WEEVIL TOLERANT CULTIVARS AND OKLAHOMA COMMON ALFALFAS AS SOURCES OF RESISTANCE FOR THE SPOTTED ALFALFA APHID

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CHAPTER I

INTRODUCTION

Alfalfa, Medicago sativa L., was introduced into the United States in the early 1700's and it has since become this country's most important forage crop. Production of alfalfa hay and seed provides an important cash income for Oklahoma farmers who harvested some 200,000 hectares of alfalfa in 1979.[1]

Other than the alfalfa weevil, Hypera postica (Gyllenhal), the spotted alfalfa aphid, Therioaphis maculata (Buckton), is the most destructive insect pest of alfalfa in Oklahoma. This aphid causes economic losses in alfalfa hay and seed production by removal of plant juices, injection of a toxin, and excessive honeydew formation (Nickel and Sylvester 1959). The spotted alfalfa aphid (hereafter referred to as the SAA) caused widespread damage for a few years after it invaded Oklahoma in 1954, but economic infestations have been sporadic and unpredictable during the last 2 decades. Although heavy infestations have been reported periodically from all alfalfa growing regions, there have been years when the pest has been all but nonexistent in most areas of the state. Despite the annual inconsistencies of the SAA, several trends can be extracted from the pest's 28 year history in Oklahoma. The SAA is basically a warm, dry weather pest which develops economic

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[1] Personal communication, F. E. LeGrand, Department of Agronomy, Oklahoma State University.

infestations most commonly in the late spring and summer, although fall planted alfalfa is often attacked in October and November. Finally, the SAA appears to be a more frequent pest in the southwestern portion of Oklahoma.[2]

Control measures are necessary to insure profitable alfalfa production in the presence of SAA infestations. There are several insecticides available which provide adequate suppression of SAA (Coppock 1976), but chemical agents often produce undesirable side effects such as residues and destruction of beneficial arthropods (Fenton 1959). Furthermore, the use of insecticides can be quite costly if multiple applications are necessitated by resurgence of a previously suppressed population.

Expenses involved with insect control can be reduced and sometimes eliminated by the use of resistant cultivars. Oklahoma growers can choose from numerous cultivars with high levels of SAA resistance. There are also several cultivars available with moderate resistance to the alfalfa weevil. However, growers face a dilemma in deciding which type of resistance is the most important because the choice of commercial cultivars with proven resistance to both pests is quite limited. The alfalfa weevil has been a serious pest over the state since 1971, but damage from this species is confined primarily to the period prior to first cutting, whereas the SAA is a potential threat throughout the growing season (Berberet and Pinkston 1976). The problem of controlling both could be simplified by developing cultivars adapted to Oklahoma with combined resistance to the SAA and the alfalfa weevil; thus reducing the need of insecticides for both pests.

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[2] Personal communication, Don Arnold, Department of Entomology, Oklahoma State University.

Oklahoma Commons are naturalized alfalfa strains which comprise a large percentage of the alfalfa acreage in Oklahoma. Oklahoma Commons are preferred over commercial cultivars by many growers because of their high yield potential which results from a high degree of adaption to Oklahoma conditions. Their reaction to SAA attack has not been documented. Oklahoma Commons are not known to possess significant levels of SAA resistance from the standpoint of reducing crop damage, however, their potential as a source of SAA resistance has not been investigated.

The primary objective of my research was to screen alfalfa cultivars adapted to Oklahoma for SAA resistance. Secondly, this research was designed to develop or adapt methods and techniques for investigating SAA resistance in alfalfa. Parental clones selected from weevil tolerant cultivars were used to develop experimental alfalfas with high levels of resistance to both the SAA and the pea aphid, Acyrthosiphon pisum (Harris). In addition, several strains of Oklahoma Common alfalfa were evaluated and screened for SAA resistance.

CHAPTER II

REVIEW OF LITERATURE

The SAA was first reported damaging alfalfa in the United States in February of 1954 (Sorenson et al. 1972). Following its discovery in New Mexico this pest apparently spread quite rapidly and was reported in Oklahoma by July of that same year (Bieberdorf and Bryan 1956). By March 1955 the SAA was inflicting serious damage in south central Oklahoma; a year in which nationwide damage was estimated at $42 million (Sorenson et al. 1972). The SAA is currently distributed throughout most of the alfalfa producing regions of the United States, and it is a serious problem primarily from Nebraska southwest to Southern California (Barnes et al. 1974).

Host plant resistance is probably the most desirable alternative to chemical control because resistant cultivars provide pest protection with no additional cost to the grower for labor or materials. Painter (1951, 1958) reviewed the importance and potential of insect resistant plants. Perhaps no other insect pest has done more to enhance the credibility and feasibility of host plant resistance than the SAA. It is estimated that alfalfa growers save $35 million annually by using SAA resistant cultivars (Luginbill 1969). 'Lahontan' was the first alfalfa cultivar to exhibit resistance to the SAA (Howe and Smith 1957). Although Lahontan was developed for disease and nematode resistance, this cultivar has been grown extensively in areas of Arizona, Nevada,

and California for its coincidental resistance to the SAA. Since the advent of Lahontan over 30 SAA resistant cultivars have been released for commercial production (Nielson and Lehman 1980). 'Moapa' the first cultivar developed specifically with SAA resistance, was released to growers in the Southwest in 1957 (Smith et al. 1958), and 'Zia' was released the following year primarily for use in New Mexico (Anonymous 1968). The development cost of Moapa was estimated to be only $30,000 (Hanson 1961). 'Cody' was the first cultivar developed with SAA resistance for the Central Plains (Harvey et al. 1960). There are numerous SAA resistant cultivars presently available for Oklahoma production, and many of these are multiple pest resistant, e.g. 'Dawson', 'Riley', 'Kanza', Pioneer brand '530', and 'WL 318' (Caddel and Taliaferro 1979).

Painter (1951) classified resistance as seen in the field as either tolerance, antibiosis, or nonpreference. The literature is not conclusive in regard to the exact mechanism which imparts resistance in alfalfa to the SAA. However, studies which investigated a limited number of clones provide convincing evidence that SAA resistance affects host plant utilization, i.e. extreme nonpreference and antibiosis (Kindler and Staples 1969, Kircher et al. 1970, Kishaba and Manglitz 1965, and McMurty and Stanford 1960).

Confinement of SAA to certain resistant clones results in high mortality. However, observations of aphid behavior in the absence of confinement devices reveal that the SAA will not remain on a resistant clone and die, but rather will migrate to a suitable host (Kishaba and Manglitz 1965). Several investigations revealed that SAA confined to resistant clones and SAA maintained under starvation showed no difference in mortality when both groups were transferred to susceptible alfalfa

after 22-32 hours (Kindler and Staples 1969, Kishaba and Manglitz 1965, and McMurty and Stanford 1960). Furthermore, mortality of SAA confined to resistant clones does not exceed that of SAA confined without food (McMurty and Stanford 1960). These findings discount the presence of a plant substance toxic to the SAA and suggest that mortality results from starvation and desiccation.

Apparently resistant alfalfa does not satisfy the dietary requirements of the SAA and this results from either a deficiency of certain nutrients, the presence of a feeding deterrent, or the absence of a feeding stimulant. Kircher et al. (1970) discounted the last 2 possibilities in a study where plant juices were extracted from resistant and susceptible clones and then reciprocally infused into excised stems of each clone with no effect on aphid response. They concluded that resistance was probably due to lower levels of nutrients, but these are not known. Studies have shown that susceptible and resistant clones were equal in quantity of soluble nitrogen and carbohydrates, and amino acid composition, especially those required by phytophagous insects, did not differ sufficiently to explain aphid behavior (Kircher et al. 1970 and Marble et al. 1959). Moreover, attempts to transfer resistance and susceptibility through reciprocal grafting had no effect on aphid reaction for either the scion or stock (Harvey and Hackerott 1958). Nielson and Don (1968) suggested that resistance might be explained by the phytoalexin theory based on a chemical reaction between the plant and aphid.

Tolerance is not a predominant mechanism in resistant cultivars, but its value as a potential component for SAA resistance has been investigated (Jones et al. 1968). A relationship between tolerance and

auxin content was found to exist in reference to the absence of neutral auxins in tolerant plants (Maxwell and Painter 1962).

Environmental factors are known to affect the expression of insect resistance in plants (Painter 1951). McMurty (1962) conducted an extensive investigation of SAA resistance in relation to temperature, light duration and intensity, soil moisture, plant mineral nutrition, and physiological age of host leaves. Temperature was the most important factor, but mineral nutrition also effected the expression of resistance. In general, resistant cultivars are more effective at higher temperatures but the temperature range and degree of change in the expression of resistance is dependent on the cultivar or clone that is being tested (Hackerott and Harvey 1959, Isaak et al. 1963, and Schalk et al. 1969). It is assumed that changes in resistance at low temperatures are due primarily to changes in the plant which affect aphid survival and reproduction (McMurty 1962). Resistance in alfalfa is also known to be affected by excessive or deficient treatment with various nutrients. Resistance decreases in plants grown in a nutrient solution deficient in potassium while resistance is increased in plants receiving deficient levels of phosphorus (Kindler and Staples 1970a and McMurty 1962). Excesses of magnesium or nitrogen also decrease resistance, but various concentrations of sulfur do not alter resistance (Kindler and Staples 1970a). Humidity, photoperiod, and level of soil moisture have not been shown to alter aphid reaction to resistant alfalfa (Isaak et al. 1963, Kindler and Staples 1970b, and McMurty 1962).

