistant synthetic tracing to Ranger (17).

Several reports on the differential behavior of aphids on resistant and susceptible plants indicated that nymphs become restless on resistant plants, often leave and die (21, 27). It was observed also that aphids feeding on resistant plants preferred the leaflet midribs and stems (21). Biotypes of the spotted alfalfa aphid were reported in California. One biotype, identified in appraisal studies of Moapa, reproduced rapidly on the variety and three of its parental clones, but did not cause increased plant mortality due apparently because of a tolerance factor (33). Another biotype from an infested Lahontan field reproduced more rapidly than a greenhouse strain on a resistant parental clone of Lahontan (36). As yet there appears to be no field evidence that aphid biotypes appreciably alter resistance. Detection

of a sexual egg-laying form in 1959 was also reported (25). Its damage potential to varieties bred for resistance to the parthenogenetic form is reported in this Bulletin.

In cage experiments in California (19), variations in plant age resulted in profound differential effects on plant damage and mortality in susceptible and resistant varieties. Three seedling age groups of susceptible Caliverde and resistant Lahontan were simultaneously infested. It was observed that increased age greatly increased plant survival of Lahontan but had little influence on susceptible Caliverde under the same conditions of rapidly increasing predator-free populations. Seedling age had little influence on final survival of Caliverde.

METHODS AND MATERIALS

The research described in this Bulletin was conducted primarily in the greenhouse where temperatures were maintained at 75° ± 5°F. Supplemental lighting was used during short-day seasons. All appraisal tests were conducted with parthenogenetic females (aliencolae) cultured on tolerant alfalfa varieties grown in caged flats. Aliencolae from an egg-laying sexual strain were also involved in comparative tests. In order to maintain culture vigor, colonies were renewed annually from collections made in heavily infested fields in southwest Nebraska. Plants and clones for antibiosis tests were grown in 4″ pots and appraised only when in vigorous growing condition. Certified seed of standard varieties was used in all plantings. Plants were watered by a solid stream applied directly to the soil to avoid washing aphids from plants.

Several previously cited reports related the general methods for appraisal of alfalfa resistance to aphids. Essentially these are standardized with only minor differences in technique. Basically, two types of simple tests were employed. One designated as the "mass infestation test" in this Bulletin, sometimes called a plant-response or preference test, consisted of the exposure of varieties and breeding lines to large populations of reproducing aphids, usually under predator-free conditions. This method generally involved replicated seedings in rows 2½ to 3 inches apart in greenhouse benches, flats, or outdoor cages, such as shown in Figure 1, to provide plant densities of 35 to 50 seedlings per linear foot. These were subsequently infested by sprinkling seedlings with aphids of the desired species obtained from vigorous stock cultures maintained on alfalfa. Aphids were introduced to plants ranging in growth from the cotyledonary to the two- or three-trifoliolate stage. Number of aphids introduced per plant varied with tests, but usually averaged about five per plant. Subsequent aphid introductions were commonly made. Population increase through at least three generations was necessary to obtain a critical test. Tests involving a high proportion of entries of known or sus-

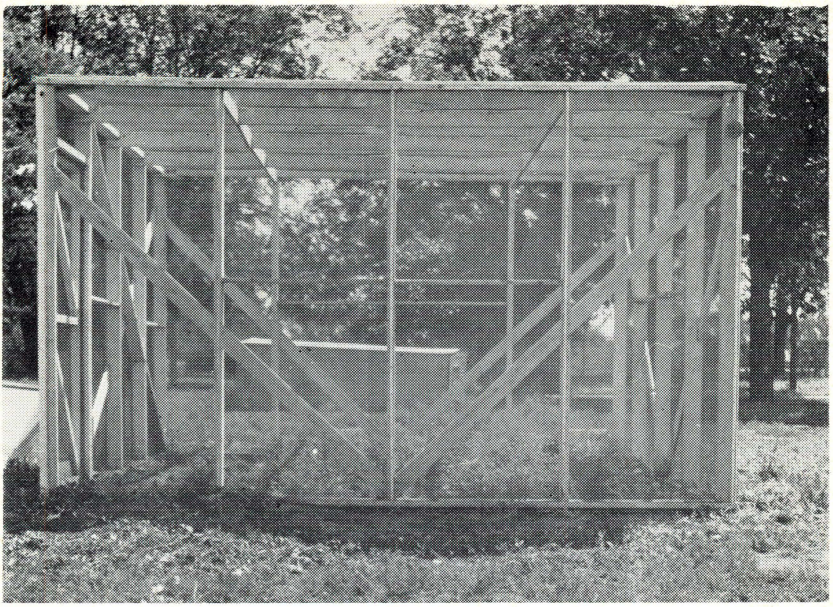

Figure 1. Outdoor cage used in appraising experimental synthetics and progenies for resistance to induced aphid populations.

pected resistance were interplanted with a variety of known susceptibility to assure sufficient populations for critical evaluation.

The mass-infestation test serves two functions: first, to provide a means for rapid screening of large numbers of plants for initial selection of potentially resistant survivors and, second, to obtain an overall appraisal of alfalfa lines and progenies under conditions of injurious infestations. Both aphid species often were used for a given mass-infestation test in initial screening or progeny evaluation. Seedlings were infested first with pea aphids and evaluated, then infested with spotted aphids and again evaluated. However, evaluation for spotted aphid damage followed by pea aphid did not prove practical since vein-clearing by spotted aphids masked the expression of yellowing by pea aphids. Appraisal methods for varietal and progeny reactions to infestation varied, but were usually made on numerical scales of 1–10 or 1–5, with the lower number indicating little or no visible injury and the higher complete mortality or severe injury. For some varieties or clones these ratings were arbitrarily converted to highly resistant (HR), resistant (R), intermediate (I), susceptible (S), and highly susceptible (HS).

The second test technique, usually designated as the "antibiosis test," is commonly used to study the effects of the plant on the biology of the aphid. Evaluation criteria consist of aphid survival and/or

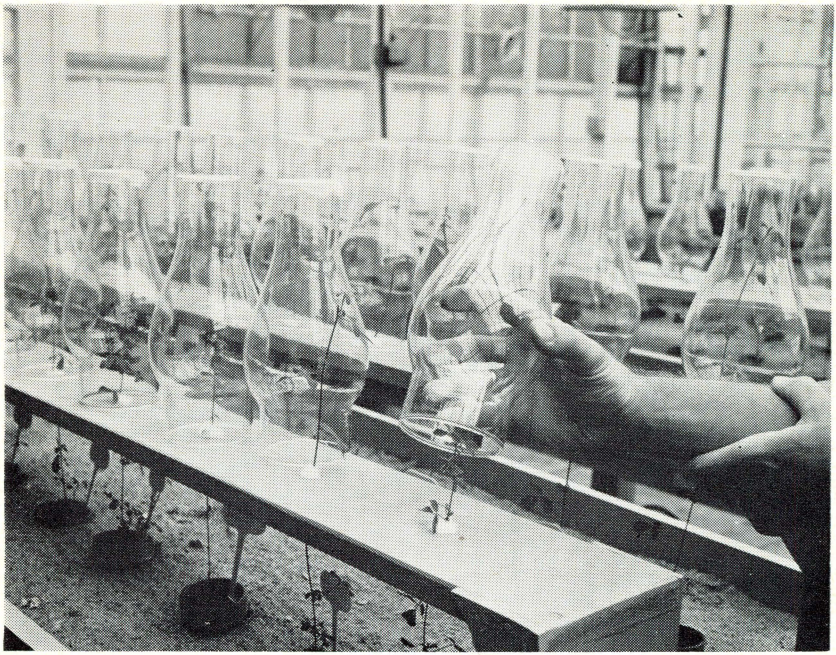

Figure 2. Lamp chimney cages used in observing aphid behavior and development for evaluating antibiosis.

