SELECTION, QUANTIFICATION, AND HERITABILITY OF TOLERANCE TO THE SPOTTED ALFALFA APHID IN ALFALFA CLONES

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CHAPTER I

Introduction

The spotted alfalfa aphid, (Therioaphis maculata (Buckton), is an insect pest of major economic importance in production of alfalfa (Medicago sativa L.). Although this perennial forage crop originated in Asia, the U.S. is now the world's leading alfalfa producer. High levels of protein and digestibility have made alfalfa the most important forage crop in the world. Unfortunately, many diseases and insect pests attack alfalfa, and this has caused breeding for pest resistance to become a major priority for improvement of alfalfa cultivars.

The spotted alfalfa aphid (SAA) is a sucking insect with a high reproductive capability (mainly by parthenogenesis) and high damage potential. The SAA presents a problem in establishment of alfalfa by readily killing small seedlings, particularly those of susceptible cultivars. In established fields the SAA not only reduces yield and quality, but it may kill susceptible plants when high aphid densities are present.

Sources of genetic resistance to the SAA were quickly identified and incorporated into improved cultivars following the arrival of the aphid in the U.S. in 1954. This resulted despite the fact that there was little knowledge of mechanisms or inheritance of resistance. As the SAA population began to adapt to the host resistance in some areas, it

has become important to investigate the nature of the resistance. New strategies for better utilization of host plant resistance have been proposed, but these cannot be implemented because of the lack of understanding the characteristics possessed by plants to impart different forms of resistance.

Tolerance is a form of resistance characterized by the plant's ability to support with a reduced level of damage an insect population that would severely damage or kill a susceptible plant. Little attention has been given to this form of resistance because it has been thought to be less desirable than forms of resistance that inhibit or avoid establishment of insect populations in crops. These forms of resistance called antibiosis and antixenosis are deemed highly desirable because they do not allow the buildup of pest infestations. These types of resistance are not absolute, however, and some insect genotypes (initially at low frequency in the population) are able to adapt and form new biotypes which can utilize previously resistant cultivars.

It is highly probable that for every gene conditioning antibiosis or antixenosis in a host species there is a corresponding gene for virulence in the pest population (Gallun and Khush 1980, Claridge and Hollander 1983). Under this insect-host interaction tolerance, which does not impose any selective pressure on the pest population, may be more desirable than previously thought.

For integrated control programs, predators and parasites may be able to reduce insect pest populations to some extent, but some level of crop tolerance is often required to minimize additionally the need for insecticide applications. Most biological control agents require time to provide effective regulation of insect populations, and some

capability in the crop to resist or tolerate insect attack would be essential if insecticide usage is to be minimized. Moderate levels of antibiosis could be complemented under field conditions by the presence of tolerance and biological control.

Tolerance to insects has been successfully used in crops in which no other forms of resistance were available, like resistance to the spittlebug, Philaenus spumarius L., in alfalfa (Wilson and David 1958). Tolerance has been associated with plant vigor and ability to repair damage in some cases. For example, stronger stalks of corn and plant's ability to wall-off the feeding areas of the European cornbore, Ostrinia nubilalis Hubner, provide good field resistance (Holbert 1946).

A major disadvantage which has restricted the use of tolerance is that it is a trait which is difficult to select for and quantify. Quantification is difficult because tolerance results from an interaction of insect feeding and plant damage symptoms and both need to be measured. Selection for tolerance to SAA requires special screening techniques because heavy infestation levels used to screen for antibiosis or antixenosis typically eliminate all but the most resistant plants. Thus, tolerant plants are usually lost in the process of selection. It is likely that tolerance is quantitatively inherited and may require more cycles of breeding to be concentrated at high levels than the qualitatively inherited traits of antibiosis or antixenosis. In alfalfa, most existing resistant cultivars possess antibiosis and/or antixenosis forms of resistance to the SAA with tolerance having been eliminated by current screening techniques.

The objectives of my studies were to select, characterize, quantify, and estimate the inheritance of tolerance to the SAA.

Progress on selection with modified screening techniques was also described.

A first stage to facilitate the utilization of tolerance is to develop practical screening techniques for the identification of germplasm and the subsequent evaluation of large quantities of segregating material. It may then be possible to include tolerant parents when implementing new strategies of host plant resistance to the SAA. For example, it will be possible to have genes for tolerance as well as different genes for antibiosis or antixenosis in the same cultivar. This will lower the chances for the SAA to break the antibiosis or antixenosis resistance; and if these do break down, tolerance will minimize damage of the SAA.

CHAPTER II

LITERATURE REVIEW

Insect, Host and Relationship

The spotted alfalfa aphid (SAA), Therioaphis maculata Buckton, was first reported in the U.S. during spring of 1954 in New Mexico (Dickson et al. 1955). By July of the same year, damage to alfalfa, Medicago sativa L., stands in Oklahoma was already evident (Bieberdorf and Bryan 1956). In 1956, the damage caused by the SAA in the U.S. was estimated at $42 million dollars (Howe et al. 1963). The aphid causes about $200 million in losses annually in Australia (Cameron et al. 1983). Host range of the SAA was extensively investigated by Peters and Painter (1957, 1958). All Medicago spp. are among the hosts; however, no Trifolium spp. were acceptable hosts.

The SAA is a soft-bodied insect with sucking mouthparts and is ca. 1-2 mm in length. It has eight rows of dark spots on the dorsal side of the body, each with a setum. Nymphs are characterized by a pale green color while the adults are yellow to light brown (Bieberdorf and Bryan 1956). Four nymphal instars are completed during development which are morphologically distinct (Ashraf 1986). Although apterous adults are most common, alate forms are regularly produced under crowded conditions, at low temperatures, or with short daylength.

The SAA reproduces mostly by parthenogenesis, but sexual reproduction has been reported in some northern states (Dickson et al. 1958, Manglitz et al. 1966, Schalk 1972). Ten hour daylengths and temperatures of less than 10°C (late fall conditions) are associated with the development of sexual forms (Walker and Cameron 1985). Also under these conditions oviparous females and alate males are produced (Cameron et al. 1983). Even though genetic recombination is very limited for the SAA in the U.S., differences in adaptability to hosts have been frequently found. Biotypes have been designated as ENT-A through ENTO-H (Pesho and Lieberman 1960, Nielson et al. 1970, 1971, Nielson and Don 1974a, Nielson and Kuehl 1982). In Oklahoma no sexual forms are produced. The SAA may survive during winter in Oklahoma by finding shelter under well-developed alfalfa crowns provided that sub-freezing temperatures do not persist for long time periods (Bieberdorf and Bryan 1956). The SAA may survive the winter as far north as Kansas (Simpson and Burkhardt 1960). If the SAA survives the winter, populations may increase rapidly in the spring during March and April and cause serious damage on spring growth of alfalfa (Berberet et al. 1983).

Development of the SAA from first instar to adult can be completed in less than 1 week during mid-summer or require a month or longer during winter. Apterous females produce an average of four nymphs per day while alate females average three per day. The highest observed number of nymphs produced by a single female has been 160, and as many as 35 generations per year may be completed (Harpaz 1955, Nielson and Barnes 1957).

The SAA is of great economic importance to alfalfa producers because small seedlings may be readily killed during stand establishment and production may be limited by destruction of plants in established stands (Bieberdorf and Bryan 1956). Field studies on yield reduction due to heavy SAA infestation have indicated that stunting of growth causes the greatest yield reduction. No carry-over effect from SAA attacks was observed on subsequent cuttings provided that plants were not killed (Burkhardt 1959, Lloyd et al. 1980). Continued withdrawal of nutrients plus a toxin injected by SAA causes stunting of growth followed by chlorosis and death of the host. Indirect damage can also occur because weakened plants are susceptible to winterkill, other insect pests, and root diseases (Bieberdorf and Bryan 1956, Berberet et al. 1983, Caddel and Porter 1984).

Chemical control has been effective with several insecticides, but continuous reinfestation of fields may be a problem (Bieberdorf and Bryan 1956).

Screening, Evaluation, and Resistant Germplasm Release

The classical book published by Painter (1951) was the first comprehensive review on the usefulness of host plant resistance to insects and on the characterization of the resistance mechanisms. Three mechanisms were proposed and characterized, each based on a type of insect-host interaction. Three possible responses of plants to insect attacks are: 1) the host can adversely affect the development or the reproduction of the insect by dietary differences or by toxic effects (antibiosis); 2) the plant can be less preferred for feeding or oviposition (antixenosis); 3) or the plant allows normal development and

reproduction of the insect population, but it sustains less damage than plants having equal or lower insect infestation levels (tolerance) (Painter 1951). Antixenosis resistance has been difficult to separate from the antibiosis form because extremely nonpreferred plants could also produce adverse effects on the insect even under no-choice conditions.

The first SAA resistant alfalfa cultivar was inadvertently being grown in the southwestern U.S. at the time the SAA arrived. The newly developed cultivar (Lahontan) had been selected for stem nematode and bacterial wilt resistance, but was the only cultivar to remain green and productive after the SAA attacks began in 1955 and 1956. It was found later that three of the five parents of Lahontan were highly resistant to the SAA (Howe and Smith 1957).

Field observations after the heavy infestations in 1956 showed that even susceptible cultivars possessed resistant plants at low frequencies. The low frequency of resistance in the populations led to high response to screening techniques in the greenhouse and one of the fastest rates of development of insect resistance ever observed in the field. Over 17 cultivars with resistance to the SAA had been developed by 1972 (Gallun 1972). Numbers of genes involved or the form of inheritance is unknown, however, the genes conditioning resistance were at low frequencies in the populations, and repeated cycles of mass selection were effective in breeding resistance to SAA (Maxwell et al. 1974). Variation in the insect population was probably low in 1956 and could also have contributed to the effectiveness and durability of resistance at that time.

Screening and evaluation of germplasm was standardized for the greenhouse at the seedling stage (Howe et al. 1963, Nielson 1984). Resistance at the seedling stage holds well in the field at all growth stages (Harvey et al. 1960, Howe and Pesho 1960). To maintain the high rate of success in developing resistant varieties, screening techniques were aimed at the elimination of low to moderate levels of resistance. Earlier it was thought that high levels of antibiosis would be desirable to eradicate the SAA from the fields and that a more moderate or tolerance type of resistance should not be desirable. To accomplish this, heavy and prolonged artificial infestation were developed to insured that only seedlings with high levels of antibiosis could survive (Howe et al. 1963).

Genetic variability of the SAA was indicated 2 years after release of the first resistant cultivars when biotype development was observed. Fields planted to Lahontan showed increasing populations of SAA, which indicated that the resistance was being overcome and members of a new biotype were increasing in numbers (Stanford and McMurtry 1959). Controlled tests in the laboratory showed that newly collected aphids were able to reproduce well on three resistant parents of the cultivar 'Moapa'. Aphids from the original collections were also tested, and the plants were still resistant. The new collection of aphids was designated as biotype ENT A, and the original collection as ENT B (Pesho and Lieberman 1960).

Further identification and characterization of biotypes have been conducted (Nielson and Barnes 1957, Nielson and Don 1974a, 1974b, Nielson and Olson 1982, Nielson and Kuehl 1982). Several biotypes were associated with specific selected parents, which suggests that a gene

for gene relationship does exist between the SAA population and the alfalfa germplasm (Nielson and Don 1974a). Alfalfa clones have shown resistance to specific biotypes, with the exception of Lahontan PGL (polygenic line), which was developed from clones that showed resistance to specific biotypes including biotype ENT H that has the most virulence. Due to this reaction it is believed that Lahontan germplasm includes several independent genes for resistance (Nielson and Don 1974a, Nielson and Olson 1982).