Perhaps the greatest single threat to the stability of plant resistance is the development of insect biotypes. Pesho and Lieberman (1960) observed the presence of a virulent biotype on Moapa alfalfa in 1958.

Presently, there are 7 biotypes of the SAA recognized in the southwestern United States (Nielson and Lehman 1980). Although the use of insecticides has undoubtably contributed to biotype development, several of the biotypes appear to have resulted directly from the selective pressure of resistant plants (Nielson et al. 1971). Nielson and Don (1974) investigated the interrelationship between virulence of the SAA and resistance in alfalfa. However, there has been no evidence of biotype development to resistant cultivars commonly grown in the Central Plains.

Improvement through selection is the fundamental breeding concept in alfalfa. Busbice et al. (1972) reviewed selection under 2 broad categories: selection after progeny testing and selection without progeny testing. In the latter method selection is based strictly on the phenotype of the plant and is only effective for those characters that are highly heritable, e.g. disease and insect resistance. Nevertheless, it often requires more than 1 cycle of selection (recurrent phenotypic selection) to develop a synthetic population with the desired gene frequency (Hunt et al. 1971). Selection after progeny testing provides at least some idea of the genotypic potential or breeding value of a plant. Both the S_1 and polycross progeny tests have been utilized in the development of numerous alfalfa cultivars (Sherwood et al. 1967). Johnson (1968) reported the value of S_1 progeny testing for disease and insect resistance. However, the polycross and especially the S_1 progeny tests require a large expenditure of resources and are practical only after most of the population has been eliminated by phenotypic selection.

Selection for SAA resistance within an adapted cultivar has been used successfully and is probably a more expedient method of breeding

than trying to transfer resistance from foreign sources (Harvey et al. 1960). Although most sources of alfalfa contain some resistant plants, higher levels of resistance and higher gene frequencies for resistance have been found in cultivars involving Turkestan heritage (Hackerott et al. 1958).

Selection for SAA resistance has been conducted in both the greenhouse and field with equal success (Howe et al. 1965). Hackerott and Harvey (1959) showed a similar relationship in differences between resistant and susceptible plants in the greenhouse and those in the field. Greenhouse screening is usually preferred at least in the initial phases of selection because factors such as timing and degree of infestation can be controlled.

Although different stages of plant growth have been utilized, the seedling stage has proved the most expedient because large numbers of plants can be screened in a short period of time (Harvey et al. 1960). Initial screening usually involves infesting flats of seedlings with large numbers of SAA (preference test or a mass infestation test) (Howe et al. 1965). Seedlings which survive mass infestation are routinely retested individually in a more advanced stage of growth to verify resistance (Howe and Pesho 1960). Mass infestation survivors include plants with various degrees of resistance and possibly susceptible escape plants (Harvey and Hackerott 1956). Harvey et al. (1960) demonstrated that mass infestation as a sole source of selection does not increase resistance in 1 cycle of selection as effectively as when seedling survivors are retested. Furthermore, the mass infestation test measures only plant response while the individual plant test also analyzes aphid response in respect to the effect of the plant on the

biology of the aphid (Howe et al. 1965). The individual plant test, also referred to as an antibiosis test, has been conducted on whole plants and plant parts, e.g. single stems and trifoliolate leaves (Harvey et al. 1960 and Howe et al. 1965). Confinement devices such as chimney lamps and clear plastic cages have been utilized in antibiosis tests to provide more accurate measurements of aphid survival and reproduction (Howe et al. 1965 and Peters and Painter 1958). Use of excised plant parts often enhances ease of screening and evaluation, but Thomas and Sorensen (1971) have shown that excised plant parts may tend to underestimate the resistance level of a plant.

Polycross and S_1 seedling progenies can be effectively evaluated in the greenhouse (Howe et al. 1965). Harvey et al. (1960) failed to show a significant correlation between polycross progeny seedling survival and antibiosis results for 22 clones which were all designated as highly resistant. However, this relationship was favorable when 2 groups of clones with marked differences in resistance were compared.

In view of increasing production costs it is highly desirable to develop cultivars with resistance to more than 1 pest. Moreover, multiple pest resistance can function as an integral and compatible component of an integrated pest management system because several pests can be controlled by simply utilizing the appropriate cultivar. Sorensen et al. (1972), Kehr et al. (1972), and Hunt et al. (1972) reviewed the progress made in host plant resistance for insect, disease, and nematode pests of alfalfa. Other than the SAA, the most spectacular advances in insect resistance have been made with the pea aphid. Breeders have had considerable success in combining high levels of resistance to both species of aphids, e.g. Kanza, Dawson, and 'Washoe' (Kindler and Schalk 1975).

Furthermore, Nielson and Lehman (1977) have developed a cultivar, 'CUF-101', with combined resistance to the pea aphid, the SAA, and the blue aphid, Acyrthosiphon kondoi Shinji, a relatively new pest of alfalfa in this country. The alfalfa weevil is probably the most destructive pest of alfalfa in the United States, but efforts to develop a high level of resistance to it have not been successful (Nielson and Lehman 1980). 'Team', 'Arc', and 'Liberty' are 3 released cultivars which were selected from Starnes germplasm (Nielson and Lehman 1980). These cultivars exhibit a moderate degree of tolerance to weevil feeding, and they also contain high levels of resistance to the pea aphid. However, they are highly susceptible to the SAA. Considering the insect pest problems in Oklahoma, growers need alfalfa cultivars with at least some degree of resistance to both the alfalfa weevil and the SAA.

CHAPTER III

SELECTION OF SPOTTED ALFALFA APHID RESISTANCE FROM ARC

Introduction

Arc alfalfa is a high yielding multiple pest resistant cultivar. Arc possesses high resistance to the pea aphid and anthracnose, Colletotrichum trifolii Bain, and moderate resistance to the alfalfa weevil and bacterial wilt, Corynebacterium insidiosum (McCull) (Devine et al. 1977). All of these characters are important to yield and stand longevity, but weevil resistance is perhaps the most outstanding feature of Arc because of the widespread economic impact of the pest. Weevil resistance is expressed in the form of a moderate tolerance which is attributed to the rapid and early spring growth. Arc has heavy stem terminals and well developed axillary buds which enable the crop to continue growth under moderate weevil infestations (Devine et al. 1977). However, Arc is highly susceptible to the SAA and this is perhaps its major deficiency for production of alfalfa in Oklahoma. The objective of this study was to develop an experimental alfalfa similar to Arc but with resistance to the SAA.

Methods and Procedures

Arc was screened for resistance to the SAA in 1 cycle of selection. The cycle consisted of a sequence of 3 selection processes in which the

initial population was progressively narrowed to a relatively small number of plants with SAA resistance. All 3 of the processes were conducted in the greenhouse. The SAA used in screening were reared in the greenhouse on susceptible alfalfa cultivars. The aphid colony was periodically renewed with progeny of field collections from various locations in Oklahoma.

It was not possible to maintain a constant temperature or a consistent range of temperatures in the greenhouse during this study. Temperatures in the late spring, summer, and early fall varied considerably according to outdoor temperatures, i.e. 18^{o}-38^{o} C. The greenhouse was heated during the winter months and other intermittent cold periods to provide a relatively warm environment, i.e. $27^{o}\pm5^{o}$ C.

The initial step of the selection cycle was a mass infestation or preference test. The objective of this procedure was to allow the aphids to kill the susceptible seedlings so that resistant candidates could be identified. Selection was based on a qualitative criterion in that plants either lived or died and those which were not killed apparently possessed some degree of resistance. Approximately 24,000 Arc plants were screened during the summer of 1978. The test alfalfa was grown in metal flats (35x50x9 cm), and each flat contained ca. 1,000 plants. The soil medium consisted of a mixture of 4 parts sandy loam, 1 part sand and 1 part peat which was mulched with ca. 2 cm of vermiculite after seeds were planted. Flats were fertilized once with a soluble, complete fertilizer with trace elements.

Each flat had 9 rows of alfalfa which were evenly spaced 5 cm apart. A total of 8 rows contained Arc seedlings and one row contained 'Buffalo', a susceptible standard which was planted in half rows of 7 and 3. This

flat design was altered for the last 6 flats to include Kanza, a resistant standard of known performance. Row 5 contained a half row of Kanza and a half row of Buffalo, while the remaining 8 rows contained Arc. The resistant and susceptible standards were used to gauge aphid damage within each flat. Six flats were planted on each of 4 planting dates; May 15, May 25, June 1, and June 6. The flats were infested with aphids when the seedling plants were in the single trifoliolate leaf stage. Approximately 1 cc of aphids (nymphs and adults) were evenly distributed over the 9 rows of a flat. Some of the flats received a subsequent infestation during the course of the test to facilitate screening.

Flats were not exposed to aphids for a uniform number of days, but rather duration of screening was determined primarily by the performance of the susceptible check. In most instances flats were sprayed with a short residual insecticide (e.g. malathion) when the Buffalo was 98% dead. The performance of the resistant check as well as the performance of the test alfalfa relative to its expected reaction to SAA were also observed for indications of screening effectiveness. Duration of screening ranged from 21-67 days and averaged 44 days per flat. After the aphid infestation was terminated each flat was set aside in isolation for 2-3 weeks so that seedling survivors could recover and resume growth.