reproduction. Single or multiple aphid introductions were made on individually caged leaflets, terminals, or entire seedlings, and appropriate survival and reproduction data were recorded. Numerous types of cages were used by various workers, as recorded in literature previously cited. In these studies lamp-chimney cages placed on adjustable racks, shown in Figure 2, isolated individual stems of potted plants (17). In most tests three to five nymphs were introduced, with survival and/or reproduction data recorded at predetermined intervals. Plants which failed to support aphids were reinfested a second or third time to insure definite evidence of high antibiosis. Arbitrary antibiosis classifications into highly resistant, resistant, intermediate, susceptible, and highly susceptible were made on the basis of survival and reproduction of aphids. A classification of highly resistant generally indicated that aphids did not survive or reproduce even after mutiple introductions. In both types of tests standards of known reaction were included.

Sequences in which the two types of tests were used varied, depending on plant material under study, its previous appraisals for resistance, and evaluations desired. Mass-infestation tests on progenies were considered important in evaluating promising clonal germ plasm, particularly for pea aphid resistance.

Previous intensive screening, selection, and progeny and varietal appraisals for spotted alfalfa aphid resistance conducted at the Nebraska Agricultural Experiment Station provided spotted alfalfa aphid resistant plant sources with a broad genetic base for further selection and breeding for pea aphid resistance. These, along with other plant sources, were utilized heavily in the diverse selection and breeding program for combined resistance described under Results and Discussion.

RESULTS AND DISCUSSION

Selection for Combined Resistance

Resistance in alfalfa to both aphid species was sought in Ranger and its parental strains, foreign plant introductions, and among Central Alfalfa Improvement Conference clones and progenies. The most intensive selection was conducted among the latter plant materials because these included disease resistance and favorable agronomic characteristics along with climatic adaptability. For simplicity and conciseness, varied selections made and breeding procedures employed are outlined separately for each plant source. Results of more than one test are often compiled in a single table.

Ranger Alfalfa

Previously cited reports by several authors indicated that Ranger might constitute a favorable source of resistance to both aphid species. Thus, Ranger was chosen for initial studies because information on the nature and frequencies of spotted aphid resistance in its parental strains had been previously investigated (17) and plant selections of known resistance and susceptibility to the spotted alfalfa aphid were available for pea aphid evaluation. An exploratory test of pea aphid antibiosis was conducted among plants both resistant and susceptible to spotted alfalfa aphid to identify combined aphid resistance and explore the frequency of pea aphid resistance in each spotted aphid reaction category.

Test Methods, Selections, and Breeding Sequences

Test A: Spotted alfalfa aphid antibiosis (1957–58).

Plant sources: Ranger and parental strains of Ranger, A110, A111, A116, A117, and A119.

Selections: 69 resistant and 35 susceptible (Table 1).

Test B: Pea aphid antibiosis (1959).

Plant sources: Resistant and susceptible selections from test A above.

Selections: 28 plants with antibiosis to both aphids. To clonal nursery for seed production (Table 1).

Test C: Pea aphid and spotted alfalfa aphid mass infestation (1959). Consecutive tests on same planting.

Table 1. Tests for pea aphid antibiosis in alfalfa clones of Ranger origin with high and low antibiosis to the spotted alfalfa aphid.

Spotted alfalfa aphid antibiosis	Number of plants	Pea aphid antibiosis							
		Highly resistant		Resistant		Intermediate		Susceptible	
		No.	%	No.	%	No.	%	No.	%
High	69	7*	10.1	21*	30.4	22	31.9	19	27.5
Low	34	2	5.7	11	31.4	15	42.9	6	17.1

* Resistant to both aphids—Total 28.

Plant sources: Open-pollinated progeny from 8 of 28 clones with dual resistance.

Selections: Progeny of No. 3309 resistant to both aphids. Progeny of No. 3291 resistant to spotted alfalfa aphid and intermediate to pea aphid (Table 2).

Classification of the plants for resistance was based on antibiosis tests only (Table 1). Reaction to spotted alfalfa aphid appeared to have little significant influence on the frequency distribution of pea aphid antibiosis evaluations. The 28 plants classed as resistant on the basis of antibiosis were propagated vegetatively and placed in a clonal open-pollination nursery during the summer of 1959. Unfortunately,

Table 2. Comparative pea aphid and spotted alfalfa aphid injury to progenies of clones which showed antibiosis to both aphids.

Nebraska clone No.	Source[a]	Progeney reaction[b]	
		Pea aphid	Spotted alfalfa aphid
3285	A-111	91.4	50.6
3291	A-110	50.4	36.3
3298	A-111	67.2	37.9
3309	A-111	19.6	36.6
3311	A-111	83.3	33.3
3319	A-116	87.6	39.8
3337	A-119	85.3	38.5
3343	A-119	83.3	36.1
Varieties			
Lahontan		79.3	33.9
Ranger		87.7	83.7
Buffalo		93.8	96.0

[a] Ranger parental strain.

[b] 0 = no injury; 100 = complete kill. Overall progeny damage appraisal based on weighted injury of individual plants calculated as follows:

$$\left[\frac{(N_1 \times 1) + (N_2 \times 2) + (N_3 \times 3) + (N_4 \times 4) + (N_5 \times 5)}{\Sigma\ N_1 + N_2 + N_3 + N_4 + N_5}\right] \quad 10 = \text{Index of injury}$$

Where N_1 = No. of seedlings with little or no visible injury
N_2 = No. of seedlings with light injury
N_3 = No. of seedlings with moderate injury
N_4 = No. of seedlings with severe injury
N_5 = No. of seedlings dead

seed in sufficient quantity for greenhouse pea aphid and spotted alfalfa aphid progeny tests was available from only eight clones. Plantings of the open-pollinated seed were made in flats during the winter of 1959–1960 in two to four replicates depending upon the amount of seed available.

Differences in progeny appraisal to both aphid species recorded in Table 2 were based on a weighted index involving examination of individual plants and calculated as shown in footnote 2 of the Table. This method for evaluating is considered the most accurate, but was too time-consuming for further use.