Field tests for dry matter production under SAA infestation showed that rating of resistance based on seedling survival correlated well with productivity of mature plants, but other indices of tolerance may be plant height and extent of chlorosis (Lloyd et al. 1980, Turner et al. 1981). In 2 years of field studies of resistant vs. susceptible cultivars, estimates showed that the SAA can decrease forage dry matter production by 38% in a susceptible cultivar and by 19% in one with resistance (Kindler et al. 1971). Forage quality measured together by protein and carotene content was reduced by 48% in the susceptible and 24% on the resistant cultivar. It is difficult to estimate the benefits of resistant cultivars, but funds invested for their development have been quite limited and returns have been impressive.

Development of biotypes and vulnerability of narrow genetic base cultivars are strong disadvantages to a high intensity selection process. Heavy and prolonged artificial infestations eliminate moderate antibiosis or antixenosis as well as tolerant plants. The high intensity of selection given by the current screening techniques has resulted in selection of levels of resistance significantly higher than needed in the field under natural infestations (Lloyd et al. 1980,

Lupton 1984). Even with artificial infestations in the field, the percent survival of seedlings is significantly higher than seedling survival of the same cultivar at the greenhouse. Low to moderate degrees of resistance which are ignored during screening may be quite adequate when compared to highly resistant plants under field conditions. Furthermore, moderate levels of resistance may be enhanced in the field by natural controls (rain, predators, and parasites) and more plant tolerance at later stages of plant development (Lodge and Greenup 1983). Adjusting plant age at infestation could be important in the modification of the current techniques to be able to screen for low to moderate levels of resistance. Tolerance has been shown to increase with plant age while high degrees of antibiosis were not affected (Lloyd et al. 1983).

Physical and Chemical Characterization of Mechanisms of Resistance to the Spotted Alfalfa Aphid

During the early development of resistant cultivars, it was observed that there are probably three forms of resistance to the SAA; one is tolerance, and the others are antibiosis and antixenosis (nonpreference) (Harvey and Hackerott 1956, Peters and Painter 1958, Howe et al. 1963, Kindler and Staples 1969, Nielson and Olson 1982). Most investigations into mechanisms of resistance to the SAA have been directed toward understanding more about antibiosis and antixenosis, as well as possible toxins introduced by the aphids while feeding (Kishaba and Manglitz 1965, Manglitz and Kehr 1984, McMurtry and Stanford 1960). Studies of the antibiosis and antixenosis resistance have indicated that extreme antixenosis is more likely to be the reason for the slow

development of aphid infestations in these plants (McMurtry and Stanford 1960, Kishaba and Manglitz 1965, Kindler and Staples 1969, Nault and Styer 1972, Lorenz DeVries and Manglitz 1982, Manglitz and Kehr 1984). Moderate levels of antixenosis (nonpreference) have also been observed in highly pubescent alfalfa (Ferguson et al. 1982). Tolerance to the SAA has been observed in the field, but little is known of the basis or the extent of this type of resistance.

With free choice of susceptible and resistant plants, aphids showed equal preference at first, but 4 hours later all aphids moved to the susceptible plants. Aphids that had fed on the resistant plants did not show any carry-over effect once they moved to the susceptible plants, which suggested that no toxicity was involved in the mechanism of resistance. It was also shown that the life expectancy for an aphid confined on a resistant plant was not significantly different from that for an aphid without food, which indicated a mechanism of antixenosis (Kishaba and Manglitz 1965). Trichomes have been shown to provide a moderate degree of antixenosis at the seedling stage when pubescence is dense; but later, as pubescence becomes less dense, the level of the resistance decreases under a no-choice situation (Manglitz and Kehr 1984).

Feeding sites within the plant tissues have been investigated by killing or anesthetizing aphids while feeding and using histological techniques to reveal the location of the stylets (Diehl and Chatters 1956). Staining of the aphids' salivary sheaths in plant tissues has been used to trace the sites of stylet penetration (McMurtry and Stanford 1960). First studies indicated that the SAA feeding sites were in the parenchymal cells and the vascular bundles of the phloem (Diehl

and Chatters 1956). Principal site of feeding appeared to be in sieve elements of the phloem.

On resistant plants, aphids penetrated the phloem and sieve elements less frequently. This indicated that, even though aphids on resistant plants were in the feeding position from 1 to 4 hours, little ingestion from the phloem took place. Limited phloem ingestion may explain observations of low honeydew production of aphids feeding in resistant plants (McMurtry and Stanford 1960). Limited phloem ingestion of SAA in resistant plants was confirmed by the use of an electronic feeding monitor which was used to separate different biotypes of the SAA. Nonvirulent biotypes recorded little phloem ingestion. Virulent biotypes recorded long periods of phloem ingestion (Nielson and Don 1974b).

On resistant plants, petioles or stems were more attractive as feeding sites than leaves, flower petals are readily fed upon. The degree of acceptability of different plant parts changed as the nutritional quality of the plants changes with age (Kindler and Staples 1969). Other studies have shown that excesses or deficiencies of calcium, magnesium, nitrogen, potassium, or phosphates, in plant tissue, had no significant effect on aphid feeding on susceptible plants. Deficient levels of calcium or potassium increased aphid reproduction on resistant plants. Phosphorous deficiency on resistant plants caused depressed aphid reproduction. Nitrogen deficiencies had no effect on aphid reproduction, but excesses of nitrogen or magnesium enhanced reproduction on resistant plants (Kindler and Staples 1970b).

Reciprocal grafts of susceptible and resistant plants indicated that resistance cannot be translocated, but is manifested at particular

plant parts (Harvey and Hackerott 1958). Resistance, however, can be completely lost upon excision of plant parts (trifoliolates) and gradually recovered after 6 days in distilled water when clones are moderately resistant. Trifoliolates of a highly resistant clone, however, did retain full resistance on the same day excision was performed (Thomas and Sorenson 1971). The instability of resistance upon excision suggested that the mechanism may not be morphological, but probably resulted from specific interactions between insect and host at the time of feeding. Plant fluids from resistant and susceptible stems have been interchanged by infusion. Resistant plants have retained resistance, and susceptible plants did not obtain resistance from fluids of a resistant plant (Kircher et al. 1970).

An environmental factor that affects the resistance to the SAA to a great extent is temperature. Resistance in the form of antibiosis or antixenosis disappeared at temperatures below 15°C and was recovered at temperatures above 21°C (Hackerott and Harvey 1959). Free choice tests at different temperatures indicated that resistant clones were nonpreferred at 27°C, but at 10°C susceptible and resistant clones are equally preferred. However, even at the higher temperature the nonpreference also breaks down upon excision (Schalk et al. 1969). The interaction of clones with temperature is significant when wide ranges of resistance are tested. Highly resistant clones are resistant at 10°C, while other clones that are less resistant lose their resistance at 21°C (Isaak et al. 1963).

Susceptible plants became more susceptible as temperature increased from 12.5°C to 21°C due to more SAA reproduction. Reproduction of SAA was depressed at 29.5°C and further reduced with increased humidity from

75% to 90% (Issak et al. 1963). Maximum SAA reproductive rates on susceptible clones were observed at constant 20-22°C. Fluctuations of diurnal temperature with means at 20°C, 22°C, and 24°C, and a daily range of 8°C, enhanced fecundity of the SAA on susceptible plants compared with same, but constant temperatures. Aphids on resistant plants showed no response to fluctuating temperatures (Kindler and Staples 1970a).

Biochemical interactions of the SAA and alfalfa have been investigated mainly because of possible toxins injected by aphids and possible disruption of hormonal balance in the host. Attempts have been made to isolate a toxic substance that could be responsible for the interveinal chlorosis and subsequent necrosis on the leaves of alfalfa caused by the SAA (Paschke and Sylvester 1957, Nickel and Sylvester 1959). Veinbanding may be observed on newly developed leaves after 10 hours of aphid feeding, and the intensity of this systemic reaction increases as a function of feeding time. Early instars did not cause veinbanding as extensively after 10 hours of feeding as third and fourth instars did. Large variations in the intensity of veinbanding do occur, and they are probably related to differences in plant tolerance and growing conditions of the plant (Nickel and Sylvester 1959). Because of the observed variation of veinbanding it may be possible to used this systemic symptom as a basis for selection of resistant plants (Harvey and Hackerott 1956). Susceptible plants recovered from veinbanding symptoms soon after feeding stopped, indicating no residual effect.

Salivary sheaths formed by aphids about their stylets as they feed are comprised of proteinaceous substances that are secreted mainly at the time of intracellular penetration, and small amounts of sheath

material are also released during phloem ingestion. Numbers of salivary sheaths present in a plant were not related to the intensity of veinbanding. Aphid salivary sheaths have been extracted, but no injected toxin was identified (Nickel and Sylvester 1959).

Plants have been inoculated with a homogenate of crushed aphids, but typical feeding symptoms in the plants were not reproduced. Chemical investigations have continued with the objective of isolating a toxin, but the limitations of the small amounts of salivary secretion and methods of inoculation which seem to alter the composition of the substances are difficult to overcome (Paschke and Sylvester 1957).

The pea aphid, Acyrthosiphon pisum Harris, has occurred in the U.S. in association with alfalfa for a much longer time than the SAA. Plants with resistance to the pea aphid have a low ratio of nitrogen to sugar content, which suggests that low levels of nitrogen in the phloem could be the cause for low fecundity of the pea aphid on these plants (Maltais and Auclair 1957). Some of the pea aphid resistant cultivars have been shown to be susceptible to the SAA (Sandmeyer et al. 1971). Identification and ratios of different amino acids in the pea plant, Pisum sativum L., and the pea aphid have also been investigated (Auclair 1958).

In alfalfa concentration of amino acids is higher in SAA resistant plants than in susceptibles. Proline is an amino acid found in resistant plants, but not in susceptibles. Hemolymph of SAA feeding on resistant plants showed a high ratio of serine to ethanolamine and a low content of B-alanine relative to SAA feeding on susceptible plants. Asparagine is the most abundant amino nitrogen present in susceptible plants. Asparagine could serve as a base for SAA to metabolize

increasing concentrations of other amino acids. Aphid hemolymph of the SAA contains five times the concentration of free amino acids as the susceptible plants they had fed on (Marble et al. 1959).

Excised trifoliolates were placed in solutions of different concentrations and combinations of amino acids and sugars, and behavior of aphids feeding on them was observed. Inclusion of cysteine, 4-aminobutyric acid, homoserine, proline, tyrosine, and melezitose solutions allowed aphids to feed longer on resistant leaves than was the case when trifoliolates were placed in water only. Resistance or susceptibility was not modified by any sugars or amino acid combinations (Kircher et al. 1970).

Amino acid content was equally reduced on susceptible and resistant plants under water stress, but no effects were observed on aphid fecundity due to plant stress. Relative concentrations of protein-bound amino acids were not different between susceptible and resistant plants when stressed or not stressed. Resistant plants showed higher concentrations of proline and valine under water stress. These results indicated that differences in amino acid content, in general, were not great enough to produce differential reactions to SAA on susceptible and resistant plants (Lorenz DeVries and Manglitz 1982).

Differences in the levels of free auxins have been found between tolerant and susceptible wheat plants, Triticum aestivum L., to the greenbug, Schizaphis graminum (Rondani). Low levels of free auxins in the tolerant plants were probably due to high utilization of auxins in the plant to repair damage caused by the greenbug. Effects of free auxins seem to be related to tolerance and not to the antibiosis mechanism of resistance to greenbug (Maxwell and Painter 1962).