The primary purpose of the individual plant test was to eliminate any susceptible plants which escaped infestation during the mass infestation test and to eliminate plants with marginal levels of resistance. A maximum of 20 plants were selected from each flat, and these plants were transplanted into plastic pots 15 cm in diameter. The soil medium consisted of a mixture of 2 parts sand, 2 parts perlite, 1 part peat, and 1 part soil. Plants received 1 application of a soluble, complete

fertilizer with trace elements. Selection of the 20 seedlings from each flat for transplanting was conducted with preference given to those plants which exhibited superior size, vigor, and root system development. Seedling survivors were retested for SAA resistance after they had adjusted to the transplant and resumed normal growth. Plants were always tested in groups according to the flat where they originated.

Aphid infestation was initiated by placing 10 adult, apterous aphids at the base of each plant. Plants were inspected at weekly intervals for 4 weeks. Aphid counts were made at the first 2 inspections, and damage ratings were made at all 4 inspections. Aphid counts were limited to 50 at the first inspection and 75 the following week. Plants which supported aphid numbers in excess of the limit were so designated. Damage ratings were made on a scale of 0-10 with 0 representing a dead plant and 10 indicating a plant with no evidence of SAA damage. The 8 and 9 ratings represented vigorous plants which exhibited various degrees of localized chlorosis and vein banding. Plants rated a 7 exhibited generalized chlorosis but no wilting. Ratings 4-6 included plants with extreme chlorosis and various degrees of wilting. Plants in the 1-3 range exhibited extreme wilting and various degrees of necrosis and leaf drop. In most instances plants with a damage rating of 8 or better at the final inspection were selected for further evaluation. Plant selections were sprayed with a systemic insecticide (e.g. dimethoate) and then transplanted into plastic pots 18 cm in diameter.

The final step in the selection of SAA resistance from Arc was a self-pollinated (S_1) progeny test. The progeny test is perhaps the best method of determining the merit of a plant selection because it tests the heritability of the character for which the parent plant was selected.

Plants were self-pollinated in the greenhouse to produce S_1 seed for progeny testing. Due to the number of plants and the labor requirements involved with self-pollination, the plants were divided into 2 groups. Seed was produced in the first group during the fall and winter (1978-1979), and in the second group during the winter and spring (1979). These 2 groups of plants will hereafter be referred to as Arc I and Arc II, respectively. During selfing the plants were maintained under a 16 hour photoperiod with florescent lighting. Several stems (ca. 4-6) of each plant were supported with bamboo stakes embedded in the pot, while additional stem growth was periodically cut back near the crown of the plant. Plants were fertilized periodically with a soluble, complete fertilizer with trace elements.

Selfing was initiated at the onset of flowering and was continued until plants had either set a sufficient number of seed for progeny testing or it became apparent that they would not produce self-pollinated seed. Flowers were tripped by inserting a flat toothpick into the throat of a flower causing the sexual parts to snap forward and allow pollen to come in contact with the stigma (Barnes and Stephenson 1971). Plants were inspected at 2 day intervals and all new flowers present were tripped.

During the selfing process plant selections were cloned by taking 5 vegetative cuttings from each plant. A cutting consisted of a segment of stem 8-12 cm in length which had 2 nodes. The trifoliolate leaf was removed from the lower node. The stem was dipped in Rootone® and then inserted into a sand medium until the lower node was beneath the surface. Cuttings were established in plastic flats (28x54x6 cm) which contained 10 rows spaced 4 cm apart. The flats were watered 3 or 4 times daily

with a fine mist hose nozzle until it was evident that root system development had been initiated.

Plant selections were progeny tested in 2 groups which corresponded with the self-pollination grouping. S_1 progeny of the Arc I plants were tested in the spring and the Arc II plants were progeny tested in the summer. Each parent plant was progeny tested 3 times provided that sufficient self-pollinated seed was available. Labor and facility limitations permitted planting of 2 flats every 2-3 days. S_1 progeny families were grown in metal flats and evaluated in the seedling stage for their reaction to SAA infestation. Each flat contained 9 rows which were spaced 5 cm apart. Entries were assigned rows in flats at random for each replication. Rows 2, 5, and 8 contained commercial cultivars of known performance, and these were used to gauge SAA intensity within the flat. Rows 2 and 8 contained Arc, the parent cultivar. Row 5 contained a half row of Kanza, the resistant standard, and a half row of Buffalo, the susceptible standard.

Sufficient seed was planted to insure at least 50 plants per entry in rows 1-4 and 6-9, while sufficient seed was planted in row 5 to insure at least 25 plants per entry. S_1 seed was hand scarified with coarse sand paper to eliminate hard seed dormancy. The soil medium consisted of a mixture of 4 parts sand, 2 parts clay loam, and 1 part peat, and this mixture was covered with ca. 2 cm of vermiculite after the seed was planted. Flats were fertilized once with a soluble, complete fertilizer with trace elements. Stand counts were taken for all entries 7-10 days after planting. Two days prior to infestation rows 1-4 and 6-9 were thinned to 50 plants, and row 5 was thinned to 25 plants per entry.

The flats were infested with SAA when plants were in the single

trifoliolate leaf stage. Approximately 1 cc of SAA (adults and nymphs) were evenly distributed over the 9 rows. When the Buffalo appeared 90% dead the SAA infestation was terminated by spraying the flat with a systemic insecticide (e.g. dimethoate). At this point all entries except for the susceptible standards received an appearance grade for the surviving plants. Rows were graded on a scale of 1-9 with 1 representing a row of plants with extreme damage and 9 representing a row of plants with no apparent aphid damage. In general, rows which received a rating in the range of 7-8 exhibited various degrees of vein banding and localized chlorosis. Rows rated in the 4-6 range exhibited various degrees of generalized chlorosis and minimal wilting. Rows rated in the 1-3 range exhibited extreme chlorosis, wilting, and partial necrosis. The number of living plants per entry were counted 4 days after the flat was sprayed with insecticide. The purpose of the 4 day interval between spraying and counting was to reduce indecision in determining living plants from those that were dead.

The final selection for SYN 0 plants was based on progeny test results. Plants which averaged ca. 50% or more progeny survival were used to develop 2 new alfalfa populations with improved SAA resistance. SYN 0 plants from both groups were transplanted to Cow Creek Bottom, Agronomy Farm, Stillwater, during 1979. Selections from Arc I were transplanted in late spring, and selections from Arc II were transplanted in early summer. Each group of SYN 0 plants was placed in a separate crossing block both of which contained 6 rows spaced 59 cm apart, and each row was 6.1 m long with plants spaced 31 cm apart. It was not possible to provide equal replication for all clones of the first group due to problems in timing and logistics. Consequently, some of the first

group clones were replicated twice while others were replicated 3 times. Each clone of the second group was replicated twice in the crossing block.

Honey bees, Apis mellifera (L.), were used for cross-pollinating the parent clones. Each crossing block was enclosed with a metal frame cage (3.7x7.4x2.5 m) which was covered with a fine mesh screen so as to prevent contamination from foreign sources of pollen. A bee hive which contained 10,000-15,000 bees was placed in each cage when the majority of the plants began flowering and was removed when it appeared that flowering had terminated on most of the plants. After seeds had matured, plants were harvested with a hedge trimmer which was used to remove all plant material ca. 10 cm above the crown. Foliage of each was bagged and placed in a drying room where the temperature ranged from 32^{o}-35^{o} C. The seed was threshed after the foliage and seed pods were sufficiently dry.

Honey bees were placed in the Arc I cage on June 14 and removed on July 20. The plants were harvested on July 30. The seed pods were manually removed from the stems and the seed was threshed with a rubber matted scrub board as described by Harlan and Ahring (1960). The seed was cleaned with a Model B South Dakota® seed blower and then packaged by clones. Seeds from each clone (2 g) were mixed to form the experimental germplasm OK 1 SYN 1.

Honey bees were placed in the Arc II cage on July 27, the hive was removed on August 24, and the plants were harvested on September 1. The seed was threshed with a hammer mill and cleaned with a Model B South Dakota® seed blower. The seed was bulked according to clones and then blended to form the experimental germplasm OK 2 SYN 1.

OK 1 SYN 1 was evaluated for SAA resistance in the greenhouse.

Kanza was used as the resistant standard and the parent cultivar was used as a susceptible standard. The 3 cultivars were planted in metal flats and each flat was divided into 3 blocks of 3 rows each. Arc was planted in the middle row of each block (rows 2, 5, and 8) so that susceptible alfalfa would be evenly spaced in the flat. Kanza and OK 1 SYN 1 were randomly assigned to the remaining 2 rows of each block. This design was used in 3 flats which were all planted on the same date. Stand counts were made 10 days following planting and each row was subsequently thinned to 50 plants. Each flat was infested with ca. 1 cc of aphids (adults and nymphs) when plants were in the single trifoliolate leaf stage. The number of living plants were counted and recorded for each row after 2 and 3 weeks of infestation. OK 2 SYN 1 was evaluated for SAA resistance in a manner similar to that described above except that only 1 flat was planted due to the limited seed supply.