The severe pea aphid damage to progenies of most entries indicated that previously cited evidence of antibiosis in the clone was not always reflected by improved progeny performance. In contrast, high antibiosis to the spotted alfalfa aphid in the clone was consistently correlated with low progeny damage—usually only slightly higher than that of the aphid resistant variety Lahontan. Nebraska clone 3309 appeared to be extremely promising as a source of combined aphid resistance. Increased growth under high population levels is shown in Figure 3. Clone 3291 appeared to possess some degree of tolerance or resistance and was retained also as a source of resistance for future breeding. The importance of progeny appraisal, particularly for pea aphid resistance, was clearly demonstrated in this experiment.

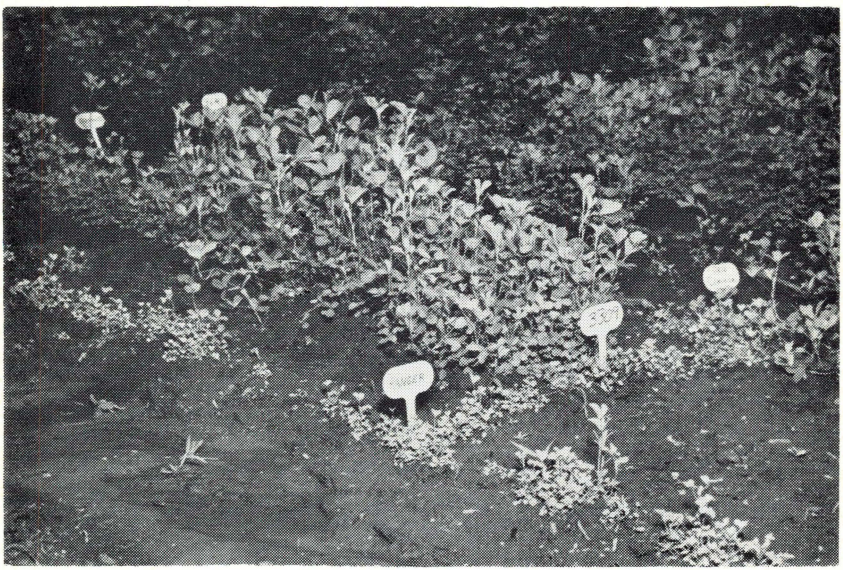

Figure 3. Differential growth of progeny of Nebraska clone 3309 compared with Ranger (left) and Oklahoma common (right) after exposure to mass infestation of pea aphids in a field cage. Progenies of clone 3309 displayed similar growth after exposure to the spotted alfalfa aphid.

Foreign Plant Introductions

A search for combined resistance among foreign plant introductions was conducted to broaden the genetic base for future breeding. The test sequences represented the first attempt to make preliminary selection for combined aphid resistance in a single planting by consecutive mass infestation tests by the two aphid species.

Test Methods, Breeding, and Selection Sequences:

Test A: Pea aphid mass infestations (1959).
Plant sources: Plant introductions from Afghanistan, France, Algeria, Iraq, Turkey, India, Italy, England, Canada, Peru, Portugal, Russia, Iran, Greece, Yugoslavia, New Zealand, South Africa, Guatemala, Uruguay, Belgium, and Cyprus.
Selections: 65 plants marked *in situ* for subsequent test. Nebraska clones 4550–4615.

Test B: Spotted alfalfa aphid mass infestation test in same planting after a 1-week insect-free recovery period.
Plant sources: 65 survivors from test A.
Selections: 28 resistant.

Test C: Spotted alfalfa aphid antibiosis.
Plant sources: 28 selections from test B above.
Selections: 6 Nebraska clones 4553, 4554, 4556, 4571, 4578, and 4588 (Table 4).

The frequency distribution of all introductions into injury classes for both aphids is shown in Table 3. A total of 185 entries was intermediate or susceptible to both aphids. Only two entries were classed as resistant to both aphids. The selection of only six promising plants, identified in Table 4, from about 10,000 plants representing 203 introductions indicated the infrequency of combined aphid resistance among a wide array of diverse lines. Introductions of Near East origin, Afghanistan and Iran, where the spotted alfalfa aphid is native, were sources of 5 of the 6 resistant selections. Two introductions from Afghanistan, PI 220299 and PI 220668, displayed less injury upon exposure to populations of aphid species; however, because of poor growth no selections were made for combined resistance. The injury

Table 3. Distribution of foreign plant introductions into pea and spotted alfalfa aphid reaction classes based on responses to mass infestations.

Spotted alfalfa aphid reaction	Plant injury class—number of plants Pea aphid reaction			
	Resistant	Intermediate	Susceptible	Totals
Resistant	2	2	7	11
Intermediate	5	18	27	50
Susceptible	2	23	117	142
Totals	9	43	151	203

Table 4. Source of clones classed as resistant to pea aphid and spotted alfalfa aphid, and overall damage to foreign introduction sources by both aphid species.

Nebraska clone	Plant introduction number	Origin	Plant injury classification	
			Pea aphid	Spotted alfalfa aphid
4553	211609	Afghanistan	I	I
4554	211608	Afghanistan	R	I
4556	207494	Afghanistan	S	S
4571	234205	Iran	I	R
4578	217419	Denmark	I	I
4588	201864	Iran	I	R

classification of all introductions tested is recorded in Table 1 in the Appendix.

Central Alfalfa Improvement Conference Clones and Progenies

Sources of combined aphid resistance were sought most intensively among a select group of Conference clones, designated "C" clones in this Bulletin. A sequence of 10 appraisal, selection, and breeding tests involved these clones and their polycross progenies. These are outlined in test sequences A through J.

Test Methods, Breeding, and Selection Sequences:

Test A: Spotted alfalfa aphid antibiosis, greenhouse tests.

Plant sources: C clones (143) largely of Central Conference origin listed in appendix Table 2.

Selections: Highly resistant: C3, C7, C27, C32, C40, C84, C89, C93, C176, C218, C220, C242, C607, C616, C634, C900, (Table 2, Appendix).

Test B: Field appraisal of C clones in heavily infested clonal nursery—Bakersfield, California, based on combined appraisals of aphid population, honeydew production, and plant injury including stunting and regrowth ability (1960)[5]

Plant sources: C clones, essentially same as in test A.

Selections: Resistant, same as in test A plus C45, C113, C114, C223. (These 4 clones were classed as susceptible in test A). (Table 2, Appendix).

Test C: Spotted alfalfa aphid mass infestation (1959).

Plant sources: Polycross progenies of selected C clones.

Selections: Many based on tall, uninjured growth of survivors.

Test D: Spotted alfalfa aphid antibiosis.

Plant sources: Survivors from test C with least injury.

[5] The authors gratefully acknowledge the assistance of F. V. Lieberman, Entomology Research Division, Agr. Res. Serv., U.S.D.A., Tucson, Arizona, for the comprehensive and accurate field appraisal of spotted alfalfa aphid resistance in 136 of the above clones in replicated tests.

Selections: 158 plants with high antibiosis. Assigned Neb. clone numbers 3613 through 3770. To polycross nursery March, 1959 for seed production (Tables 7 & 8).

Test E: Pea aphid mass infestation (1959).