The effect of sinigrin, a mustard oil glucoside, on feeding behavior of several aphid species, not including the SAA, appears to be of importance. In the pea aphid, presence of sinigrin appears to increase salivation and improve stylet orientation to the phloem in alfalfa (Nault and Styer 1972).

An electronic feeding monitor developed by McLean and Kinsey (1964) has been used to trace the feeding sites of the pea aphid in alfalfa. Electrical waves produced by the aphids at probing or feeding are amplified and recorded in a strip chart recorder. Different wave shapes have been identified and correlated with stylet location at the time the particular wave is given. This device has made it possible to study the feeding behavior of several sucking insects and their interactions with their hosts (McLean and Kinsey 1967, Nielson and Don 1974b, Kennedy et al. 1978, Kawabe et al. 1981, Campbell et al. 1982, Khan and Saxena 1984, Velusamy and Heinrichs 1986).

The effect of sinigrin has been investigated with the use of an electronic monitor on the pea aphid. Aphids were anesthetized and their stylets amputated at the time a particular wave shape was given by the monitor. Stylets of the pea aphid on resistant pea plants were found on the parenchymal cells and the xylem at the time the monitor gave a nonphloem ingestion wave. A phloem ingestion wave was produced after the plants were treated with sinigrin and the stylets were found in the sieve elements in the phloem. These results confirmed the correlation of wave-shape to stylet site previously reported in alfalfa for the pea aphid (McLean and Kinsey 1967). It was suggested that there is a lack of stylet orientation toward the sieve elements of the resistant plants,

and it was temporarily corrected by the application of sinigrin (Nault and Styer 1972).

The electronic feeding monitor was used to separate biotypes of the SAA (Nielson and Don 1974b). The monitor has not been used, however, for the investigation of the different mechanisms of resistance to the SAA.

Tolerance and Host Plant Resistance

Attempts to measure tolerance when present in a plant along with antibiosis have been made by maintaining certain numbers of aphids with replacement of those which died due to the antibiosis or antixenosis resistance (Ortman and Painter 1960, Schuster and Starks 1973). With this technique all three mechanisms of resistance have been identified in the same plant when studying greenbug resistance in sorghum, Sorghum bicolor L., and wheat. Phloem ingestion from an antibiotic or antixenotic plant, however, may be limited, and measuring tolerance in plants having these forms of resistance may not be adequate.

Tolerance as a type of resistance in the field has received little attention because it is difficult to measure and in some cases greatly affected by the environment (Painter 1951). There are several examples in the literature, however, where tolerance has been an effective mechanism of host plant resistance. In sorghum and corn, Zea mays L., tolerant cultivars to the chinch bug, Blissus leucopterus leucpterus (Say), have been successfully grown for many years (Painter et al. 1935, Snelling et al. 1937). In potatoes, Solanum tuberosum L., tolerance to psyllid yellows was the primary mechanism of resistance (Painter 1951).

The ability of corn to wall-off the feeding areas of the corn borer, Diatraea sp., was incorporated into early corn hybrids (Holbert 1946).

Moderate levels of antibiosis or antixenosis can be reinforced with tolerance in the host (Painter 1951). The long-lasting resistance in barley, Hordeum vulgare L., to all the biotypes of the greenbug is due mainly to high levels of tolerance (Painter 1960). Resistance to the meadow spittlebug, Philaenus spumarius L., in alfalfa is mainly due to tolerance (Wilson and Davis 1958). Tolerance to the potato leafhopper, Empoasca fabae (Harris), in alfalfa is a component of resistance in the field (Newton and Barnes 1965). A cotton, Gossypium hirsutum L., strain with tolerance to stem weevil, Pempherulus affinis L., damage has been released (Bagavandoss and Nataraj 1960).

The physiological explanation for plant tolerance to insects and other pests has been generally attributed to plant vigor (Smith and Harris 1952). However, specific mechanisms may give plants the ability to avoid (detoxify insect secretions) or to repair damage. Certain plant hormones seem to be important to the mechanism of tolerance to sucking insects. Aphid damage was suppressed by application of plant hormones, especially auxins which are found at low levels in susceptible plants (Allen 1947). High amounts of auxins have been found in the honeydew of the SAA after these had fed on susceptible and tolerant plants. Tolerant plants, however, seemed to be able to maintain enough auxin levels for new growth and repair of damage (Maxwell and Painter 1962).

Tolerance of Alfalfa to the Spotted Alfalfa Aphid

Tolerance to the SAA has been mentioned in the literature since early investigations of resistance (Bieberdorf and Bryan 1956, Harvey and Hackerott 1956, Hackerott et al. 1958, Howe and Pesho 1960, Howe et al. 1963, Ridland and Berg 1981, Lloyd et al. 1983). In most instances, however, tolerance has only been observed and its expression investigated. The physiological and genetic basis of tolerance to the SAA, as well as the selection procedures, have not been reported.

Identification of tolerance in one study was done on the basis of discarding plants that did not support SAA on single excised alfalfa leaflets (Jones et al. 1968, Jones 1969). Plants supporting aphids were also discarded if the leaflets were killed by the aphids in a short time (susceptibles). Selected tolerant plants were self-pollinated ,and intercrossed to investigate the degree of tolerance transmitted to the progenies. Degree of tolerance was measured by the percent of the total leaflet area that showed chlorosis in subsequent feeding trials. Tolerance was separated into high, intermediate, and low levels. The same three levels of tolerance were observed in the selfed-progeny, indicating that the tolerance was inherited. Broad sense heritability using parent offspring regression analysis was 0.69 (Jones et al. 1968, Jones 1969). This was the first time that tolerance to the SAA was experimentally identified, but no reports are found on the development of screening techniques necessary to select tolerant seedlings from large germplasm collections.

Wide ranges of tolerance were observed based on the degree of chlorosis; however, better quantification is needed to standardize the trait and measure its heritability. Other symptoms to the SAA are

interveinal chlorosis and stunted growth, But these traits could not be measured in this study because it was limited to excised leaflets. Tolerance to the SAA in the absence of antibiosis/antixenosis was also measured by Thomas and Sorensen (1970) and found to be lower on excised leaves than on attached leaves.

CHAPTER III

SCREENING AND QUANTIFICATION OF TOLERANT ALFALFA CLONES TO THE SPOTTED ALFALFA APHID

Introduction

Importance of polygenic resistance in alfalfa, Medicago sativa L., to the spotted alfalfa aphid (SAA), Therioaphis maculata (Buckton), has long been recognized (Painter 1951, Barnes et al. 1977, Nielson and Olson 1982, Lupton 1984). Polygenic resistance in a cultivar can provide stability of yield for many years in spite of the occurrence of biotypes or races (Van der Plank 1963). This type of resistance has been demonstrated with 'Lahontan', a cultivar which showed uniformly high resistance levels for several biotypes of the SAA (Nielson and Olson 1982). This cultivar was derived from sources with several genes conditioning antibiosis or antixenosis and, probably good levels of tolerance as some SAA biotypes developed relatively high population densities on it but no damage was observed. Cultivars possessing several genes for resistance may provide effective protection from SAA for many years (Pesho and Lieberman 1960). Resistance which remains stable for many years is also referred to as durable resistance (Robinson 1973, Lupton 1984).

Current screening methods for SAA resistance are based on the survival of seedlings after a relatively lengthy period of heavy infestation in the greenhouse (Nielson 1984). The technique eliminates tolerant plants as well as those with moderate levels of other forms of resistance because the test stops when only highly resistant plants remain. Seedling survival to SAA in the field is sometimes much higher than survival predicted from greenhouse evaluations of resistant and susceptible cultivars. Some seedlings survive moderately high field infestations without apparent damage, indicating that some degree of tolerance exist (Lodge and Greenup 1983). It is important to determine at what intensity of infestation in the greenhouse it may be possible to identify these tolerant seedlings and intermediate levels of other forms of resistance among susceptible plants. In current greenhouse screening techniques, age at infestation varies from emergence to 10 days later. Length of exposure averages 21 days or when only plants not supporting aphids remain alive (Howe et al. 1963).

Howe and Pesho (1960) reported that a resistant cultivar sustained less plant mortality when infested 14 days after emergence than when infested at 3 days of age in the field. Susceptible and tolerant clones showed no effect of age at infestation for plant mortality except when the exposure was short (9 days), in which case older susceptible plants had a better survival than those infested when younger. This type of investigation is needed in the greenhouse with known levels of infestation and better quantification of plant damage other than survival. It would be desirable to develop methods to regulate the infestation levels to give maximum separation of tolerant plants and those which are susceptible (Dahms 1972). To obtain this type of

controlled screening it is necessary to closely evaluate the effect of aphid density and duration of infestation at different growth stages of the host.

For tolerant plants, it is helpful to quantify plant damage on the basis of plant symptoms and aphid numbers. An early symptom of SAA feeding is veinbanding on newly developed leaves, followed by localized chlorosis, stunted growth, necrosis of leaves and plant death (Cameron et al. 1983). Plant injury due to greenbug, Schizaphis graminum (Rondani), feeding has been quantified by measuring plant height of wheat, Triticum aestivum L., and sorghum, Sorghum bicolor L. (Ortman and Painter 1960, Schuster and Starks 1973). Quantification of plant damage in alfalfa due to the SAA has been done in terms of percent chlorosis of excised leaves with classification of damage levels into high, intermediate, and low (Jones et al. 1968). A better quantification of tolerance that combines all plant symptoms due to the SAA damage in more continuous increments is needed.

The objectives of this study were to investigate screening methods that would allow the selection of alfalfa seedlings with tolerance to the SAA and to quantify levels of tolerance in these plants at the mature stage under similar SAA infestations in the greenhouse.

Materials and Methods

Screening

A susceptible cultivar 'OK08' and a resistant cultivar 'Riley' were planted in 15 cm pots and after emergence, plants were thinned to 10 seedlings per pot. Seedlings were infested with apterous adult SAA at

three seedling ages after emergence; cotyledon (day 0), unifoliolate (day 3), and first trifoliolate (day 8). The initial infestations were completed by placing 40 aphids per pot on seedlings with a camel's hair brush to insure a uniform infestation of four adult aphids per seedling. Plants were infested with aphids for five time periods (2, 4, 6, 8, and 10 days). Equal numbers of uninfested pots were maintained so that growth rates of plants without aphids could be measured. Infested and uninfested seedlings were placed in cages (1m X 5m) covered with a fine meshed cloth. Photophase was maintained at a minimum of 16 hours by use of artificial lighting. After the designated exposure times, pots were sprayed with phosphorothionate insecticide and placed in an uninfested greenhouse.

The experiment was a 3x5x2 factorial including the three ages at infestation, five exposure times, and two cultivars. There were a total of eight replications arranged in a randomized complete block design completed during spring and summer of 1983.

Plant measurements taken as all seedlings reached 25 days of age included stem height, dry stem weight, and dry root weight. Analysis of variance was calculated for the total root weight in each pot (10 seedlings), total stem weight per pot, total stem height, and the survival percent of each pot. From the cultivar OK08, a total of 54 seedlings (out of 2400 seedlings tested) were selected as possible sources of tolerance on the basis that these had survived the 6, 8, 10 day exposures with aphids on them. Dry weights of selected seedlings were estimated from the fresh weight by using the average percent dry matter per pot.

After selected transplants had formed crowns, six stem cuttings were rooted from each. The four most uniform clones from each were saved for the clonal test. Stem cuttings from plants known to be susceptible to the SAA were also rooted at this time to serve as standards in later studies.

Clonal test

This experiment was designed to compare the capabilities of clones selected for "apparent tolerance" to support aphids in numbers not significantly different from susceptible clones under a free choice arrangement. Additionally, several plant injury symptoms were measured so that tolerance could be quantified in terms of numbers of aphids present and plant damage caused by aphid feeding in mature plants.