OK 1 SYN 1 was also evaluated for pea aphid resistance in the greenhouse. The primary purpose of this experiment was to determine if this characteristic had been significantly affected by selection strictly for SAA resistance from the parent cultivar, Arc. The experiment design was similar to that used for the SAA evaluations except that the resistant and susceptible cultivars were Arc and Buffalo, respectively. Stand counts were made 7 days after seeds were planted and each entry was subsequently thinned to 60 plants. One flat was evenly infested with ca. 8 cc of pea aphids (adults and nymphs) when plants were in the unifoliolate leaf stage. The flat was reinfested with 2 cc of pea aphids 1 week later. After 3 weeks of infestation stand counts were made and living plants were classified as either damaged or undamaged. Plants classified as damaged were those which exhibited obvious wilting, stunting,

and chlorosis.

Results and Discussion

Mean seedling survival in the mass infestation procedure was 7.0% (s=2.5) and ranged from 2.2-11.6% for all flats. It is the author's opinion that this survival rate was generally too high and resulted in a greater expenditure of resources in subsequent screening procedures to identify resistant plants. A total of 465 plants (1.9% of the initial test population) were selected from among the mass infestation survivors and subsequently retested individually. Appendix A presents individual plant test data for 60 seedling survivors which were selected from 3 flats. These results are more or less representative of the entire group. The individual plant test identified 162 resistant candidates or 0.7% of the initial test population.

Table I summarizes the S_1 progeny test results for the 133 plants which produced sufficient seed for at least 1 progeny test. OK 1 SYN 0 selections were made from 87 of these plants (Arc I group) and OK 2 SYN 0 selections were made from the remaining 46 plants (Arc II group).

A negative correlation ($P<0.01$) was found to exist between 2 week aphid counts (individual plant test) and progeny test results ($r=-0.5$), while a positive correlation ($P<0.01$) was found between 4 week damage ratings (individual plant test) and progeny test results ($r=0.42$). However, these correlations used only plants which appeared resistant and thus did not represent the full array of independent variables. That is, plants with damage ratings below 8 or aphid counts in excess of 75 were not included.

It is difficult to formulate specific conclusions from the progeny

test data because very little is known about the heritability of SAA resistance except that the trait is assumed to be qualitative and highly heritable. Although 74% of the plants tested averaged 50% progeny survival or greater, there was a small minority of plants with progeny survivals in the 10-49% range. This suggests the presence of SAA resistance sources in alfalfa with either low or complex heritability. Plants which averaged 0-9% progeny survival were probably plants with pseudoresistance. That is, plants were either not infested adequately or expressed a transitory form of resistance induced by environmental conditions during the individual plant test.

A total of 55 clones were selected from the Arc I group, and 40 clones were selected from the Arc II group, and these were used to produce the experimental germplasms OK 1 SYN 1 and OK 2 SYN 1, respecticely. OK 1 SYN 0 selections were made after all resistant candidates were progeny tested twice, while OK 2 SYN 0 selections were made after only 1 progeny test. This was done so that the plants could be transferred to the field in time to produce seed during the 1979 season. The individual plant test performances and complete progeny test results of both groups of clones are presented in Tables II and III. Two of the OK 2 SYN 0 clones were not progeny tested.

As previously stated, the individual plant test was not designed to define resistance mechanisms. Nevertheless, the aphid counts at the 2 week inspection suggest that resistance in the majority of clones can be attributed to an aphid response mechanism, i.e. non-preference or antibiosis (Tables II and III). Plants which supported in excess of 75 aphids at the 2 week inspection might possess tolerance but this can not be firmly concluded because complete aphid counts were not taken.

Both OK 1 SYN 1 and OK 2 SYN 1 were comparable to Kanza in terms of seedling survival when evaluated for resistance to the spotted alfalfa aphid in the greenhouse (Table IV and V). From a qualitative perspective, both experimental alfalfas exhibited slightly more feeding damage and appeared to support more aphids than Kanza during the first 2 weeks of the test. Although these observations lack documentation, they suggest that OK 1 SYN 1 and OK 2 SYN 1 possess more tolerance or less aphid response resistance than Kanza.

The reaction of OK 1 SYN 1 to pea aphid attack is similar to that of Arc which is considered to be highly resistant (Table VI). This phenomenon indicates that intense selection for spotted alfalfa aphid resistance without regard for other crop characteristics did not significantly affect at least 1 of the important features of the parent cultivar. The maintenance of pea aphid resistance in OK 1 SYN 1 can probably be attributed to the large number of clones used for its production.

TABLE I

DISTRIBUTION OF ARC PLANT SELECTIONS ACCORDING TO S_1 PROGENY REACTION TO SAA INFESTATION IN THE GREENHOUSE, 1979

Mean % seedling progeny survival	No. of parent plants	% of parent plants progeny tested
90 -100	22	17
80 - 89	28	21
70 - 79	19	14
60 - 69	18	14
50 - 59	8	6
40 - 49	10	8
30 - 39	7	5
20 - 29	8	6
10 - 19	4	3
0 - 9	9	7

TABLE II

INDIVIDUAL PLANT TEST AND S_1 PROGENY TEST RESULTS FOR OK 1 SYN 0 CLONES

Clone	Individual plant test results		Progeny test results						
			% seedling survival					Appearance rating [a]	
	No. of aphids after 2 weeks	Final damage rating [b]	Test 1	Test 2	Test 3	Mean	Relative to Kanza [c]	Mean	Kanza [d]
A1204	>75 [e]	9	72	86	74	77	93	6.3	6.3
A1218	>75	8	62	56	52	57	74	5.7	6.3
A1313	0	10	100	92	84	92	115	7.7	7.0
A1405 [f]	14	10	62	82	76	73	92	7.0	7.3
A1503 [f]	12	10	98	-	-	98	111	8.0	7.0
A1505 [g]	3	10	68	90	100	86	106	7.7	7.7
A1601 [g]	>75	8	80	78	-	79	84	7.0	7.0
A1607	>75	8	57	84	86	76	92	6.7	7.3
A1612	3	10	72	80	90	81	100	8.0	7.7
A1615	>75	9	82	78	43	68	81	6.0	6.7
A2101	0	10	100	90	94	95	104	8.3	8.0
A2104	47	9	82	84	80	82	98	6.3	6.7
A2105	0	10	69	84	96	83	100	7.7	7.7
A2106	0	10	88	86	96	90	102	7.7	7.7
A2108	39	10	76	74	58	69	83	6.0	6.3
A2112	26	10	68	64	78	70	86	5.7	6.3
A2216	8	10	84	100	92	92	100	7.7	7.7
A2301	>75	8	86	88	90	88	94	7.3	7.0
A2305	38	10	78	78	92	83	114	7.0	7.0
A2315	6	10	96	100	100	99	108	7.3	7.7
A2401 [f]	0	10	88	84	94	89	105	7.7	7.3
A2411 [f]	14	9	54	-	-	54	90	7.0	7.0
A2413 [f]	20	10	80	-	-	80	133	7.0	7.0
A2419	35	9	59	88	61	69	86	6.0	7.7
A2502 [g]	3	10	90	78	68	79	98	7.0	7.0
A2506 [g]	>75	8	90	64	-	77	103	7.0	7.0
A2515	12	8	72	68	70	70	92	7.0	7.0
A2518	43	8	82	66	62	70	88	6.0	6.0
A2609	12	9	98	94	100	97	110	6.7	7.0
A3106	>75	8	74	74	53	67	80	5.6	7.0

A3206 [f]	3	10	86	-	-	86	98	8.0	8.0
A3216 [g]	13	10	50	41	-	46	62	4.5	7.0
A3303	52	9	76	78	47	67	105	6.0	6.6
A3310	4	9	98	100	100	99	114	7.0	7.0
A3314	33	8	94	58	86	79	95	5.7	7.0
A3411	>75	8	42	66	40	49	56	6.0	7.0
A3413	7	10	74	64	55	64	80	5.0	7.0
A3415	32	10	98	84	86	89	110	6.7	7.3
A3417	7	10	96	82	66	81	107	7.0	6.3
A3419 [f]	16	10	98	-	-	98	111	7.0	7.0
A3501 [f]	5	10	68	-	-	68	73	6.0	7.0
A3510	1	9	76	100	83	86	113	7.3	7.3
A3515	7	9	84	64	73	74	91	7.3	6.7
A3516	7	10	100	96	100	97	117	8.0	7.3
A3518	53	9	94	84	72	83	105	7.3	7.3
A3601	17	10	70	74	74	73	95	6.3	5.7
A3604	16	10	89	100	100	96	122	8.3	7.3
A4103	6	10	100	96	98	96	122	8.3	7.0
A4108	>75	9	54	56	28	46	54	4.3	7.0
A4109	3	9	82	98	96	98	110	6.7	7.3
A4113 [g]	31	9	72	42	-	57	77	6.0	7.0
A4411	2	10	74	72	70	72	82	6.0	6.3
A4501	27	8	86	82	90	86	98	7.0	7.3
A4510	4	9	100	100	92	97	110	7.3	7.3
A4520	5	8	90	71	96	86	99	6.6	7.3

[a] Rated 1-9; 1=100% defoliation and 9=no damage.

[b] Rated 0-10; 0=dead plant and 10=plant with no SAA damage.