Plant sources: Polycross progenies from selections made from test D above. First cycle recurrent selection.

Selections: 119 with most vigorous, least injured growth. Assigned Neb. clone numbers 4380–4498. Progenies from clones 3638, 3640, 3641, 3649, 3651, 3683, 3692, 3694, 3702 classed as resistant to pea aphid (Tables 7 & 8).

Test F: Spotted alfalfa aphid antibiosis (1959).

Plant sources: 119 selections 4380–4498 made in test D.

Selections: 78 with high antibiosis (Table 10).

Test G: Pea aphid antibiosis (2 tests) (1960).

Plant sources: 9 selections from clonal series 3613–3770 which demonstrated high antibiosis in test D, and subsequently produced progenies resistant to the pea aphid in test E.

Selections: High antibiosis to pea aphid: 3638 3640, 3641, 3692, 3694, 3702. Low antibiosis: 3651, 3683. Lost: 3649 (Table 9).

Test H: Pea aphid antibiosis and agronomic data (1960).

Plant sources: 78 clones from first cycle recurrent selection in pea aphid mass infestation test E. High antibiosis to spotted alfalfa aphid in subsequent test F.

Selections: 29 with high antibiosis to pea aphid.

Test I: Pea aphid mass infestation (1961).

Plant sources: Open-pollinated progenies of 29 selections of clonal series 4380–4498 made in test H. All portrayed high antibiosis to spotted alfalfa aphid.

Selections: No further selections made. All demonstrated good growth under induced pea aphid attack.

Test J: Spotted alfalfa aphid mass infestation (1960).

Plant sources: Same as in test I.

Selections: No further selections made. All 29 progenies performed well (Table 10).

Test A, based on greenhouse antibiosis, and test B, a nursery appraisal conducted in California, provided valuable information on spotted alfalfa aphid resistance in a wide range of genetically desirable breeding sources. The classifications assigned individual clones in both tests are shown in Appendix Table 2. The comparative appraisals in the two tests summarized in Table 5 indicated a remarkable degree of correlation between two quite diverse evaluation methods.

Subsequent tests and selection involved a portion of the C clones with favorable agronomic and other characteristics in a Central Alfalfa Improvement Conference polycross nursery. Polycross progenies were subjected to intensive selection in mass infestation and antibiosis tests in sequences C and D which resulted in the selection of 158 clones—

Table 5. A comparison of classifications of C clones for spotted alfalfa aphid injury in greenhouse antibiosis tests and subsequent injury appraisal under nursery conditions of high field infestation.

Nebraska antibiosis classification	California nursery appraisal						
	No.	HR	R	I	S	HS	UNK
HR	16	14	2	0	0	0	0
R	6	3	3	0	0	0	0
I	6	0	2	1	1	1	1
S	24	1	3	5	6	7	2
HS	91	0	1	2	15	68	5

assigned Nebraska numbers 3613 through 3770. Tests D and E provided further information on the reaction of their progenies to the spotted alfalfa aphid and pea aphid. The dual reaction of progeny of selected clones is summarized in Table 6.

The large proportion of clones—104 of 111—with highly resistant or resistant progenies demonstrates the validity of previous spotted alfalfa aphid selection methods and appraisals. Distribution of progenies into various pea aphid reaction categories was expected since no selection for pea aphid had been made. Polycross progenies of the 158 clones selected in test D constituted the first cycle and were utilized in a first-cycle recurrent selection pea aphid mass infestation appraisal outlined in test E. It yielded 119 healthy survivors assigned clone numbers 4380 through 4498, which were further screened for spotted alfalfa aphid antibiosis in test F. Tables 7 and 8 record the selections and their sources. Progenies of one clone of Ranger origin, 3309, were also included, from which five selections were made.

A subsequent test for pea aphid antibiosis continued the appraisal of clonal series 3613 through 3770 involved in test series D, E, and G. The three tests resulted in selection of six clones of polycross origin and two of Ranger origin with high levels of antibiosis to both aphid species. Selected clones produced progenies which performed well under infestations of each aphid. Table 9 gives a summary of these appraisals, including certain agronomic data. The eight clones comprised the

Table 6. Spotted and pea aphid reaction of open-pollinated progeny of clones having spotted aphid resistance of the antibiosis type, selected from polycross progenies of C clones in a Central Alfalfa Improvement Conference Polycross Nursery.

Spotted alfalfa aphid injury class	Number of progenies in each pea aphid injury class					
	HR	R	I	S	HS	Totals
HR	6	20	12	10	5	53
R	7	10	14	16	4	51
I	0	1	1	0	1	3
S	0	0	0	4	0	4
HS	0	0	0	0	0	0
Total	13	31	27	30	10	111

Table 7. Pea aphid injury to polycross or Ranger progenies of clones selected for high antibiosis to the spotted alfalfa aphid and subsequent selections with high antibiosis to spotted alfalfa aphid.

Clone No.	Source	Progeny reaction to pea aphid RESISTANT		
		Resistant survivors to high pea aphid populations		
		Number of selections	High antibiosis to spotted alfalfa aphid	
			No. retained	Nebraska clone number
		North Central polycross origin		
3638	C-601	6	2	4415, 4416
3640	C-601	6	1	4408
3641	C-601	7	7	4401–4407
3649	C-605	6	5	4430–4435
3651	C-605	6	5	4424–4428
3683	C-614	6	5	4481–4486
3692	C-616	6	3	4470, 4474, 4476
3694	C-616	4	3	4463, 4464, 4465
3702	C-27	6	3	4443, 4445, 4446
		Ranger origin		
3309	A-111	6	5	4544, 4545, 4547–4549
3291	A-110		no test	

Table 8. Pea aphid injury to polycross progenies of clones selected for high antibiosis to the spotted alfalfa aphid and subsequent selections with high antibiosis to spotted alfalfa aphid.

Clone No.	Source	Progeny reaction to pea aphid INTERMEDIATE		
		Resistant survivors to high pea aphid populations		
		Number of selections	High antibiosis to spotted alfalfa aphid	
			No. retained	Nebraska clone number
3625	C-223	2	2	4419, 4420
3646	C-605	1	1	4400
3666	C-610	1	0	—
3671	C-613	2	1	4421
3678	C-614	1	1	4479
3682	C-614	1	1	4480
3684	C-614	6	5	4487, 4488, 4490–4492
3685	C-615	2	1	4478
3691	C-616	6	4	4493–4496
3693	C-616	3	1	4467
3701	C-27	1	1	4447
3703	C-27	4	3	4437–4439
3709	C-27	1	1	4436
3720	C-40	1	0	—
3721	C-63	1	1	4455
3728	C-193	3	3	4452–4454
3732	C-193	2	2	4450, 4451
3734	C-196	1	1	4449
3735	C-196	1	1	4448
3752	C-236	1	1	4462
3753	C-244	2	2	4460, 4461
3759	C-603	1	1	4459
3760	C-603	1	0	—
3766	C-608	1	1	4457