For this experiment plants were arranged in four replicates, each replicate consisting of the 54 selected clones and four susceptible checks. All plants were cut back at the bloom stage and uniformly fertilized. Ten days later the longest stem on each plant was staked and the height recorded. Numbers of trifoliolates were also counted on these stems prior to infestation. All other stems were clipped near the crown. Ten large SAA nymphs were placed on or near the terminal of each stem. Plants were examined 1 and 2 days after the initial infestation and aphids were added as necessary to assure that each had at least 10.

Aphids per plant were counted on days 5, 10, and 19 post-infestation. At day 19 aphids from each clone were collected and stored in 95% alcohol and counted. After aphids had been collected, stem lengths, numbers of trifoliolates, numbers of chlorotic leaves, and stem weights were recorded. Numbers of trifoliolates added (or lost due to

leaf drop) and stem elongation during the infestation period were obtained by subtracting the initial record from that taken after infestation. In most replicates, the four susceptible checks were killed by the aphids between days 10 and 19, but plant measurements were taken regardless. Quantification of plant damage under similar SAA infestation levels was measured by combining measurements of stem height, number of trifoliolates, and percentage of chlorotic leaves. Each of these plant traits is related to tolerance to SAA, but an index considering all traits was necessary. Statistical analysis was obtained for each of the traits measured and for an index that included three of the traits. Susceptible checks were included in each analysis and used for statistical comparison of aphid densities and plant damage against the selected 'tolerant plants'. The first comparison was on aphid densities after 10 days of infestation to assure that only plants with similar infestations were compared for plant damage.

Results

Results of seedling development revealed that on uninfested seedlings there were no significant differences among the cultivars, overall means for the uninfested seedlings are presented (Table 1). On infested seedlings, differences in stem heights, stem weights, root weights, and survival percentages were significant ($P < 0.01$) among cultivars (Table 13, Appendix). The interaction of cultivar by exposure and/or age were also significant ($P < 0.01$ and $P < 0.05$, respectively) and the results are presented individually for each cultivar.

Table 1. Seedling development (25 days after emergence) of uninfested OK08 and Riley (totals for 10 seedlings in each of 8 replicates.)

	Plant Parameters (Totals for 10 seedlings)			
	Survival %	Stem Height (cm)	Stem Weight (g)	Root Weight (g)
Mean	96	149.36	1.42	0.70
CV (%)	15	19	30	36

The cultivar Riley showed significant ($P < 0.05$) differences due to age at infestation only for stem heights and stem weights, (Table 14, Appendix). Exposure time caused no significant ($P > 0.05$) differences in any of the parameters measured on Riley seedlings. Overall mean survival was 96% (Table 2).

For the cultivar OK08, effects of age at infestation, and exposure time to SAA were highly significant ($P < 0.01$) (Table 15, Appendix). The interaction of age at infestation with duration of exposure to SAA infested OK08 was not significant ($P > 0.05$)

Plant heights for 10 seedlings were significantly ($P < 0.05$) reduced after 6 days of SAA exposure when compared to the 2 and 4 days exposures (Table 3). Seedlings infested at the cotyledon stage were also significantly ($P < 0.05$) shorter than those infested at the trifoliolate stage. Stem weights per 10 seedlings were significantly ($P < 0.05$) reduced after seedlings were exposed for 4 or more days. Root weights per pot were significantly ($P < 0.05$) reduced after the seedlings had been exposed to SAA for 4 days or more.

Infestation at the cotyledon stage caused significantly ($P < 0.05$) lower root weights across all exposure periods than when seedlings were infested at latter stages. Survival percentages were significantly ($P < 0.05$) lower after six days of SAA exposure. Survival percentages were similar for all three ages at infestation.

Clonal test

Nearly all susceptible clones included as checks were killed by the aphids between days 10 and 19 in all four replicates. The highest count of aphids on these checks was obtained at day 10.

Table 2. Seedling development (25 days after emergence) of Riley after SAA infestation (total for 10 seedlings in each of 8 replicates).

	Plant Parameters (Totals for 10 seedlings)			
	Survival %	Stem Height (cm)	Stem Weight (g)	Root Weight (g)
Mean	96	136.15	1.19	.52
CV (%)	13	18	31	29

Table 3. Seedling development (25 days after emergence) of OK08 as affected by age at infestation and exposure time to SAA

	Plant parameters			
	Survival (%)	Stem Height[a] (cm)	Stem Weight[a] (g)	Root Weight[a] (g)
Exposure (Days)				
2	98.7	130.5	1.10	0.57
4	95.8	118.5	0.84	0.42
6	78.0	82.8	0.62	0.32
8	69.0	62.8	0.40	0.17
10	52.0	40.1	0.27	0.12
LSD (P = 0.05)	11.9	20.5	0.24	0.13
Stage at Infestation				
Cotyledon	81.0	70.1	0.24	0.44
Unifoliolate	71.0	85.1	0.35	0.69
Trifoliolate	83.0	105.7	0.37	0.78
LSD (P = 0.05)	9.2	15.9	0.19	0.10
C.V (%)	26	41	67	69

[a] Totals for 10 seedlings per replicate.

After 10 days of SAA exposure, significantly ($P < 0.05$) lower SAA population densities were observed on 21 clones than on the susceptible checks. It was clear that moderate levels of antibiosis or antixenosis were present in some of these clones because two reinfestations were given to five of these clones. Since a second objective of the clonal test was to quantify the damage on susceptible and tolerant clones that were relatively free of other forms of resistance, the 21 clones which exhibited antibiosis and/or antixenosis were discarded from further analyses. At day 5 of infestation, these 21 clones had the same or lower numbers of aphids than the initial infestation numbers (Figure 1). At day 10, aphid numbers were even lower (<10 per plant), however at day 19 numbers of aphids increased (>300 per plant) on these clones due to the movement of alate aphids from dying susceptible plants. On the susceptible checks and the remaining 33 selected (tolerant) clones, aphid numbers were similar at day 5 of infestation (mean of 68 per plant) (Figure 1). At day 10 aphid numbers were high (>400 per plant) and still similar for susceptibles and the remaining 33 'tolerant' clones. At day 19, however, the number of aphids on the susceptible clones had declined (<400 per plant) due to plant mortality and was actually lower than in tolerant clones (>800 per plant) which had continued to support aphids well.

Under similar SAA infestations, most selected clones had means for stem height, stem weight, and number of trifoliolates that were significantly ($P < 0.05$) higher than the susceptible checks (Table 4). There were high variations on the levels of tolerance among the selected clones. Percentages of chlorotic leaves exceeded 90% on susceptible checks and ranged from 10 to 90% on selected clones. The high incidence

Figure 1. SAA Population Density at Three Counts on Susceptible, Tolerant, and Antibiotic and/or Antixenotic Plants

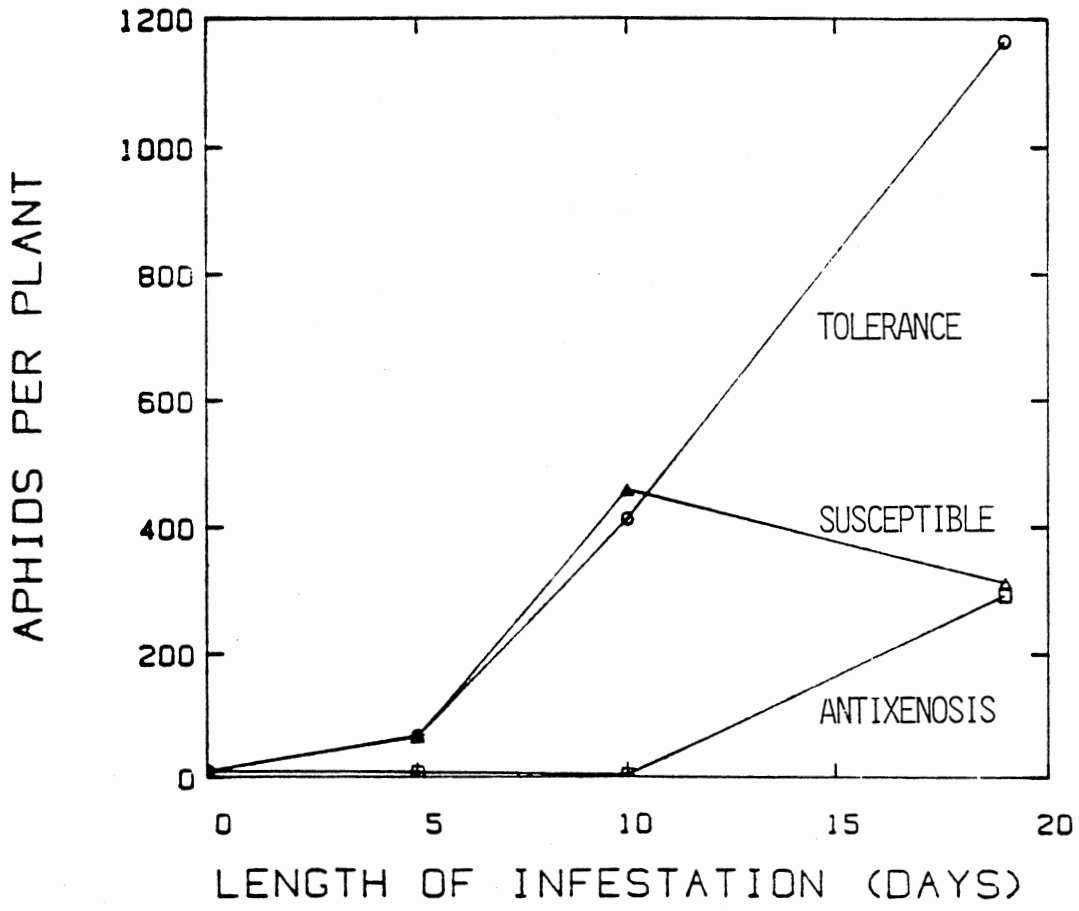

APHIDS PER PLANT
1200
1000
800
600
400
200
0
0
5
10
15
20
LENGTH OF INFESTATION (DAYS)
TOLERANCE
SUSCEPTIBLE
ANTIXENOSIS

Table 4. Plant parameters for 33 tolerant and 4 susceptible clones after 19 days of SAA infestation

Clone Number	Stem Height(cm)	Stem Weight(g)	Trifoliolate Number	Chlorotic Leaves(%)
1	54.0	0.69	14.0	80.5
4	27.0	0.48	7.5	92.8
5	45.3	1.13	24.5	75.0
7	60.1	0.86	17.0	87.8
8	34.6	0.67	10.5	59.1
9	38.5	1.02	23.8	39.4
10	41.6	0.81	22.8	48.6
11	36.3	0.90	27.5	20.0
12	47.3	1.32	15.8	65.9
13	43.6	1.24	33.8	27.5
14	29.8	0.97	22.0	28.8
15	43.9	1.40	25.3	48.6
16	45.3	1.18	30.8	24.7
18	54.3	1.41	30.5	29.0
20	36.3	1.13	27.5	48.9
25	39.3	0.93	29.3	34.2
28	40.5	1.30	25.3	50.3
29	48.5	1.06	18.8	64.1
30	53.9	1.56	28.3	43.2
32	41.4	0.74	12.0	46.5
33	29.6	1.09	34.8	40.9
35	51.1	1.26	29.8	54.6
37	35.4	1.01	31.8	53.7
38	43.5	0.62	11.0	88.0
40	29.8	0.48	9.8	81.5
41	51.4	0.91	18.0	76.9
43	27.6	0.39	6.8	98.1
44	43.0	0.43	3.0	100.0
45	72.6	2.24	21.3	43.7
48	41.5	0.94	24.0	45.3
49	32.0	0.46	15.3	29.6
55	46.9	1.86	30.0	30.2
56	42.9	0.89	23.8	51.0
81(susc check)	30.4	0.38	7.5	100.0
82(susc check)	18.4	0.22	4.3	98.0
83(susc check)	21.9	0.30	7.8	100.0
84(susc check)	33.6	0.47	4.0	100.0
LSD(P=0.05)	16.2	0.64	14.4	29.0
CV (%)	28	49	53	35

of chlorotic leaves on some tolerant clones was offset by the ability to produce new leaves from leaf axils. Since percent chlorosis was the ratio of the number of chlorotic leaves to total number of leaves, the possibility that development of new leaves is a mechanism of tolerance was taken into account. The number of leaves produced during infestation was negative for some susceptible clones because of a net loss of leaves, and for these case numbers of leaves were recorded as one in the damage index below. A functional classification for tolerant and susceptible plants was obtained by the following index of plant damage:

$$\text{DAMAGE INDEX} = \frac{\text{Stem height + numbers of trifoliolates}}{\text{Chlorosis (\%)}}$$

Index values for this experiment ranged from 7.85 for clones with minimum damage to 0.23 for clones with high damage (susceptibles) (Table 5). Using LSD = 3.2 ($P < 0.05$), five of the experimental clones had significantly higher index values than the susceptible checks (Table 5). At a probability of 0.30 (LSD= 1.68), an additional ten of the selected clones had also significantly higher index values (low damage).