[c] Figure calculated by dividing mean percent seedling survival by the mean percent seedling survival of Kanza entries from the flats in which the respective clone was tested.

[d] Mean appearance rating for the Kanza entry from the flats in which the respective clone was tested.

[e] Aphid counts were not made for those plants which supported in excess of 75 aphids.

[f] Insufficient seed for 2 progeny tests.

[g] Insufficient seed for 3 progeny tests.

TABLE III

INDIVIDUAL PLANT TEST AND S_1 PROGENY TEST RESULTS FOR OK 2 SYN 0 CLONES

Clone	Individual plant test results		Progeny test results						
			% seedling survival					Appearance rating [a]	
	No. of aphids after 2 weeks	Final damage rating [b]	Test 1	Test 2	Test 3	Mean	Relative to Kanza [c]	Mean	Kanza [d]
A1316 [e]	47	9	-	-	-	-	-	-	-
A2202	2	9	66	65	72	68	80	5.3	7.3
A2207 [f]	0	10	82	89	78	83	106	7.7	7.3
A2209	0	9	100	84	-	92	107	7.0	6.5
A2212	>75 [g]	9	84	54	12	50	59	5.7	7.7
A2217 [h]	5	10	68	84	58	70	97	6.7	7.3
A2402	0	10	60	-	-	60	68	6.0	8.0
A2410	0	9	62	52	57	57	70	6.0	7.3
A2412	8	10	72	89	80	80	123	6.7	7.0
A2414 [f]	1	10	92	78	-	85	106	8.0	8.0
A2415	0	10	70	54	58	61	73	5.7	7.3
A2520 [e]	5	10	-	-	-	-	-	-	-
A3202	0	10	82	88	96	88	106	8.0	7.3
A3209	1	10	96	100	74	90	107	8.0	7.7
A3210	3	10	84	87	74	82	122	7.3	7.3
A3211 [f]	1	10	98	95	-	97	111	7.5	7.5
A3212	0	10	80	86	90	85	110	6.3	7.0
A3214	1	10	83	70	52	68	85	7.0	7.3
A3218	1	10	90	80	76	82	101	6.7	7.0
A3220	4	10	82	78	60	73	90	6.3	7.0
A3512 [h]	14	9	94	-	-	94	107	7.0	8.0
A3603	17	9	86	84	80	83	105	7.0	7.3
A3606	75	8	78	58	66	67	81	6.7	7.3
A4204	0	10	100	100	88	96	110	8.0	7.7
A4206	0	10	90	94	98	94	116	7.7	7.3
A4207	2	10	96	84	78	86	128	7.3	7.3
A4208	26	8	70	100	82	84	112	7.7	7.7
A4220	1	10	62	88	88	79	98	7.0	7.7
A4309 [h]	15	9	95	-	-	95	108	6.0	8.0
A4311	1	10	70	64	60	65	81	6.7	8.0
A4312	37	9	74	68	48	63	91	6.7	7.0

A4313	0	10	86	77	85	83	105	7.0	7.3
A4315	5	10	70	56	56	61	84	7.0	7.3
A4317	70	8	82	80	68	77	89	6.7	7.3
A4403	51	9	86	86	78	83	100	7.0	7.7
A4404	17	9	66	74	63	68	88	6.0	7.3
A4406	9	10	74	86	64	75	100	6.0	7.7
A4407	3	9	84	100	86	90	117	7.3	7.3
A4416	50	9	86	82	82	83	104	7.3	7.3
A4511	>75	8	44	72	79	65	75	5.7	7.7

[a] Rated 1-9; 1=100% defoliation and 9=no damage.

[b] Rated 0-10; 0=dead plant and 10=plant with no SAA damage.

[c] Figure calculated by dividing mean percent seedling survival by the mean percent seedling survival of Kanza entries from the flats in which the respective clone was tested.

[d] Mean appearance rating for the Kanza entries from the flats in which the respective clone was tested.

[e] Insufficient seed for 1 progeny test

[f] Insufficent seed for 3 progeny tests.

[g] Aphid counts were not made for those plants which supported in excess of 75 aphids.

[h] Insufficient seed for 2 progeny tests.

TABLE IV

GREENHOUSE EVALUATION OF OK 1 SYN 1 FOR SAA RESISTANCE, 1979

Cultivar	Mean % seedling survival after 2 weeks [a]	Mean % seedling survival after 3 weeks [a]
OK 1 SYN 1	82	70
Kanza	84	76
Arc [b]	7	3

[a] F values not significant at the 5% level of probability for cultivars.

[b] Not included in the statistical analysis.

TABLE V

GREENHOUSE EVALUATION OF OK 2 SYN 1 FOR SAA RESISTANCE, 1980

Cultivar	Mean % seedling survival after 2 weeks [a]	Mean % seedling survival after 3 weeks [a]
OK 2 SYN 1	77	62
Kanza	71	59
Arc [b]	5	1

[a] F values not significant at the 5% level of probability for cultivars.

[b] Not included in the statistical analysis.

TABLE VI

GREENHOUSE EVALUATION OF OK 1 SYN 1 FOR PEA APHID RESISTANCE, 1980

Cultivar	Seedling reaction after 3 week infestation		
	% undamaged	% damaged	% killed [a]
OK 1 SYN 1	70	6	25
Arc	72	3	24
Buffalo [b]	9	17	74

[a] F value not significant at the 5% level of probability for cultivars.

[b] Not included in the statistical analysis.

CHAPTER IV

SELECTION OF SPOTTED ALFALFA APHID RESISTANCE FROM TEAM

Introduction

Team alfalfa was the first cultivar developed for resistance to the alfalfa weevil (Barnes et al. 1970). Team has vigorous spring growth along with heavy stem terminals and well developed axillary buds. These characters impart a moderate tolerance to the weevil in that the cultivar maintains a greater capacity to withstand or recover from pest damage. Team also possesses desirable agronomic traits, high resistance to the pea aphid, and moderate resistance to several diseases including common leaf spot, Pseudopezia medicaginis (Lib.), and Stemphylium leaf spot, Stemphylium boryosum Wallr. (Barnes et al. 1970). Despite this breeding accomplishment in the area of multiple pest resistance, Team has a major deficiency in that it is highly susceptible to the SAA. The objective of this study was to develop an experimental alfalfa similar to Team but with resistance to the SAA.

Methods and Procedures

Team was screened for SAA resistance in 1 cycle of selection which was conducted in the greenhouse. The cycle consisted of a series of 3 selection procedures (i.e. mass infestation test, individual plant test, and progeny test) which were performed in a manner virtually identical

to those described in Chapter III. Selection was conducted with greenhouse reared SAA which were a composite of field collections from Oklahoma.

Approximately 24,000 Team seedlings were mass infested with SAA during the summer of 1978. The design of flats was the same as that described for the last 6 flats of Arc which were mass screened. A total of 24 flats were planted on 5 planting dates, i.e. June 14, June 22, July 6, July 14, and July 31. Each flat was infested with 2 cc of SAA, twice that used for Arc. The purpose of intensifying the initial infestation was to increase the speed and effectiveness of mass screening. Duration of screening ranged from 11-21 days and averaged 15 days per flat.

Seedling survivors were retested in an individual plant test which was modified slightly to insure that susceptible escape plants would be detected. Plants which supported less than 10 aphids at the first inspection were subsequently reinfested by placing 10 adult, apterous aphids on the plant. The reinfested plants were held over an additional week for a fifth inspection which consisted of a damage rating.

Plants selected in the individual plant test were self-pollinated during the winter and spring (1979) to produce seed for S_1 progeny testing. During this process plants were cloned by taking vegetative cuttings and rooting them in sand.

Plants which averaged ca. 50% or more progeny survival were selected as SYN 0 plants and these were transplanted to the Agronomy Farm during the summer of 1979. The methods and procedures used in producing OK 3 SYN 1 seed, the experimental germplasm, were virtually identical to those described for OK 2 SYN 1. Honey bees were placed in the cage on July 24

and removed on August 24, and the plants were harvested on September 1.

OK 3 SYN 1 was evaluated in the greenhouse for SAA resistance in a manner similar to that described for OK 2 SYN 1 except that Team was used as the susceptible check. OK 3 SYN 1 was also evaluated for pea aphid resistance using techniques previously described for OK 1 SYN 1 except that Team was used as the resistant check.

Results and Discussion

Mean seedling survival in the mass infestation test was 1.1% (s=1.3) and ranged from 0.2-6.3% for all flats. A total of 206 mass infestion survivors (0.9% of the initial base population) were retested to verify resistance. The data for 40 of these plants are presented in Appendix B. The individual plant test identified 70 resistant candidates or 0.3% of the initial test population.

Table VII sumarizes the progeny test results for the 67 plants which produced sufficient seed for at least 1 progeny test. Approximately 85% of the plants tested had 50% or greater progeny survival.

A negative correlation ($P<0.01$) was found to exist between 2 week aphid counts (individual plant test) and progeny test results ($r=-0.37$), while a positive correlation ($P<0.01$) was found between 4 week damage ratings (individual plant test) and progeny test results ($r=0.4$). However, these correlations used only plants which appeared resistant and thus did not represent the full array of independent variables. That is, plants with damage ratings below 8 or aphid counts in excess of 75 were not included.

