THE EFFECT OF SPIREA APHID (HOMOPTERA: APHIDIDAE) FEEDING AND NITROGEN FERTILIZATION ON THE GROWTH OF YOUNG APPLE TREES, WITH COMPARISONS TO APPLE APHID

by

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(ABSTRACT)

The overall goal of this research was to determine the effects of spirea aphid, Aphis spiraecola Patch, feeding and nitrogen fertilization on net photosynthesis (Pn), leaf chlorophyll content and greenneess, growth, dry matter accumulation, and carbohydrate concentrations of young apple trees, with comparisons to apple aphid, Aphis pomi DeGeer. Trees were artificially infested and grown in an unheated greenhouse with screened ends.

The spirea aphid responded differently to various nitrogen treatments. Aphid density increased at a faster rate on trees receiving higher nitrogen application. The leaf nitrogen concentration increased significantly and linearly with increasing amount of urea application in both infested and control leaves. Also, a significant difference in leaf nitrogen concentration was found at each urea application rate between infested and control leaves.

Spirea aphid feeding and sooty mold accumulations caused significant reductions in photosynthetic rates, leaf chlorophyll content, and greenness. Pn increased linearly with increasing chlorophyll content and greenness; nitrogen rates caused an increase in

Pn and leaf greenness. Aphid-days accumulations were strongly correlated to Pn and greenness at each nitrogen rate applied. Accumulation of callose at the phloem sieve plates in response to spirea aphid feeding occurred but to a lesser degree than from other aphids reported on apple and pecan leaves.

Accumulation of fresh and dry weights in all tree parts (leaves, lateral shoots, trunk, rootstock, and roots) during the growing season were affected by both spirea aphid and nitrogen fertilization. The spirea aphid reduced accumulation of fresh and dry weights in all tree partitions when trees were harvested at the end of the first growing season. These reductions were still lower than the control when trees were harvested at the ten-leaf stage the following spring. The spirea aphid caused a significant reduction in lateral shoot growth at the end of the growing season and at the ten-leaf stage. Fresh and dry weights of all tree partitions tended to increase with increasing rates of nitrogen.

The percentage and the amount of nonstructural carbohydrates (NSC) in all tree partitions were reduced by spirea aphid feeding and were positively related to nitrogen rate. At the ten-leaf stage in the second season, similar results were obtained.

Development of spirea aphid and apple aphid was similar on trees fertilized with a moderate rate of nitrogen. Pn and leaf greenness declined to a similar extent with accumulated aphid-days, for both aphid species. Aphid species did not affect any of tree growth or NSC accumulation.

To my lovely wife, Wafa

for her thoughtfulness, warmth, and understanding

for her good advice, tender, and loving care

for all the treasured memories she's always quick to share

To my wonderful daughters, Yaman and Rola

who are smart and pretty and as sweet as they can be

To my terrific son, Anas

who filled a home with smiles, making

each day happier with the wonderful things

To all of them

I dedicate this dissertation

without asking their permission

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GENERAL INTRODUCTION AND OBJECTIVES

The ubiquitous aphids, with their capacity for explosive increase and potential for transmitting diseases, have been the subject of investigations by many entomologists. Their interesting and often complicated biologies have attracted the attention of not only entomologists, but workers in the larger fields of zoology, general biology, and agriculture.

Apple is a high-value crop. The annual cash receipt from apples in Virginia accounts for 40-60 million dollars annually (3-4% of the agricultural income)(Virginia 1985). Three species of Aphididae are commonly reported overwintering as eggs on apple (Malus domestica Borkhausen): Aphis pomi DeGeer (apple aphid), Dysaphis plantaginea (Passerini) (rosy apple aphid), and Rhopalosiphum fitchii (Sanderson) [the same as R. insertum (Walker) of European workers], the apple grain aphid) (Matheson 1919, Lathrop 1928, Brunner & Howitt 1981). However, the spirea aphid, Aphis spiraecola Patch, has also been recorded from apple in Virginia and elsewhere (Parrella et al. 1981, Blackman & Eastop 1985). The spirea aphid, which is the most common aphid pest of citrus in both Nearctic and Palaearctic regions (Neubauer et al. 1981), greatly outnumbers apple aphid in most of the apple orchards sampled in Virginia, West Virginia and Maryland (Pfeiffer et al. 1989).

Aphids feed by inserting their long, thin stylets deep into the phloem tissues of their plant hosts (Kennedy & Stroyman 1959), injecting watery saliva into each cell on which they feed, and sucking out the contents (Miles 1972). As soon as they start to feed, they excrete a honeydew which attracts ants, bees, and other insects, and supports the growth of sooty mold (Swart 1978).

Hall & Ferree (1976) simulated insect feeding injury on apple net photosynthesis (Pn) using a cork borer to remove sections of leaves and found that a 10% or greater

loss in leaf area caused a reduction in Pn. Both Hall & Ferree (1976) and Poston et al. (1976) found that it is important to avoid the midrib or the main lateral veins of the leaf to simulate damage by insects that feed in the interveinal area because veinal injury causes a large reduction in Pn. Ferree & Hall (1981) studied the influence of physical stress and the effects of simulated insect injury on Pn of apple leaves and stated that the amount of cut surface exposed by injury was more important than the amount of leaf area removed.

The effects of aphids on Pn and leaf chlorophyll content have been reported (Wood et al. 1985, Varn et al. in press). A study was conducted by Wood et al. (1985) to determine the feeding influence of three pecan aphid species on Pn of pecan seedlings (Carya illinoensis Wangenheim K. Koch). Increasing populations of any of the three species resulted in up to 50% reduction in Pn and increased accumulation of callose at the phloem sieve plates. Pecan aphids also caused a reduction in Pn in mature pecan trees (Wood & Tedders 1982). Recently, Varn et al. (in press) indicated that rosy apple aphid reduced Pn of apple leaves by 50-55%.

Leaf chlorophyll content is an important plant component that provides a measure of plant photosynthetic potential. Wood et al. (1985) reported that pecan aphids reduced the chlorophyll content of pecan leaves. The feeding by rosy apple aphid, the most damaging of the aphids infesting apple leaves, significantly reduced leaf chlorophyll content of apple leaves (Varn et al. in press).

Research on the effects of apple aphid on wood formation by mature apple trees has been restricted to the measurement of terminal shoot growth (Oatman & Legner 1961, Hamilton et al. 1986). Dixon (1971a & b) investigated effects of aphids on wood formation in sycamore and European linden saplings. Drepanosiphum platanoides

(Schrank) reduced sycamore (Acer pseudoplatanus (L.)) leaf size by 40 % and the production of stem wood by 62 % (Dixon 1971a). European linden saplings (Tilia vulgaris Hayne) infested with Eucallipterus tiliae L. increased less in weight because of poor root growth (Dixon 1971b).

Three pecan-infesting species were reported to significantly reduce dry matter accumulation in stems and roots of potted seedlings grown in a greenhouse (Tedders et al. 1982). A reduction in root dry weight (approximately 7-10%) in apple trees infested by Panonycus ulmi (Koch) for three seasons was reported by Hull et al. (1986), but there was no significant difference from the control in the dry weights of aerial portions of the trees. Varn & Pfeiffer (1989) indicated that rosy apple aphid significantly reduced accumulation of dry weight in all portions of apple trees during the first season's growth (i.e. after arrival from the nursery). At the ten-leaf stage of the second season, the dry weight of trees infested with rosy apple aphid during the previous year were still significantly lower than those of control trees. The spirea aphid, at a single population density, did not reduce dry weight accumulation of the young trees.

The nitrogen state of the host plant affects photosynthetic activity (Longstreth & Nobel 1980, DeJong 1983, Evans 1983, Syvertsen 1985, 1987) and carbohydrates (Smith 1966, Ramamurthy & Ludders 1982). The effects of spirea aphid and nitrogen fertilization on net photosynthsis, leaf chlorophyll content, growth, dry matter accumulation, and carbohydrate concentration in young apple trees have not been evaluated. Correlation between orchard pests and physiological parameters have centered mainly on mites and more recently on leafminers. The overall goal of this research is to determine the effects of feeding by spirea aphids on the growth of young apple trees before establishing an Integrated Pest Management (IPM) program. No

thresholds have been determined for this pest on bearing or nonbearing apple trees. A long-term goal is to derive a realistic economic injury level for this pest on apple. To achieve this goal, experiments were designed to gather information based on the following aspects:

1. Determination of spirea aphid population abundance and the morphs present on young apple trees during the growing season (before leaf abscission), and the effect of tree fertilization (foliar nitrogen) on aphid population growth.

2. Determination of the effects of spirea aphid feeding and nitrogen fertilization on a variety of plant physiological and morphological characters (i.e. net photosynthesis, leaf chlorophyll content and continuity of the phloem).

3. Determination of the effects of spirea aphid and nitrogen fertilization on the growth, dry matter accumulation, and carbohydrate concentration of young apple trees.

The relative impact on tree parameters from feeding by A. spiraecola vs. A. pomi is considered in the above studies.

LITERATURE REVIEW

Introduction

The spirea aphid or the green citrus aphid, Aphis spiraecola Patch (previously reported as Aphis citricola Van der Goot), ranks as a major agricultural insect pest today because of its widespread distribution and the considerable damage inflicted on citrus trees and other cultivated plants. This species was observed by many entomologists over a period of 70 years (Gillette 1910, Patch 1914, Dickson et al. 1956, Tao & Tan 1961, Brooks 1968, Bullock 1972, Porath et al. 1975, Furk 1979, Barbagallo & Patti 1982, Komazaki 1983, Zehavi & Rosen 1987).

The spirea aphid is distributed on citrus in both Nearctic and Palaearctic regions. Through the movement of nursery stock, the spirea aphid has spread far outside its natural range and several separate accidental importations account for its appearance on the North American continent (Neubauer et al. 1981, Barbagallo & Patti 1982, University of California 1984).

The first North American record of the spirea aphid was from bridal wreath, Spiraea prunifolia Siebold & Zuccarini, in Colorado in 1907 (Gillette 1910) and subsequently from spirea in Washington D. C., New York, and Michigan in 1909 (Wolcott 1954). Gillette (1910) tentatively identified the species collected from Colorado as Aphis spiraeella Schoutenden. Van der Goot (1912) described A. citricola from Citrus in Chile. Patch (1914) recognized that two similar aphid pests were present on fruit trees and ornamental shrubs in North America and were confused under the name Aphis pomi DeGeer (the apple aphid); she then erected A. spiraecola Patch. In 1923, Patch considered A. spiraecola Patch to be only a race of A. pomi because of their morphological similarities and because both colonize spirea as well as apple.

Geographic origins are probably European for A. pomi (Matheson 1919) and Far Eastern for A. spiraecola (Blackman & Eastop 1985). A. spiraecola achieved pest status in 1924 and caused a marked curling and rosetting of active shoots of citrus (Wolcott 1954). Hille Ris Lambers (1975) slide-mounted some of the damaged specimens from the original collection of A. citricola and reported that 'as suspected' they were the species previously known as A. spiraecola. Since then A. spiraecola has most frequently been referred to as A. citricola. Eastop & Blackman (1988) studied the slide-mounted aphids from Hille Ris Lambers'collection and they reported that they were not A. spiraecola but Aphis fabae Scopoli. Eastop & Blackman stated that the name A. citricola should be regarded as synonym of A. fabae rather than the valid name for the citrus pest, which should again be known as A. spiraecola Patch.

Biology of The Spirea Aphid

Various aspects of the life history of the spirea aphid have been studied on citrus by many workers (Higuchi & Miyazaki 1969, Tanaka 1976, Dzhibladze & Kokhreidze 1979, Komazaki et al. 1979, Barbagallo & Patti 1982, Komazaki 1983, Komazaki et al. 1985, Zehavi & Rosen 1987). The spirea aphid has two different life cycles varying in relative importance in different parts of its range. In temperate parts of the world a holocyclic life cycle occurs. In the holocyclic life cycle, oviparae lay overwintering eggs on spirea (primary host), and colonies derived from these eggs produce alatae in spring which migrate to citrus and other food-plants (secondary hosts) in the vicinity. This part of the population may play a major role in the spring infestation of citrus trees (Komazaki et al. 1979, Barbagallo & Patti 1982). In many countries of the world where the winters are relatively warm, the life cycle is anholocyclic. In this cycle, asexual forms overwinter in colonies on weeds and crops and produce dispersing alatae the following

spring. The development of aphid colonies on citrus (as secondary host plants) is dependant upon the presence of tender shoots. Therefore, the maximum population density is reached late in spring (May or early June). The survival of the spirea aphid during the winter is probably enabled by the permanence of small colonies on occasional tender shoots of the same plants. An economic threshold of 10% infested shoots for the spirea aphid on young citrus trees is suggested (Delrio et al. 1981, Barbagallo & Patti 1982).

The spirea aphid was discovered to overwinter on citrus in Japan in 1979 (Komazaki 1983). Although the spirea aphid has long been known to colonize apple as a secondary host (Patch 1923, Parrella et al. 1981, Blackman & Eastop 1985), only recently it was reported using Malus as a primary host (Pfeiffer et al. 1989). Pfeiffer et al. (1989) showed that spirea aphid greatly outnumbers apple aphid in early summer in apple orchards of Virginia, West Virginia, and Maryland.

The most detailed study of the holocyclic life cycle of the spirea aphid was conducted in Japan by Komazaki (1983), who clarified the differences in the overwintering habits of this species on citrus (Citrus unshiu Marc.) and spirea (Spiraea thunbergii Sieb.) and also compared how the two plants affect the spring infestation in citrus groves. Komazaki found that the aphid overwintered holocyclically and anholocyclically on spirea but only holocyclically on citrus. The aphids were more densely populated on spirea than on citrus; spirea is probably more suitable as a winter host than citrus. On spirea, viviparous nymphs and adults were observed at the end of January in 1980. The aphid population decreased until mid-February, when it began to increase. On citrus, the percentage of sexual morphs was highest in mid to late November, and decreased to zero by early February. Nymphal oviparae and adult males

began to appear in October on spirea and early in November on citrus. Oviparae began to oviposit in mid or late November and lasted until late December or January.

The egg hatching time on spirea differs from that on citrus even in the same district. On spirea, eggs began to hatch late in January, while on citrus they began to hatch in early or mid-March when hatching on spirea had already finished (Komazaki 1983).

The sexuales of spirea aphid overwintering on citrus and spirea were experimentally crossed by Komazaki (1986). The eggs of crossed stains hatched between the time of field egg hatch on citrus and that on spirea. This hatching sequence was retained for over one year in the inbred populations. The egg hatching time is thought to be genetically determined and these populations differing in overwintering host are defined as the biotypes. It was suggested by Komazaki (1986) that the host preference of these biotypes may differ.

In spring, the population rises again until the alatae appear from May onward, when their dispersal and a strong buildup of natural enemies cause a sharp reduction in numbers. The appearance of sexual forms on spirea was more than 20 days earlier than on citrus when the citrus grove invasion began in mid-May. The first peak of alate infestation in groves was between late May and early June. Komazaki (1983) stated that the period of invasion of alatae overlapped more with the period of the presence of alatae in the overwintering population on citrus than with that in the overwintering population on spirea. It was suggested that the spirea emigrants might not play a major role in the spring infestation in citrus groves but that the citrus overwintering population might be important in this infestation.

The specific tissue feeding site for spirea aphid on citrus or pome fruits is not known; however, in general, aphids feed by inserting their long, thin stylets deep into the phloem tissues of their plant hosts (Kennedy & Stroyman 1959, Auclair 1963), injecting watery saliva into each cell on which they feed, and sucking out the contents (Miles 1972). As soon as they start to feed, they excrete honeydew which attract ants, bees, and other insects, and supports the growth of sooty mold (Swart 1978).

The spirea aphid causes a marked curling and rosetting of the new flushes of growth of citrus trees. No other aphid produces such obvious distortion of citrus leaves (Watson & Beyer 1925, Cermeli 1969, Raccah et al. 1978). The crumpling and distortion of the leaves by spirea aphid that are so characteristic of infestations on citrus were barely perceptible on the tender terminal leaves of Prunus occidentalis and had been completely outgrown or had disappeared on the older leaves (Wolcott 1954). The spirea aphid also attacks the young leaf flush in mandarin (Citrus reticulata Blanco) groves in India and causes crinkling, curling and sometimes premature leaf-abscission (Singh & Rao 1978).

Mature citrus leaves are unsuitable for feeding by spirea aphid. Upon maturity, the cuticle and cell walls of citrus leaves develop a condition that renders them repellent to aphids and this condition accounts for the brevity of the probes on these leaves (Zettler et al. 1969). Takeda (1980) showed that the spirea aphid prefers the immature leaves on apple to the mature ones and that leaf selection is closely related to the nitrogen content of the leaf.

In citrus growing regions this aphid has attracted considerable attention in recent years, as it has been shown to be a vector of various plant viruses, the most serious disease being tristeza virus of citrus (Norman & Grant 1961, Servazzi et al. 1967, Adlerz

1976, Raccah et al. 1976 & 1978, Adlerz 1987, Yokomi & Garnsey 1987, Raccah & Singer 1987). Table 1 shows a list of plant virus diseases transferred by spirea aphid and the transmission type of the virus. The spirea aphid's role in disease transmission in pome fruits is unknown.

Rubbing behavior by spirea aphid was observed by Kubota (1985). This species produced sound audible to the human ear. The rubbing motions of aphid were observed with a stereoscopic microscope and the vibration produced by the rubbing motions was detected with a stereo sound system. The spirea aphid rubs the abdomen against the hind tibia with raising and lowering the abdomen with all legs fixed on the plant or rubs the hind leg against the plant with scraping the hind leg on the plant after raising the leg.

Host Plants of The Spirea Aphid

Patch (1938) published a list of 65 plant genera as hosts of spirea aphid compiled from the world literature. Because spirea aphid dispersed to the semi-tropics, and shortly thereafter to the tropics, it required a complete change in hosts. Some demographic and phenological parameters of aphid populations were used to study the performance of the spirea aphid on different species and biotypes have been shown to vary with the choice of host plant (Guiterez et al. 1971, Frazer 1972, Naidu 1980, Neubauer et al. 1981, Komazaki 1983).

Narrowleaf meadowsweet Spiraea alba DuRoi, and Meadowsweet S. latifolia Borkh. were the original hosts of spirea aphid (Wolcott 1954). Infestation on the tender leaves of hog plum, Spondias mombin L. was found (Wolcott 1954). The spirea aphid is known to infest citrus and also Tridax procumbens, a weed common in citrus groves.

Table 1. Transmission of plant virus diseases by spirea aphid and the transmission type of the virus.

Virus Diseases	Type of Transmission	Reference
Sharka (Plum pox)	Non-persistant	Leclant 1973
Papaw mosaic	Non-persistant	Adsuar 1950 Martorell & Adsuar 1952
Citrus tristeza	Probably Non-persistant	Kennedy et al. 1962, Naidu 1980, Barbagallo & Patti 1982 Raccah & Singer 1987
Citrus vein enation	Unknown type	Kennedy et al. 1962
Abaca mosaic	Non-persistant	Kennedy et al. 1962
Pea mosaic	Probably non-persistant	Kennedy et al. 1962
Beet mosaic	Probably non-persistant	Kennedy et al. 1962
Bean common mosaic	Probably non-persistant	Kennedy et al. 1962
Asystasia mottle virus	Non-persistant	Thouvenel et al. 1988
Cowpea mosaic virus	Non-persistant	Atiri et al. 1986
Cucumber mosaic virus	Non-persistant	Raccah et al. 1985 Fang et al. 1985
Potato virus Y	Non-persistant	Raccah et al. 1985
Tobacco vein-banding mosaic virus	Non-persistant	Fang et al. 1985
Telfairia (fluted pumpkin) mosaic virus	Non-persistant	Shoyinka et al. 1987

This aphid was also observed to attack Eupatorium odoratum , Malpighia glabra , and Chromolaeria odorata (Singh & Rao 1978, Naidu 1980, Stary' & Zeleny' 1983). A wide range of secondary host plants were reported from the Rosaceae, Compositae, Rutaceae and Umbelliferae (Moritsu 1954, Wolcott 1954, Higughi & Miyazaki 1969, Sorin 1975, Richards 1976, Tanaka 1976).

The spirea aphid is known to feed on woody ornamental plants: spirea, Spiraea vanhouttei Zabel; viburnum, Viburnum suspensum Lindl.; ixora, Ixora chinesis Lam., and gardenia, Gardenia jasminoides Ellis. These plants support dense aphid colonies on their succulent young shoots; they become unable to support aphids as these shoots become sclerotic. Evans & Zettler (1968) used the herbaceous ornamental dieffenbachia, Dieffenbachia picta Schott., as a laboratory host for maintaining the spirea aphid. The aphid appears to colonize new economic host plants, thereby increasing its pest status.

Control of The Spirea Aphid

Since spirea aphid is one of the most important pests on citrus in the world and also damages many orchard trees, vegetables and ornamentals both directly and by transmitting numerous viral diseases, it is a pest requiring well organized control strategies.

A- Chemical Control

In 1961, in Taiwan, methyl-demeton and thiometon in emulsion sprays were used to suppress populations of spirea aphid on citrus trees (Tao & Tan 1961). These compounds resulted in greater mortality than malathion. Treatment of tristeza-infested plants, which are characterised by irregular and earlier bloom and flush, is advocated as

the initial step in reducing the spread of the virus by eliminating aphid migration to healthy plants, which bloom and flush later.