Other clones were moderately to highly damaged by the infestation and their index values were not statistically different from the index value of susceptible checks. The inclusion of stems dry matter produced in the index did not alter the relative classification of clones and it was unnecessary to include it in the damage index.

The five selected clones with significantly higher index values (low damage), had aphid numbers at day 10 that were very similar to those for susceptible checks (Table 5). As a result of less uniform aphid populations at day 5, some selected clones showed higher

Table 5. SAA counts at day 10 of infestation, aphid population increase, and index of plant damage of 33 tolerant and 4 susceptible clones

Clone #	Aphid[a] Density	Population[b] Increase	Damage[c] Index
01	578	7.1	0.98
04	484	7.2	0.40
05	700	11.3	1.10
07	471	5.4	0.88
08	592	7.2	0.97
09	529	8.7	2.13
10	396	6.1	1.47
11	246	4.4	5.24*
12	604	10.7	2.29
13	218	4.8	3.85*
14	268	4.8	1.77
15	339	6.7	1.89
16	435	6.8	5.54*
18	412	7.8	7.85*
20	309	6.3	1.31
25	451	6.1	2.14
28	577	14.8	2.30
29	391	6.4	2.63
30	366	7.0	1.93
32	178	3.9	2.41
33	320	4.8	3.85*
35	324	4.5	1.55
37	549	6.3	2.25
38	518	5.8	0.67
40	445	5.6	0.53
41	525	7.1	1.04
43	608	10.9	0.35
44	535	6.5	0.46
45	204	5.2	2.84
48	368	5.7	1.76
49	219	5.4	2.04
55	294	5.6	2.73
56	289	4.3	3.44
81(susc check)	465	5.7	0.38
82(" ")	412	6.8	0.23
83(" ")	520	7.4	0.25
84(" ")	501	7.3	0.38

[a]Number of aphids per plant at day 10
[b]Population increase from day 5 to day 10 (X 5 day population)
[c]Damage index = $\frac{\text{plant height + \# trifoliolates}}{\text{Chlorosis \%}}$
*Significantly higher index than susceptible checks LSD (0.05) = 3.2

population increases at day 10 than the susceptible checks. Clones 32, 11, 45, and 55 had relatively low aphid numbers at day 10, but populations that had increased many fold.

Discussion

Infestation of seedlings at the unifoliolate stage with four SAA per seedling for 10 days seems adequate to eliminate susceptible plants and identify those with detectable levels of tolerance to the SAA. To exclude other forms of resistance, seedlings with low number of aphids (less than initial infestation) should be discarded or separated. The cultivar Riley had lower survival percentages when infested at the cotyledon stage and may indicate that the antibiosis form of resistance was not fully expressed at this stage. Some clones that were selected after being infested at the cotyledon stage and showed capability for supporting aphids, expressed moderate levels of antibiosis or antixenosis as mature plants.

Infestation at the unifoliolate and first trifoliolate stages required longer periods of exposure to aphids, to adequately separate susceptible seedlings from tolerant ones either by survival or plant damage. At the cotyledon stage susceptible seedlings were killed by the 6th day of exposure, but at the unifoliolate stage 8 days were required. The clonal test with mature plants indicated that some seedlings selected after only 6 days of exposure showed low levels of tolerance, but those selected after 10 days of exposure were highly tolerant. These results indicated that plants with minimal tolerance levels would not survive the 10 days of exposure at the seedling stage.

Plant height can be used effectively to separate different levels of tolerance at the seedling stage. Extent of veinbanding at the seedling stage was not estimated in this experiment, but susceptible seedlings seem to show this symptom sooner than seedlings with low levels of tolerance. It may be possible that reduced veinbanding can be used as a selection criterion for tolerance. An index that could consider veinbanding, seedling height, and number of leaves may also work well to quantify seedling damage criteria.

The clonal experiment confirmed that plants which were selected as tolerant at the seedling stage; in many cases possessed this trait as mature plants. The study also showed that tolerance is expressed not only by reduced chlorosis as measured previously by Jones (1969), but also by higher numbers of leaves produced, and greater stem growth. The results also emphasize the need to test whole plants when testing for tolerance rather than excised leaves as previously used in an antibiosis test (Thomas and Sorensen 1971).

The development of an index to quantify plant damage has taken in consideration three traits (stem height, numbers of leaves, and chlorosis) that had previously been known to be affected by the SAA. These studies isolated SAA tolerant clones by a greenhouse screening technique and quantified this trait so progress of selection could be monitored.

CHAPTER IV

PROBING BEHAVIOR OF THE SPOTTED ALFALFA APHID AS AFFECTED BY TYPE OF RESISTANCE IN ALFALFA

Introduction

An electronic feeding monitor which gives a graphic representation of feeding activity of sucking insects was developed by McLean and Kinsey (1964). Feeding activity and location of stylets within plant tissues can be determined from current passing through the plant and insect and recorded as wave-shapes on a strip chart recorder. Histological examinations (McLean and Kinsey 1967) were used to verify that specific wave-shapes corresponded with sites of feeding of the pea aphid, Acyrthosiphon pisum (Harris), in alfalfa, Medicago sativa L. More work has been done on dicotyledons than in monocotyledons, but wave-shapes have been generally the same for various aphids and for leafhoppers feeding on different crops (McLean and Kinsey 1967, Brown and Holbrook 1976, Kennedy et al. 1978, Campbell et al. 1982, Khan and Saxena 1984, Velusamy and Heinrichs 1986, Ryan et al. 1987). The monitor has been used for the spotted alfalfa aphid (SAA), Therioaphis maculata (Buckton), on susceptible and resistant alfalfa clones to identify SAA biotypes (Nielson and Don 1974). Wave-shapes were the same as those reported for the pea aphid (McLean and Kinsey 1967).

Location of stylets within plant tissues has been determined by staining of salivary sheaths of the SAA (Diehl and Chatters 1956, McMurtry and Stanford 1960, Kindler and Staples 1969). The stylets are inserted intercellularly between epidermal and mesophyll cells and then intracellulary into the phloem. Feeding sites suggested by the electronic monitor and confirmed by histological examinations show that phloem ingestion is limited for SAA on resistant alfalfa plants. On resistant plants, termination of stylet penetration was found mostly in paranchyma cells and xylem, but feeding at these sites was not verified by histological techniques. The electronic monitor, however, indicated that the SAA ingests fluids for short periods of time at sites other than the phloem cells, especially in resistant hosts (Nielson and Don 1974).

Most studies involving electronic monitors indicate this instrument could aid in identifying different forms of resistance in plants if the resistance affects feeding behavior. In alfalfa, forms of resistance to the SAA have been observed which fit descriptions of antibiosis, antixenosis, and tolerance (Howe et al. 1963, Kishaba and Manglitz 1965, Jones et al. 1968). In previous studies (Chapter III), I have identified susceptible clones to SAA by progeny tests; identified possible tolerant clones by measuring aphid numbers and plant damage; and antibiotic and/or antixenotic clones by measuring reduced aphid development and reproduction.

The objective of this research is to describe to some extent the feeding behavior of SAA on plants which appear to have antibiosis, and/or antixenosis, and tolerance forms of resistance as compared to susceptible plants, and to determine the capability of the electronic

insect feeding monitor in separating these different insect-plant interactions. It could be more practical to identify antibiosis or antixenosis resistance by abnormal aphid probing behavior than by a more lengthy bioassay procedure. The monitor allowed also the comparison of probing behavior patterns on susceptibles plants and those that I had classified as tolerance on the basis that these supported SAA infestations with minimal damage.

Material and Methods

The test clones included six highly susceptible, six highly tolerant, and six moderately antibiotic and/or antixenotic (referred to jointly as antixenotic in this experiment) clones. All clones had been selected from the cultivar 'OK08'. Tolerant plants were characterized by the ability to support SAA infestations with minimal plant damage. Antixenotic plants had been shown to slow the rate of increase in SAA numbers relative to those on tolerant and susceptible plants (see Chapter III). A total of 36 apterous SAA adults were randomly selected from a greenhouse colony for these experiments.

Each monitor was equipped with two 9 volt batteries, an output copper wire carrying 0.5 volts to a potted plant. Aphids were attached to a 5 cm length of 14 K gold wire (10μm dia.) using silver glue and placed on the test plant just below the highest visible node. An input amplifier received the current back from the aphid (2u volts) when it attempted to feed and thus completed a circuit. A strip chart recorder was operated at 0.5 cm per minute to print wave forms generated as aphids fed. Electrical interference was reduced by a wire screen that absorbed static electricity around the work area.