A total of 60 clones, 2 of which were not progeny tested, were selected to produce the experimental germplasm, OK 3 SYN 1. Selection

was made after resistant candidates were progeny tested once so that plants could be transferred to the field in time to produce seed during the 1979 season. The individual plant test and progeny test performances of each clone are presented in Table VIII. The aphid count records suggest that the source of resistance for the majority of these clones was antibiosis or non-preference.

The level of SAA resistance in OK 3 SYN 1 was found to be similar to that of Kanza after 3 weeks of infestation (Table IX). Undocumented observations revealed that OK 3 SYN 1 appeared to support more aphids than Kanza and exhibited more aphid damage when the test was terminated. In general, aphid resistance in OK 3 SYN 1 is probably similar to that of OK 1 SYN 1 and OK 2 SYN 1.

Evaluation of OK 3 SYN 1 for pea aphid resistance indicated that plant mortality was significantly higher than that of Team, the parent cultivar. Nevertheless, differences between OK 3 SYN 1 and Buffalo, the susceptible check, were quite striking in % undamaged plants and % mortality. In the author's opinion, these data indicate that OK 3 SYN 1 possesses an acceptable level of pea aphid resistance.

TABLE VII

DISTRIBUTION OF TEAM PLANT SELECTIONS ACCORDING TO S_1 PROGENY REACTION TO SAA INFESTATION IN THE GREENHOUSE, 1979

Mean % seedling progeny survival	No. of parent plants	% of parent plants progeny tested
90 -100	13	19
80 - 89	16	24
70 - 79	14	21
60 - 69	11	16
50 - 59	3	5
40 - 49	5	8
30 - 39	2	3
20 - 29	1	1
10 - 19	1	1
0 - 9	1	1

TABLE VIII

INDIVIDUAL PLANT TEST AND S_1 PROGENEY TEST RESULTS FOR OK 3 SYN 0 CLONES

Clone	Individual plant test results		Progeny test results						
			% seedling survival					Appearance rating [a]	
	No. of aphids after 2 weeks	Final damage rating [b]	Test 1	Test 2	Test 3	Mean	Relative to Kanza [c]	Mean	Kanza [d]
T1305	52	10	74	52	62	63	77	6.3	7.0
T1308	>75 [e]	8	60	68	76	68	96	5.3	7.3
T1309	20	10	72	81	72	75	99	8.0	8.0
T1313	8	10	96	88	90	91	118	7.7	7.3
T1315	13	9	76	56	58	63	82	6.7	7.0
T1401	>75	8	78	86	77	80	99	7.3	7.3
T1416	6	10	84	96	100	93	104	7.3	7.7
T1501	57	9	90	92	78	87	118	7.7	7.0
T1502	>75	9	90	66	72	76	102	7.0	7.3
T1604	>75	8	86	98	72	85	112	7.7	7.7
T1605	41	10	60	67	84	70	93	6.7	7.3
T1608 [f]	67	9	86	80	74	80	95	6.7	6.7
T1618 [f]	>75	8	64	-	-	64	70	6.0	8.0
T1620 [f]	>75	8	98	84	76	86	109	7.7	7.3
T2101 [f]	29	8	62	-	-	62	67	7.0	8.0
T2201 [f]	3	10	68	60	58	62	80	6.0	7.7
T2603 [f]	>75	9	85	-	-	85	118	7.0	7.0
T2604	4	10	85	65	52	67	87	7.3	7.3
T3303	0	10	90	95	80	88	110	8.0	7.7
T3307	8	10	88	80	87	85	110	7.0	7.3
T3314	26	10	80	82	72	78	101	6.0	8.0
T3501	24	9	90	62	64	72	96	6.7	6.7
T3502 [g]	7	9	-	-	-	-	-	-	-
T3503 [f]	0	9	80	-	-	80	105	6.0	7.0
T3504 [h]	8	8	82	76	-	79	106	6.0	7.0
T3508 [h]	0	10	88	98	-	93	119	7.5	7.0
T3103	>75	8	41	64	54	53	71	5.4	7.3
T3104	1	9	92	98	74	88	110	6.7	6.7
T3204	58	8	72	42	46	53	70	4.7	6.7
T3403	14	9	74	76	84	78	101	5.7	7.7
T3406 [f]	49	8	92	-	-	92	121	7.0	7.0
T5110 [f]	3	10	88	-	-	88	116	7.0	7.0

T5111	4	9	94	88	86	89	126	7.3	7.0
T5112 [h]	11	10	96	82	94	91	113	7.3	7.7
T5114 [h]	2	10	73	83	-	78	111	6.3	7.0
T5118 [h]	13	10	72	64	-	68	90	5.3	6.7
T5120	34	9	94	96	88	93	120	8.0	8.0
T5201	9	10	62	69	58	63	83	6.7	7.3
T5205	0	10	92	96	100	96	122	7.0	7.3
T5207	0	10	100	94	84	93	120	7.0	7.3
T5208	0	10	80	68	74	74	96	7.7	7.0
T5210	32	10	98	100	94	97	114	7.7	7.7
T5213	14	8	62	54	67	61	93	4.3	6.7
T5220 [h]	48	8	60	72	72	68	93	6.0	6.7
T5302 [h]	62	9	88	77	-	83	114	7.0	7.0
T5602	0	10	94	98	80	91	111	7.0	7.3
T5606	8	9	100	88	100	96	106	8.0	7.3
T5607	2	9	92	98	72	89	119	6.7	7.3
T5608 [g]	0	10	84	86	68	79	103	7.0	6.7
T5502 [g]	0	9	-	-	-	-	-	-	-
T6101	2	10	100	92	76	89	120	7.7	7.7
T6103	2	9	84	66	60	70	94	6.7	7.0
T6106	2	10	77	66	70	71	100	6.0	7.3
T6201	10	9	90	83	72	82	114	7.3	7.0
T6202	1	10	80	60	76	72	90	7.3	7.0
T6203	0	10	94	90	86	90	113	7.7	7.7
T6301	4	9	92	92	100	95	127	8.3	7.7
T6302	3	8	74	48	72	65	87	6.0	7.0
T6401	35	8	62	62	23	49	59	6.0	7.7
T6502	17	9	82	94	58	78	94	5.7	7.3

[a] Rated 1-9; 1=100% defoliation and 9=no damage.

[b] Rated 0-10; 0=dead plant and 10=plant with no SAA damage.

[c] Figure calculated by dividing mean percent seedling survival by the mean percent seedling survival of Kanza entries from the flats in which the respective clone was tested.

[d] Mean appearance rating for the Kanza entry from the flats in which the respective clone was treated.

[e] Aphid counts were not made for those plants which supported in excess of 75 aphids.

[f] Insufficient seed for 2 progeny tests.

[g] Insufficient seed for 1 progeny test.

[h] Insufficient seed for 3 progeny tests.

TABLE IX

GREENHOUSE EVALUATION OF OK 3 SYN 1 FOR SAA RESISTANCE, 1979

Cultivar	Mean % seedling survival after 2 weeks [a]	Mean % seedling survival after 3 weeks [a]
OK 3 SYN 1	82	67
Kanza	81	69
Team [b]	3	2

[a] F values not significant at the 5% level or probability for cultivars.

[b] Not included in the statistical analysis.

TABLE X

GREENHOUSE EVALUATION OF OK 3 SYN 1 FOR PEA APHID RESISTANCE, 1980

Cultivar	Seedling reaction after 3 week infestation		
	% undamaged	% damaged	% killed [a]
OK 3 SYN 1	63	6	31
Team	85	4	10
Buffalo [b]	5	9	86

[a] F value significant at the 5% level of probability for cultivars.

[b] Not included in the statistical analysis.

CHAPTER V

COMPARATIVE REACTION OF OK 1 SYN 1, OK 2 SYN 1, AND OK 3 SYN 1 TO SPOTTED ALFALFA APHID INFESTATION

Introduction

OK 1 SYN 1, OK 2 SYN 1, and OK 3 SYN 1 are experimental germplasms of weevil tolerant parentage which are resistant to the SAA. When evaluated in separate experiments, each of these cultivars had a level of resistance similar to Kanza after 3 weeks of infestation. These findings suggested that the 3 experimental alfalfas did not differ significantly among themselves in SAA resistance. However, this conclusion might be presumptuous because they were not evaluated in the same test. Therefore, a study was designed to provide a direct comparison of OK 1 SYN 1, OK 2 SYN 1, and OK 3 SYN 1 for SAA resistance.

Methods and Procedures

This study was conducted in a manner similar to previous SAA evaluations described in Chapter III. Kanza and Riley were included as resistant standards, and Team and Arc were utilized in susceptible check rows. Each resistant entry was replicated 5 times, with flats representing replications. Resistant entries were randomly assigned to rows 1, 3, 5, 7 and 9. Each flat contained 2 rows of both Arc and Team, and these were randomly assigned to rows 2, 4, 6 and 8.

Stand counts were made 2 and 3 weeks following infestations. In an effort to further qualify the resistance level of entries, 2 additional measurements were taken from 3 of the flats after the infestation was terminated. First, each row of resistant alfalfa was given an appearance grade relative SAA damage. The scoring system utilized was identical to that described in Chapter III for progeny rows. Secondly, each row of resistant seedlings was harvested and weighed. Rows were harvested by clipping each seedling at the base of the cotyledons, and bulk row weight was converted to grams per seedling survivors.