Pinnock et al. (1974) have described the suppression of populations of spirea aphid with soap spray. Formulated soap spray effectively removed spirea aphid from various flowering shrubs, particularly Pyracantha varieties. The maximum soap concentration tested, 0.1% removed 72% of the aphids. But water alone removed 46-47% of the population.

In Italy, applications of pirimicarb and ethiofencarb were required in both May and June against spirea aphid, which appeared in dense colonies and damaged 50% of the orange buds (Ortu & Proto 1981). In Spain, ethiofencarb, phosphamidon, menazon, oxydemeton-methyl and pirimicarb gave good control for the spirea aphid on citrus (Melia & Blasco 1982).

Spraying lime trees with clay suspensions containing kaolin or mixtures of kaolin and bentonite reduced the rate of natural colonization by alatae of spirea aphid. This reduction was due to the white coating of clay deposited on the leaves; bentonite increased the adhesion of the kaolin deposits (Bar-Joseph & Frenkel 1983). Also, partial bait sprays containing a small quantity of malathion gave good control for the spirea aphid (Uygun & Sekeroglu 1984).

In Korea, efficacy of several insecticides against each of the dominant aphids on apple was investigated in field trials with potted trees. Methomyl, deltamethrin, cypermethrin, and methidathion were most effective (over 90% mortality) on spirea aphid and phosphamidon, monocrotophos, dimethoate, acephate and chlorpyrifos had moderate effects (81-89% mortality) (Lee et al. 1986).

A study of foliar residues and toxicity of three systemic insecticides (aldicarb, dimethoate and ethiofencarb) applied to the soil for controlling spirea aphid was performed by Neubauer et al. (1982). The study showed that more aldicarb than dimethoate or ethiofencarb was found in the leaves. The differences in effectiveness of the three treatments seem to have resulted from this difference in accumulation rather than intrinsic differences in their toxicity to aphids. In a synthetic diet, all three compounds showed similar toxicity, whereas in the leaves, ethiofencarb was less effective than dimethoate or aldicarb. The authors also stated that these synthetic diet studies alone may not be sufficient as a screening test for systemic insecticides, but taken together with experiments with detached leaves, can serve as a valuable screening test for systemic insecticides.

Spirea aphid infestations were controlled in citrus groves in 1968 by painting portions of trunks with a product containing 60% monocrotophos diluted in an equal volume of water. The best results were obtained when the trunks were painted at the start of flushing and again two weeks later (Tao & Wu 1968).

Aldicarb was applied as a 10% granular formulation to the soil under the canopy of two-year-old navel orange trees. This compound showed considerable promise for controlling spirea aphid (Tashiro et al. 1969).

Systemic organophosphorous and carbamate compounds, such as dimethoate, croneton, and aldicarb when applied to the soil were successful in controlling this pest without affecting the biological balance with natural enemies in the citrus grove (Aharonson et al. 1979). A similar study also has indicated the effectiveness of these pesticides on nonbearing orange trees (Neubauer et al. 1982).

B- Natural Enemies

Numerous attempts have been made to control spirea aphid populations through the manipulation of biological control agents such as, parasites and pathogens; less is known of the use of predators.

1- Predators

Little is known in the literature on predators of spirea aphid. Barbagallo & Patti (1982) reported that the most important predators of citrus aphids in Italy are Scymnus spp. and several other species of coccinellids, the most common of which is Coccinella septempunctata L.

2- Parasites

In Australia, Aphelinus asychis Walker (Hymenoptera: Braconidae) readily parasitized spirea aphid, and most of its progeny ceased development in this aphid before reaching the mummification stage and died within the dead or dying nonmummified aphid host (Carver & Woolcock 1985). The common species, Lipolexis scutellaris Mack (Hymenoptera: Aphidiidae) has a promising host range for biological control. L. scutellaris is a relatively oligophagous species on Aphis species such as spirea aphid and other aphids (Stary' & Zeleny' 1983).

In Japan, Lysiphlebus japonicus Ashmed (Hymenoptera: Braconidae) was reported to parasitize spirea aphid (Kato 1974). Lysiphlebus fabarum (Marshall) and Lysiphlebus testaceipes (Cress.) have been introduced into Australia as biological control agents (Carver 1984). Although both species develop to the adult stage in some

Aphis spp. and Toxoptera spp., they were unable to complete their development in spirea aphid since it caused the death of the host before mummification (Miller 1929, Tremblay & Barbagallo 1982). Furthermore, Charips sp., a hyperparasite of Lysiphlebus, also failed to complete its development (Schlinger & Hall 1960). There was little parasitism of spirea aphid by any of the indigenous parasites, accounting for the status of this aphid as the main pest of citrus in southern Italy (Trembly et al. 1978).

Stary' et al. (1988) reported that L. testaceipes , which had been introduced from Cuba (via Czechoslovakia) to southern France in 1973 and 1974 to control the exotic aphid Toxoptera aurantii (Boy.) and the spirea aphid on citrus, had established over the whole of Mediterranean France and has become the predominant parasitoid of a number of indigenous pest aphids (incomplete parasitism on the spirea aphid).

3- Pathogens

The fungus Entomophthora fresenii (Nowakowski) was reported as a parasite on spirea aphid in Africa, Europe, and North America and also on many other citrus aphids (Ramaseshiah 1968). Also, the entomopathogenic fungi, Zoophthora orientalis Ben-Zeev' & Kenneth was found infecting spirea aphid on citrus in Israel (Ben-Zeev' and Kenneth 1981).

Finally, the fungus Triplosporum fresenii (Nowakowski) attacks spirea aphid in many parts of the world (Gilbert & Kuntz 1926, Ramaseshiah 1968, Nemoto 1973). In Israel, this fungus attacks many species of aphids (Kenneth et al. 1971, Kenneth & Olmert 1975, Bitton 1978). It was encountered by Bitton et al. (1979) in citrus orchards and was connected with spirea aphid mortality and epizootics.

THE EFFECT OF NITROGEN FERTILIZATION ON POPULATION ABUNDANCE OF SPIREA APHID (HOMOPTERA:APHIDIDAE) ON YOUNG APPLE TREES, WITH COMPARISONS TO APPLE APHID

Introduction

Three species of Aphididae are commonly reported overwintering as eggs on apple (Malus domestica Borkhausen): Aphis pomi DeGeer (apple aphid), Dysaphis plantaginea (Passerini) (rosy apple aphid), and Rhopalosiphum fitchii (Sanderson) (apple grain aphid) (Matheson 1919, Lathrop 1928, Brunner & Howitt 1981). However, the spirea aphid, Aphis spiraecola Patch, has also been recorded from apple in Virginia and elsewhere (Parrella et al. 1981, Blackman & Eastop 1985). The spirea aphid, which is the most common aphid pest of citrus in both Nearctic and Palaearctic regions (Neubauer et al. 1981), greatly outnumbers apple aphid in most of the apple orchards sampled in Virginia, West Virginia and Maryland (Pfeiffer et al. 1989).

In commercial apple production, young trees are normally heavily fertilized to optimize vegetative growth, but little is known about the plant-arthropod-nitrogen interactions. Nitrogen is the primary nutritional element recommended for apple production in Virginia. There is usually a positive correlation between nitrogen application rate, foliar nitrogen content, and development of leaf feeding insects. White (1970a) implicated nitrogen in feeding and oviposition site selection of the psyllid Cardiaspina densitexta Taylor on Eucalyptus fasciculosa F.V.M.. Poor nitrogen supply was thought to be responsible for nymphal death in C. densitexta (White 1970b). Population dynamics and physiology of other homopterans have been known to be affected by nitrogen fertilization. Pear psylla, Psylla pyricola Foerster, egg and nymph densities increased at a faster rate and reached higher levels on orchard pear, Pyrus communis L., trees receiving higher nitrogen application. Also, nitrogen application rate had greater effect on psylla numbers than time of application (dormant vs. late summer) (Pfeiffer & Burts 1983, 1984).

Effects of nitrogen fertilization on development of several mite species have been studied. Putman (1964) reported no association between leaf nitrogen level and fecundity or rate of development of European red mite, Panonychus ulmi (Koch) on peach. Similar results were obtained by van de Vrie et al. (1972). Storms (1969) and Wermelinger et al. (1985) indicated that twospotted spider mites, Tetranychus urticae Koch, respond to higher foliar nitrogen by increased fecundity. Recently Wilson et al. (1988) studied the relation of leaf nitrogen to population growth of the Pacific spider mite, Tetranychus pacificus (McGregor) on grape in the greenhouse. They found that Pacific spider mite responded to increasing foliar nitrogen with significantly ($P < 0.05$) increased fecundity and shorter immature developmental time.

Spirea aphid preferred the immature leaves on an apple branch to the mature ones and the leaf selection by the aphid is closely related to the nitrogen content of the leaf (Takeda 1980). The purpose of this study is to determine the effect of nitrogen fertilization on population abundance of the spirea aphid on young apple trees in the greenhouse.

Materials and Methods

A- 1986 Growing Season Experiments:

Thirty-seven one-year-old 'Redchief Delicious' apple trees on Malling Merton 26 rootstock were pruned in mid-June, 1986 to 63 cm above the graft union. The trees were weighed and planted at a uniform depth in 20-liter pots. The potting medium contained horticultural vermiculite, perlite, and sphagnum peatmoss (1:1:1 by volume) and was adjusted to pH 6.8 with dolomitic lime. Fertilizer was applied over the soil surface of each pot after planting, with 20N - 10P - 20K Peter's soluble fertilizer (W.R.

Grace, Fogelsville, Pa) at a rate of 483 ppm N, and 110 ml of a solution containing 1.25g soluble trace element mix. Each pot also received 28g of 18N - 18P - 18K Osmocote slow release fertilizer (Sierra Chem. Co., Melpitas, CA). The trees were watered when the soil was dry to the touch. Trees were grown in an unheated greenhouse with screened ends (Fig. 1) to maintain near-ambient air temperature. The plastic of the greenhouse reduced light transmission by 23%. Trees were ranked by weight and were sequentially assigned to treatments (spirea aphid-infested, n = 20, and non-infested control, n = 17), resulting in uniform distribution of weights. Trees were assigned to two treatments (spirea aphid-infested, n = 20, and non-infested control, n = 17). Trees in each treatment were arranged as single-tree-replicates in a randomized block design. Extra trees were infested to assure that enough aphids would be available for reinfestation when needed. Infested and control trees (n = 10 each) were harvested at the end of the 1986 growing season (December 4) and the remaining trees (infested n = 10, control n = 7) were kept over the winter and harvested at the ten-leaf stage (May 5) for studies on dry matter accumulation and carbohydrate concentration (chapter 3).

Aphids were obtained from orchards located in Botetourt County, Virginia. Two mature viviparae (Fig. 2) were transferred from freshly excised leaves with a fine pair of forceps and placed on the upper surface of two leaves located on upper and lower terminal shoots of each tree. All trees were infested in early August, 1986.

Four lateral shoots were maintained on each tree. Aphids were counted from the top six unfolded leaves on these shoots (the top two or three attached leaves were considered one leaf until they expanded). Thus, a total of 24 leaves per tree were checked two times per week from late July to early November. All alate and apterous aphids were counted and the average numbers of aphids per shoot were calculated.

Fig. 1. Young apple trees grown in 20-liter pots in a greenhouse with screened ends.

Fig. 2. Mature vivipara on the upper surface of young terminal apple leaf at the beginning of the infestation.

Because of the dispersal ability of alatae, they were removed at each counting date to avoid confused counts. Aphid populations were expressed as spirea aphid-days (SAD) per shoot for each treatment following the procedures of Sances et al. (1981) using the equation:

SAD = [(no.aphids day1 + no.aphids day2)/2] x no.of days between day1 and day2

Cumulative SAD were determined by totalling the SAD for each counting period. Use of aphid-days rather than aphids/shoot provides an estimate of extent and duration of aphid infestation.

B- 1987 Growing Season Experiments:

Sixty-four one-year-old 'Redchief Delicious' apple trees were planted in 3.8-liter plastic pots, grown in the greenhouse as the previous year, and were assigned to two groups (n = 32 each). The first group was infested with spirea aphids and the second group was kept free of aphids. Three weeks after planting, four treatments consisting of four nitrogen rates (0.0, 0.5, 1.0, and 2.0 g urea per tree or 0.0, 0.23, 0.45, and 0.90 g actual nitrogen per tree) were applied to both infested and control trees every three weeks. The experimental design was a randomized complete block design with a 2x4 factorial arrangement of treatments (eight treatments) with eight-single-tree replicates each. Trees were ranked by weight and were sequentially assigned to treatments, resulting in uniform distribution of weights. Half of the trees (n = 4) from each treatment were harvested at the end of 1987 growing season and the remaining trees (n = 4) were kept over the winter and harvested at the ten-leaf stage in 1988 (for study 3). Trees were infested with spirea aphids as the previous year. Aphids were counted twice per week, from the top six unfolded leaves of four shoots per tree. Density and duration of infestation were recorded using SAD as above. Mid-shoot leaves were sampled for nitrogen analysis in early November of 1987 and consisted of four selected

leaves per replicate. Four replicates for each nitrogen level for both infested and control leaves were used. A total of 16 leaves per treatment were analyzed for nitrogen content. Preparation of all leaves included oven-drying to a consistent weight for several days in a 70 ^{o}C forced-air oven and grinding through a Wiley mill with a 40-mesh sieve. Total nitrogen content of mature leaves (infested and controls) was evaluated by modified Kjeldahl type digestion procedure for total nitrogen (foliar %N) as described by Bremner and Breitenbeck (1983) (Appendix A).

Population abundance of spirea aphid vs apple aphid:

The development of both aphids on young apple trees were determined and compared. Twenty-four one-year-old apple trees were planted and grown in spring, 1987. Trees were assigned to three groups (spirea aphid, apple aphid, and control) with eight single-tree replicates for each group. Four trees from each group were harvested at the end of first season and those remaining were harvested at the ten-leaf stage. A single nitrogen fertilization rate (1.0 g urea per tree) was applied at three-week intervals and total leaf nitrogen content was evaluated.

Statistical Analysis:

Data were analyzed by analysis of variance and regression analysis (SAS Institute 1985). Numbers of aphids per shoot were transformed to log (x + 1) to normalize the data. The significance of differences among aphid populations treated with different nitrogen rates was assessed using the Tukey's student range test ($P < 0.05$) (Zar 1984). The relation between the number of aphids and their alates was analyzed and fitted to the general linear model (REG) and Correlation Coefficients procedures from SAS (SAS Institute 1985).

Results and Discussion

A- 1986 Growing Season:

All terminal leaves were infested three weeks after inoculation. Young, actively growing, terminal leaves were more susceptible to colonization than mature, old leaves. The spirea aphid tend to colonize on the underside of the upper, young leaves and may distribute over the whole leaf. In case of severe infestation, a few colonies could be found on the stem (between nodes) as well. A few colonies also developed on the lower, oldest leaves.

Aphid density and accumulated SAD per shoot during the growing season, for trees harvested at the end of first growing season, are presented in Figure 3. SAD accumulation increased rapidly during September then gradually leveled off as density declined for four weeks following population peak.

A total of 11,516 aphid-days per shoot accumulated during the season. Average aphid densities during aphid infestation, which lasted three months, are presented in Table 2. The highest densities occurred between September 10-20. Average aphid densities reached a maximum of 445 aphids per shoot (74 per leaf) (Fig. 4) before declining in late September. The aphid population decreased until all aphids died by early November.

The first few alatae were observed two weeks after inoculation. The percentage of alatae (relative to total aphids) (9.48%) was highest in October when the aphid population began to decline (Table 2). A strong positive correlation was observed ($r^2 = 0.97$) between aphid numbers per shoot and their alatae (Figure 5). Mittler & Kleinjan

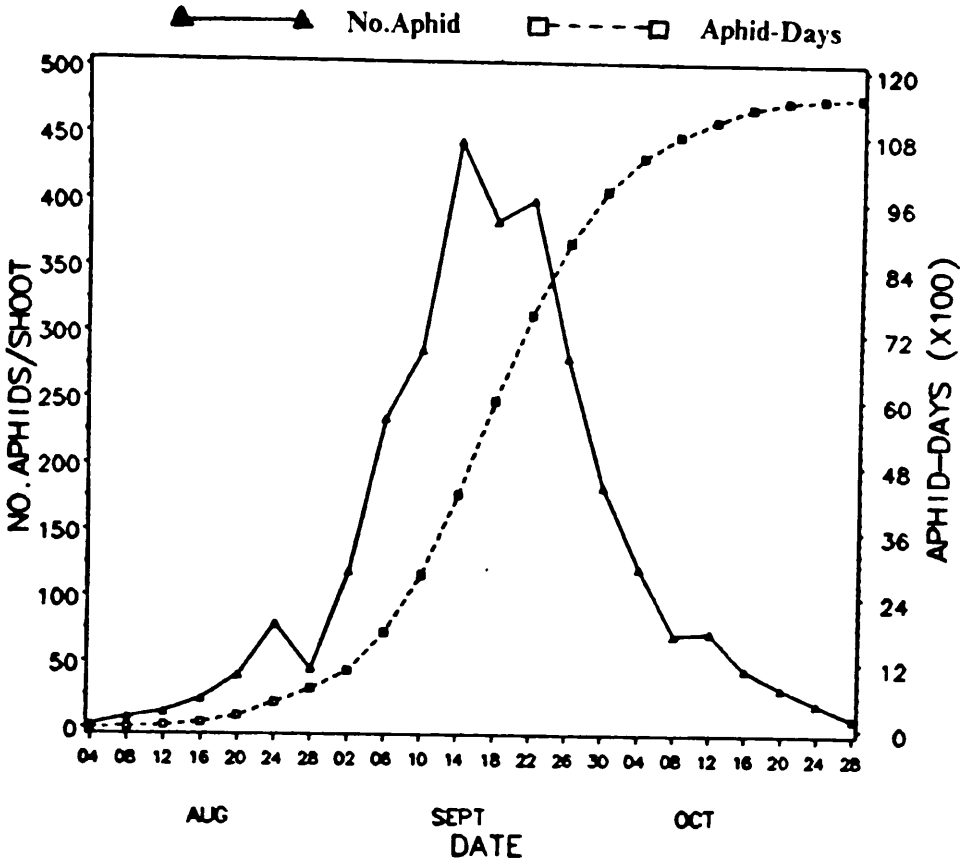


Fig. 3. Spirea aphid density and cumulative aphid-days per shoot during 1986 growing season for trees harvested at the end of the growing season.

Table 2. The average number of spirea aphids per shoot and the percentage of alatae during 1986-87 growing season (trees harvested at the end of first growing season in 1986 and harvested at the ten-leaf stage in 1987).

	End of first season (1986)		Ten-leaf stage (1987)	
Sampling date	No. aphids per shoot*	Winged aphids(%)	No. aphids per shoot*	Winged aphids(%)
August	30.0 (10.2)	8.17	42.0 (13.0)	7.85
September	292.3 (39.8)	8.21	299.6 (41.9)	8.59
October	52.7 (14.8)	9.48	53.3 (16.2)	12.33

* Transformation to log (x + 1) was performed to normalize the data. Nontransformed means are presented. Numbers in parentheses represent the SEM.

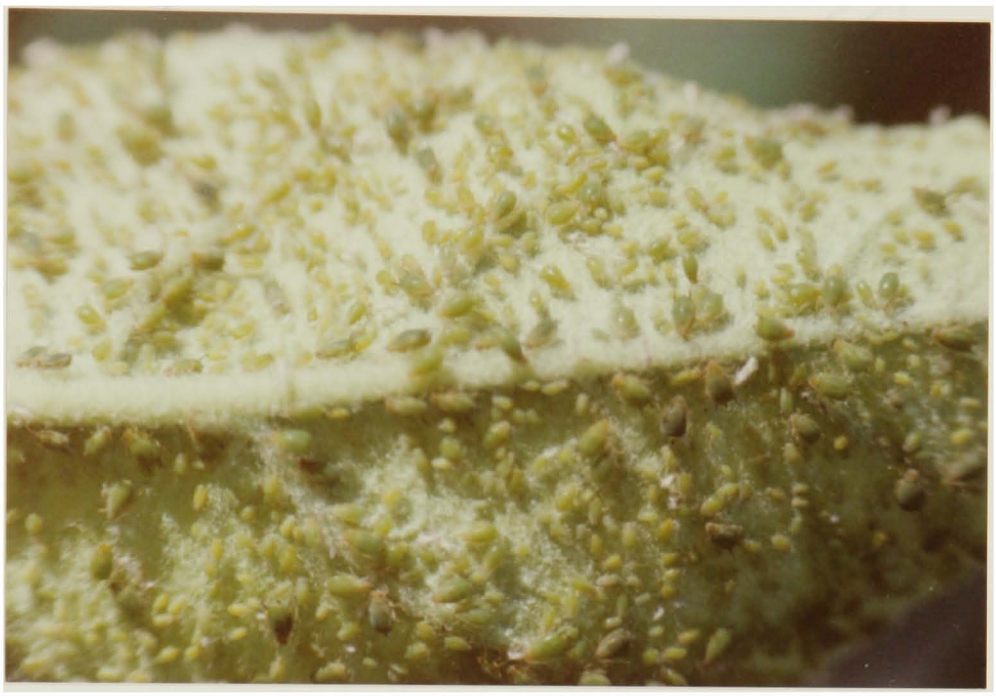


Fig. 4. Young spirea aphid colony on the underside of young terminal apple leaf at the peak stage of infestation.