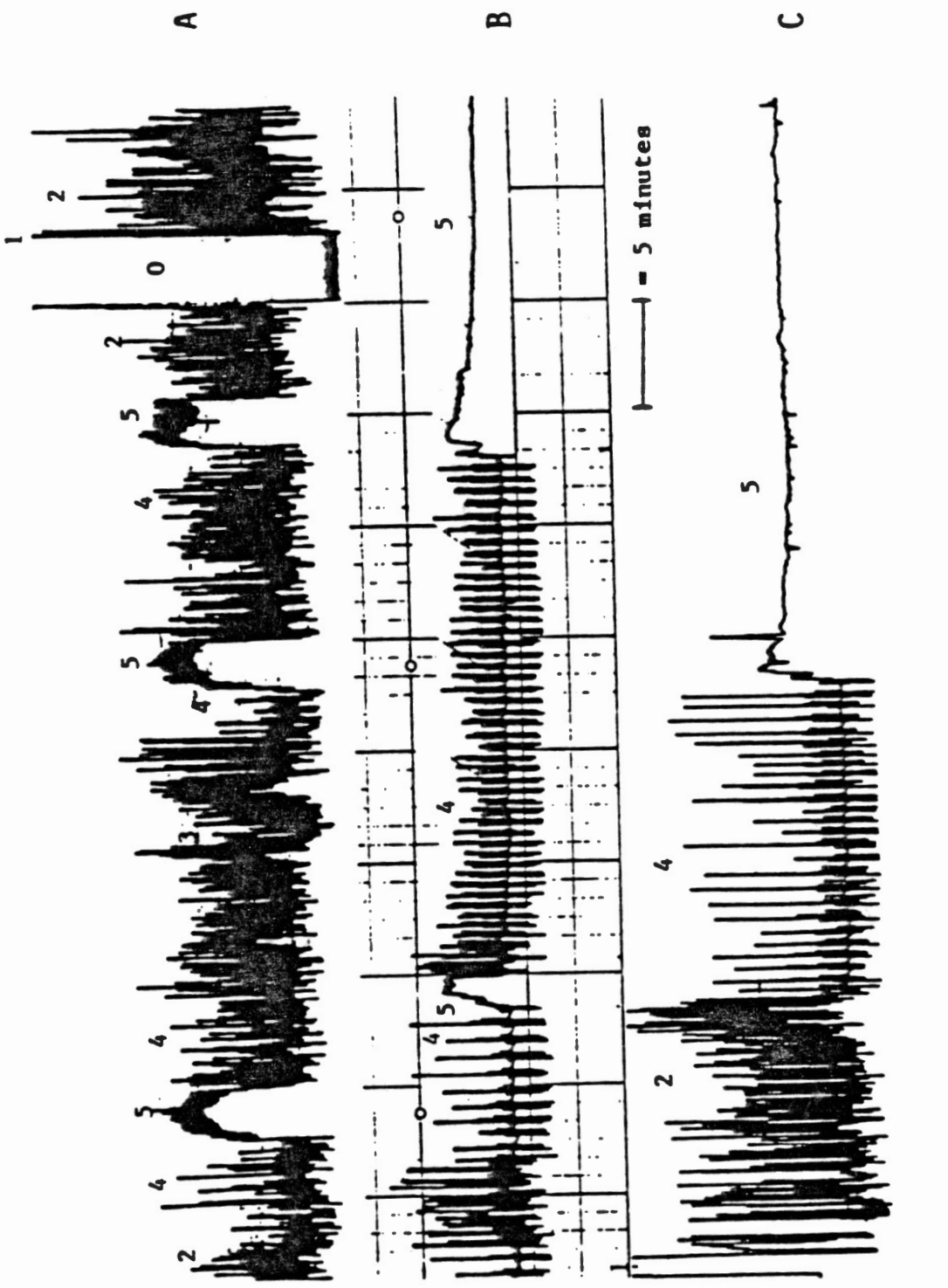
During the first four replications, aphids were monitored for 24 h, but it was realized that continuous close supervision was necessary and that 8 h of monitoring was an adequate time to establish clear cut patterns. Subsequent test were done for 8 h of monitoring and all statistical analysis involved this length of time.

As had been reported by McLean and Kinsey (1967) six different wave shapes were recorded in monitoring of the SAA (Fig. 2). These were: (0) the base line with no feeding activity; (1) the probe wave which denotes the first penetration of the aphid mouth parts into plant tissue (allowing passage of current through the monitor); (2) the salivation wave during intercellular and intracellular penetration of stylet occurs; (3) the nonphloem ingestion wave during feeding; (4) the phloem penetration as stylets penetrate phloem; (5) and phloem ingestion wave during which there was a relatively consistent passage of current.

Waves were measured in centimeters of chart movement from the time a particular wave began to the time it ended. The time devoted to each form was accumulated over the 8 h of the test. Centimeters were converted to minutes by multiplying by two, and data were analyzed as percent of total minutes for each of the particular waves (activities) during the 8 h of the test. Percentages were transformed using square root and the inverse sine funtion before A.O.V. analysis (Steele and Torrie 1980). In addition to the portion of time for each wave, frequency of occurrence was determined from the number of times each wave pattern was recorded.

From recordings I determined the number of successful probes which were defined as a sequence of waves that begins with a probe wave followed by a salivation wave, a phloem penetration wave, and a phloem

Figure 2. SAA feeding wave shapes recorded on antixenotic (A), tolerant (B), and susceptible (C) plants from an aphid feeding monitor. Wave codes are base line (0), probe (1), salivation (2), nonphloem ingestion (3), phloem penetration (4), and phloem ingestion (5). Paper reads from left to right.


A
B
C
= 5 minutes

ingestion wave (probing behavior). To study feeding behavior, I counted the probes that had phloem ingestion for a period longer than 15 minutes which is referred to as committed phloem ingestion (CPI).

The experiment was conducted in a randomized complete block design. A block consisted of three treatments (a susceptible, a tolerant, and an antixenotic plant) with three randomly selected apterous adult aphids on three electronic monitors. There were a total of 12 replications conducted during daytime hours at an average temperature of 22+5^{o}C. Analysis of variance was calculated for the percent time spent on each of the six behavioral patterns and the frequency of occurrence of each. The percentage of total probes that were successful probes and the percentage of the successful probes that resulted in a CPI were also analyzed by an A.O.V after transformation of percentages (Steele and Torrie 1980).

Results

Analysis of variance for the percentage time spent in each of the six feeding behavioral patterns indicated that significant ($P < 0.05$) differences due to plant genotype (e.g., susceptible, resistant, tolerant) occurred in three of the feeding behavior patterns. Analysis of the frequency of occurrence of each behavioral pattern indicated significant ($P < 0.05$) differences due to plant genotype for two of them.

Mean percent of inactive time (base line) during the 8 h of monitoring was significantly ($P < 0.05$) higher for aphids on antixenotic plants than for those on susceptible plants (Table 6). The coefficient of variation was relatively high for inactive time, apparently due to

Table 6. Percentages of monitoring time devoted to different feeding behavioral patterns of the SAA depending on host resistance of alfalfa.

Host Type	Wave Patterns (Percentage of Total Time)					
	Base Line	Probing	Salivation	Nonphloem Ingestion	Phloem Penetration	Phloem Ingestion
Susceptible	4.7[a]	0.9[a]	24.4[a]	6.4[a]	14.9[a]	48.7[a]
Tolerant	7.4[ab]	1.1[a]	34.0[a]	5.1[a]	22.0[a]	30.3[a]
Antixenotic	15.6[b]	1.3[a]	48.4[b]	12.7[a]	20.6[a]	1.8[b]
C.V.(%)	73	49	29	117	32	57

*Mean values having a common letter within a column were not significantly ($P < 0.05$) different under the inverse sine scale.

different levels of antixenosis resistance within this group of plant entries. Percent time spent probing was relatively low for all aphids regardless of plant entry. Time for salivation waves was affected significantly ($P < 0.05$) by the plant entry. Aphids on antixenotic plants produced salivation waves 48% of the time, which was significantly higher ($P < 0.05$) than for those on either tolerant or susceptible plants (33 and 23% respectively).

Percentage of time devoted to nonphloem ingestion was highly variable. Although this behavior accounted for a great expenditure of time for aphids on antixenotic plants, differences between these and other entries were not significant ($P > 0.05$).

Time spent on phloem penetration (X-wave) was relatively high for aphids feeding in each of the plant entries. Phloem penetration time was not statistically different among plant entries ($P > 0.05$).

Time devoted to phloem ingestion was significantly ($P < 0.01$) reduced by antixenosis resistance in plants. Aphids on susceptible plants spent 48% of the monitoring time in this behavioral pattern. Aphids on tolerant plants spent 27% engaged in phloem feeding. Aphids on antixenotic plants spent only about 0.9% of their time in this behavior.

The mean frequency of occurrence of probe waves and inactive behavior were not significantly ($P > 0.05$) affected by plant genotype (Table 7). Frequency of salivation waves was significantly ($P < 0.05$) lower on susceptible plants than on antixenotic plants. Frequency of nonphloem ingestion waves was significantly ($P < 0.05$) higher for aphids on antixenotic plants than for those on either tolerant or susceptible plants. There were no significant ($P > 0.05$) differences in the

Table 7. Frequency (number of times) of behavioral patterns of SAA depending on host resistance of alfalfa entries.

Alfalfa Genotype	Behavioral Patterns (number of occurrences)					
	Inactive	Probing	Salivation	Nonphloem Ingestion	Phloem Penetration	Phloem Ingestion
Susceptible	6.2[a]*	9.0[a]	8.0[a]	1.2[a]	3.2[a]	2.0[a]
Tolerant	8.7[a]	6.7[a]	12.6[ab]	1.4[ab]	6.2[b]	2.9[a]
Antixenotic	8.8[a]	10.2[a]	14.6[b]	3.3[b]	5.9[b]	1.9[a]
LSD ($P < 0.05$)	4.4	4.4	4.2	1.9	2.4	1.5
C.V. (%)	67	61	42	112	54	75

*Mean values having a common letter within a column were not significantly ($P < 0.05$) different.

frequency of occurrence of phloem ingestion waves.

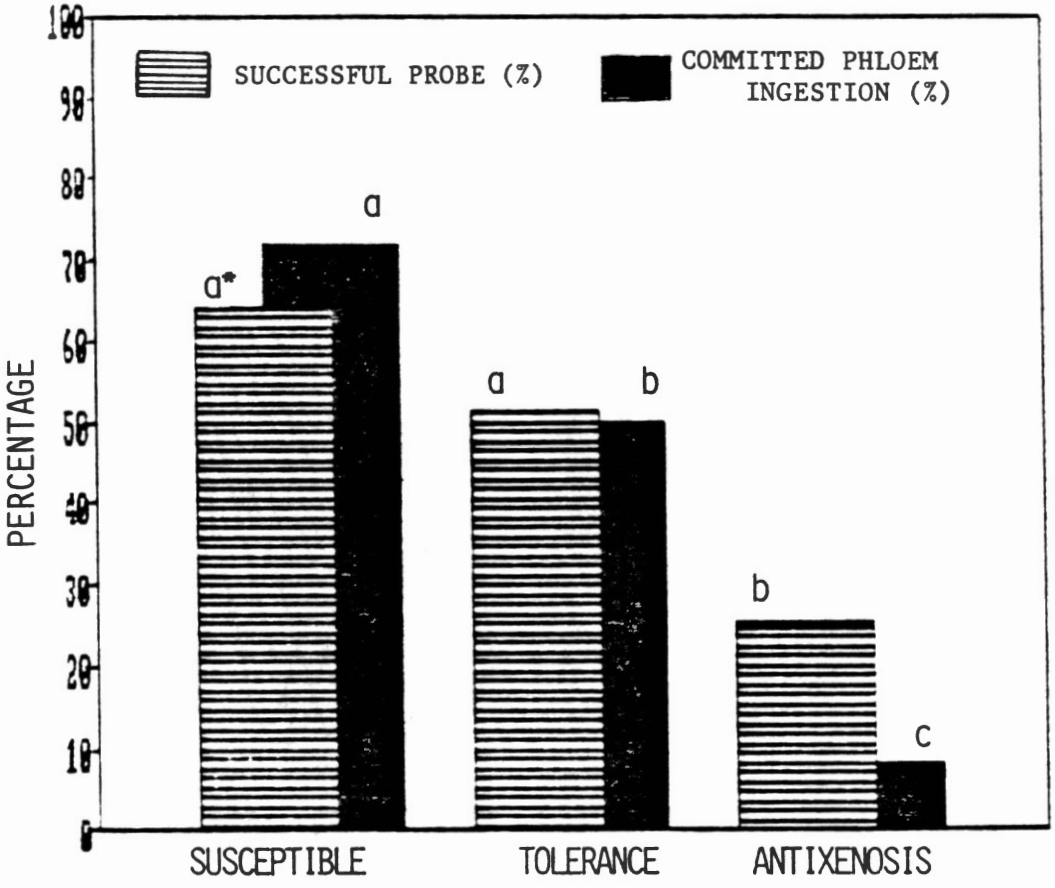
Mean percentage of successful probes for aphids feeding in susceptible plants was significantly ($P < 0.05$) higher than for aphids on antixenotic plants, while there were no differences among those on tolerant and susceptible plants (Fig. 3). Percentage of the successful probes that resulted in a CPI was significantly ($P < 0.01$) lower for aphids on antixenotic plants than for those on susceptible or tolerant plants.