Results and Discussion

There were no significant differences among the 3 experimental germplasms in percent survival and seedling survivor weights (Table XI). However, OK 1 SYN 1 had a slightly higher percent survival after 3 weeks and was noticeably higher in seedling weight. These numerical differences were accompanied by a higher appearance rating for OK 1 SYN 1. There were no significant differences between the experimental germplasms and Kanza; however, Riley had significantly higher seedling survivals and seedling weight.

OK 1 SYN 1, OK 2 SYN 1, and OK 3 SYN 1 were produced with clones which were not evaluated for agronomic quality nor were they tested for reaction to other insect and disease pests. Consequently, the criterion for SAA resistance was kept low (i.e. 50% progeny survival) so as to utilize as many clones as possible. In effect, the objective was to develop SAA resistant alfalfa populations while maintaining the overall quality of the parent cultivars, Team and Arc. Higher levels of SAA resistance could probably be obtained by eliminating certain clones according to S_1 progeny test results.

TABLE XI

COMPARATIVE REACTION OF OK 1 SYN 1, OK 2 SYN 1, AND OK 3 SYN 1 TO SAA ATTACK

Cultivar	Mean % seedling survival after		Seedling survivor quality	
	2 weeks [a]	3 weeks [a]	Row damage rating [b]	Weight (g.)/10 seedlings [a]
OK 1 SYN 1	80 b	67 b	6.3	0.593 b
OK 2 SYN 1	78 b	64 b	5.7	0.350 b
OK 3 SYN 1	84 b	62 b	5.3	0.330 b
Kanza	81 b	74 b	6.3	0.493 b
Riley	92 a	87 a	7.7	0.783 a
Team [c]	8	2	-	-
Arc [c]	9	2	-	-

[a] Means (vertical) followed by the same letter do not differ significantly at the 5% level of probability by Murphy's studentized maximum gap test.

[b] Rated 1-9; 1=100% defoliation and 9=no damage.

[c] Not included in the statistical analysis.

CHAPTER VI

SELECTION OF SPOTTED ALFALFA APHID RESISTANCE FROM OKLAHOMA COMMONS

Introduction

Oklahoma Commons are heterogeneous populations of alfalfa which have been grown in Oklahoma for many generations, and thus have been exposed to a wide array of natural selection forces. The Oklahoma Commons apparently descended from the Chilean type of alfalfa which was introduced from South America into California in the mid 1800's (Caddel and Taliaferro 1979). Although the Commons are viewed by growers as stable and consistent alfalfas which are well adapted for the Oklahoma environment, they are not known to possess high levels of resistance to any major pest. Each of the common strains used in this research is designated by the name of the family which has maintained it for most or all of the time since its introduction into the state. The strains are further defined by a date which indicates the probable date of introduction. The Oklahoma Commons used in this research include: Elsner (1902), Schroeder (1904), Graham (1918), Kohler (1921), Givens (before 1930), and Spradlin (before 1930). In addition to the 6 Oklahoma Common strains, the research included 2 experimental polycrosses which were developed by the Oklahoma Agricultural Experiment Station. APC-76 is bulked polycross seed of multicultivar parentage which includes several SAA resistant varieties. OK-PC-SYN-1 is a polycross which was produced

by intercrossing 50 Oklahoma Common clones selected for good agronomic quality. Although APC-76 lacks true Oklahoma Common parentage, the 8 test entries will be collectively termed the Commons.

Methods and Procedures

The Commons were concurrently evaluated and screened for SAA resistance in 1 cycle of selection. The cycle consisted of a sequence of 2 phenotypic selection procedures which were conducted in the greenhouse during 1979. The SAA used for testing were greenhouse reared and originated from field collections from various locations in Oklahoma.

A mass infestation test was utilized to initiate screening. The test alfalfa was grown in metal flats (35x50x9 cm) which had 9 rows spaced 5 cm apart. The soil mixture consisted of 4 parts sand, 2 parts clay loam, and 1 part peat and this mixture was mulched with 4 cm of vermiculite after the seed was planted. The Commons were randomly assigned to rows 1-4 and 6-9 in each flat. Sufficient seed was planted to insure at least 100 plants per entry. Row 5 contained a half row of Kanza as a resistant standard and a half row of Arc as a susceptible standard. Sufficient seed was planted in the indicator row to insure 50 plants per entry. Three flats were planted on each of 9 dates which occurred at 2-3 day intervals over a 3 week period. Flats were infested with ca. 1 cc of aphids (nymphs and adults) when plants were in the single trifoliolate leaf stage. When the half row of Arc appeared ca. 96% dead (12-18 days after infestation), stand counts were made for all entries in a flat. This provided relative comparison of percent survival of test entries. At this point some of the surviving plants showed obvious aphid damage and some were still infested with aphids which were

actively feeding. Therefore, flats were not sprayed at the time of evaluation, but rather the aphid infestation was allowed to continue until each flat was thoroughly screened. The point of screening termination was a subjective decision based on the general condition of the surviving plants in a flat. A flat was considered thoroughly screened when the SAA infestation had all but disappeared and when seedling survivors had begun to stabilize and recover. This signaled a termination in further plant mortality from SAA attack. At this point the flats were sprayed with a short residual insecticide (e.g. malathion), and a second stand count was made for all entries. Duration of screening ranged from 19-33 days and averaged 24 days per flat. The performance of the resistant standard was used as a safety valve to prevent excessive screening.

A maximum of 20 plants were selected from each flat for further testing. Criteria were adopted to provide a somewhat systematic approach for determining which plants would be selected and which plants would be discarded. First, at least 1 plant was selected from each entry providing that survivors were available. Secondly, the number of plants selected from each entry was in proportion to their respective percent survival, e.g. more plants were selected from entries with high percent survivals. Finally, selection among plants of an entry was based on the general vigor, size, and root system development of the surviving plants. It was not possible to make every plant selection in strict accordance with these 3 criteria. However, the criteria served as guidelines for conducting selection with some degree of consistency. Plant selections were transplanted into cups 9 cm in diameter for further evaluation.

The individual plant test was used to confirm resistance in seedling

survivors. Since the selection cycle did not include an S_1 progeny test, precautionary steps were included in the normal test procedure to prevent further selection of susceptible escape plants. In general, plants which supported low numbers of aphids were reinfested to insure that the absence of aphids was due to antibiosis or nonpreference and was not due to inadequate infestation. The individual plant test was conducted in a manner similar to that described for Arc except for those plants which supported less than 10 aphids at the first inspection. These plants were immediately reinfested by placing 10 adult, apterous aphids on the plant. The reinfestation process was repeated at each succeeding inspection provided that aphid counts remained below 10. Plants which possessed less than 10 aphids at the fourth inspection were selected and designated as having high levels of antibiosis or nonpreference. Plants which had more than 10 aphids following a reinfestation were evaluated for an additional 3 weeks with normal individual plant test procedures.

The criterion for final plant selection was a plant damage rating of 8 or better. Those plants which appeared to have at least a moderate level of antibiosis or nonpreference were selected as SYN 0 plants and transferred to a crossing block on the Agronomy Farm for future SYN 1 seed production.

Results and Discussion

Results from the mass infestation test confirmed SAA susceptibility in the Commons (Table XII). However, significant differences in seedling survival among the Commons were found at both inspections. This connotes that small differences in gene frequencies for resistance exist among the various strains of Oklahoma Common alfalfa. Furthermore, it appears

that some Commons have higher gene frequencies for resistance than some commercial varieties which are classified as highly susceptible, i.e. Arc and Team.

A total of 501 plants were selected from the mass infestation survivors and retested to verify resistance. Of the 256 plants which received a damage rating of 8 or better, 135 were chosen as SYN 0 plants. The individual plant test data indicated that these plants possessed moderate-high levels of antibiosis or nonpreference. The number of SYN 0 selections per Common was more or less in proportion to their respective percent survival in the mass infestation test, although this was not predetermined (Table XII). None of the APC '76 plants were selected as SYN 0 plants because this cultivar lacked true Oklahoma Common parentage.

TABLE XII

SELECTION OF SPOTTED ALFALFA APHID RESISTANCE FROM OKLAHOMA COMMON ALFALFAS

Cultivar	% seedling survival in mass infestation test		Individual plant test		SYN 0 plants
	1st inspection [a]	2nd inspection [a]	Tested	Selected	
Spradlin (OC) [b]	23 a	14 a	155	80	49
Givens (OC)	13 b	7 c	67	36	25
APC '76	13 b	10 b	95	62	0
OK-PC-SYN 1	10 b	6 c	58	29	26
Elsner (OC)	7 c	3 d	47	14	10
Schroeder (OC)	7 c	2 d	40	20	13
Grahm (OC)	4 d	2 d	24	10	8
Kohler (OC)	2 d	1 d	15	5	4
Arc [c]	3	1	-	-	-
Kanza [c]	79.1	69.7	-	-	-

[a] Means (vertical) followed by the same letter do not differ significantly at the 5% level of probability by Murphy's studentized maximum gap test.

[b] OC = Oklahoma Common.