Table 9. Pea aphid and spotted alfalfa aphid resistance classifications, and agronomic data on initial clonal selections of polycross or Ranger origin.[a]

Clone number	Greenhouse data				Field data		
	Antibiosis		O. P. Progeny reaction				
	Spotted aphid	Pea aphid	Spotted aphid	Pea aphid	Seed (gms/plot) 1961	Growth habit[b]	Rate of recovery[c]
			Clones of Polycross Origin				
3638	R	HR	1.5	1.0(HR)	3	6	5
3640	R	HR	1.5	1.0(HR)	5	8	4
3641	R	R	1.5	1.5(HR)	6	6	4
3692	R	HR	2.0	2.5(HR)	5	7	6
3694	R	IR	2.0	2.5(R)	4	7	6
3702	R	HR	1.0	1.5(HR)	5	6	4
			Clones of Ranger Origin				
3291	R	R	2.5	2.0(R)	2	5	5
3309	R	HR	2.0	1.0(R)	1	6	6

[a] These 8 clones comprise the parentage for Neb. Syn 27.
[b] 1—erect; 9—prostrate (10/31/61).
[c] 1—most rapid; 9—least rapid to recover after cutting (8/13/62).

parents of Nebraska Synthetic 27, considered in the next section of this Bulletin.

Pea aphid antibiosis and pea and spotted alfalfa aphid mass-infestation tests H, I, and J, provided data supporting heritable combined resistance in the 29 selections comprising the first cycle recurrent selections made in test E. These data are compiled in Table 10 with agronomic data on seed production, growth habit, and rate of recovery.

Relationship of Aphid Resistance to Other Characteristics

Correlations were calculated to determine the degree of association among pea and spotted alfalfa aphid progeny reactions, certain agronomic characteristics, and injury by the potato leafhopper *Empoasca fabae* (Harris). One analysis involved clones and progenies selected in test sequences D and E, with appraisals shown in Tables 9 and 10.

Results presented in Table 11 showed significant positive correlations among spotted aphid reaction of progenies and both fall growth habit and rate of recovery of clones. The correlations were too small to be of predictive value, however. All progenies were either resistant or highly resistant to both aphids. No significant correlations were found between pea aphid reaction and any of the above agronomic characteristics or potato leafhopper injury. Spotted and pea aphid reactions were independent.

A second series of correlation analyses was made between spotted alfalfa aphid and pea aphid injury, reaction to certain diseases, agronomic characteristics, and potato leafhopper reaction. Aphid injury by both species was based on damage to open-pollinated progenies of spotted alfalfa aphid-resistant clones while other characteristics were

Table 10. Pea aphid and spotted alfalfa aphid resistance classifications and agronomic data on first cycle recurrent selections.

Clone number	Greenhouse data				Field data		
	Antibiosis		O. P. Progeny reaction				
	Spotted aphid	Pea aphid	Spotted aphid	Pea aphid	Seed (gms/plot) 1961	Growth habit 10/31/61	Rate of recovery 8/13/62
4400	HR	R	1.5	1.5R	9	6	5
4402	R	R	1.5	1.0R	4	6	4
4407	R	R	1.0	1.5R	8	7	5
4408	HR	R	1.5	2.0R	3	6	4
4419	HR	R	1.0	1.3R	5	5	3
4421	R	R	2.0	1.0R	4	6	3
4425	HR	R	1.5	1.8R	7	5	4
4433	HR	R	1.5	1.3R	5	7	4
4434	HR	R	1.0	1.3R	5	6	5
4436	HR	R	1.0	1.5R	1	7	6
4443	R	R	1.0	1.0R	3	6	5
4445	HR	R	—	[a]	1	7	4
4446	HR	R	2.0	2.0R	2	7	6
4447	HR	R	2.0	1.3R	3	6	5
4450	HR	R	2.0	1.3R	9	6	4
4451	HR	R	2.0	1.0R	3	6	5
4452	HR	R	1.0	1.5R	3	6	4
4453	HR	R	1.0	1.0R	3	6	3
4454	HR	R	2.0	1.5R	9	6	4
4455	HR	R	2.0	1.0R	7	6	4
4462	HR	R	1.0	1.5R	3	7	4
4463	R	R	1.0	1.0R	7	6	5
4464	HR	R	1.0	2.5R	6	6	4
4467	R	R	2.0	1.5R	9	6	6
4470	HR	R	1.0	2.0R	11	6	4
4476	HR	R	1.0	1.5R	1	5	5
4479	R	R	1.0	1.8R	5	6	4
4480	HR	R	2.0	1.5R	3	5	6
4492	HR	R	1.5	1.5R	5	5	3

[a] Insufficient seed.

Table 11. Correlations between spotted alfalfa aphid and pea aphid reactions of open-pollinated progeny of clones with combined resistance to spotted alfalfa and pea aphids selected from miscellaneous sources, and other characteristics of clones and their open-pollinated progeny.

Characters correlated	Spotted aphid reaction		Pea aphid reaction	
	df	r	df	r
Clones in replicated nurseries				
Fall growth habit	42	.31*	42	.16
Leafhopper reaction	42	−.11	42	−.06
Rate of recovery	42	.32*	42	.04
Seed production	42	−.02	42	.09
Open-pollinated progeny in replicated greenhouse tests				
Pea aphid reaction	42	.24	....	....

* Significant at the 5-percent level.

Table 12. Correlations between spotted alfalfa aphid and pea aphid reactions of open-pollinated progeny of spotted alfalfa aphid resistant clones selected from polycross progeny of C clones, and other characteristics of clones and their open-pollinated progeny.

Characters correlated	Spotted aphid reaction		Pea aphid reaction	
	df	r	df	r
Clones in replicated nurseries				
Blackstem reaction[a]	63	.00	45	−.11
Blackstem reaction[b]	97	.08	76	−.13
Bloom	149	−.11	115	.04
Common leafspot reaction[a]	62	.15	44	−.10
Fall growth habit	149	.01	115	.10
Fall vigor	149	.03	115	.02
Leafhopper reaction	149	−.08	115	−.02
Percent stand	149	.02	115	−.33**
Rate of recovery[a]	65	.04	45	.30*
Seed production	149	.03	115	.08
Spring vigor[a]	59	.06	43	.12
Winter injury[a]	92	.02	70	.03
Open pollinated progeny in replicated greenhouse tests				
Bacterial wilt reaction	93	.00	82	.21
Pea aphid reaction	109	.19	....	....

* Significant at the 5-percent level.
** Significant at the 1-percent level.
[a] Data obtained by L. J. Elling and F. I. Frosheiser, Minnesota Agr. Expt. Station.
[b] Data obtained by C. P. Wilsie, Iowa Agr. Expt. Station.

based on both clones and their open-pollinated progenies. The analyses were made on data recorded from a Central Alfalfa Improvement Conference uniform clone test. Nebraska clones 3613–3770 ("C" clone origin), 3499–3505 (central polycross and Lahontan origin), and C10, C53 and C199 were included.