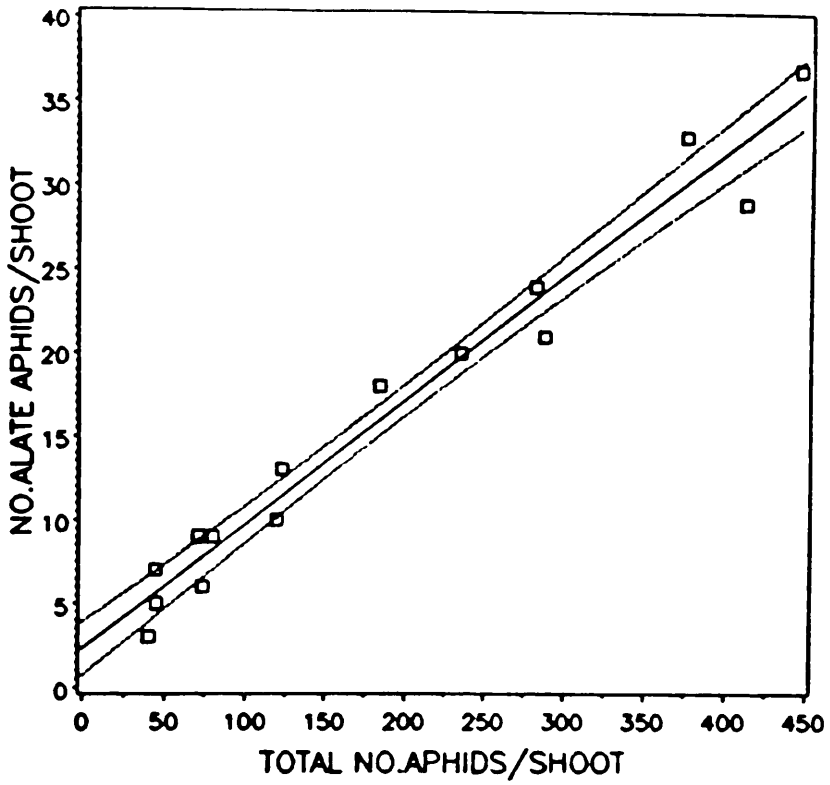


Fig. 5. Relation between spirea aphid numbers per shoot and alatae (trees harvested at the end of first growing season in 1986). Broken lines represent 95% confidence limits about the regression line. The general linear regression equation (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 values are:

$$Y = 2.29 + 0.274x \qquad r^2 = 0.97$$
$$(2.1)\ (0.061)$$

(1970) reported that aphids would produce alatae due to nutritional conditions of the host plant. Leaf age is considered to be a factor that affects food quality and in turn reduced aphid numbers (Kennedy & Booth 1954, Gibson 1971a & b).

Aphid populations for trees kept over the winter and harvested at the ten-leaf stage in 1987 followed the same trend in 1986 as those trees harvested at the end of the 1986 growing season (Fig. 6). A total of 11,622 SAD per shoot was accumulated at the end of the season. The average number of aphids during the infestation and the percentage of winged forms to the total aphids is presented in Table 2. A highly significant positive correlation between aphid numbers and alatae was found ($r^2 = 0.96$) (Fig. 7).

B- 1987 Growing Season:

Different patterns of population growth were observed in the 1987 growing season due to the effect of low, medium, and high N fertilization. Aphid density and accumulated SAD per shoot during the growing season for trees harvested at the end of 1987 are illustrated in Figure 8.

Aphid infestation extended over a period of three months, from late July until early November. During this period, trees fertilized with higher nitrogen supported greater aphid densities. The first two applications of urea had no or little effect on aphid abundance until the third week of infestation when populations started to increase from August 24 and reached maximum levels during the second week of September. Monthly percentage of aphids (to the total number of aphids during the season) was highest in September (72%). By the end of October and early November, few aphids remained on the trees. The decline in aphid numbers may have been accelerated by leaf age at the

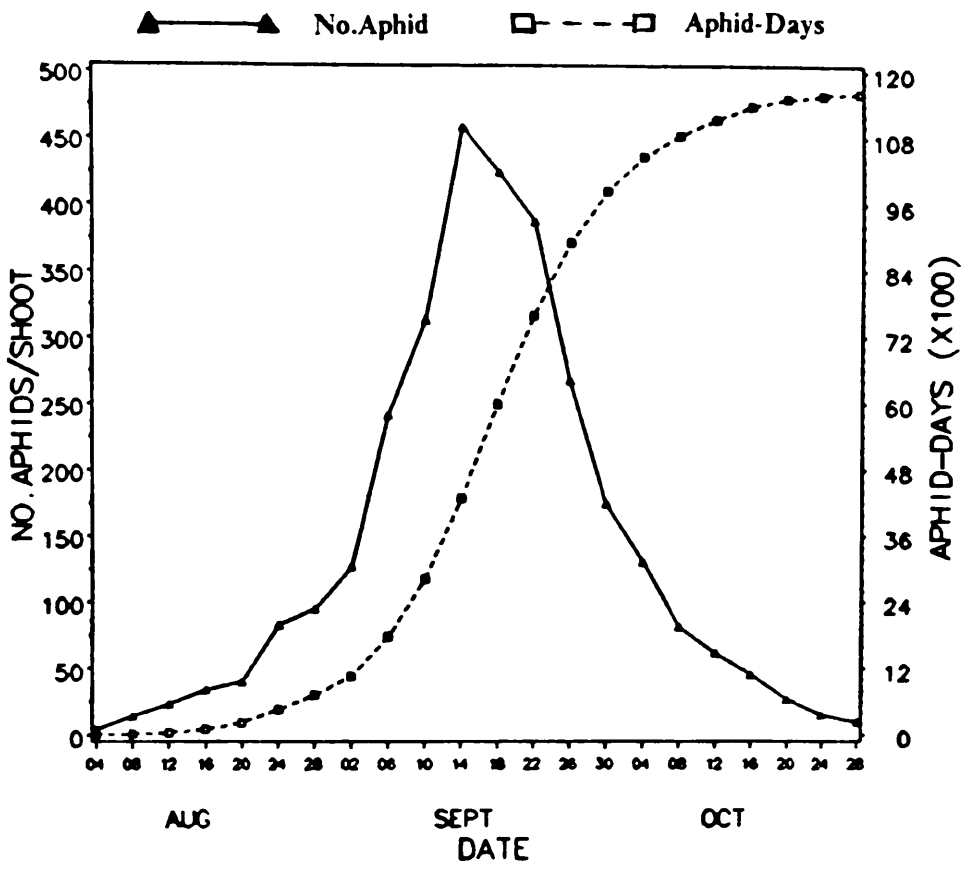


Fig. 6. Spirea aphid density and cumulative aphid-days per shoot during 1986 growing season for trees harvested in the second growing season at the ten-leaf stage in 1987.

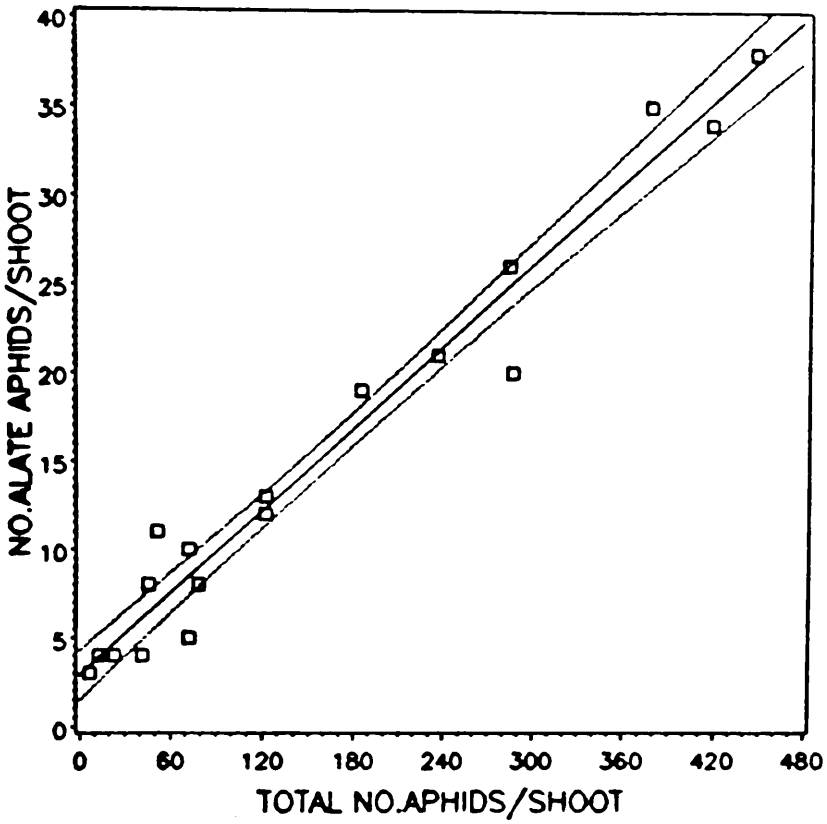
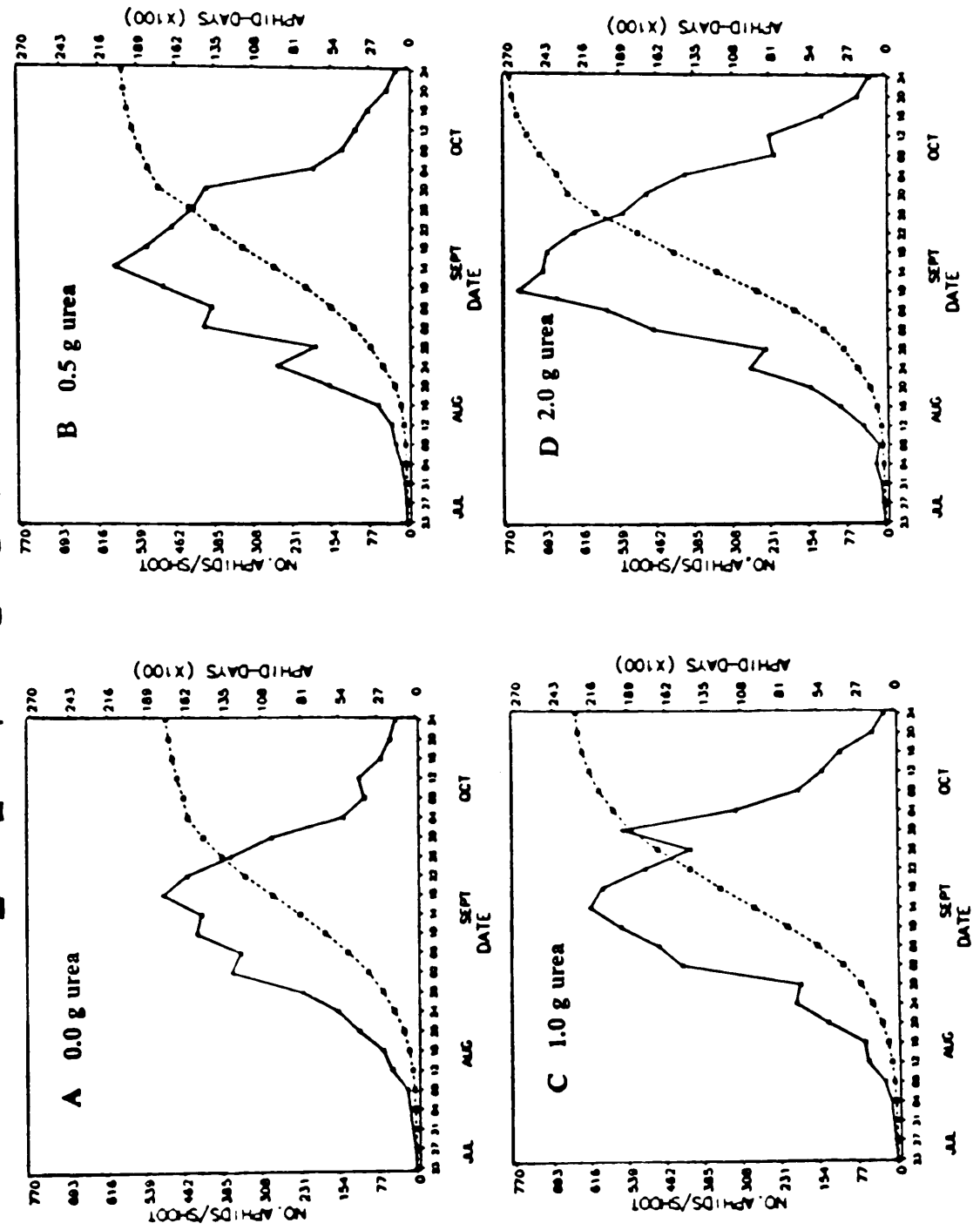


Fig. 7. Relation between spirea aphid numbers per shoot and alatae (trees harvested in the second growing season at the ten-leaf stage in 1987). Broken line represent 95%confidence limits about the regression line. The general linear regression equation (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 value are:

$$Y = 2.89 + 0.077x \qquad r^2 = 0.96$$
$$(2.42)\ (0.007)$$

Fig. 8. Spirea aphid density and cumulative aphid-days per shoot during 1987 growing season with four different nitrogen rates (trees harvested at the end of first growing season in 1987). A,B,C, and D represent urea application rates of 0.0, 0.5, 1.0, and 2.0g urea/tree respectively.


No.Aphid
Aphid-Days
A 0.0 g urea
B 0.5 g urea
C 1.0 g urea
D 2.0 g urea
NO. APHIDS/SHOOT
APHID-DAYS (X100)
JUL
AUG
SEPT
OCT
DATE

end of the growing season.

Aphid numbers per shoot differed for various nitrogen treatments (Table 3). Aphid numbers were positively related to nitrogen level. During the infestation period, 17,428 SAD were accumulated in the untreated control (0.0 g urea) compared to 19,922, 22,568, and 26,756 SAD with 0.5, 1.0, and 2.0 g urea, respectively. The percentage of alatae to the total number of aphids with different nitrogen levels is also presented in Table 3. Alatae increased with an increase in the total number of aphids at all nitrogen levels (Fig. 9). The proportion of alatae did not increase with increasing nitrogen rate. The slopes of the regression equations did not differ statistically; therefore there was no interaction between nitrogen and aphids.

The mean number of aphids at the peak stage was significantly higher at the highest nitrogen rate (Table 4). Population maxima occurred significantly earlier with higher nitrogen rate. These results indicate that the nitrogen rate applied to the trees has a positive influence on the development of aphids.

Aphids on trees harvested in the second growing season (at the ten-leaf stage) followed similar population trends to those on trees harvested at the end of the first season. Aphid density and accumulated SAD per shoot under different nitrogen levels are illustrated in Figure 10. The numbers of aphids at the peak stage and during the season are presented in Tables 5 and 6. A positive correlation was observed between nitrogen rate and the number of aphids. In the untreated control (0.0 g urea), 16,654 aphid-days were accumulated compared to 19,934, 21,654, and 26,978 SAD with 0.5, 1.0, and 2.0 g urea/tree, respectively. The percentage of total aphids with wings is presented in Table 5. There was a positive correlation between the number of aphids and their alatae (Fig. 11).

Table 3. The effect of nitrogen fertilization on the number of spirea aphids per shoot and the percentage of alatae (to the total aphid number) during 1987 growing season (trees harvested at the end of first growing season in 1987).

	No.aphids per shoot at four nitrogen rates *			
Sampling date	0.0 g	0.5 g	1.0 g	2.0 g
July	4.0 (2.11)a	3.3 (1.45)b	4.3 (1.76)a	4.3 (1.76)a
August	91.7 (29.6)b	104.3 (36.4)b	96.0 (29.3)c	116.7 (39.6)a
September	399.8 (24.5)d	460.0 (24.0)c	516.4 (26.4)b	601.0 (36.8)a
October	87.5 (14.6)d	95.2 (17.8)c	137.3 (22.9)b	177.8 (29.6)a
Mean for Season	182.4 (34.7)d	208.0 (40.2)c	235.0 (45.0)b	279.4 (52.0)a
	% alatae (to the total)			
July	0.00 a	0.00 a	0.00 a	0.00 a
August	4.71 a	7.94 a	7.43 a	6.97 a
September	8.31 a	8.29 a	8.12 a	9.13 a
October	13.70 a	11.40 a	10.56 a	10.68 a
Mean for Season	8.34 a	8.61 a	8.40 a	9.09 a

* Transformation to log (x + 1) was performed to normalize the data. Nontransformed means are presented. Means (SEM in parentheses) in the same row followed by d2e same letter are not significantly different ($P < 0.01$) using Tukey's studentized range test.

Fig. 9. Relation between spirea aphid numbers per shoot to alatae, during 1987 growing season, with four different nitrogen rates (trees harvested at the end of first growing season in 1987). A,B,C, and D represent urea application rates of 0.0, 0.5, 1.0, and 2.0g urea/tree respectively. Broken lines represent 95% confidence limits about the regression line. The slopes of the regression equations are not significantly different from each other ($P < 0.05$). The general linear regression equations (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 values in the same order are:

A--- $Y = 2.25 + 0.077x$ $r^2 = 0.86$
(2.24) (0.008)

B--- $Y = 3.84 + 0.075x$ $r^2 = 0.95$
(1.45) (0.005)

C--- $Y = 2.82 + 0.076x$ $r^2 = 0.96$
(1.48) (0.004)

D--- $Y = 1.88 + 0.087x$ $r^2 = 0.97$
(1.80) (0.004)

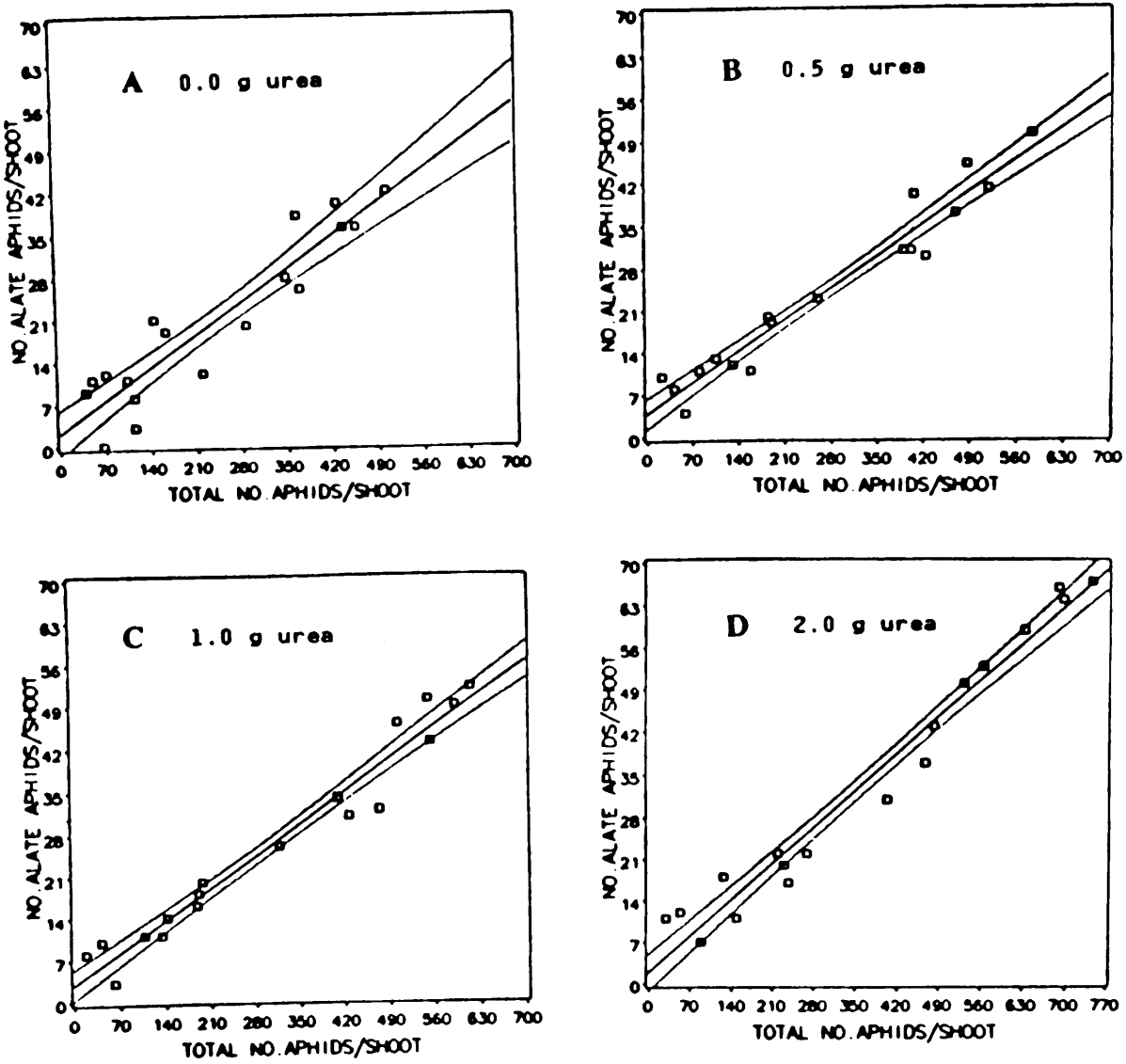

A 0.0 g urea
B 0.5 g urea
C 1.0 g urea
D 2.0 g urea
NO. ALATE APHIDS/SHOOT
TOTAL NO. APHIDS/SHOOT
0 7 14 21 28 35 42 49 56 63 70
0 70 140 210 280 350 420 490 560 630 700
0 70 140 210 280 350 420 490 560 630 700 770

Table 4. The effect of nitrogen fertilization on the peak spirea aphid densities (Sept. 10-18) for trees harvested at the end of 1987 growing season.