Discussion

Electronic feeding monitors allowed the examination of the probing and feeding patterns of the SAA on plants with different types of resistance. The monitors indicated that most SAA feeding activities were altered by the existence of antibiosis or antixenosis and to a lesser extent by what I have termed the tolerance form of resistance. Aphids on tolerant plants showed the same probing behavior as on susceptible plants when measured as the percentage of successful probes. Aphids on susceptible and tolerant plants showed a normal wave sequence as described by McLean and Kinsey (1967) and Nielson and Don (1974). This sequence started with a probe wave, followed by salivation wave, phloem penetration wave (X-wave), and a phloem ingestion wave. Aphids on antixenotic plants did not complete this normal sequence because they seldom achieved phloem ingestion.

In general, aphids on antixenotic plants produced the phloem penetration wave (X-wave), but had difficulty ingesting from the phloem. In most cases these aphids ceased their attempts at phloem penetration and produced the salivation waves again, followed at times by nonphloem

Figure 3. Graphical representation for mean percentages of successful probes and committed phloem ingestion of SAA feeding on susceptible, tolerant, and antixenotic plants.



* Bars with a common letter were not significantly ($P < 0.05$) different on the inverse sine scale.

ingestion waves. In only two cases in the entire experiment did aphids on the antixenotic plants ingest from the phloem for longer than 15 minutes. In most cases aphids on these plants quit probing after 4 to 5 h and began walking over the surface of the plant producing base line waves. In some instances, they attempted to probe again before the test was terminated. This agrees with previous visual observations in which aphids had abandoned resistant plants after 4 h and moved to susceptible plants when they were available (Kindler and Staples 1969).

Most aphids on susceptible plants produced phloem ingestion waves quickly and continued CPI for a lengthy period, at times for the duration of the test. For this reason aphids on susceptible plants had the lowest number of probes and the longest periods of phloem ingestion. Aphids on tolerant plants also produced phloem ingestion waves quickly, but CPI's were frequently interrupted, especially during the first 3 h of the test. This resulted in less time devoted to phloem ingestion for these aphids.

Feeding behavior on antixenotic plants was clearly distinctive from that on susceptible or tolerant plants as delineated by the electronic feeding monitor. Additionally aphids on the antixenotic plants often failed to produce committed phloem ingestion waves in several hours of attempted feeding. As a result it was also possible to separate this mode of resistance from tolerance on the basis of probing behavior.

CHAPTER V

EXPRESSION AND HERITABILITIES OF TOLERANCE TO THE SPOTTED ALFALFA APHID IN AN ALFALFA POPULATION

Introduction

Development of alfalfa, Medicago sativa L., cultivars resistant to the spotted alfalfa aphid (SAA), Therioaphis maculata (Buckton), provides a classic example of the economic importance of host plant resistance to insects (Maxwell et al. 1974). Losses to SAA were estimated at 42 millions dollars for U.S. in 1956 before resistant cultivars were planted (Howe et al. 1963). All three mechanisms of resistance to insects as described by Painter (1951) have been reported in alfalfa resistance to the SAA (Harvey and Hakerott 1956, Peters and Painter 1958, Howe et al. 1963, Kishaba and Manglitz 1965, Kindler and Staples 1969, Nielson and Olson 1982). The antibiosis or antixenosis mechanisms of resistance are qualitatively inherited and are relatively easy to manipulate by breeders and entomologists. These types of resistance have made possible the quick and successful development of resistant alfalfa cultivars to SAA. Because these forms of resistance are controlled by one or a few major genes, they are likely to be overcome by SAA biotypes. Different alfalfa cultivars resistant to SAA

have shown differential reactions to biotypes of SAA (Nielson and Don 1974).

Tolerance is a form of insect resistance characterized mainly by the lack of differential reaction to insect populations compared to those on susceptible plants. Thus, this resistance causes no selection pressure to be exerted on the insect population. Tolerance is defined as the plant's ability to support insect infestations with less damage than occurs on susceptible plants (Horber 1980).

A way to prevent the development of SAA biotypes on presently resistant alfalfa cultivars may be to incorporate different genes conditioning antibiosis and/or antixenosis and to complement these forms of resistance with genes conditioning tolerance. It is possible to isolate sources for tolerance and then to include these sources in the parentage of new synthetics or strain crosses during the development of new alfalfa cultivars (Bingham 1979, Elgin et al. 1983). Tolerance is quantitatively inherited, however, and the rate of genetic improvement in a breeding population depends largely on its heritability (Gallun and Khush 1980).

Identification and quantification of plant expression of tolerance are necessary for estimation of its heritability. Stunting of growth and chlorosis are the most obvious symptoms observed before plants die due to SAA infestation (Lloyd et al. 1980). Taller plants with reduced chlorosis and accelerated development of trifoliolates have been associated with tolerance to SAA (Cameron et al. 1983). Tolerance to SAA, however, has only been measured by reduced chlorosis Jones (1969). Tolerance of mature plants to the SAA was investigated by testing excised leaflets for the amount of chlorosis produced by SAA feeding in

the laboratory. Leaflets from different plants were infested with SAA and expressed wide variation in the amount of chlorosis produced and were grouped into four classes by Jones et al. (1968). Reduced chlorosis under SAA feeding was inherited by the selfed-progeny of tolerant plants. Jones (1969) reported broad sense heritability to be 0.69 for this trait. Similar studies were needed for whole plants. Additional research to identify other more quantitative plant responses indicating tolerance to SAA was needed. Other traits such as stem height and number of trifoliolates can be more quantitatively measured than estimation of chlorosis.

The objectives of this investigation were to measure the expression of tolerance to artificial SAA infestations in the greenhouse by plant growth of alfalfa, estimate its heritability in selfed and polycrossed progenies, and to calculate realized heritability with selection of tolerant plants. Stem length, number of trifoliolates, and percent of leaves showing chlorosis were investigated to determine their suitability as selection criteria for extent of tolerance present.

Materials and Methods

Experiments

A sample (2400 seedlings) from the cultivar 'OK-08', a registered Oklahoma common cultivar, had been exposed to SAA infestations and stem length from the soil surface to the growing point was measured in all seedlings. Fifty-four seedlings that survived 6 to 10 days of SAA infestation were tested at the mature stage in a clonal test and showed wide ranges of tolerance to SAA as measured by stem length, stem weight, number of trifoliolates, and number of chlorotic leaves (Chapter III).

This population was reduced from 54 to 48 after discarding three plants that did not support aphids well, and three other plants that died later.

The 48 plants with varying levels of tolerance to SAA were self-pollinated and randomly cross pollinated. Ten seedlings from each of the 48 selfed progeny (full-sib family) and 10 seedlings from each of the 48 polycrossed progeny (half-sib family) were initially infested with four adult SAA each and held for 12 days. Seedlings were infested at the unifoliolate stage, and reinfested if needed during the first 3 days of infestation. From each family 20 seeds were planted in 15 cm pots and, upon emergence, were thinned to 10 seedlings per pot.

Half-sib families were tested during the spring of 1986 and full-sibs during the fall of 1986. Both tests were conducted at approximately 25 $\pm$ 5^{o}C and 16 h day length maintained with artificial lighting. I included 100 seedlings from the original OK08 population in 10 other pots to obtain the mean of the original population under the same environmental conditions as when the progenies were tested.

Aphids per seedling were counted at the end of the test and averaged for each pot. The average stem length, number of trifoliolates, and number of chlorotic leaves per plant were obtained for each of the families. The averages of stem length and numbers of trifoliolates of a family were regressed on the values of the parents to estimate heritability of these two plant traits under SAA infestation. These expressions of tolerance were also combined into a single value (stem length plus numbers of trifoliolates), and regression analysis based on this value of parents and progeny was also obtained.

Parent-offspring regression is regularly used for the estimation of narrow sense heritability, which is the ratio of additive variance to the phenotypic variance (Falconer 1983). Because of self-fertilization of the parents to obtain the selfed progeny, estimation of additive variance by regression analysis may be biased due to dominant effects. These effects could be further intensified by the autotetraploid nature of alfalfa. As a result I have referred to the selfed progeny test as the estimation of heritability in the broad sense, which is the ratio of genetic variance, including dominant effects, to the phenotypic variance (Falconer, 1983).

From the polycrossed progeny test, I selected 75 plants, including one or two from each half-sib family. Some progenies with low levels of tolerance had only one reasonably healthy survivor. These newly selected plants (second cycle) were then randomly polycrossed by hand and the resulting progenies were similarly tested with SAA as the first polycrossed progeny test. This time, however, when two progenies had originated from the same selected plant, seeds were mixed and tested as one family. Again, a sample of 100 seedlings from the original OK08 cultivar was included to obtain the mean of the original population.

Heritability Estimates

Broad sense heritability was estimated using two procedures from two separate experiments. First by partition of the mean square of the A.O.V.'s obtained for four traits under SAA infestation in a clonal test. The clonal test was designed to quantify the levels of tolerance by testing four well-established stem cuttings per genotype (Chapter III) but also allowed for the estimation of broad sense heritability.

The error mean square was the estimate of variation due to environment, and the treatment (clones) mean square estimated both environmental and genetic variance (Table 8). Heritability was the ratio of genetic variance to phenotypic variance. A second estimate was the coefficient of regression of full-sib families (self-progeny) on the parents for stem height and number of trifoliolates after SAA infestation.

For the estimation of heritability in the narrow sense, it is necessary to exclude from the genetic variance all dominant effects leaving only additive variance. In autotetraploid species estimation of the additive variance without any dominant effects cannot be obtained as in diploid species (Jacquard 1983). With low inbreeding, and with polygenic inheritance of genes with minor additive effects, twice the coefficient of the regression of parents on their half-sib families can be considered a good estimate of narrow sense heritability (additive variance/phenotypic variance) (Levings and Dudley 1963). I expected tolerance to the SAA to be mainly due to the effect of many genes with minor additive effects and inbreeding to be low in the polycrossed progenies.

Narrow sense heritability was estimated by the coefficient of regression of means for plant parameters measured for half-sib families and those recorded for the parents at the time these were selected. This regression coefficient estimates only half of the heritability since gene effect of the maternal parent is reduced by half in the progeny. The coefficient of regression was multiplied by two to obtain the full estimate.

Realized heritability which measures the actual gains from selection and intercrossing (polycross) was calculated for the two

Table 8. Partition of mean squares for the estimation of broad sense heritability (h^2_{BS}) , by a clonal test (4 cuttings per each of 50 clones)

Source	D.F	A.O.V. Mean square	Expected mean squares
Total	131		
Blocks	3	M_1	$\sigma_E^2 + 50\,\sigma_C^2$
Among clones	32	M_2	$\sigma_E^2 + 4\,\sigma_G^2$
Within clones Error	96	M_3	σ_E^2
Genetic variance		M_4	$M_2-M_3/4$
Evironmental variance			M_3
Phenotypic variance		M_5	M_3+M_4

h^2_{BS} = Genetic variance/Phenotypic variance

cycles of selection by the following procedure. Selection differential (S) was the mean deviation of the selected parents from the unselected OK08 population. Response to selection (R) was the mean deviation of the half-sib families from the mean of the unselected OK08 population. Selection differential was calculated for each cycle of selection.