Results presented in Table 12 show significant correlations among pea aphid injury, percent clonal stand, and rate of recovery. Recovery ability probably contributes appreciably to pea aphid resistance. The correlations were too small to be of predictive value, however. Spotted aphid reaction was independent of all other characteristics.

Appraisal of Experimental Synthetics for Combined Resistance

The previous breeding and selection programs identified sources of combined aphid resistance which appeared to possess adequate heritable resistance for the development of two experimental synthetics. Selections which originated from polycross test sequences were reduced to six, as shown in test sequence G, after final antibiosis and mass infestation tests. Clones 3309 and 3291 of Ranger origin performed well in the same test. These six clones of polycross origin were assigned Nebraska clone numbers 3638, 3640, 3641, 3692, 3694, and 3702. These eight clones were vegetatively propagated by stem cuttings. Rooted cuttings were transplanted in a cage in California to obtain seed. Honeybees were the pollinating agents. The narrow

polycross seed was mixed to produce the Syn-1 generation of Nebraska Synthetic 27. The differential growth of progenies from clones 3309 and 3641 after exposure to high pea aphid infestations in a field cage is shown in Figures 3 and 4, respectively.

Another synthetic similarly developed and comprised of eight clones entirely of Ranger parental origin was designated Nebraska Synthetic 28. The parental clones selected for Neb. Syn. 28 were 3292, 3296, 3303, 3308, 3312, 3317, 3318, and 3319. All produced resistant reactions in spotted alfalfa aphid antibiosis tests and 6 were resistant to the pea aphid. Clones 3308 and 3312 were classed as moderately resistant to the pea aphid.

These two experimental synthetics were appraised for spotted alfalfa aphid and pea aphid reactions in the greenhouse. Also included in the tests were Nebraska Synthetic 16, developed for qualities other than aphid resistance, and Nebraska synthetics 19, 20, and 21 developed for spotted alfalfa aphid resistance. Ratings recorded in Table 13 show that all synthetics, with the exception of N.S. 16 and N.S. 19 Syn-2, performed at least as well as Cody and Lahontan when subjected to high populations of spotted alfalfa aphids. Superior growth and low injury of Synthetics 27 and 28 were also indicated when these were subjected to pea aphid infestations. The improved pea aphid resistance in the synthetics over released varieties considered partially

Figure 4. Differential growth of progeny of Nebraska clone 3641 compared with Du Puits (center) and Vernal (left) after exposure to induced mass pea aphid infestation in a field cage.

Table 13. Greenhouse evaluations of experimental alfalfa synthetics and check varieties under severe pea aphid and spotted alfalfa aphid infestations.[a]

Entry	Spotted alfalfa aphid		Pea aphid
	Visual rating[b]	Percent resistant plants	Visual rating[b]
Aphid Susceptible Synthetics			
N.S.-16, Syn-1	4.5	6.1	4.0
N.S.-16, Syn-2	4.0	9.6	4.0
Aphid Resistant Synthetics			
N.S.-19, Syn-1	2.0	60.7	3.0
N.S.-19, Syn-2	4.0	37.6	4.0
N.S.-20, Syn-1	2.0	52.3	3.5
N.S.-20, Syn-2	2.5	42.3	3.0
N.S.-21, Syn-1	2.5	57.4	2.0
N.S.-21, Syn-2	2.0	53.6	3.0
Experimental Synthetics or Populations Developed for Combined Resistance			
N.S.-27, Syn-1	2.0	45.3	1.0
N.S.-28, Syn-1	2.0	44.8	1.5
California E-10[c]	NT[d]	NT	1.0
Kansas Syn 6, Syn-1[e]	NT	NT	1.0
Nevada Syn-T, Syn-1[f]	NT	NT	1.0
Check Varieties			
Oklahoma Common[g]	....	..	5.0
Buffalo	4.3	6.4	5.0
Cody	2.5	47.3	3.5
Culver	4.2	20.8	....
Du Puits	....	...	3.0
Lahontan	2.6	43.8	3.2
Ranger	3.6	11.2	5.0

[a] Permission to evaluate the experimental materials of out-State origin is gratefully acknowledged.
[b] 1 = No detectable damage; 2 = 25% damage or stunting; 3 = 50% damage or stunting; 4 = 75% damage or stunting; 5 = 100% damage or stunting.
[c] Narrow polycross seed of Clone E-10 produced by and obtained from M. H. Schonhorst, University of Arizona. This clone was selected by W. F. Lehman of the University of California. Evaluations of this clone involved G. R. Pesho, Entomology Research Division, Agr. Res. Serv., U.S.D.A., M. W. Nielsen and Vincent Roth of Arizona. Clone E-10 is one of 9 clones in Arizona–California experimental Syn. A.
[d] No test.
[e] An experimental synthetic developed through the cooperation of E. L. Sorensen, Crops Res. Div., Agr. Res. Serv., U.S.D.A., R. H. Painter, H. L. Hackerott, and T. L. Harvey of the Kansas Agr. Expt. Sta.
[f] An experimental synthetic developed through the cooperation of H. L. Carnahan and R. N. Peaden Crops Res. Div., Agr. Res. Serv., U.S.D.A., University of Nevada, and F. V. Lieberman, Entomology Research Division, Agr. Res. Serv., U.S.D.A., Tucson, Arizona.
[g] The sample of Oklahoma Common obtained by a farmer after a heavy infestation of pea aphid had presumably killed all susceptible plants, was obtained from W. R. Kneebone, formerly with Crops Res. Div., Agr. Res. Serv., U.S.D.A., Woodward, Oklahoma.

resistant by various researchers in the past was striking. Nebraska synthetics 27 and 28 were about equal in resistance to both aphids and to three other experimentals: California E-10, Kansas Syn. 6, and Nevada Syn-T, developed at other locations for combined resistance to spotted alfalfa and pea aphids. A sample of Oklahoma Common, as well as standard varieties Buffalo, Cody, and Ranger, were heavily damaged.

Synthetics developed for combined aphid resistance were also evaluated for response to high pea aphid populations in a replicated

Table 14. Appraisal of various parental progenies, synthetics, and checks in a field cage after exposure to heavy induced pea aphid infestation.