Nitrogen rate (g urea)	No. aphids/shoot[1]	Date of peak infestation[2]
0.0	504 (14.1) c	Sept. 18 c
0.5	582 (13.2) b	Sept. 14 b
1.0	612 (14.1) b	Sept. 14 b
2.0	744 (14.3) a	Sept. 10 a

[1] Transformation to log (x + 1) was performed to normalize the data. Nontransformed means are presented. Means (SEM in parentheses) in the same column followed by the same letter are not significantly different ($P < 0.01$) using Tukey's studentized range test.

[2] Statistical analysis is based on comparisons of cumulative aphid-days until the peak stage.

Fig. 10. Spirea aphid density and cumulative aphid-days per shoot during 1987 growing season with four different nitrogen rates (trees harvested in the second growing season at the ten-leaf stage in 1988). A,B,C, and D represent urea application rates of 0.0, 0.5, 1.0, and 2.0g urea/tree respectively.

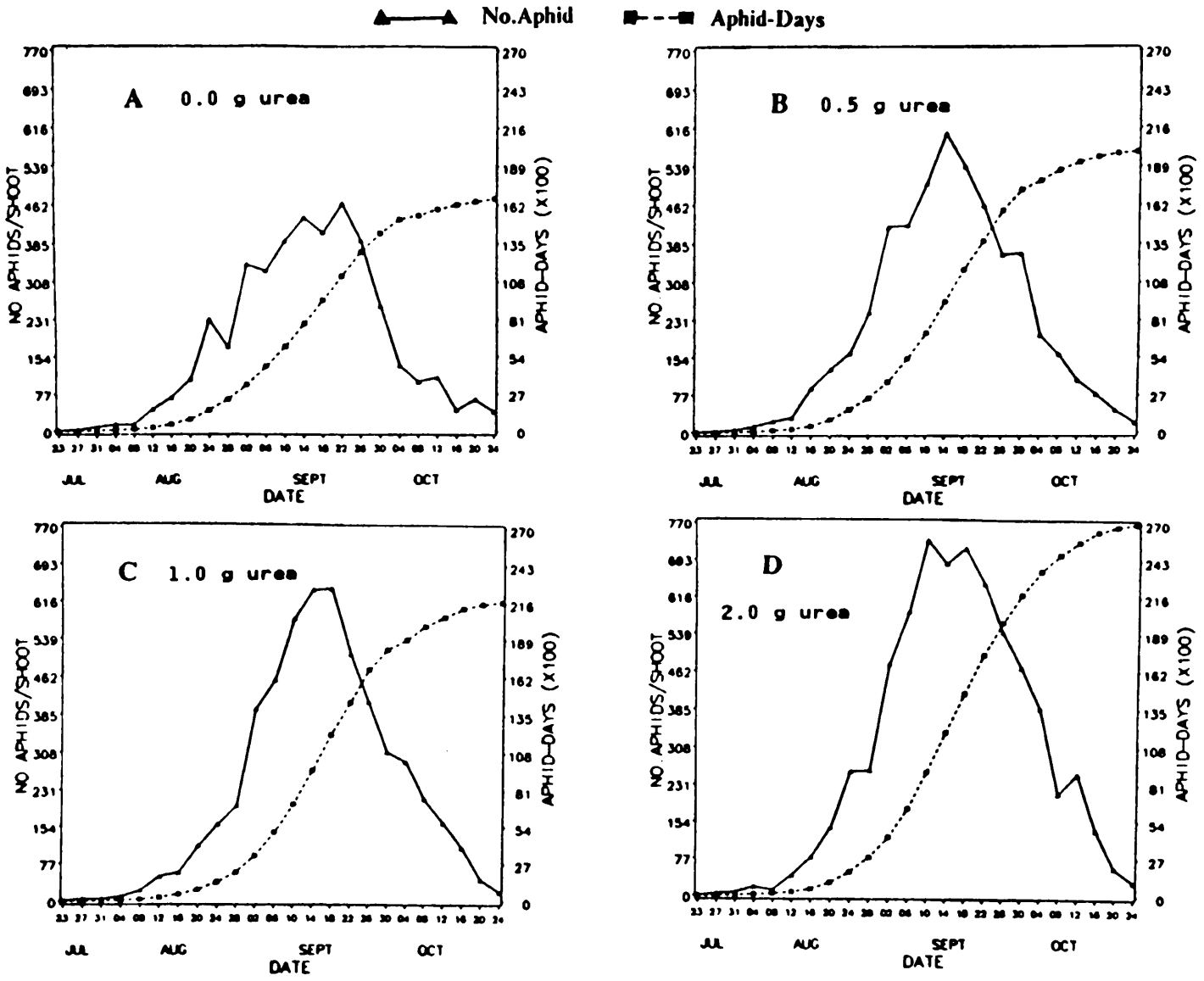

No.Aphid
Aphid-Days
A 0.0 g urea
B 0.5 g urea
C 1.0 g urea
D 2.0 g urea
NO APHIDS/SHOOT
APHID-DAYS (X100)
JUL
AUG
SEPT
OCT
DATE

Table 5. The effect of nitrogen fertilization on the number of spirea aphids per shoot and the percentage of alatae (to the total aphid number) during 1987 growing season (trees harvested in the second growing season at the ten-leaf stage in 1988).

	No.aphids per shoot at four nitrogen rates *			
Sampling date	0.0 g	0.5 g	1.0 g	2.0 g
July	4.0 (2.08)a	2.7 (1.20)b	3.7 (1.33)a	4.3 (1.76)a
August	93.0 (31.3)b	98.7 (32.8)b	87.6 (26.9)c	115.7 (40.5)a
September	378.0 (23.2)d	460.0 (30.1)c	494.5 (43.0)b	607.5 (36.8)a
October	83.2 (15.7)d	102.5 (27.9)c	146.5 (41.8)b	179.3 (54.6)a
Mean for Season	174.3 (32.8)d	208.1 (40.5)c	227.5 (44.4)b	287.6 (53.3)a
	% alatae (to the total)			
July	0.00 a	0.00 a	0.00 a	0.00 a
August	5.99 a	7.81 a	7.99 a	7.40 a
September	8.77 a	8.53 a	8.39 a	9.07 a
October	14.03 a	11.87 a	10.35 a	11.15 a
Mean for Season	8.94 a	8.83 a	8.64 a	9.19 a

* Transformation to log (x + 1) was performed to normalize the data. Nontransformed means are presented. Means (SEM in parentheses) in the same row followed by the same letter are not significantly different ($P < 0.01$) using Tukey's studentized range test.

Table 6. The effect of nitrogen fertilization on peak spirea aphid densities (Sept. 10-22) for trees harvested in the second growing season at the ten-leaf stage in 1988.

Nitrogen rate (g urea)	No. aphids/shoot[1]	Date of peak infestation[2]
0.0	438 (11.6) d	Sept. 22 c
0.5	600 (10.7) c	Sept. 14 b
1.0	648 (11.2) b	Sept. 18 a
2.0	720 (6.6) a	Sept. 10 a

[1] Transformation to log (x + 1) was performed to normalize the data. Nontransformed means are presented. Means (SEM in parentheses) in the same column followed by the same letter are not significantly different ($P < 0.01$) using Tukey's studentized range test.

[2] Statistical analysis is based on comparisons of cumulative aphid-days until the peak stage.

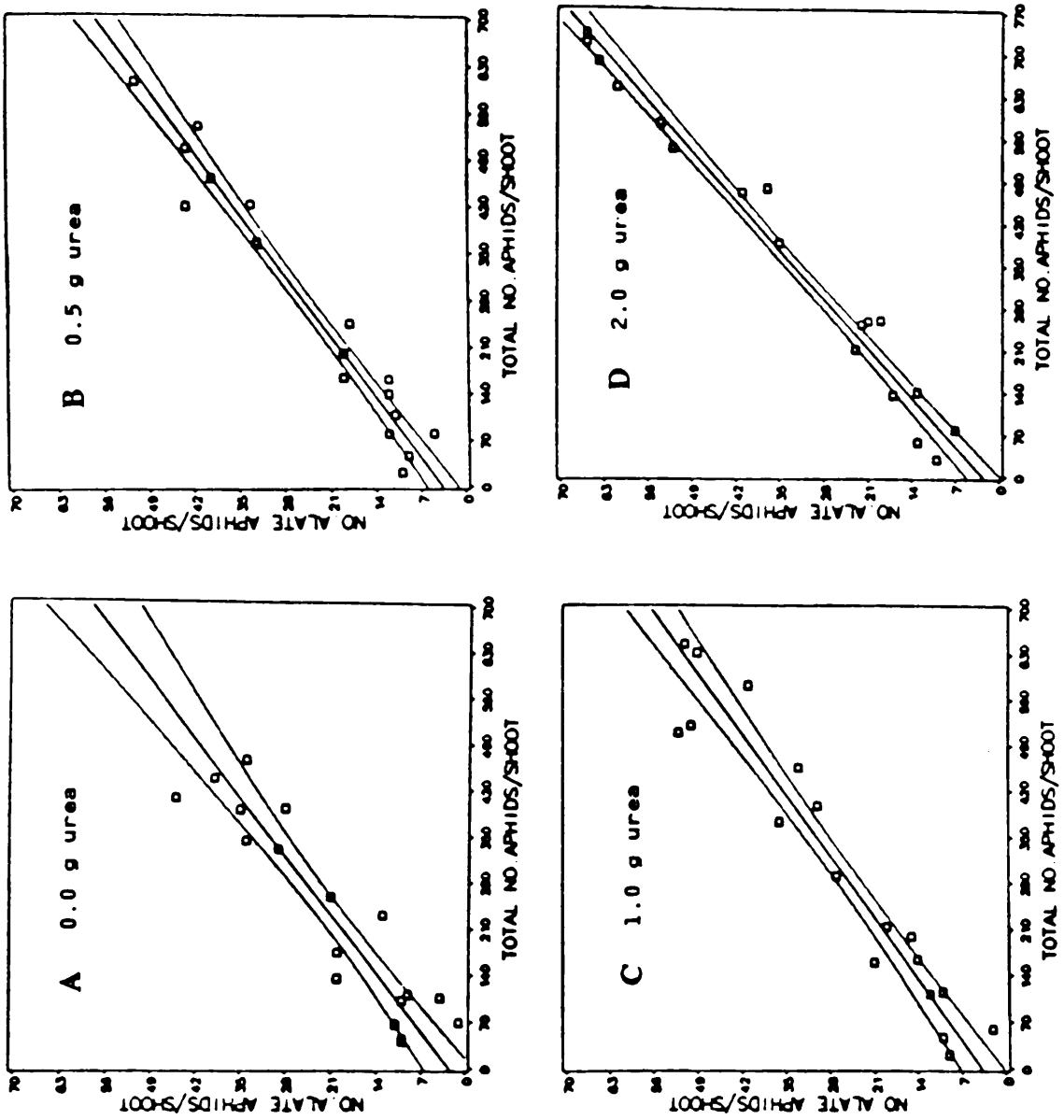
Fig. 11. Relation between spirea aphid numbers per shoot to alatae, during 1987 growing season, with four different nitrogen rates (trees harvested in the second growing season at the ten-leaf stage in 1988). A,B,C, and D represent urea application rates of 0.0, 0.5, 1.0, and 2.0g urea/tree respectively. Broken lines represent 95% confidence limits about the regression line. The slopes of the regression equations are not significantly different from each other ($P < 0.05$). The general linear regression equations (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 values in the same order are:

A--- $Y = 2.58 + 0.079x$ $r^2 = 0.84$
(2.33) (0.009)

B--- $Y = 3.67 + 0.076x$ $r^2 = 0.95$
(1.41) (0.004)

C--- $Y = 3.82 + 0.075x$ $r^2 = 0.92$
(2.25) (0.006)

D--- $Y = 2.57 + 0.080x$ $r^2 = 0.97$
(1.58) (0.004)


A 0.0 g urea
B 0.5 g urea
C 1.0 g urea
D 2.0 g urea
NO. ALATE APHIDS/SHOOT
TOTAL NO. APHIDS/SHOOT

The development of the spirea aphid vs apple aphid, at a single nitrogen rate, was determined on trees harvested at the end of the first season. Rate of population development did not differ significantly between species. Figure 12 shows the aphid density and accumulated aphid-days per shoot. Populations peaked at 612 and 648 aphids per shoot (102 and 108 aphids per leaf) for spirea aphid and apple aphid, respectively. There was no significant difference in aphid numbers between species during September (the heaviest infestation period); 86.1 and 88.3 aphids per leaf were found for spirea aphid and apple aphid, respectively. Alatae increased with increasing total number of aphids in both species (Figure 13).

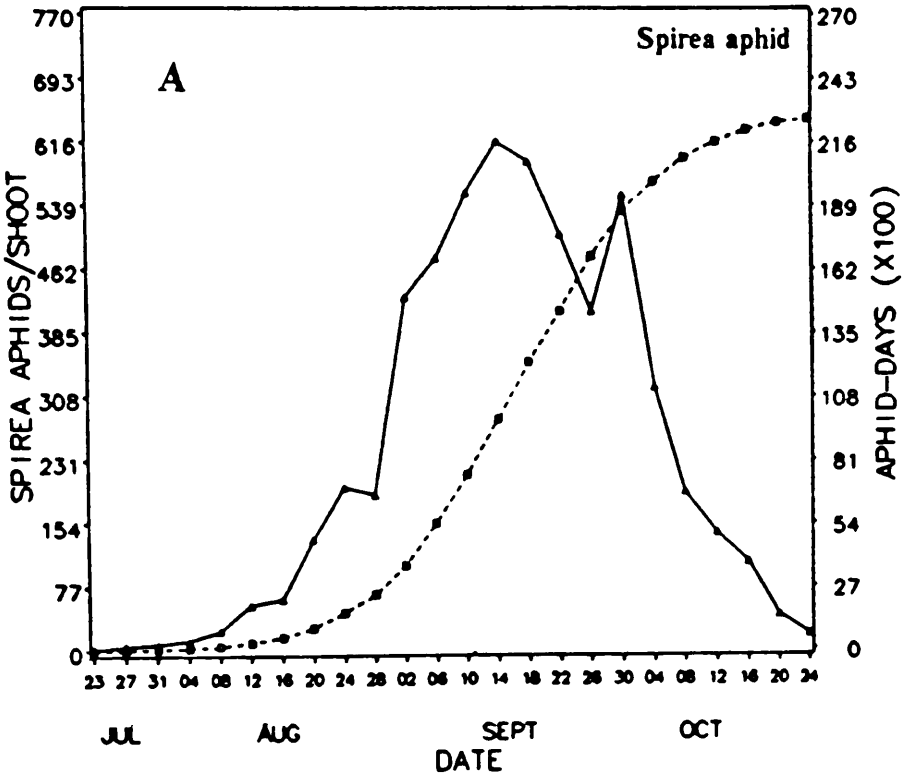
Similar results were obtained comparing the two species on those trees harvested at the ten-leaf stage in 1988. Figure 14 illustrates the population trends of both species. Populations of the spirea aphid and apple aphid peaked at 648 and 654 aphids per shoot (108 and 109 aphids per leaf) in September 18 and 14, respectively. Figure 15 shows the relation between the number of aphids and their alatae for the two species.

The effect of changes in the physiological conditions of the trees and their impact on the development of aphids should be considered. In this study, spirea aphid populations responded to nitrogen treatments; population growth was significantly greater on the leaves with higher nitrogen.

Correlation between nitrogen application and apple leaf nitrogen content:

Total leaf nitrogen was calculated on a dry-weight basis. Nitrogen content of apple leaves at the end of the first season is presented in Table 7. Leaf nitrogen concentration increased significantly ($P < 0.05$) and linearly with increasing urea application in both infested and control leaves (Fig. 16). Also, a significant difference was found at each urea application rate between infested and control leaves.

Fig. 12. Aphid density and cumulative aphid-days of spirea aphid (A) and apple aphid (B) during 1987 growing season (trees harvested at the end of first growing season in 1987).


A
Spirea aphid
SPIREA APHIDS/SHOOT
APHID-DAYS (X100)
770
693
616
539
462
385
308
231
154
77
0
270
243
216
189
162
135
108
81
54
27
0
23 27 31 04 08 12 16 20 24 28 02 06 10 14 18 22 26 30 04 08 12 16 20 24
JUL
AUG
SEPT
OCT
DATE

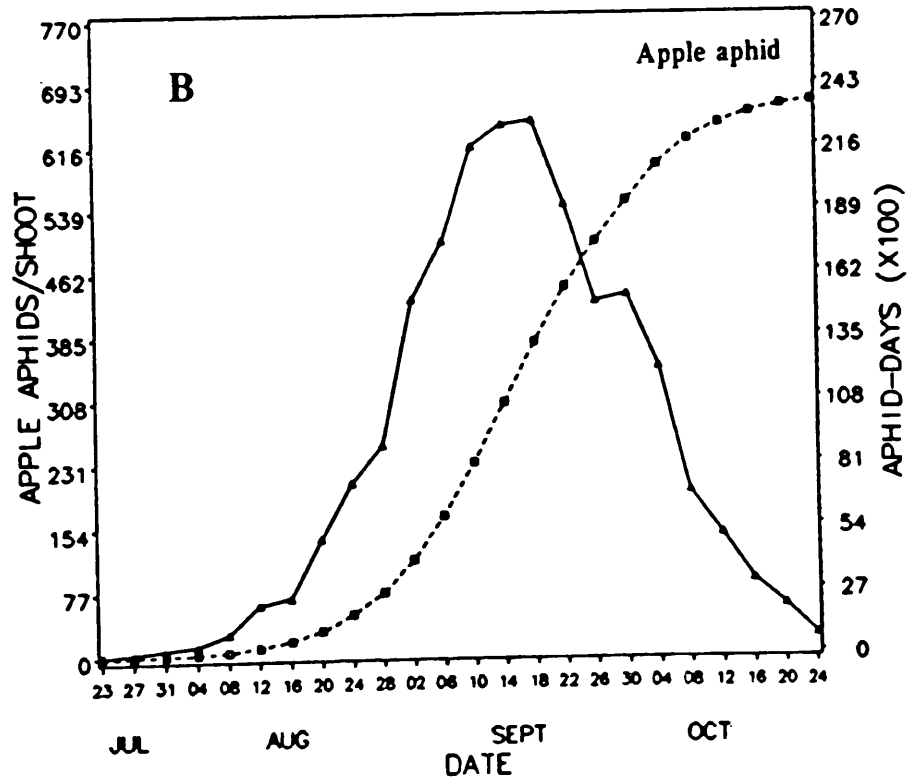
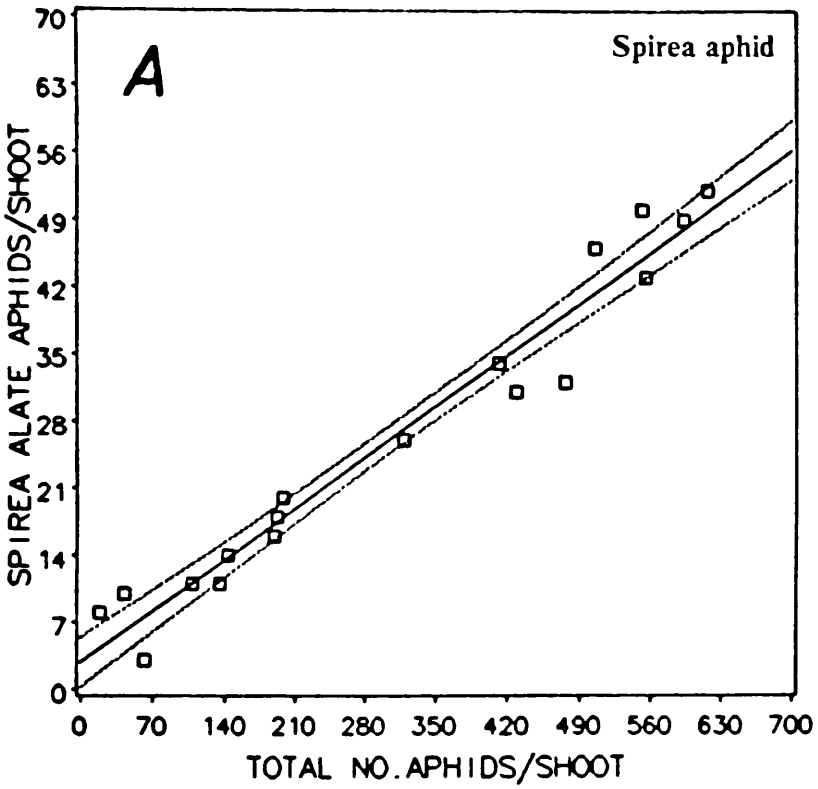

B
Apple aphid
APPLE APHIDS/SHOOT
APHID-DAYS (X100)
770
693
616
539
462
385
308
231
154
77
0
270
243
216
189
162
135
108
81
54
27
0
23 27 31 04 08 12 16 20 24 28 02 06 10 14 18 22 26 30 04 08 12 16 20 24
JUL
AUG
SEPT
OCT
DATE

Fig. 13. Relation between spirea aphid (A) and apple aphid (B) numbers per shoot to their alatae (trees harvested at the end of first growing season in 1987). Broken lines represent 95% confidence limits about the regression line. Slopes and intercepts of the regression equations are not significantly different from each other ($P < 0.05$). The general linear regression equations (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 values in the same order are:

A--- $Y = 2.92 + 0.076x$ $r^2 = 0.96$
(1.48) (0.004)

B--- $Y = 4.07 + 0.072x$ $r^2 = 0.93$
(1.83) (0.005)


A
Spirea aphid
SPIREA ALATE APHIDS/SHOOT
0
7
14
21
28
35
42
49
56
63
70
0 70 140 210 280 350 420 490 560 630 700
TOTAL NO. APHIDS/SHOOT

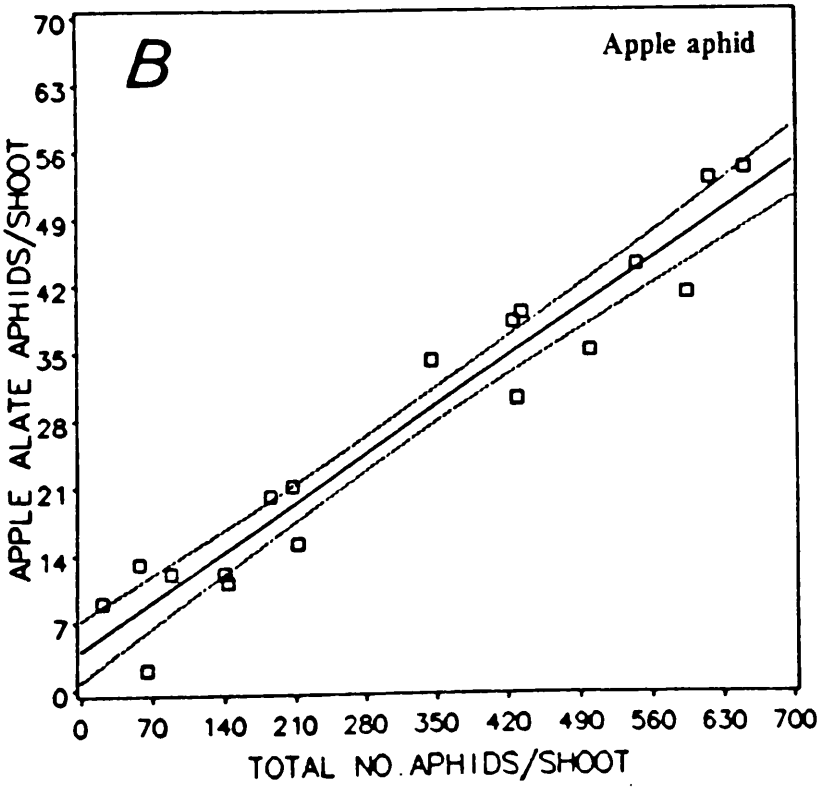
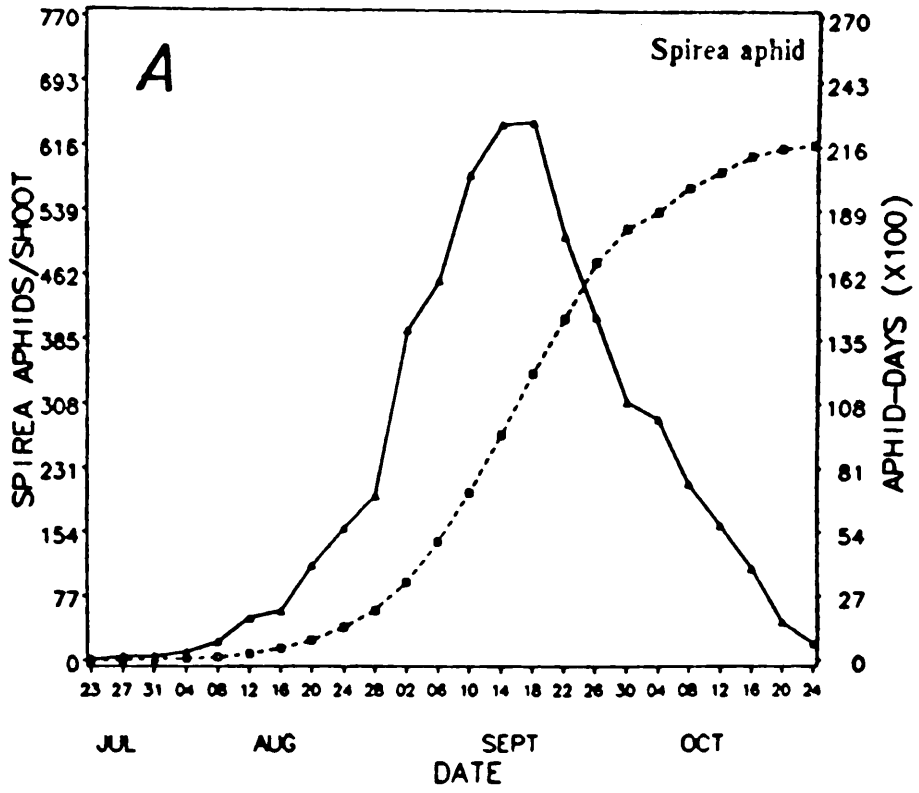

B
Apple aphid
APPLE ALATE APHIDS/SHOOT
0
7
14
21
28
35
42
49
56
63
70
0 70 140 210 280 350 420 490 560 630 700
TOTAL NO. APHIDS/SHOOT

Fig. 14. Aphid density and cumulative aphid-days for spirea aphid (A) and apple aphid (B) during 1987 growing season (trees harvested in the second growing season at the ten-leaf stage in 1988).


A
Spirea aphid
SPIREA APHIDS/SHOOT
APHID-DAYS (X100)
770
693
616
539
462
385
308
231
154
77
0
270
243
216
189
162
135
108
81
54
27
0
23 27 31 04 08 12 16 20 24 28 02 06 10 14 18 22 26 30 04 08 12 16 20 24
JUL
AUG
SEPT
OCT
DATE

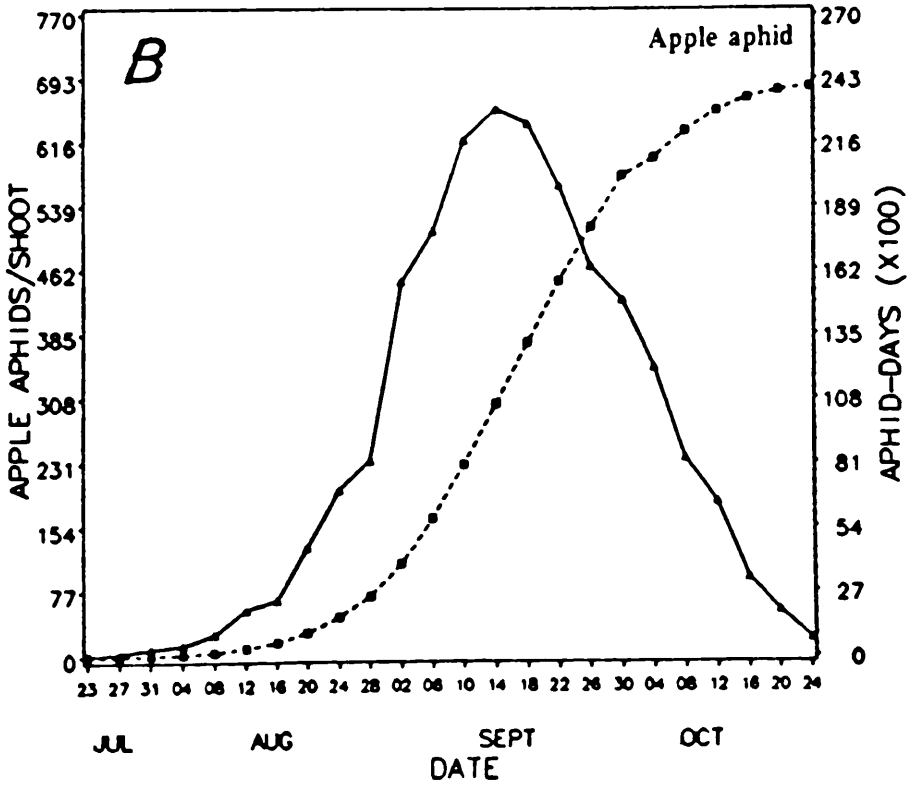
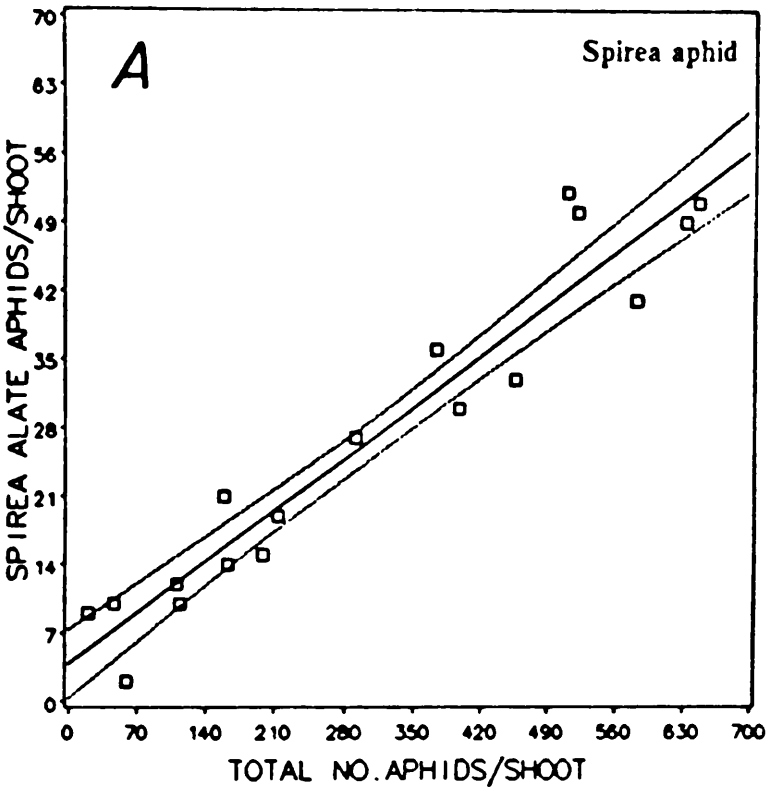

B
Apple aphid
APPLE APHIDS/SHOOT
APHID-DAYS (X100)
770
693
616
539
462
385
308
231
154
77
0
270
243
216
189
162
135
108
81
54
27
0
23 27 31 04 08 12 16 20 24 28 02 06 10 14 18 22 26 30 04 08 12 16 20 24
JUL
AUG
SEPT
OCT
DATE

Fig. 15. Relation between spirea aphid (A) and apple aphid (B) numbers per shoot to their alatae, during 1987 growing season, with a 1.0g urea/tree (trees harvested in the second growing season at the ten-leaf stage in 1988). Broken lines represent 95% confidence limits about the regression line. Slopes and intercepts of the regression equations are not significantly different from each other ($P < 0.05$). The general linear regression equations (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 values in the same order are:

A--- $Y = 3.82 + 0.075x$ $r^2 = 0.92$
(2.25) (0.006)

B--- $Y = 3.11 + 0.073x$ $r^2 = 0.93$
(1.87) (0.005)


A
Spirea aphid
SPIREA ALATE APHIDS/SHOOT
0
7
14
21
28
35
42
49
56
63
70
0
70
140
210
280
350
420
490
560
630
700
TOTAL NO. APHIDS/SHOOT

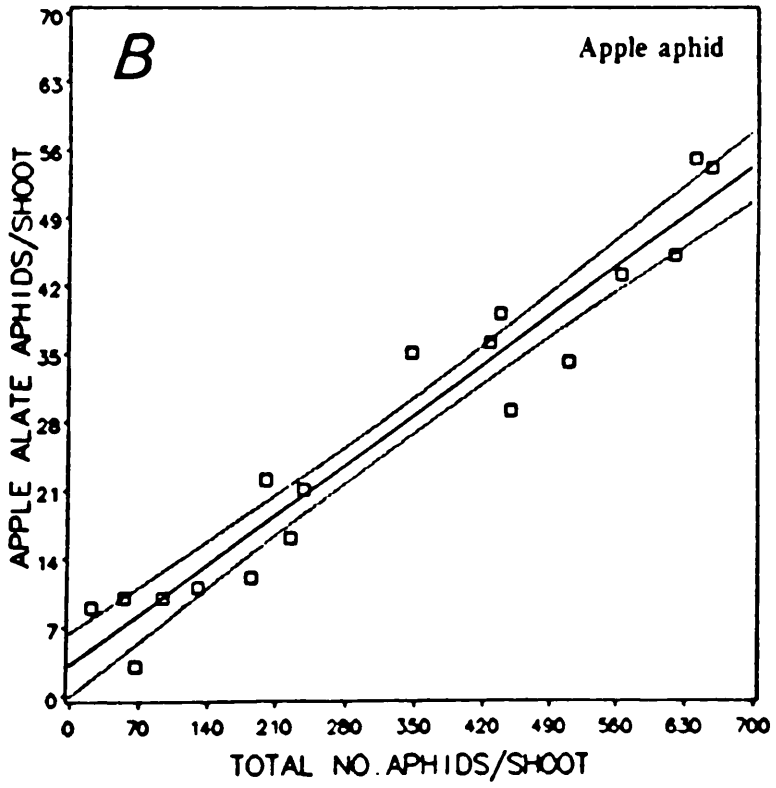

B
Apple aphid
APPLE ALATE APHIDS/SHOOT
0
7
14
21
28
35
42
49
56
63
70
0
70
140
210
280
350
420
490
560
630
700
TOTAL NO. APHIDS/SHOOT

Table 7. Percent nitrogen content (%) of one-year-old apple trees with different urea application rates (1987).

Treatment[1]	Urea Application Rate			
	0.0 g	0.5 g	1.0 g	2.0 g
Control	1.51 (0.007) c	1.55 (0.009) c	2.21 (0.011) b	2.71 (0.014) a
	*	*	*	*
Spirea Aphid	1.38 (0.006) d	1.42 (0.009) c	1.89 (0.014) b	2.12 (0.007) a

[1] Means in the same row followed by the same letter are not significantly different ($P < 0.05$, using Tukey's test). $n = 16$ for each level. Asterisk indicates a significant difference between treatments at one level ($P < 0.05$, using LSD test).

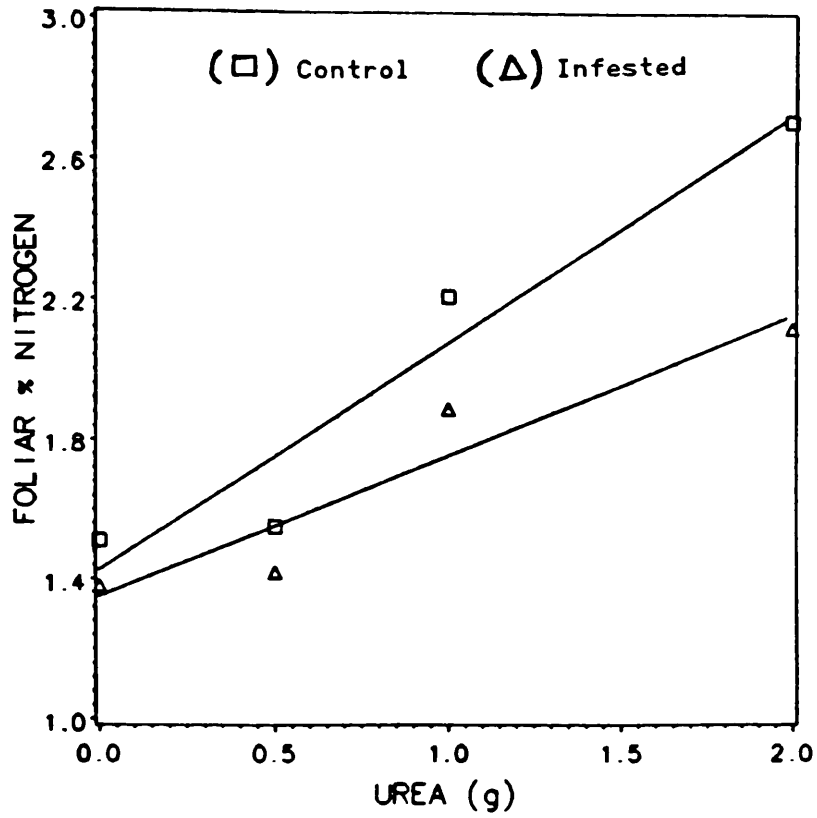


Fig. 16. Relationship between amount of urea applied per tree and foliar percent nitrogen in apple leaves. A = control trees and B = infested trees with spirea aphid. The slope of the regression equation (A) is significantly different from the other ($P < 0.05$). The general linear regression equations (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 values in the same order are:

A $Y = 1.43 + 0.65x$ $r^2 = 0.94$
(0.14) (0.123)

B $Y = 1.35 + 0.40x$ $r^2 = 0.90$
(0.11) (0.094)

In the control group, differences in percent nitrogen among the lowest rates (0 and 0.5 g urea) were not significant. The highest nitrogen rate (2.0 g) had the greatest foliar % nitrogen at the end of the season. In this study, leaf nitrogen levels were similar to the 1.5 to 3.5% nitrogen range reported for apple trees by Westwood (1978).

Changes in the chemical composition of the leaves might have an important role in controlling aphid populations. Other factors should be considered in further studies on aphid-apple interaction. The effect of water content of the leaves may also influence the development of aphids. It would be worthwhile to extend the investigation of other mineral nutrients and their interactions on aphid populations.

THE EFFECT OF SPIREA APHID AND NITROGEN FERTILIZATION ON NET PHOTOSYNTHESIS AND LEAF CHLOROPHYLL CONTENT OF APPLE LEAVES, WITH COMPARISONS TO APPLE APHID

Introduction

Many investigators, including entomologists, are interested in measuring the primary growth process of plants, photosynthesis. The effects of leafhopper feeding (Typhlocyba pomaria McAtee and Erythroneura hartii Fitch) on apple (Malus domestica Borkhausen) Pn were studied by Marshall et al. (1942). Feeding by both species reduced Pn by 25 percent at the rate of 100 leafhoppers per leaf for three days. Infestations of 15 twospotted spider mites (Tetranychus urticae Koch) per leaf significantly reduced apple Pn as early as seven days after treatment (Hall and Ferree 1975). Reduced Pn of impatiens, Impatiens wallerana Hook f., and peach, Prunus persica L. Batsch, by a leaf-feeding thrips, Echinothrips americanus Morgan, was also reported by Buntin et al. (1988).

A study was conducted by Wood et al. (1985) to determine the feeding influence of three pecan aphid species (blackmargined aphid, Monelliopsis nigropunctata (Granovsky); yellow pecan aphid, Monellia caryellaa (Fitch); and black pecan aphid, Melanocallis caryaefoliae (Davis)) on phloem integrity of pecan seedlings (Carya illinoensis (Wangenh.) K. Koch. Increasing populations of any of these species resulted in up to 50% reduction in Pn and increased accumulation of callose at the sieve plates. Pecan aphids also reduced Pn in mature pecan trees (Wood and Tedders 1982). The recent study by Varn et al. (in press) demonstrated that the rosy apple aphid, D. plantaginea , reduced Pn of apple leaves by 50-55%.

Leaf chlorophyll content provides a measure of plant photosynthetic potential; since the chlorophylls are the light-harvesting and reaction center components of the photosynthesis electron transport chain, the extensive loss of chlorophyll must reduce

the efficiency of electron transport in the leaf. Wood et al. (1985) reported that pecan aphids reduced the chlorophyll content of pecan leaves. The feeding by rosy apple aphid, the most damaging of the aphids infesting apple leaves, reduced leaf chlorophyll content of apple leaves by 25 percent (Varn et al. in press).

The nutritional state of the host plant affects photosynthetic activity (Barker 1979, Longstreth & Nobel 1980, DeJong 1983, Syvertsen 1987). The effects of spirea aphid and nitrogen fertilization on Pn, chlorophyll content, and greenness of apple leaves have not been studied. The purpose of this study is to determine these effects on various parameters of leaf function, and to describe the relationship between Pn and chlorophyll to see if a higher level of chlorophyll content could increase the Pn rates.

Phloem tissue is concerned with translocation of photosynthates (Zimmermann 1971). The principal conducting elements are the sieve tubes which are joined end to end by sieve plates. When an aphid inserts its stylets into a single sieve-tube element, the sieve tube reacts by plugging the pores in the sieve plates with callose (a β-1-3, glucose polysaccharide; Kessler 1958). Another objective of this study was to observe the effect of aphid feeding on the continuity of the phloem.

Materials and Methods

A- 1986 Growing Season Experiments:

Net photosynthesis : One fully expanded mid-shoot leaf infested with spirea aphids was selected from each of six one-year-old 'Redchief Delicious' apple trees to determine Pn. Trees were planted and grown in an unheated greenhouse with screened ends and leaves were inoculated with aphids in early August. Fully expanded leaves were selected

and were assumed to have similar potential photosynthetic rate. Another six leaves with sooty mold (cover > 70% of leaf surface area) were selected from the middle portion of the shoot (approximately 11-13 leaves from the shoot apex) to obtain relatively comparable values for photosynthesis. One leaf was selected from each of six noninfested trees to serve as controls. Forty-five days after introduction of viviparae, leaf gas exchange rates were determined in the greenhouse on intact individual leaves with an Analytical Development Corporation Portable CO_2 Analyzer (Fig. 17). Pn was based on the amount of CO_2 the leaf fixed in the light and was determined by measuring the difference in CO_2 concentration entering and leaving the chamber (Pn = mg CO_2 dm^{-2} hr^{-1}). The CO_2 analyzer measures the concentration of CO_2 directly after placing the leaf in the chamber for 25 seconds. The air flow rate into the chamber was 400 ml min^{-1} and photosynthetic photon flux density was approximately 950-1150 μ mols s^{-1} m^{-2}. Air temperature in the leaf chamber was 30 ^{o}C $\pm$ 2 ^{o}C. The relationships between aphid-days per leaf, sooty mold levels, and Pn were determined by regression analysis.