[c] Not included in the statistical analysis.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Team and Arc were screened for SAA resistance in 1 cycle of selection which included a mass infestation test, individual plant test, and S_1 progeny test. On the basis of progeny test results, 95 Arc plants and 60 Team plants were selected as parental clones, and these were used to develop 3 experimental alfalfas. Individual plant test data implied that most of these clones possess various levels of antibiosis or non-preference, although a few may possess tolerance. OK 1 SYN 1 and OK 2 SYN 1 were developed with Arc clones, while OK 3 SYN 1 was developed with Team clones.

Greenhouse evaluations revealed no significant differences in seedling survival among the 3 experimental alfalfas. This level of resistance (62-70% seedling survival) was comparable to that of Kanza in all evaluations, although it was significantly less than Riley. The experimental alfalfas appeared to support more SAA, and thus exhibited slightly more feeding damage (chlorosis). This suggests that they possess more tolerance or less aphid response resistance than the commercial standards. Nevertheless, it is the author's opinion that this type of resistance would provide adequate SAA protection, especially in Oklahoma where economic infestations are somewhat sporadic. Low populations of SAA present in these cultivars, while not causing serious damage, would attract beneficial arthropods and thus encourage biological control. An established

predator population could act as a deterrent to other pests which might otherwise reach economic levels of infestation.

Pea aphid resistance is an important characteristic of Arc and Team. Greenhouse evaluations showed that OK 1 SYN 1 and OK 3 SYN 1 also possessed a relatively high level of resistance to this pest, i.e. 76% and 69% seedling survival, respectively. This phenomenon can probably be attributed to the large number of clones utilized in producing SYN 1 seed.

The experimental alfalfas should be evaluated for their reaction to other key pests, e.g. alfalfa weevil, blue aphid, anthracnose, and bacterial wilt. Although significant levels of blue aphid resistance are highly unlikely, this germplasm may provide an excellent source of resistant plants.

Seven strains of Oklahoma Common alfalfa were evaluated and screened for SAA resistance in 1 cycle of selection without progeny testing. Although all strains were SAA susceptible, the evaluation data showed significant differences in percent seedling survival (2-23%). In general, the Commons proved to be an excellent source for SAA resistance. The individual plant test identified 135 plants which appeared to possess antibiosis or nonpreference. These were transplanted to the field for future SYN 1 seed production. This synthetic should provide a source of SAA resistant germplasm which is highly adapted to Oklahoma. This would be an excellent base population to screen for resistance to other important pests, e.g. blue aphid.

During the course of this study over 900 plants were evaluated in the individual plant test. This process revealed that alfalfa demonstrates an extensive range of responses to SAA infestation. Although many of the plants were either susceptible or highly resistant, it was

not uncommon to observe plants with marginal or sub-optimal degrees of resistance. In general, SAA resistance in alfalfa appears to range from a weak form of tolerance to high levels of antibiosis or nonpreference.

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APPENDIX A

INDIVIDUAL PLANT TEST DATA FOR SIXTY SAA

RESISTANT CANDIDATES SELECTED FROM ARC

Plant	Aphid count		Plant damage rating [a]			
	1 week	2 weeks	1 week	2 weeks	3 weeks	4 weeks
A2501 [b]	8	32	10	10	9	7
A2502 [b]	0	3	10	10	10	10
A2503 [b]	23	>75 [c]	10	10	9	4
A2504 [b]	50	12	10	10	10	8
A2505 [b]	47	>75	10	8	8	7
A2506 [b]	12	>75	10	10	9	9
A2507	>50 [d]	>75	10	8	7	5
A2508	45	67	9	6	0	0
A2509 [b]	20	75	10	10	8	7
A2510 [b]	20	45	10	9	9	9
A2511	41	>75	10	9	8	6
A2512	>50	>75	10	8	3	0
A2513	>50	>75	9	8	4	0
A2514 [b]	10	73	10	10	9	5
A2515 [b]	2	12	10	10	10	8
A2516 [b]	3	7	10	10	9	8
A2517 [b]	>50	>75	10	9	7	0
A2518 [b]	26	43	10	10	9	8
A2519 [b]	14	>75	10	9	9	7
A2520 [b]	10	5	10	10	10	10
A3501 [b]	0	5	10	10	10	10
A3502	35	>75	9	10	8	8
A3503	36	>75	8	8	1	0
A3504	6	42	10	10	6	5
A3505	43	>75	10	8	4	0
A3506	30	>75	10	9	9	6
A3507	17	22	10	10	9	7
A3508	36	>75	9	9	5	2
A3509 [b]	0	4	10	10	9	7
A3510 [b]	0	1	10	10	10	9
A3511 [b]	10	68	10	9	9	8
A3512 [b]	19	14	10	10	9	9
A3513	9	>75	10	10	6	0

APPENDIX A (Continued)

Plant	Aphid count		Plant damage rating [a]			
	1 week	2 weeks	1 week	2 weeks	3 weeks	4 weeks
A3514 [b]	32	>75	10	8	5	4
A3515 [b]	1	7	10	10	10	9
A3516 [b]	0	7	10	10	10	10
A3517 [b]	>50	>75	9	8	3	0
A3518 [b]	0	53	10	10	9	9
A3519	>50	>75	10	5	0	0
A3520 [b]	43	>75	10	8	3	0
A4501 [b]	0	27	10	10	10	8
A4502	29	>75	10	9	7	1
A4503 [b]	8	14	10	10	8	5
A4504 [b]	1	2	10	10	10	10
A4505	38	>75	10	9	7	4
A4506	40	>75	9	8	7	7
A4507	47	>75	10	9	7	4
A4508	14	46	10	9	9	6
A4509 [b]	1	59	10	9	8	4
A4510 [b]	0	4	10	10	9	9
A4511 [b]	17	>75	10	10	8	8
A4512	0	>75	10	9	6	3
A4513	>50	>75	9	8	1	0
A4514	33	>75	9	7	2	0
A4515	33	>75	9	8	6	4
A4516	>50	>75	9	8	7	6
A4517	9	41	10	9	7	4
A4518	42	>75	9	7	1	0
A4519 [b]	36	>75	10	9	8	3
A4520 [b]	0	5	10	10	10	8

[a] Rated 0-10; 0=dead plant and 10=plant with no SAA damage.

[b] Plant selected for progeny testing.

[c] Aphid counts were not made for plants supporting in excess of 75 aphids at the second inspection.

[d] Aphid counts were not made for plants which supported in excess of 50 aphids at the first inspection.

APPENDIX B

INDIVIDUAL PLANT TEST DATA FOR FORTY SAA

RESISTANT CANDIDATES SELECTED

FROM TEAM

	Aphid count		Plant damage rating [a]				
Plant	1 week [b]	2 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks [c]
T1501 [d]	>50 [e]	57	9	9	9	9	-
T1502 [d]	>50	>75 [f]	10	10	10	9	-
T1503	>50	>75	10	10	5	1	-
T3101	>50	>75	10	8	2	0	-
T3102	>50	>75	10	9	5	2	-
T3103 [d]	16	>75	10	9	8	8	-
T3104 [d]	0	1	10	9	9	9	9
T3105	>50	>75	10	10	5	2	-
T3301	>50	62	9	4	0	0	-
T3302	13	>75	9	8	2	0	-
T3303 [d]	4	0	10	10	10	10	10
T3304	>50	>75	10	8	7	3	-
T3305	50	>75	9	8	1	1	-
T3306	>50	>75	9	6	1	0	-
T3307 [d]	0	8	10	10	10	10	10
T3308	>50	>75	9	7	2	0	-
T3309	45	>75	10	8	8	4	-
T3310	>50	>75	9	8	8	7	-
T3311	47	>75	9	8	8	2	-
T3312	>50	>75	9	7	1	0	-
T3313	>50	>75	9	8	6	3	-
T3314 [d]	11	26	10	10	9	10	-
T3501 [d]	13	24	9	10	9	9	-
T3502 [d]	2	7	10	10	9	10	9
T3503 [d]	0	0	10	10	10	9	9
T3504	0	8	10	10	9	8	8
T3505 [d]	4	>75	9	10	8	9	9
T3506	>50	>75	9	8	7	1	-
T3507	49	>75	9	7	7	1	-
T3508 [d]	2	0	10	10	10	10	10
T5301	>50	>75	9	9	8	4	-

APPENDIX B (Continued)

Plant	Aphid count		Plant damage rating [a]				
	1 week [b]	2 weeks	1 week	2 weeks	3 weeks	4 weeks	5 weeks [c]
T5302 [d]	45	62	10	9	9	9	-
T5303	28	23	9	5	5	1	-
T5304	>50	7	9	3	1	0	-
T5501 [d]	39	>75	10	9	9	9	-
T5502 [d]	0	0	10	10	9	9	9
T5503	>50	>75	9	8	6	4	-
T5504	41	>75	9	8	4	0	-
T6401 [d]	>50	35	9	8	8	8	-
T6402	>50	>75	10	7	1	0	-

[a] Rated 0-10; 0=dead plant and 10=plant with no SAA damage.

[b] Plants which supported less than 10 aphids were reinfested with 10 aphids.

[c] Plants reinfested at the first inspection received a 5 week damage rating.

[d] Plant selected for progeny testing.

[e] Aphid counts were not made for plants which supported in excess of 50 aphids at the first inspection.

[f] Aphid counts were not made for plants which supported in excess of 75 aphids at the second inspection.

VITA

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Doctor of Philosophy

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