Entry[a]	Visual field rating[b]	Yield		Stand remaining after infestation	
		Green wt. (in grams)[c]	Duncan's multiple range	Percent stand	Duncan's multiple range
Narrow Polycross Progenies of Parental Clones of Nebraska Syn. 27					
15-3291	1.0	42.5	f–k	100	e
15-3309	1.0	47.0	i–k	100	e
15-3638	1.0	40.5	f–k	94	d–e
15-3640	1.5	38.5	f–k	100	e
15-3641	1.5	32.0	e–j	100	e
15-3692	1.5	24.5	b–f	92	c–e
15-3694	1.5	27.0	c–h	92	c–e
15-3702	1.0	45.0	g–k	96	c–e
Average	1.2	37.1		97	
Open-pollinated Progenies of Parental Clones of Nebraska Syn. 27					
19-3291	3.0	17.5	a–e	100	e
19-3309	2.0	28.0	d–i	91	c–e
19-3638	1.0	39.0	f–k	94	d–e
19-3640	2.5	26.0	c–f	92	c–e
19-3641	2.0	27.5	d–i	100	e
19-3692	2.5	26.0	c–k	92	b–e
19-3694	2.5	24.5	b–f	99	d–e
19-3702	2.0	26.5	c–g	100	e
Average	2.2	26.9		96	
Experimental Synthetics or Populations Developed for Combined Resistance					
N.S.-27 Syn-1	1.0	41.0	f–k	100	e
N.S.-28 Syn-1	4.0	8.0	a–c	89	b–e
California E-10	1.0	46.0	h–k	92	c–e
K.S.-6 Syn-1	1.0	55.0	k	100	e
Nevada Syn-T Syn-1	1.0	51.5	j–k	99	d–e
Average	1.6	40.3		96	
Experimental Synthetics Bred for Spotted Alfalfa Aphid Resistance Only					
N.S.-20 Syn-2	5.0	2.0	a	71	a–e
N.S.-21 Syn-1	3.5	12.5	a–d	99	d–e
Average	4.2	7.2		85	
Checks					
Buffalo	5.0	1.5	a	57	a
Cody	5.0	3.0	a	66	a–c
Du Puits	4.5	2.0	a	46	a–b
Ladak	5.0	1.0	a	64	a–c
Lahontan	3.5	17.5	a–e	92	c–e
Okla. Common	5.0	1.5	a	83	a–d
Ranger	5.0	1.5	a	59	a–b
Vernal	5.0	2.0	a	94	d–e
501 Tourneur	4.5	1.0	a	78	a–d
Average	4.7	3.4		71	

[a] Seeded: April 23, 1962. Infested: May 5, 1962. Completed: June 5, 1962.
[b] 1 = Resistant, no damage or stunting; 5 = 100% Susceptible, damage or stunting.
[c] Center 18" of plot.

test in a field cage (Figure 1). Additional varieties and narrow polycross and open-pollinated progenies of parental clones of Nebraska Syn. 27 also were included. The heavy infestation induced by repeated aphid introductions resulted in a critical test. Visual ratings on uniform stands are in Table 14. Generally there was good agreement between greenhouse and field reactions, except for such entries as Nebraska Syn. 20 and Du Puits, which were intermediate in the greenhouse but susceptible in the field cage. Neb. Syn. 28 appeared nearly susceptible in the field cage but resistant in the greenhouse. Differences in reaction between these greenhouse and field tests may have been due to greater infestation intensity in the field cage than in the greenhouse, which reduced the tolerance factor of certain entries. Lahontan and Neb. Syn. 21 responded similarly to the pea aphid at both locations.

Higher levels of resistance were present in narrow polycross progenies than in open-pollinated progenies (Table 14). However, levels of resistance in progenies from open-pollinated seed were well above the intermediate and susceptible standards. It appeared that open-pollinated seed could be used to index progenies for pea aphid reaction.

Green weights taken when seedlings were 6 weeks old agreed with visual ratings, as would be expected. Susceptible entries produced from 1 to 3 (avg. 1.7) grams per plot; intermediate entries, 8 to 17.5 (avg. 13.9) grams, and resistant entries 24.5 to 55 (avg. 36.2) grams per plot. Resistant entries produced an average of 21.2 times more forage per plot than suceptible entries.

In many cases, large portions of susceptible rows were killed by aphids. The stand of susceptible entries ranged from 46% to 94%, intermediate from 89% to 100%, and resistant from 91% to 100%. Average stands of suceptible, intermediate, and resistant entries were 68.7%, 95.0%, and 96.5% respectively. The average stand of resistant entries was 140% that of the suceptible entries.

Comparisons of Alfalfa Resistance to Asexual and Sexual Egg-Laying Strains of the Spotted Alfalfa Aphid

Detection of an egg-laying sexual strain of the spotted alfalfa aphid in central Nebraska (25) and its subsequent spread exposes alfalfa in this area to damage by a distinctly different biotype. Recombinations of genes which may have produced the sexual strain could have resulted in genotypically different parthenogenetic forms (aliencolae) which cause the major injury to alfalfa.

To determine possible differences in plant response to injury and aphid antibiosis reactions by aliencolae originating from the sexual strain and those from the normally holoparthenogenetic strain, tests involving important spotted alfalfa aphid-resistant and appro-

priate susceptible materials were conducted. Cultures of aliencolae from the sexual strain were increased in the early spring of 1962 from field-collected stem mothers confined on susceptible alfalfa in fine-mesh cages. The usual parthenogenetic strain of Southwest Nebraska origin was used for comparison in two test series by using the standard methods previously described. Antibiosis tests of the two aphid strains were conducted simultaneously on spotted alfalfa aphid-resistant clones. The reactions of certain progenies, experimental synthetics, and varieties also were compared in concurrent tests with both aphid strains. The results of both teests, recorded in Tables 15 and 16, indicated few or no distinct differences in overall appraisals. Aliencolae of sexual and asexual origin cause similar damage to the open-pollinated progenies of parental clones of N.S. 27 in mass infestation tests as shown in Table 16. No observations were made on the feeding behavior and development of sexuals on resistant and susceptible plants.

Table 15. Antibiosis reaction of selected clones to the parthenogenetic and sexual strains of the spotted alfalfa aphid.

Clone No.	Rating	
	Parthenogenetic strain	Sexual strain
	Some of the Parental Clones of N.S. 20	
Ranger 12	HR	HR
Ranger 47	HR	HR
Ranger 57	HR	HR
Ranger 62	HR	HR
	Parental Clones of N.S. 21	
3121	HR	HR
3125	HR	HR
3127	HR	HR
3129	HR	HR
3130	HR	HR
3144	HR	HR
	Some of the Parental Clones of N.S. 27	
3640	HR	HR
3641	HR	HR
3692	HR	HR
3694	HR	HR
3702	HR	HR
	Some of the Parental Clones of N.S. 28	
3292	HR	HR
3308	HR	HR
3318	HR	HR
	Check Clones	
3309	HR	*HR*
Caliverde Selection	S	S

Table 16. Greenhouse reaction of progenies, experimental synthetics, and varieties to the parthenogenetic and sexual strains of the spotted alfalfa aphid.

Clone No.	Rating	
	Parthenogenetic strain	Sexual strain
Open-pollinated Progenies of Parental Clones of N.S. 27		
19-3291	2.5	1.8
19-3309	1.0	1.3
19-3638	1.5	1.3
19-3640	1.5	1.0
19-3641	1.5	1.3
19-3692	2.0	1.3
19-3694	2.0	1.3
19-3702	1.0	1.0
Experimental Synthetics		
N.S. 19 Syn-1	2.0	1.5
N.S. 20 Syn-1	2.0	2.0
N.S. 21 Syn-1	2.5	1.3
N.S. 27 Syn-1	2.0	1.0
N.S. 28 Syn-2	2.0	1.8
Varieties		
Buffalo	4.3	4.5
Ranger	3.6	4.3
Cody	2.5	2.0
Lahontan	2.6	1.8

APPENDIX

Table 1. Identification, origin, and classification of foreign plant introductions based on response to pea and spotted alfalfa aphid in mass infestation tests.