Leaf greenness and chlorophyll content : The same six leaves were selected as above. Ten leaf discs (8.0 mm diam.) were cut from the interveinal area on both sides of the midrib per leaf using a common paper punch (Fig. 18a). The discs were placed immediately in 10 ml of 80 % methanol in darkness at room temperature for 48 hours (Marini & Marini 1983). Concentrations of extracted chlorophyll a and b in the methanol were determined from absorption values at 645 nm and 663 nm using a double beam spectrophotometer (Fig. 18b). Total chlorophyll content per unit-leaf area were calculated using the formula:

$$\text{Total chlorophyll (mg/ m}^2\text{)} = (7.11\ A_{663} + 16.8\ A_{645})[(V*10^4)/N\pi r^2)]$$

where A = the absorbance of the extract at a given wavelength, V = the volume in liters, N = the number of leaf disks, and r = the radius of the disk (Proctor 1981).

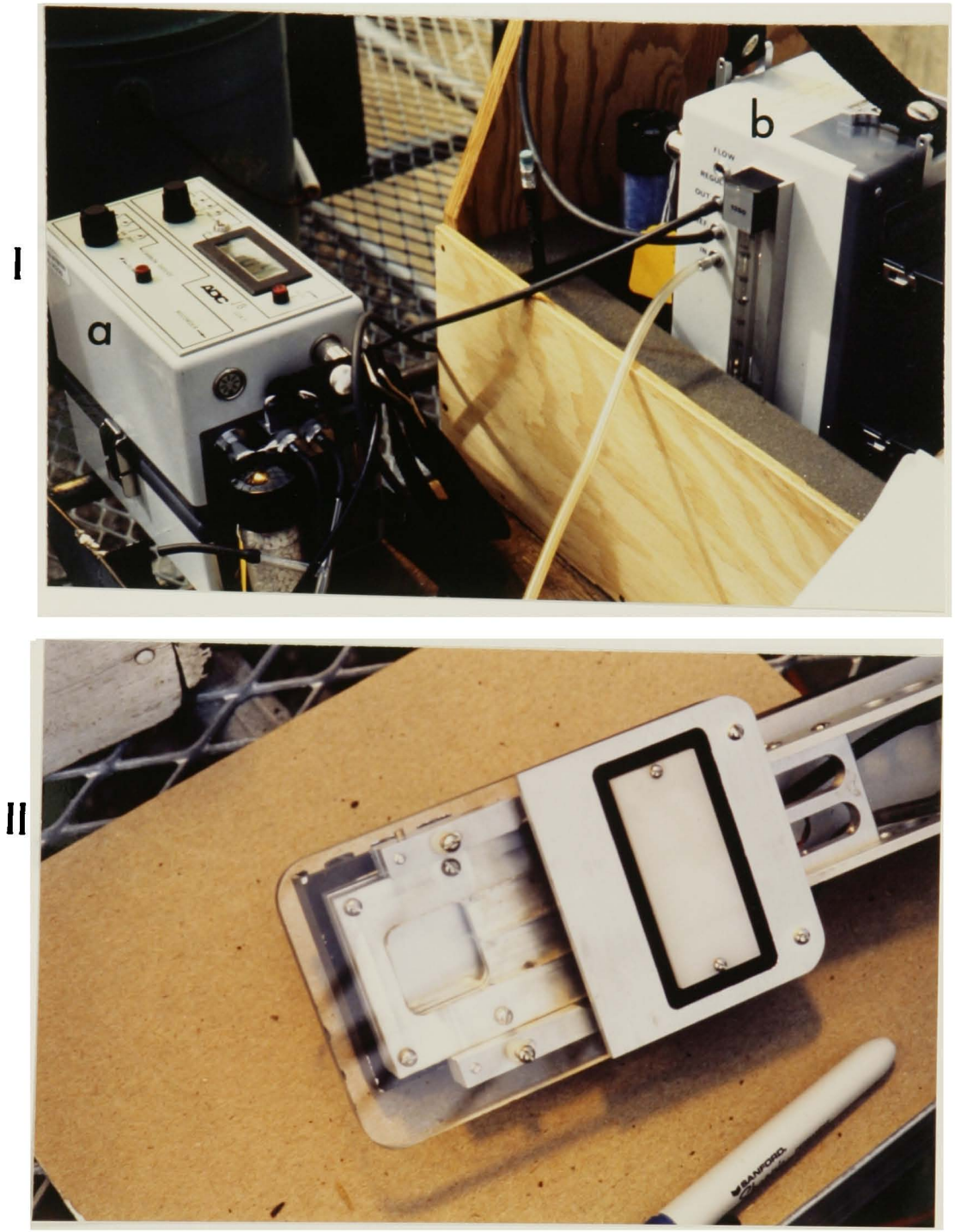


Fig. 17. The Analytical Development Corporation (ADC) CO_2 Analyzer for measuring the effect of feeding by spirea aphid on net photosynthesis of apple leaves. I: (a) a portable infrared gas analyzer, and (b) an Air Supply Unit (ASU). II: the Parkinston Leaf Chamber.

a

b

Fig. 18. (a) Apple leaf with discs removed for measuring the total chlorophyll content per unit-leaf area. (b) a double beam spectrophotometer.

Leaf greenness was measured using the Minolta SPAD 501 Portable Chlorophyll Meter (Fig. 19) (Yadava 1986). Four upper leaf surface readings were recorded on each leaf; two on each side of the leaf midrib. The data from the meter were compared and correlated with total chlorophyll concentrations obtained by the extraction method. The relationship between Pn and chlorophyll content was evaluated.

Histological examination : In order to determine callose accumulations at phloem sieve plates, apple leaves with active colonies of spirea aphids were removed and immediately fixed in acetic acid alcohol (1:3 glacial acetic acid: 95 % ethanol) and were cooled to approximately -74 °C with dry ice (Eschrich & Currier 1964). The leaves and fixative were allowed to return to ambient temperature in the laboratory. Several fixed pieces of midrib of leaves were dehydrated by transferring them at 24-hour intervals through a graded ethanol/butanol series : 35% EtOH, 9 parts 45% EtOH: 1 part 1-butanol , 8 parts 62% EtOH : 2 parts 1-butanol, 6.5 parts 77% EtOH : 3.5 parts 1-butanol, 4.5 parts 90% EtOH : 5.5 parts 1-butanol, 2.5 parts 90% EtOH : 7.5 parts 1-butanol, 1-butanol. Dehydrated pieces of midrib were then infiltrated with 2 parts 1-butanol : 8 parts Tissue Mat for 48 h and Tissue Mat for 48 h. Infiltrated specimens were embedded and sectioned longitudinally at 10 μm by Minot's Rotary Microtome (Eaton 1983). The sections were rehydrated by transferring them at 2-min intervals through xylene 1, xylene 2, 100% EtOH, 70% EtOH, 30% EtOH, and water (Gray 1964). The sections were then stained for 20 min in resorcinol blue at 1/1 stain/tap water. The stock solution of resorcinol blue was prepared according to Eschrich & Currier (1964) (Three grams of resorcinol were dissolved in 200 ml of distilled water and added to 3 ml of concentrated ammonia. The mixture was heated for 30 min in a steam bath until a dark, bluish color appeared. The hot solution was filtered into an evaporating vessel and heated until no significant amounts of NH_3 were given off).

Fig. 19. SPAD Meter for measuring the greenness of apple leaves.

The stained sections were dehydrated by transferring them at 2-min intervals through water, 30% EtOH, 70% EtOH, 100% EtOH 1, 100% EtOH 2, xylene 1, and xylene 2. Sections were mounted in Permount and kept dry for 24 h until examination (Gray 1964). The same staining procedures were used for leaves not fed on by spirea aphids.

B- 1987 Growing Season Experimentss:

Net photosynthesis : Sixty-four one-year-old 'Redchief Delicious' apple trees were planted and grown in the greenhouse as the previous year and were assigned to two groups (n = 32 each). The first group was infested with spirea aphids and the second group was kept free of aphids. Three weeks after planting, treatments consisting of four nitrogen rates (0.0, 0.5, 1.0, and 2.0 g urea per tree) were applied to both infested and control trees at three weeks intervals. The experimental design was a randomized complete block design with a 2x4 factorial arrangement of treatments (eight treatments) with eight single-tree replications each. The effect of nitrogen on the tree's response to spirea aphid feeding in terms of net photosynthesis and leaf greenness was measured repeatedly, for each nitrogen treatment, on three mid-shoot leaves per tree. Four trees per treatment were selected. Throughout the infestation of spirea aphids, seasonal Pn changes were determined for the same leaves, the first measurement being 10 days after the introduction of mature viviparae. Pn was determined using a portable CO_2 analyzer. The air flow rate into the chamber and photosynthetic photon flux density were approximately the same as in the previous year. Changes in Pn of apple leaves infested with spirea aphid and 30 minutes after the removal of aphids from the leaf surface by washing were determined.

Level of sooty mold (SM) accumulation was evaluated visually for each measurement on a scale from 0 to 3, where 0 = no SM; 1,2, and 3 represent low, medium, and high SM accumulations, respecively (Fig. 20-22). These levels corresponded to ca. trace-30%, 30-70%, and 70-100% of leaf surface area covered with SM. Twelve leaves were selected per SM level from 6 trees. SM was washed off with water and leaves were left to dry. Pn was remeasured after 30 minutes and the difference between the first and second measurements was calculated as the percentage change in light transmission.

The effect of feeding by spirea aphid vs apple aphid, at a single nitrogen rate, on Pn during the growing season were evaluated and compared. Twelve one-year-old apple trees were planted in spring of 1987 as above. Trees were assigned to three groups (spirea aphid-infested n = 4, apple aphid-infested n = 4, and non-infested control n = 4). Three mid-shoot leaves per tree were selected and changes in Pn during the infestation period were determined for the same leaves using the ADC CO_2 analyzer.

Leaf greenness : Changes in leaf greenness in relation to nitrogen rates and spirea aphid population were measured on the same three selected leaves/tree as in Pn experiment (12 replicates/treatment). The effect of feeding by spirea aphid vs apple aphid, at a single nitrogen rate, on leaf greenness during the growing season was evaluated and compared as in the Pn experiment.

Statistical Analysis

Data in 1986 were analyzed using analysis of variance (ANOVA). Data in 1987 were analyzed using Tukey's studentized range test ($P < 0.05$) to separate treatment means within measurement dates. Regression analyses using GLM and REG procedures

Fig. 20. Low level of sooty mold accumulation on apple leaves.

Fig. 21. Medium level of sooty mold accumulation on apple leaves.

Fig. 22. High level of sooty mold accumulation on apple leaves.

of the Statistical Analysis System (SAS Institute 1985) were performed to test for significant linear and nonlinear relationships between variables.

Results

A- 1986 Growing Season:

Compared to the controls, aphids and sooty mold reduced Pn (Table 8). Pn was reduced 24 and 48% by aphid feeding and sooty mold, respectively. Pn rates decreased quadratically with increasing aphid-days per leaf ($r^2 = 0.98$) (Fig. 23). Pn was reduced 50% when a leaf was exposed to approximately 1400 aphid-days.

The data for the methods of measuring leaf greenness and chlorophyll content are presented in Table 8. Using the methanol extraction technique, chlorophyll content was reduced 30 and 26% by spirea aphid and sooty mold, respectively. Similar results were obtained with the SPAD meter, where 26 and 21% reductions in leaf greenness were caused by aphids and sooty mold, respectively. Leaf chlorophyll and greenness decreased nonlinearly with increasing aphid-days/leaf (Fig. 24). Absorbance of chlorophyll a, b, and total chlorophyll were significantly ($P < 0.05$) higher in control leaves than those infested with aphids or infected with SM (Table 9). In this study, total chlorophyll content was lower than previously reported (Proctor 1979). Extraction of chlorophyll in this study was probably less complete. Therefore, differences in chlorophyll content was relative rather than absolute.

Pn increased linearly ($P < 0.05$) with increasing chlorophyll content. This appears to be different from a previous report with peach (Marini & Marini 1983). Figures 25

Table 8. The effect of spirea aphid feeding and sooty mold accumulation on net photosynthesis (Pn), greenness, and chlorophyll content of apple leaves (1986).

Treatment*	Leaf Pn (mg CO_2 dm^{-2} hr^{-1})	Leaf Chlorophyll Content: Methanol Extraction (mg/m^2)	Leaf Greenness: SPAD Meter (relative measure)
Leaves with aphids	10.3 (1.83)	186.6 (23.6)	40.3 (1.44)
Control	13.5 (1.03)	264.9 (18.2)	54.4 (1.76)
Significance	*	*	*
Leaves with sooty mold (> 70% leaf surface covered)	8.2 (1.73)	201.6 (6.15)	43.8 (2.55)
Control	15.2 (2.43)	264.4 (18.6)	55.5 (4.39)
Significance	*	*	*

* Means (SEM in parentheses) in the same column followed by an asterisk are significantly different ($P < 0.05$) using ANOVA.

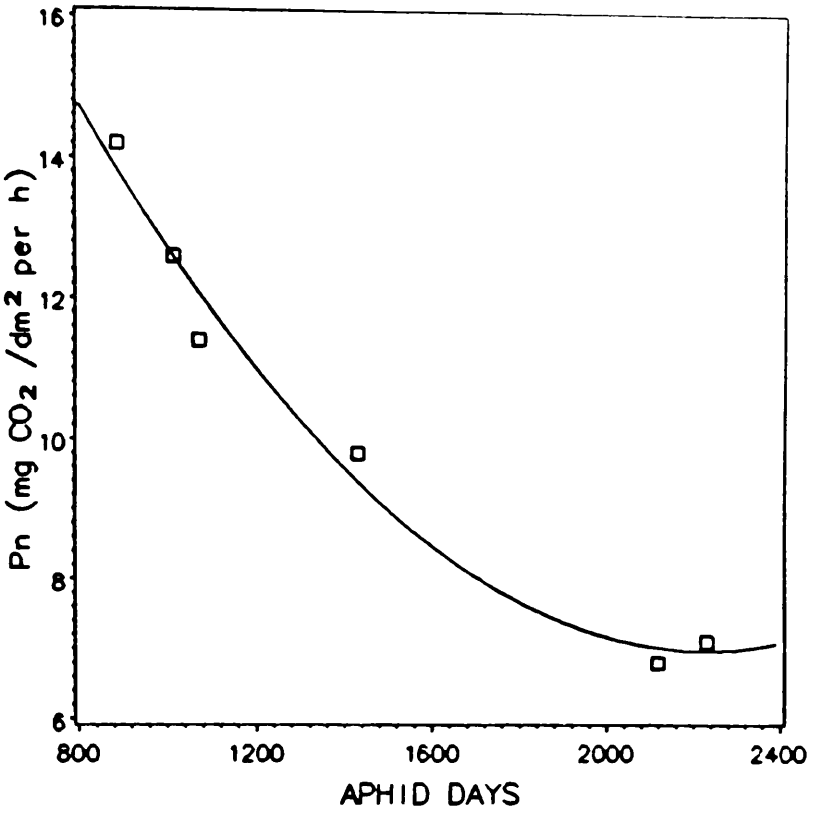
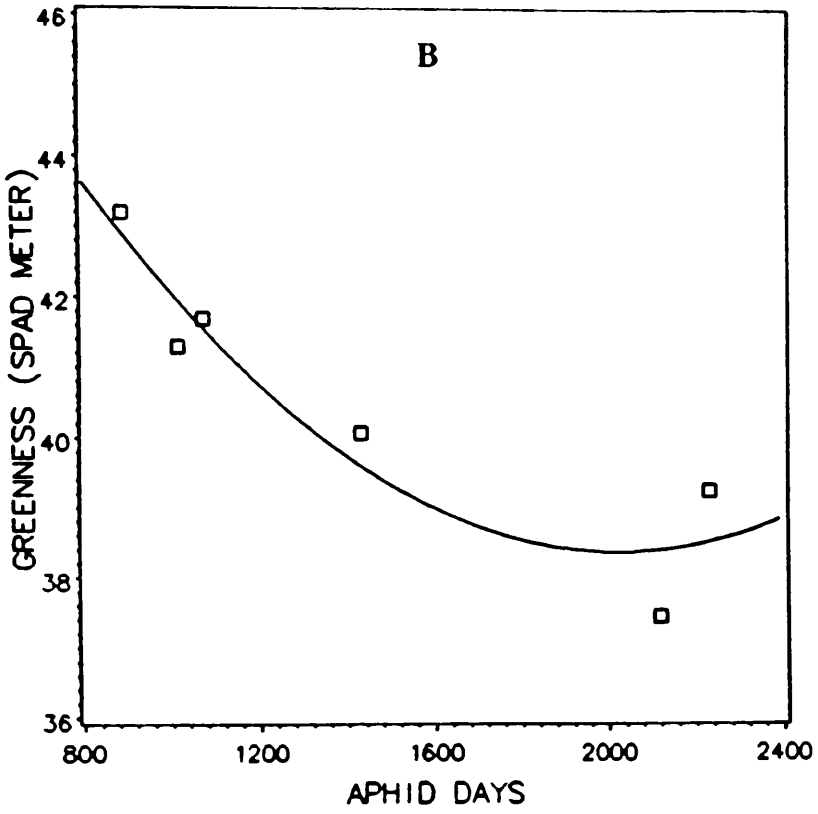


Fig. 23. Effect of cumulative aphid-days per leaf on net photosynthesis (Pn) of apple leaves (1986). The quadratic regression equation and r^2 value are:

$$Y = 25.9\text{-}0.017x + 4.0E\text{-}6\ x^2 \qquad r^2 = 0.98$$

Fig. 24. Effect of cumulative aphid-days per leaf on chlorophyll content (A) and greenness (B) of apple leaves (1986). Chlorophyll was measured by spectrophotometer and greenness was measured by the SPAD meter. The quadratic regression equations and r^2 values in the same order are:

A----	$Y = 363.7 - 0.217x + 6.0E\text{-}5\ x^2$	$r^2 = 0.92$
B----	$Y = 52.8 - 0.014x + 4.0E\text{-}6\ x^2$	$r^2 = 0.99$


B
GREENNESS (SPAD METER)
46
44
42
40
38
36
800
1200
1600
2000
2400
APHID DAYS

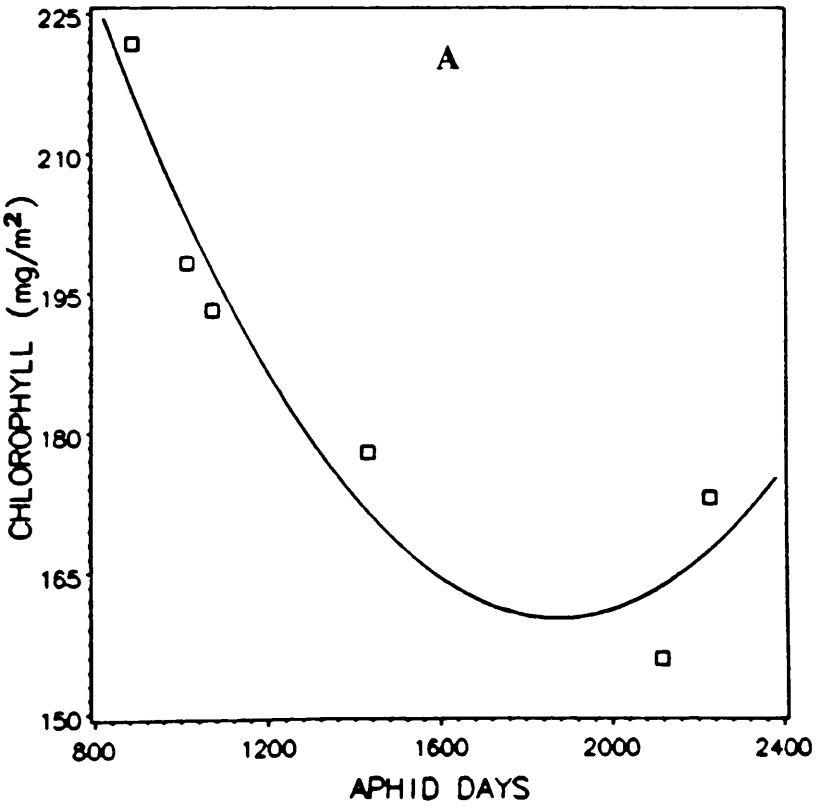

A
CHLOROPHYLL (mg/m²)
225
210
195
180
165
150
800
1200
1600
2000
2400
APHID DAYS

Table 9. Effects of spirea aphid feeding and sooty mold (SM) accumulation on chlorophyll a, b, and total chlorophyll content of greenhouse-grown young apple trees (1986).