Realized heritability was the ratio of the cumulative response to selection in the second cycle to the selection differential of the two cycles of selection as described in Falconer (1983). Only stem length, after SAA infestation, was used for the calculations of realized heritability. It was necessary to adjust the means of the first progeny test because of a nonproportional increase of stem length in the progenies relative to the unselected population. Means of the first progeny test were multiplied by the ratio of stem length increase from first selection to first progeny test of the unselected population.

Results and Discussion

Broad sense heritabilities of tolerance to SAA, as estimated by the clonal test, were relatively high for all four traits (Table 9). Tolerance to SAA as indicated by reduced chlorosis had the highest heritability (0.56), which was similar to a previous report of 0.69 by Jones et al. (1969). Heritability of a damage index (stem length + No. trifoliolates/chlorotic leaves %) to SAA was 0.52 in the broad sense by the clonal test.

Phenotypic correlation of plant measurements were highly significant and similar in the selfed and polycrossed progenies (Table 10). The highest coefficient of correlation for parents and progeny

Table 9. Mean squares from A.O.V's of clonal test and the estimates of broad sense heritability of different expressions of tolerance to SAA.

Plant parameter	Mean Squares: Phenotypic Variance	Environmental Variance	Genetic Variance	H^2 [a] BS	C.I [b] 95%
Stem height (SH)	219.8	134.2	85.6	0.39	(0.21,0.57)
Trifoliolates (TF#) number	172.9	105.8	67.1	0.39	(0.21,0.57)
Chlorosis % (CHL%)	10.0	4.4	5.6	0.56	(0.41,0.71)
Dry matter (DM)	0.4	0.2	0.1	0.40	(0.18,0.58)
$\frac{SH + TF\#}{CHL\%}$ [c]	10.8	5.2	5.6	0.52	(0.06,0.41)

[a] Broad sense heritability = $\frac{\text{Genetic variance}}{\text{Phenotypic variance}}$

[b] Confidence interval for heritability with 95% probability.

[c] Index of plant damage developed in clonal test (chapter 3).

Table 10. Correlation coefficients for parents and progenies (genetic correlation) and correlation of plant measurements (phenotypic correlation).

Plant Parameter	Genotypic (R)		Phenotypic(R)			
	Parent-Progeny		Polycrossed progeny		Selfed progeny	
	Selfed	Polycrossed	Height	Trifoliolate	Height	Trifoliolate
Height	0.29*	0.14				
Trifolates	0.15	0.32*	0.93**		0.93**	
Chlorosis	0.19	0.13	-0.75**	-0.80**	-0.74**	-0.79**

*, ** Significant (t-test) at the 0.05 and 0.01 levels of probability, respectively.

(genotypic correlation) was obtained with numbers of trifoliolates developed during SAA infestations of the half-sib families.

The coefficients of regression and their standard errors for each of the plant parameters and a combination of them are presented for the selfed and the polycrossed progenies test (Table 11). The broad sense heritability estimated by the regression coefficients of the selfed progeny test was 0.76 as measured by stem length under SAA infestation. Narrow sense heritability (polycrossed progenies) of tolerance was 0.16 as measured by stem length.

An index of stem length plus trifoliolate number showed a relatively low standard error, a high broad sense heritability, and an intermediate narrow sense heritability (Table 11). This combination seems to offset any possible differential response of stem height and number of trifoliolates when considering them individually.

Realized heritability measured by stem height under SAA infestation was 0.53 for the first cycle of selection and 0.41 for the cumulative response after two cycles of selection (Table 12). Those estimates were intermediate between the narrow sense heritability (0.16). and to the broad sense heritability (0.76) estimated by regression analysis of stem length (Table 11).

The mean stem length of new selected plants from the first polycrossed progeny test was lower than the mean of all progenies in the test (Table 12). This was probably due to the fact that seedlings supporting the highest number of aphids in each progeny, were the ones chosen to be the new parents and they had shorter stems. The lower stem length mean of new selected parents produced a lower selection

Table 11. Estimates of broad sense and narrow sense heritability of three plant traits after SAA infestations.

Plant Parameter	Broad Sense Heritability[a]	Narrow Sense Heritability[b]
Height	0.76 ± 0.36	0.16 ± 0.16
Trifoliolate	0.38 ± 0.36	0.80 ± 0.36
Height + Trifoliolates	0.83 ± 0.43	0.48 ± 0.28

[a]Broad sense = b (coefficient of regression) selfed progeny test

[b]Narrow sense = 2b (twice the coefficient of regression) of the polycrossed test, and 2X the standard error.

Table 12. Means for stem height after 12 days of SAA infestation, selection differential, response to selection, and estimation of realized heritability for this trait.

Selection Test	Stem Height (cm)					
	Unselected population	Selected parents	Progenies	S[a]	R[b]	RH[c]
First cycle	1.02	3.60		2.58		
Polycross progeny test						
Unadjusted	2.20	4.50	5.20			
Adjusted[d]	1.00	2.05	2.36		1.36	0.53
Polycross 2 progeny test	1.00		2.50	1.05	1.50	
cumulative				3.63	1.50	0.41

[a]Selection differential = mean of selected parents minus mean of unselected population.
[b]Response to selection = mean deviation of progeny from unselected population mean
[c]Realized heritability = Response to selection/Selection differential (R/S)
[d]Means of first polycross were multiplied by the increase ratio of the unselected population (1.02/2.20) to standarized an increased in stem length for first progeny test.

differential and it could inflate a little the cumulative estimation of realized heritability for the two cycles of selection (0.41).

Counts of aphid numbers at the end of each of the progeny tests indicated that the infestation levels of the unselected OK08 population were similar or somewhat lower than in the selected progenies (Table 13). Development of SAA infestations during the three progeny tests conducted in this experiment varied somewhat, but for 12 days of infestation, tolerant progenies supported higher numbers of aphids per seedling than their susceptible counterparts. For the unselected population, 12 days of infestation had eliminated many susceptible seedlings, which resulted in the lower numbers of aphids per seedling than the tolerant progenies at the end of each test.

Regression coefficients of stem length or in combination with numbers of trifoliolates gave estimates of heritabilities with lower standard errors. Expression of tolerance to the SAA in the cultivar OK08 after 12 days of infestation as measured by stem length plus trifoliolate number gave heritabilities of 0.83 (broad sense) and 0.48 (narrow sense). These estimates and the response to selection obtained in two cycles, indicated that acceptable progress in selection for tolerance to SAA can be obtain in two or three cycles of selection using the modified screening techniques developed in chapter III.

Table 13. Means (± SE) for numbers of aphids at termination of each progeny test.

Selection Cycle	Aphids per plant	
	Progenies	Unselected population
1	10.9 ± 10.0	13.9 ± 10.2
2	14.3 ± 5.2	6.7 ± 3.9
Selfed-progeny	10.0 ± 8.0	3.0 ± 5.6

CHAPTER VI

SUMMARY AND CONCLUSION

Modified greenhouse screening techniques were used to detect tolerance to the spotted alfalfa aphid, (SAA) Therioaphis maculata (Buckton), in a susceptible alfalfa, Medicago sativa L., cultivar. Screening procedures consisted of infesting seedlings at the unifoliolate leaf stage with four adult aphids for a period of up to 10 days. Susceptible seedlings rarely survived beyond 6 days of infestation and moderately tolerant seedlings lived about 8 days. Those plants with higher levels of tolerance lived 10 days.

Tolerant plants identified in the greenhouse screening, had the capability to support SAA infestations with minimal damage at the mature stage in a clonal test. This clonal test was also used to delineate three plant traits that could be used to quantify tolerance to SAA. These traits were stem height, numbers of trifoliolates produced per stem, and the percentage of leaves on each plant which exhibited chlorosis. These data were used in calculating a damage index that served in separating highly damaged plants (damage index = 0.3 to 2.1) from plants with moderate to low damage (damage index = 2.2 to 7.9) .

The most tolerant plants were compared to susceptibles and antixenotic plants by an electronic aphid feeding monitor that recorded the probing and feeding behavior of aphids. Probing patterns of SAA on

plants with antibiosis or antixenosis types of resistance were quite different than those on susceptible plants. Patterns for aphids on tolerant plants were not significantly different from those on susceptibles. Percentages of successful probes were 50 and 38% for aphids on susceptible and tolerant plants, respectively. Only 12% of the total were successful probes for aphids feeding on antibiotic or antixenotic plants. In 8 h of monitoring, aphids spent 48% of the time in phloem ingestion in susceptible plants, 30% for plants with tolerance, and 2% when plants had shown antibiosis or antixenosis resistance.

Selected tolerant plants were selfed and randomly cross-pollinated and the levels of tolerance of the progenies were tested. Heritability of tolerance to SAA was estimated using plant parameters including increased in stem height, higher numbers of trifoliolates, and lower percentage of leaves exhibiting chlorosis as indicators of tolerance. Heritability calculated by stem height comparisons was 0.16 in the narrow sense, and 0.76 in the broad sense. Realized heritabilities for two cycles of selection for tolerance to SAA using stem length was 0.41.

A second estimate of broad sense heritability using three plant parameters in an index of damage from a clonal test after 19 days of SAA infestation at the mature stage was 0.52.

These studies indicate that it is possible to select plants possessing tolerance to SAA by modification of current screening techniques. Good progress from selection can be expected after two to three cycles of selection from a susceptible cultivar. An electronic feeding monitor was useful to compare the feeding behavior of the SAA on plants possessing tolerance and antibiosis or antibiosis. This

instrument can aid in confirmation of resistance due to antibiosis or antixenosis and differentiation of these forms from resistance due to tolerance.

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APPENDIX

Table 14. F-values from A.O.V. for four plant measurements and the interactions of two cultivars, three ages at infestation, and five exposure times to SAA in the greenhouse

TREATMENT	D.F	Plant Parameters			
		Survival %	Stem Height	Root Weight	Stem Weight
Age (A)	2	1.81	13.09**	7.36**	7.77**
Exposure (E)	4	16.31	17.76**	14.19**	10.36**
Cultivar (C)	2	56.28	139.16**	55.59**	89.45**
A X E	8	1.90	1.29	0.51	0.58
A X C	2	3.10	1.80	0.42	0.51
E X C	4	13.47	15.22**	6.58**	3.29**
E X C X A	8	0.66	0.40	0.60	0.30
(Error D.F. = 203)					
C.V (%)		20	30	49	49

*, **, Significant differences at 0.05 and 0.01 leves of probability, respectively.

Table 15. F-values from A.O.V. of four plant measurements of Riley and the interaction of three ages at infestation with five exposure times to SAA in the greenhouse.

		Plant Parameters			
Treatment	D.F.	Survival %	Stem Height	Stem Weight	Root Weight
Age (A)	2	0.94	4.40*	3.16	4.14*
Exposure (E)	4	0.70	0.43	1.97	2.22
A X E	8	1.15	0.86	0.43	1.10
(Error D.F= 98)					
C.V. (%)		13	18	31	29

* Significant differenses at the 0.05 of probability

Table 16. F-values from A.O.V of four plant measurements of OK08 and the interaction of three ages at infestation with five exposure times to SAA in the greenhouse.

Treatment	D.F.	Plant Parameters			
		Survival %	Stem Height	Stem Weight	Stem Weight
Age (A)	2	3.10	10.00**	6.70**	4.64**
Exposure (E)	4	20.59**	26.60**	13.48**	16.59**
A X E	8	1.37	0.97	0.63	0.43
(Error D.F.= 98)					
C.V (%)		26	41	67	69

*, **, Significant differences at the 0.05 and 0.01 levels of probability, respectively.

VITA

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