PLANT INJURY CLASS

PEA APHID
RESISTANT
SPOTTED ALFALFA APHID

Resistant	*Intermediate*		*Susceptible*
220299 Afg.	206103 Fr.	217419 Den.	190259 Alg.
220668 Afg.	206111 Fr.	220298 Afg.	206105 Fr.
	211608 Afg.		

PEA APHID
INTERMEDIATE
SPOTTED ALFALFA APHID

Resistant	*Intermediate*		*Susceptible*	
201864 Iran	164415 Ind.	220530 Afg.	170553 Turk.	205887 Fr.
234205 Iran	170543 Turk.	220531 Afg.	190258 Alg.	205891 Fr.
	188868 Can.	220808 Afg.	199273 Port.	206100 Fr.
	201863 Iran	221469 Afg.	199274 Port.	206106 Fr.
	207775 Eng.	222733 Iran	199275 Port.	206110 Fr.
	208072 Turk.	235736 Ind.	199276 Port.	206113 Fr.
	211609 Afg.	244085 Italy	199277 Port.	208683 Alg.
	217648 Iraq		199279 Port.	215671 Swed.
	219928 Afg.		199281 Port.	233712 Italy
			204903 Turk.	

PEA APHID
SUSCEPTIBLE
SPOTTED ALFALFA APHID

Resistant	*Intermediate*		*Susceptible*		
		162787 Arg.			
207495 Afg.	204590 Turk.	167068 Turk.	206281 Turk.	222199 Afg.	
212861 Afg.	204885 Turk.	170532 Turk.	206282 Turk.	222729 Iran	
222734 Iran	205297 Turk.	170536 Turk.	206283 Turk.	222730 Iran	
223787 Afg.	206108 Fr.	170551 Turk.	206286 Turk.	222731 Iran	
228153 U.S.S.R.	206900 Turk.	179615 U.S.A.	206340 S.Af.	222732 Iran	
239950 Iran	206903 Turk.	187004 Ia.	206451 Turk.	223386 Iran	
239952 Afg.	208115 Afg.	189393 N.Z.	206452 Turk.	223387 Iran	
	211054 Afg.	193291 Yugo.	206453 Turk.	226518 Iran	
	211606 Afg.	198962 Cyp.	206454 Turk.	226684 Guat.	
	211607 Afg.	198963 Cyp.	206572 Greece	227851 Iran	
	211610 Afg.	199280 Port.	206573 Greece	228349 Iran	
	212104 Afg.	199305 Peru	206574 Greece	229570 Greece	
	212860 Afg.	202291 Arg.	206575 Greece	229954 Iran	
	220301 Afg.	204457 Turk.	206576 Greece	229955 Iran	
	222111 Afg.	204458 Turk.	206697 Turk.	230783 Ind.	
	222735 Iran	204459 Turk.	206698 Turk.	232927 Hung.	
	222999 Iran	204460 Turk.	207494 Afg.	233195 U.S.S.R.	
	223788 Afg.	204461 Turk.	208115 Afg.	233198 U.S.S.R.	
	226471 Iran	204591 Turk.	209090 Peru	233199 U.S.S.R.	
	228287 Iran	204593 Turk.	209091 Peru	233200 U.S.S.R.	
	233197 U.S.S.R.	204886 Turk.	210367 Iran	233201 U.S.S.R.	
	234438 Arg.	204890 Turk.	210440 Iraq	233713 Italy	
	241442 Austra.	204891 Turk.	210763 Spain	233714 Italy	
	243224 Iran	205198 Turk.	212105 Afg.	233715 Italy	
	244084 Italy	205329 Peru	212106 Afg.	234443 Belg.	
	244085 Italy	205634 Arg.	212367 Iraq	236607 Fr.	
		205888 Fr.	212368 Iraq	236608 Fr.	
		205889 Fr.	212512 Afg.	236614 Fr.	
		206101 Fr.	212798 Iran	236615 Fr.	
		206102 Fr.	212858 Afg.	237215 Uru.	
		206109 Fr.	212859 Afg.	239946 Turk.	
		206277 Turk.	213005 Ind.	239948 Iran	
		206279 Turk.	219927 Afg.	239951 Iran	
		206280 Turk.	220598 Afg.	239953 Alg.	
			222113 Afg.	239956 Alg.	
			222198 Afg.	244084 Italy	

Table 2. Greenhouse classifications based on antibiosis tests and field appraisal of alfalfa "C" clones to the spotted alfalfa aphid.[a]

Clone No.	Field injury California[a]	Clone No.	Field injury California	Clone No.	Field injury California
		Highly Resistant in Antibiosis Tests			
C3	HR	C89	HR	C242	HR
C7	HR	C93	HR	C607	HR
C27	HR	C176	HR	C616	HR
C32	HR	C218	HR	C634	HR
C40	R	C220	R	C900	HR
C84	HR				
		Resistant in Antibiosis Tests			
C17	HR	C219	R	C623	R
C44	HR	C236	HR	C638	R
		Intermediate in Antibiosis Tests			
C18	HS	C223	R	C611	I
C221	S	C247	....	C625	I
		Susceptible in Antibiosis Tests			
C9	S	C113	R	C231	S
C11	HS	C114	HR	C235	S
C12	R	C117	I	C240	I
C41	S	C121	HS	C244	HR
C45	R	C172	HS	C606	S
C51	I	C184	I	C615	S
C63	HS	C217	I	C627	HS
C110	I	C228	S	C631	HS
		Highly Susceptible in Antibiosis Tests			
C1	HS	C138	S	C255	S
C2	S	C141	HS	C600	HS
C5	S	C144	S	C601	HS
C8	S	C162	HS	C603	HS
C10	HS	C174	HS	C604	HS
C22	HS	C177	HS	C608	HS
C33	HS	C180	HS	C609	HS
C35	HS	C183	HS	C610	HS
C36	HS	C186	HS	C612	S
C39	HS	C187	I	C613	HS
C42	HS	C188	HS	C614	S
C46	S	C190	HS	C617	HS
C48	HS	C191	HS	C619	HS
C53	HS	C193	HS	C620	HS
C54	HS	C195	HS	C621	HS
C57	HS	C196	HS	C622	HS
C60	I	C197	I	C624	HS
C64	HS	C199	HS	C626	HS
C87	S	C216	HS	C628	HS
C88	I	C223	HS	C629	HS
C91	HS	C224	HS	C630	HS
C109	R	C225	S	C632	HS
C112	HR	C229	I	C633	HS
C115	HS	C230	HS	C635	I
C118	S	C234	HS	C636	S
C120	HS	C238	HS	C637	HS
C125	HS	C239	I	C639	HS
C126	HS	C241	HS	C640	HS
C127	HS	C245	HS	C641	HS
C130	S	C248	HS	C642	HS
C134	HS				

[a] Reference: Test sequence A.

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