Treatment	Chlorophyll[1]		
	a	b	Total
Aphid infestation	0.660	0.275	0.935
Control	0.961	0.384	1.345
% reduction	31	28	30
significance	*	*	*
SM accumulation	0.735	0.290	1.025
Control	0.983	0.373	1.356
% reduction	26	23	24
significance	*	*	*

[1] Absorbance of chlorophyll a and b was measured by a double beam spectrophotometer at 663 and 645 wavelength, respectively. Asterisk indicates a significant difference at 5% probability level.

and 26 show the relationship of Pn to chlorophyll content and Pn to leaf greeness, respectively.

Methanol extraction and SPAD readings were linearly correlated whether measured on infested, SM, or control leaves (Fig. 27). During the progress of this study, similar results using the two methods on peach and plum leaves were reported (Yadava 1986). The advantages and limitations of the SPAD meter are listed in the Minolta Corp. Manual.

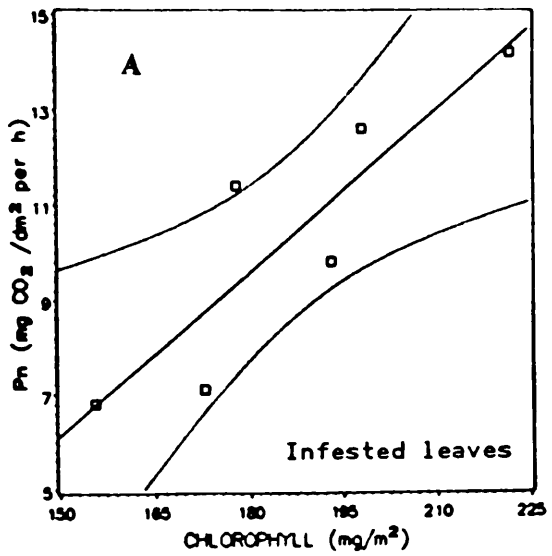
Callose accumulation at the sieve plates in response to spirea aphid feeding was observed (Fig. 28 & 29), but did not appear as severe as that caused by other aphids on apple and pecan leaves (Wood et al. 1985, Varn et al. in press).

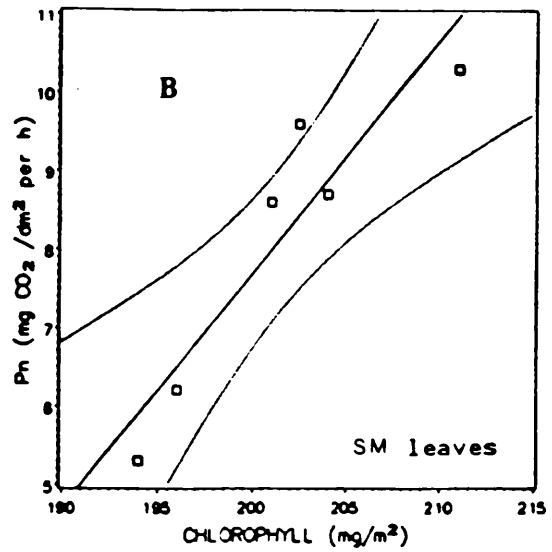
B- 1987 Growing Season:

Pn was higher for control leaves compared with infested leaves on all measurement dates (August 3 and 28, September 10 and 24, October 14). Trees treated with high N rate had higher Pn values than in trees treated with lower rates in both control and infested trees (Fig. 30). Pn values for all trees decreased after the second measurement but rates were lower for infested trees than control trees. Figure 31 shows the main effect of aphid infestation on late seasonal Pn values (for each of four N rates individually). On August 3, ten days after infestation, aphid populations had not increased sufficiently to reduce Pn. Pn values were higher in control than infested leaves across all nitrogen treatments from August 28 (second measurement) to the end of the season. After September 28, Pn declined in all treatments as a result of a leaf senescence and aphid population build up. Photosynthetic rates for all nitrogen treatments were quadratically reduced by aphid-day accumulations (Fig. 32).

Fig. 25. The relationship between chlorophyll content and rate of photosynthesis (Pn) in apple leaves during 1986 growing season. A. leaves with various levels of aphid infestation. B. leaves with accumulation of sooty mold. C. control leaves. Broken lines represent 95% confidence limits about the regression line. The slopes of the equations are not significantly different from each other. The intercept in A is different from the intercept in B or C ($P < 0.05$). The general linear regression equations (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 values in the same order are:

A---	$Y = -11.1 + 0.12x$ (5.66) (0.030)	$r^2 = 0.78$
B	$Y = -52.3 + 0.29x$ (12.1) (0.060)	$r^2 = 0.86$
C	$Y = -28.6 + 0.16x$ (8.20) (0.003)	$r^2 = 0.87$


A
Infested leaves
Pn (mg CO2 /dm2 per h)
CHLOROPHYLL (mg/m2)


B
SM leaves
Pn (mg CO2 /dm2 per h)
CHLOROPHYLL (mg/m2)

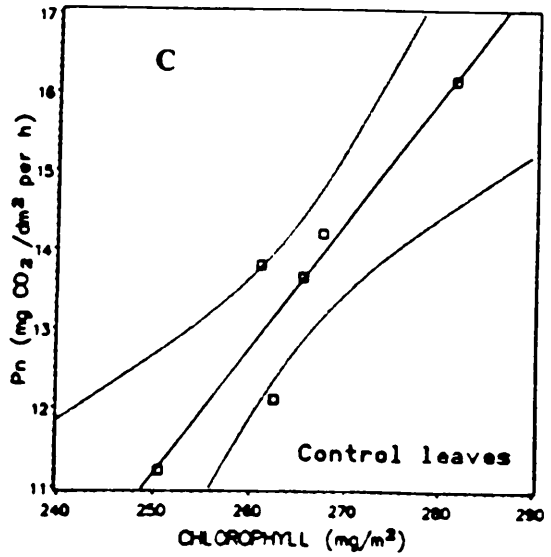
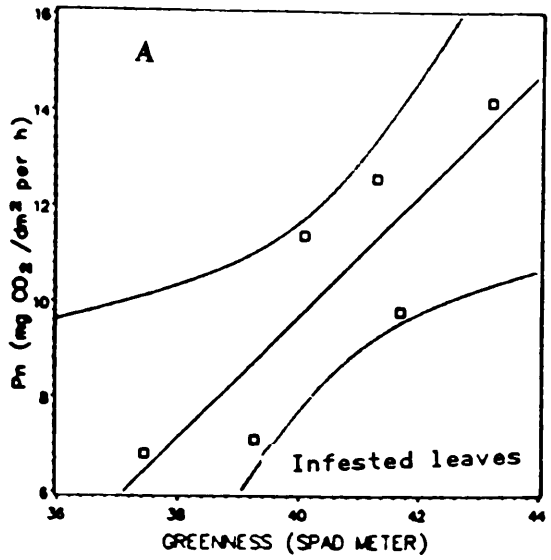
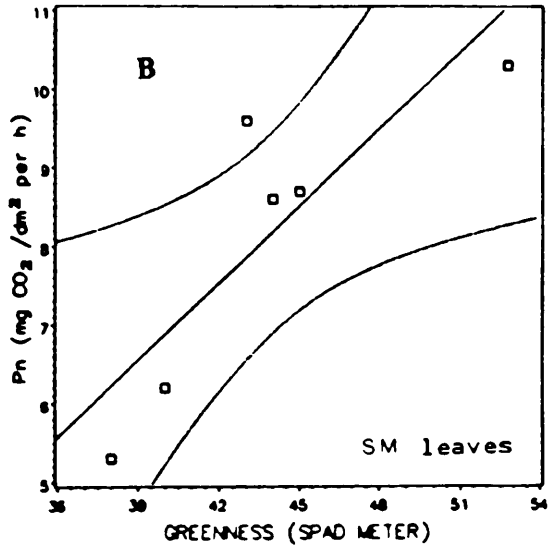

C
Control leaves
Pn (mg CO2 /dm2 per h)
CHLOROPHYLL (mg/m2)

Fig. 26. The relationship between leaf greenness (using the SPAD Meter) and rate of photosynthesis (Pn) in apple leaves during 1986 growing season. A. leaves with various level of aphid infestation. B. leaves with accumulation of sooty mold. C. control leaves. Greenness expressed as a relative measure. Broken lines represent 95% confidence limits about the regression line. Slopes and intercepts of the regression equations, B and C, are not significantly different from each other ($P < 0.05$), but the intercept of A is significanly different from that of B or C. The general linear regression equations (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 values in the same order are:

A---	$Y = -46.4 + 1.40x$ (9.50) (0.233)	$r^2 = 0.90$
B---	$Y = -6.19 + 0.33x$ (4.36) (0.099)	$r^2 = 0.73$
C---	$Y = -13.4 + 0.49x$ (2.52) (0.246)	$r^2 = 0.97$


A
Infested leaves
Pn (mg CO2 /dm2 per h)
GREENNESS (SPAD METER)


B
SM leaves
Pn (mg CO2 /dm2 per h)
GREENNESS (SPAD METER)

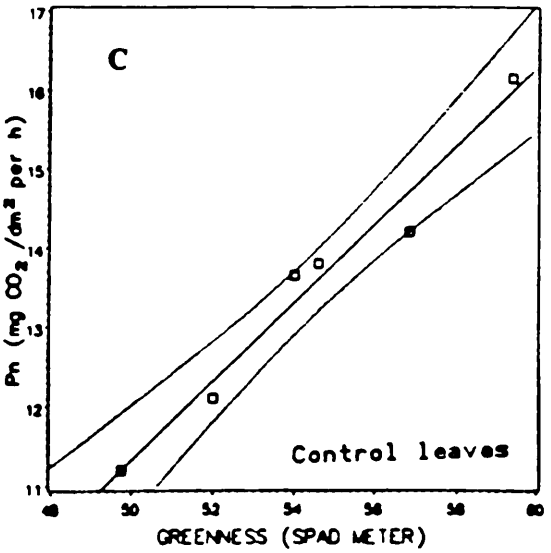

C
Control leaves
Pn (mg CO2 /dm2 per h)
GREENNESS (SPAD METER)

Fig. 27. The relationship between chlorophyll content in apple leaves using the methanol extraction technique to leaf greenness using SPAD meter (relative measure) in 1987. A. leaves with various level of aphid infestation. B. leaves with accumulation of sooty mold. C. control leaves. Broken lines represent 95% confidence limits about the regression line. The slopes of the regression equations in B and C are not significantly different from each other; but are different from the slope in A ($P < 0.05$). The intercepts of the equations are significantly different from each other. The general linear regression equations (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 values in the same order are:

A---	$Y = 264.4 + 11.1x$ $(46.2)(1.140)$	$r^2 = 0.96$
B---	$Y = 135.0 + 1.40x$ $(6.14)\ (0.139)$	$r^2 = 0.97$
C---	$Y = 115.0 + 2.75x$ $(29.3)\ (0.538)$	$r^2 = 0.88$

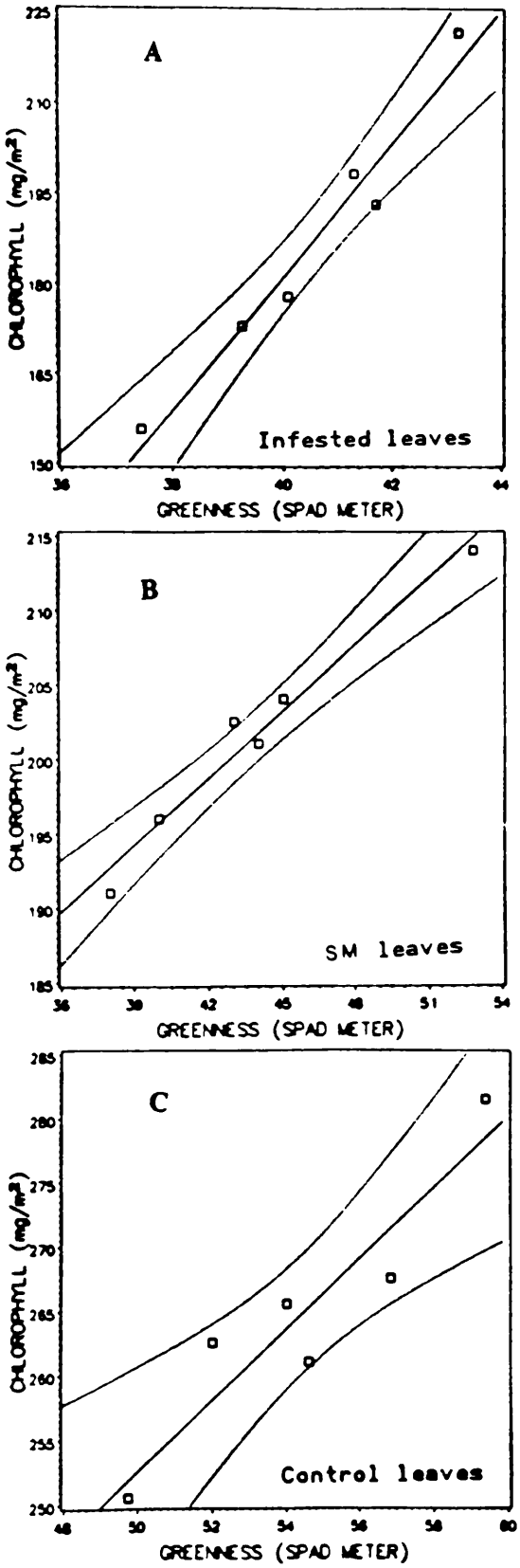
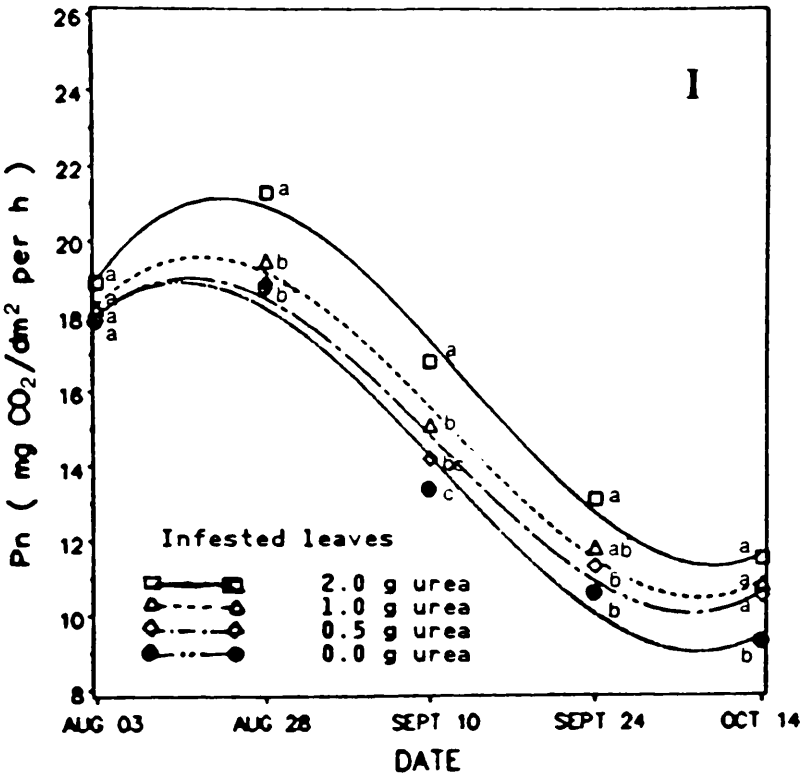

A
Infested leaves
CHLOROPHYLL (mg/m²)
GREENNESS (SPAD METER)
B
SM leaves
CHLOROPHYLL (mg/m²)
GREENNESS (SPAD METER)
C
Control leaves
CHLOROPHYLL (mg/m²)
GREENNESS (SPAD METER)

Fig. 28. Longitudinal section of midvein of apple leaves showing spirea aphid feeding injury. Callose accumulation in the phloem at the sieve plates is indicated by an arrow near center of photograph. Callose deposits are evident as red-brown stains. Top arrow bar indicates xylem, the middle arrow bar indicates phloem and bottom arrow bar indicates parenchymal cells.

Fig. 29. Longitudinal section of midvein of apple leaves free from feeding of spirea aphid. Undisrupted sieve tubes and lack of callose deposits in the sieve plates are shown in the center of photograph. Top arrow bar indicates xylem, the middle arrow bar indicates phloem and bottom arrow bar indicates parenchymal cells.

Fig. 30. Late seasonal changes in net photosynthesis (Pn) of apple leaves. I and II represent infested and control trees with four nitrogen rates during the 1987 growing season. · Each point represents the average of 12 measurements taken from four trees. Letters represent differences within measurement dates at the 0.05 level with Tukey's studentized range test. The cubic regression equations ('X' represents days after infestation) and r^2 values in the same order are:

I/	2.0 g ---	$Y = 16.6 + 0.491x - 0.020x^2 - 0.00016\ x^3$	$r^2 = 0.98$
	1.0 g ---	$Y = 16.6 + 0.491x - 0.019x^2 - 0.00016\ x^3$	$r^2 = 0.98$
	0.5 g ---	$Y = 16.8 + 0.522x - 0.022x^2 - 0.00016\ x^3$	$r^2 = 0.99$
	0.0 g ---	$Y = 17.2 + 0.629x - 0.023x^2 - 0.00017\ x^3$	$r^2 = 0.99$
II/	2.0 g ---	$Y = 16.6 + 0.551x - 0.018x^2 - 0.00013\ x^3$	$r^2 = 0.99$
	1.0 g ---	$Y = 16.7 + 0.604x - 0.019x^2 - 0.00013\ x^3$	$r^2 = 0.99$
	0.5 g ---	$Y = 16.8 + 0.671x - 0.020x^2 - 0.00014\ x^3$	$r^2 = 0.99$
	0.0 g ---	$Y = 17.7 + 0.771x - 0.022x^2 - 0.00015\ x^3$	$r^2 = 0.99$


I
Infested leaves
2.0 g urea
1.0 g urea
0.5 g urea
0.0 g urea
Pn (mg CO2/dm2 per h)
AUG 03
AUG 28
SEPT 10
SEPT 24
OCT 14
DATE

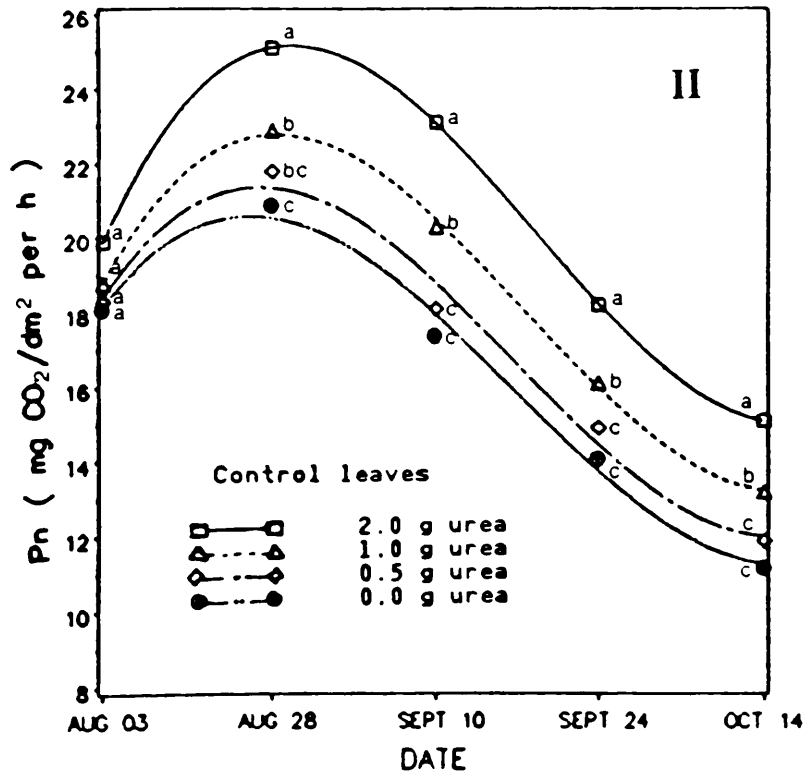

II
Control leaves
2.0 g urea
1.0 g urea
0.5 g urea
0.0 g urea
Pn (mg CO2/dm2 per h)
AUG 03
AUG 28
SEPT 10
SEPT 24
OCT 14
DATE

Fig. 31. Late seasonal changes in net photosynthesis (Pn) of apple leaves. I,II,III, and IV represent the seasonal changes at 0.0, 0.5, 1.0, and 2.0g urea/tree respectively. Solid and dashed lines represent control (CO) and infested leaves (SA), respectively. Each point represents the average of 12 measurements taken from fourfilees. Letters represent differences within measurement dates at the 0.05 level with Tukey's studentized range test. The cubic regression equations ('x' represent days after aphid infestation) and r^2 values at each N level in the same order are:

I/	CO--	$Y = 16.6 + 0.551x - 0.018x^2 - 0.00013\ x^3$	$r^2 = 0.99$
	SA--	$Y = 16.6 + 0.491x - 0.020x^2 - 0.00016\ x^3$	$r^2 = 0.98$
II/	CO--	$Y = 16.7 + 0.604x - 0.019x^2 - 0.00013\ x^3$	$r^2 = 0.99$
	SA--	$Y = 16.6 + 0.491x - 0.019x^2 - 0.00016\ x^3$	$r^2 = 0.98$
III/	CO--	$Y = 16.9 + 0.671x - 0.020x^2 - 0.00014\ x^3$	$r^2 = 0.99$
	SA--	$Y = 16.8 + 0.521x - 0.022x^2 - 0.00016\ x^3$	$r^2 = 0.99$
IV/	CO--	$Y = 17.7 + 0.771x - 0.022x^2 - 0.00015\ x^3$	$r^2 = 0.99$
	SA--	$Y = 17.2 + 0.629x - 0.023x^2 - 0.00017\ x^3$	$r^2 = 0.99$

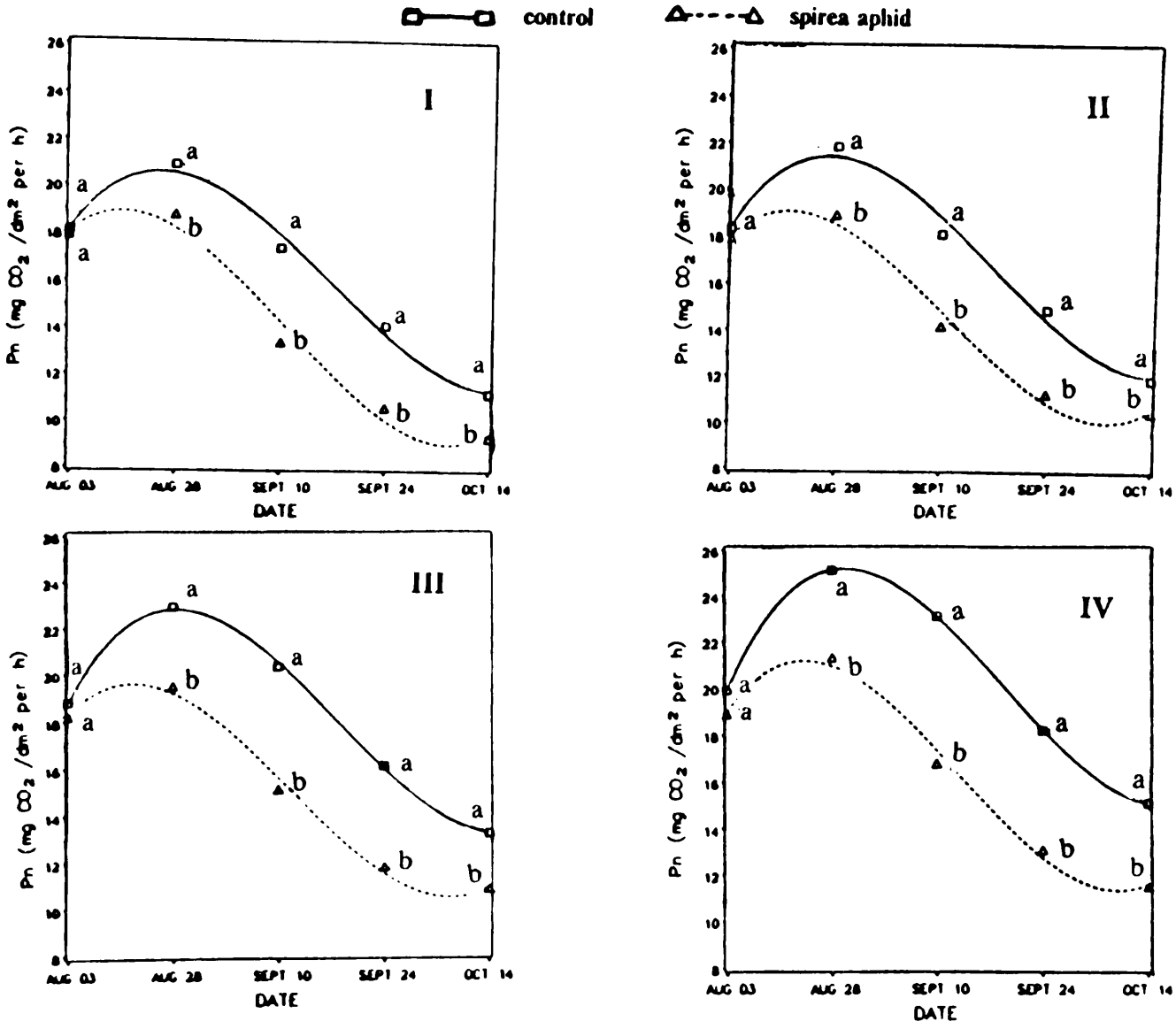
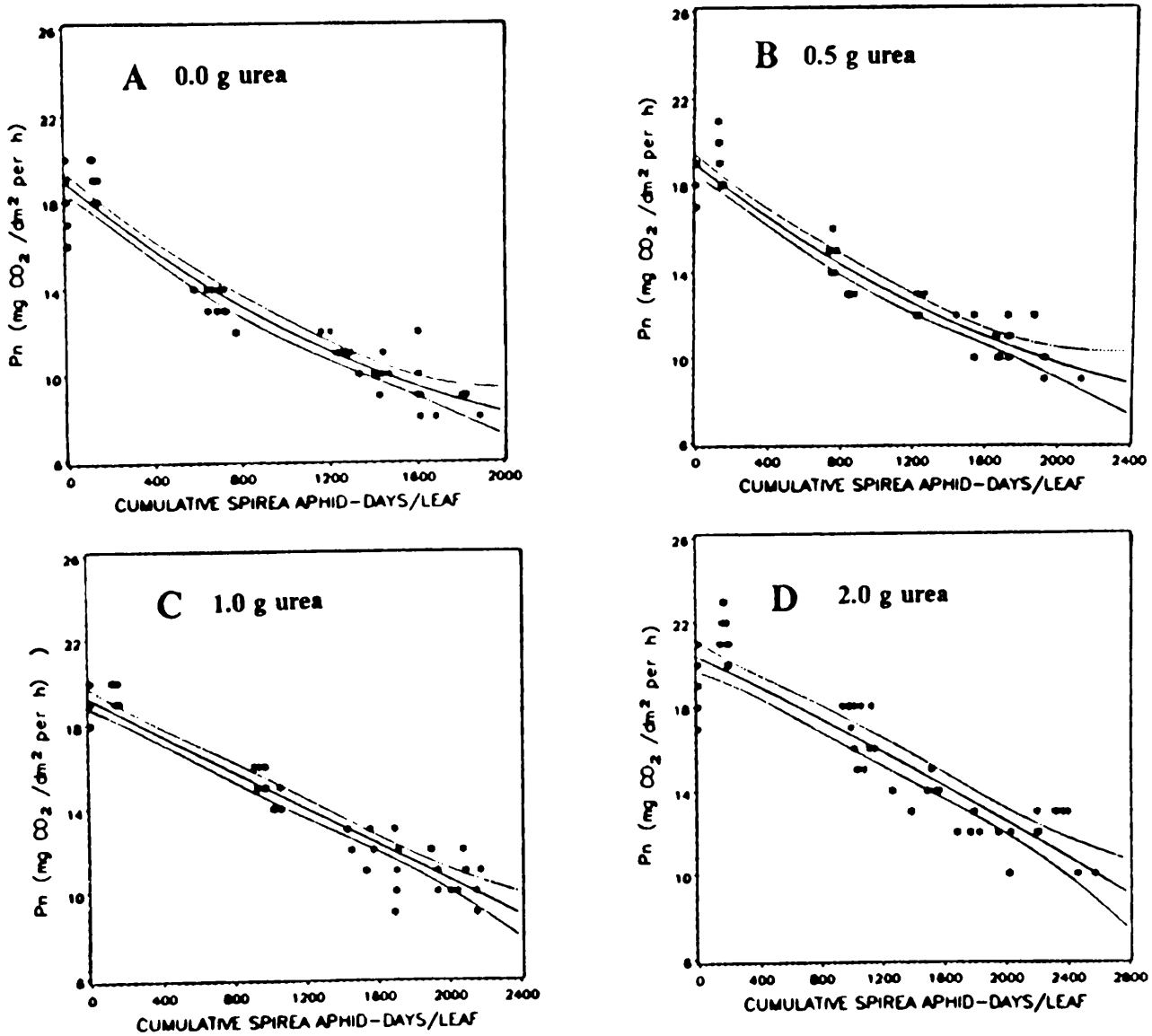

control
spirea aphid
I
II
III
IV
Pn (mg CO_2 /dm² per h)
DATE
AUG 03
AUG 28
SEPT 10
SEPT 24
OCT 14

Fig. 32. The relationship of net photosynthesis (Pn) to cumulative spirea aphid-days per leaf with four different nitrogen rates during the 1987 growing season. A,B,C, and D represent urea application rates of 0.0, 0.5, 1.0, and 2.0g urea/tree respectively. Broken lines represent 95% confidence limits about the regression line. The quadratic regression equations, r^2 values in the same order are:

A	$Y = 18.8 - 0.0082x + 1.4E\text{-}6\ x^2$	$r^2 = 0.93$
B	$Y = 18.9 - 0.0064x + 9.2E\text{-}7\ x^2$	$r^2 = 0.92$
C	$Y = 19.2 - 0.0044x + 2.3E\text{-}8\ x^2$	$r^2 = 0.92$
D	$Y = 20.4 - 0.0035x - 1.9E\text{-}7\ x^2$	$r^2 = 0.83$


A 0.0 g urea
B 0.5 g urea
C 1.0 g urea
D 2.0 g urea
Pn (mg CO2 /dm2 per h)
CUMULATIVE SPIREA APHID-DAYS/LEAF

A significant reduction in Pn of leaves infested with various levels of spirea aphid was lower than control leaves (Table 10). Low, medium, and high levels of infestation (890, 1070, 2370 aphid-days) reduced Pn by 23, 33, and 47%, respectively. Thirty minutes after aphid removal, photosynthetic rates in control leaves were similar after washing. The apparent elevated CO_2 uptake by infested leaves after washing, was probably not a result of increased Pn, but rather removal of respiring aphids. After aphid removal however, Pn was still negatively related to aphid-days. Reductions in Pn by about 15, 17, and 30% after washing were found for low, medium, and high levels, respectively. These results indicate that aphids cause a reduction in Pn which persists after aphid removal. Measuring Pn with aphids in situ yields low Pn estimates, since CO_2 evolution by aphids contributes to CO_2 concentrations used to calculate Pn.

Aphids insert their stylets deep into the phloem tissues of their plant hosts and suck out the contents. Aphids excrete honeydew which supports the growth of a sooty mold fungus (Capnodiaceae). In this study, a black layer of sooty mold fungus appeared on the upper leaf surface during the aphid infestation period. Photosynthetic rates decreased with increasing levels of sooty mold (Table 11). Low and medium levels did not differ significantly but were different from the control ($P < 0.05$). Reductions in Pn by about 21, 29, and 53% were observed in infected leaves compared with controls. Pn rates increased after removal of sooty mold, but were still about 12, 19, and 29% lower than control leaves, respectively for the three levels.

Changes in leaf greenness over time were best described by quadratic regression equations (Fig. 33). Greenness was significantly higher in control than infested leaves across all nitrogen treatments on all dates. Greenness values were similar at the begining of infestation and increased slowly in infested leaves to a maximum levels on September 10 before declining. Greenness of control leaves increased more rapidly and to a greater

Table 10. Net photosynthesis (Pn) changes of apple leaves infested with spirea aphid and 30 minutes after the removal of aphids from the leaf surface by washing (1987).

Aphid infestation level	Pn (mg CO_2 dm^{-2} hr^{-1})[1]		Preremoval level (%)	% Pn reductions	
	Aphid present	Aphid removed		In infested leaves	In washed leaves
Control (0.0 aphid-days)	21.7 (0.53) a[2]	21.3 (0.57) a[2]	98	0	0
Low (980 aphid-days)	16.8 (0.39) b	18.0 (0.37) b	107	23	15
Medium (1070 aphid-days)	14.6 (0.38) c *	17.1 (0.39) b	121	33	17
High (0.0 aphid-days)	11.5 (0.34) d *	15.1 (0.38) c	131	47	30

[1] Means (SEM in parentheses) in the same column followed by the same letter are not significantly different ($P < 0.05$) using ANOVA.
[2] Aphids were never present, the two columns indicate Pn before and after washing. Asterisk denotes a significant difference in Pn between infested and washed leaves.
[3] Reduction in Pn as compared with control.

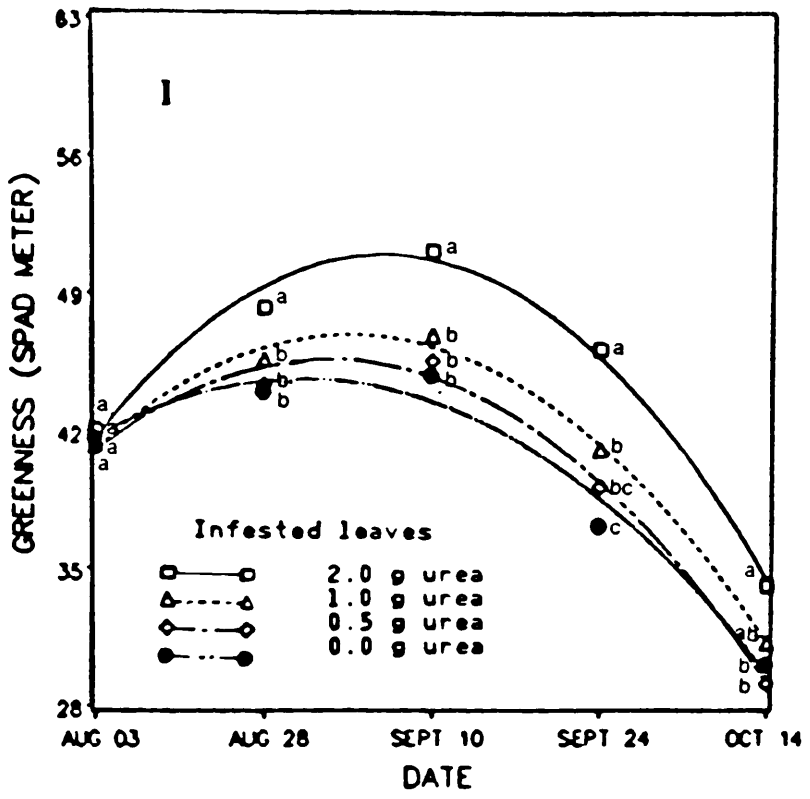
Table 11. Net photosynthesis (Pn) changes of apple leaves with sooty mold (SM) accumulation and after the removal of SM from the leaf surface by washing (1987).

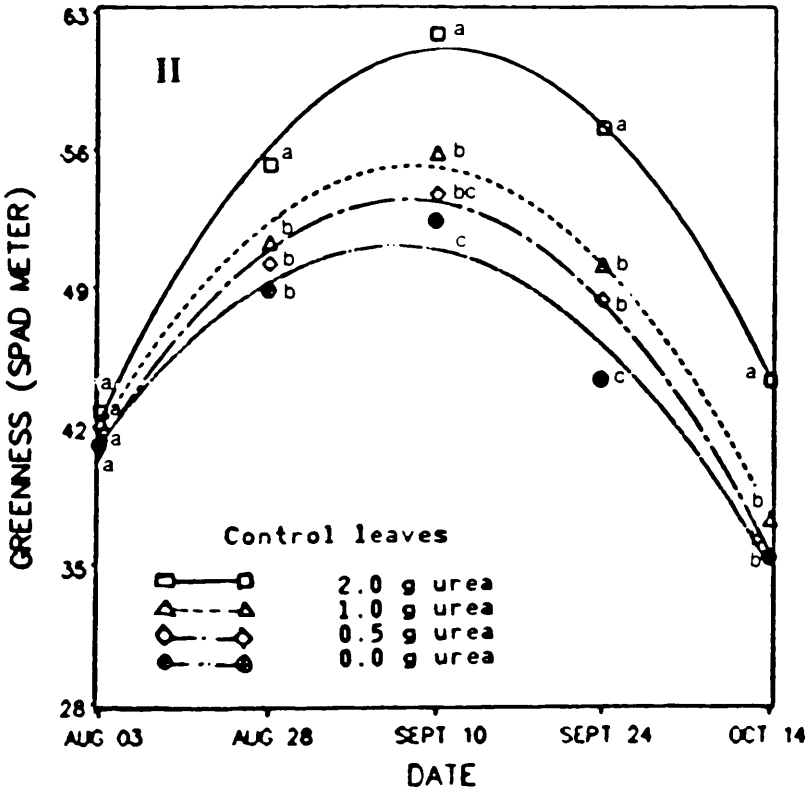
SM accumulation level	Pn (mg CO_2 dm^{-2} hr^{-1})[1] SM present	SM removed	Preremoval level (%)	% Pn reductions In infected leaves[3]	In washed leaves
Control (0.0 %)	21.7 (0.53) a[2]	21.3 (0.57) a[2]	98	0	0
Low (trace-30%)	17.2 (0.43) b	18.7 (0.43) b	109	21	12
Medium (30-70%)	15.4 (0.38) b	17.3 (0.33) b	112	29	19
High (70-100%)	10.3 (0.36) c *	15.1 (0.37) c	146	53	29

[1] Means (SEM in parentheses) in the same column followed by the same letter are not significantly different ($P < 0.05$) using ANOVA.
[2] Sooty molds were never present, the two columns indicate Pn before and after washing. Asterisk denotes a significant difference in Pn between infested and washed leaves.
[3] Reduction in Pn as compared with control.

Fig. 33. Late seasonal changes in the greenness of apple leaves. I and II represent infested and control trees with four nitrogen rates during the 1987 growing season. Greenness was measure by SPAD meter (relative measure). Each point represents the average of 12 measurements taken from four trees. Letters represent differences within measurement dates at the 0.05 level with Tukey's studentized range test. The quadratic regression equations ('x' represent days after aphid infestation) and r^2 values in the same order are:

I/	A---	$Y = 41.0 + 0.291x - 0.006x^2$	$r^2 = 0.95$
	B---	$Y = 40.0 + 0.395x - 0.007x^2$	$r^2 = 0.98$
	C---	$Y = 39.9 + 0.448x - 0.008x^2$	$r^2 = 0.98$
	D---	$Y = 39.9 + 0.619x - 0.009x^2$	$r^2 = 0.97$
II/	A---	$Y = 39.2 + 0.643x - 0.009x^2$	$r^2 = 0.93$
	B---	$Y = 39.7 + 0.775x - 0.011x^2$	$r^2 = 0.97$
	C---	$Y = 39.4 + 0.808x - 0.011x^2$	$r^2 = 0.96$
	D---	$Y = 39.2 + 1.015x - 0.013x^2$	$r^2 = 0.96$


I
GREENNESS (SPAD METER)
63
56
49
42
35
28
Infested leaves
2.0 g urea
1.0 g urea
0.5 g urea
0.0 g urea
AUG 03
AUG 28
SEPT 10
SEPT 24
OCT 14
DATE


II
GREENNESS (SPAD METER)
63
56
49
42
35
28
Control leaves
2.0 g urea
1.0 g urea
0.5 g urea
0.0 g urea
AUG 03
AUG 28
SEPT 10
SEPT 24
OCT 14
DATE

extent than for infested leaves, but peaked on the same date. After September 10, greenness declined in both groups, with lower rates in infested leaves. Figure 34 shows the comparison in the changes in greenness of control and infested leaves at each nitrogen level.

Leaf greenness and aphid-days were related quadratically for each nitrogen level (Fig. 35). Greenness rose in early September, and declined as leaves ages and the extent of infestation increased. Greenness and Pn were linearly related (Fig. 36), as was found in the previous year. Pn increased linearly with increasing greenness in all nitrogen treatments ($P < 0.05$).

Spirea aphid and apple aphid affected Pn similarly throughout the season (Fig. 37). Apple aphid reduced Pn slightly more than the spirea aphid on the last two measurement dates (September 24 and October 14). On September 24, spirea aphid and apple aphid reduced about 27 and 34%, respectively. On October 14, Pn was reduced about 19 and 27% by spirea aphid and apple aphid, respectively. The greater number of aphid and their greater reproductive rate early in the season might have been responsible for the Pn differences late in the season. Pn declined quadratically with increasing accumulated aphid-days for both species (Fig. 38).

Leaf greenness declined nonlinearly with time (Fig. 39) and with accumulated aphid-days for both species (Fig. 40). Pn increased positively with greenness of apple leaves infested with spirea and apple aphids (Fig. 41). Overall, there were no significant interspecific differences in effects on Pn rates and greenness of apple leaves.

Fig. 34. Late seasonal changes in the greenness of apple leaves. I,II,III, and IV represent changes at 0.0, 0.5, 1.0, and 2.0g urea/tree respectively. Solid and dashed lines represent control (CO) and infested leaves (SA), respectively. Greenness was measured by SPAD meter (relative measure). Each point represents the average of 12 measurements taken from four trees. Letters represent differences within measurement dates at the 0.05 level with Tukey's studentized range test. The quadratic regression equations ('x' represent days after aphid infestation) and r^2 values at each N level in the same order are:

I/	CO---	$Y = 39.2 + 0.643x - 0.009x^2$	$r^2 = 0.93$
	SA---	$Y = 41.0 + 0.291x - 0.006x^2$	$r^2 = 0.95$
II/	CO---	$Y = 39.7 + 0.775x - 0.011x^2$	$r^2 = 0.97$
	SA---	$Y = 40.0 + 0.395x - 0.007x^2$	$r^2 = 0.98$
III/	CO---	$Y = 39.4 + 0.808x - 0.011x^2$	$r^2 = 0.96$
	SA---	$Y = 39.9 + 0.448x - 0.008x^2$	$r^2 = 0.98$
IV/	CO---	$Y = 39.2 + 1.015x - 0.013x^2$	$r^2 = 0.96$
	SA---	$Y = 39.9 + 0.619x - 0.009x^2$	$r^2 = 0.97$

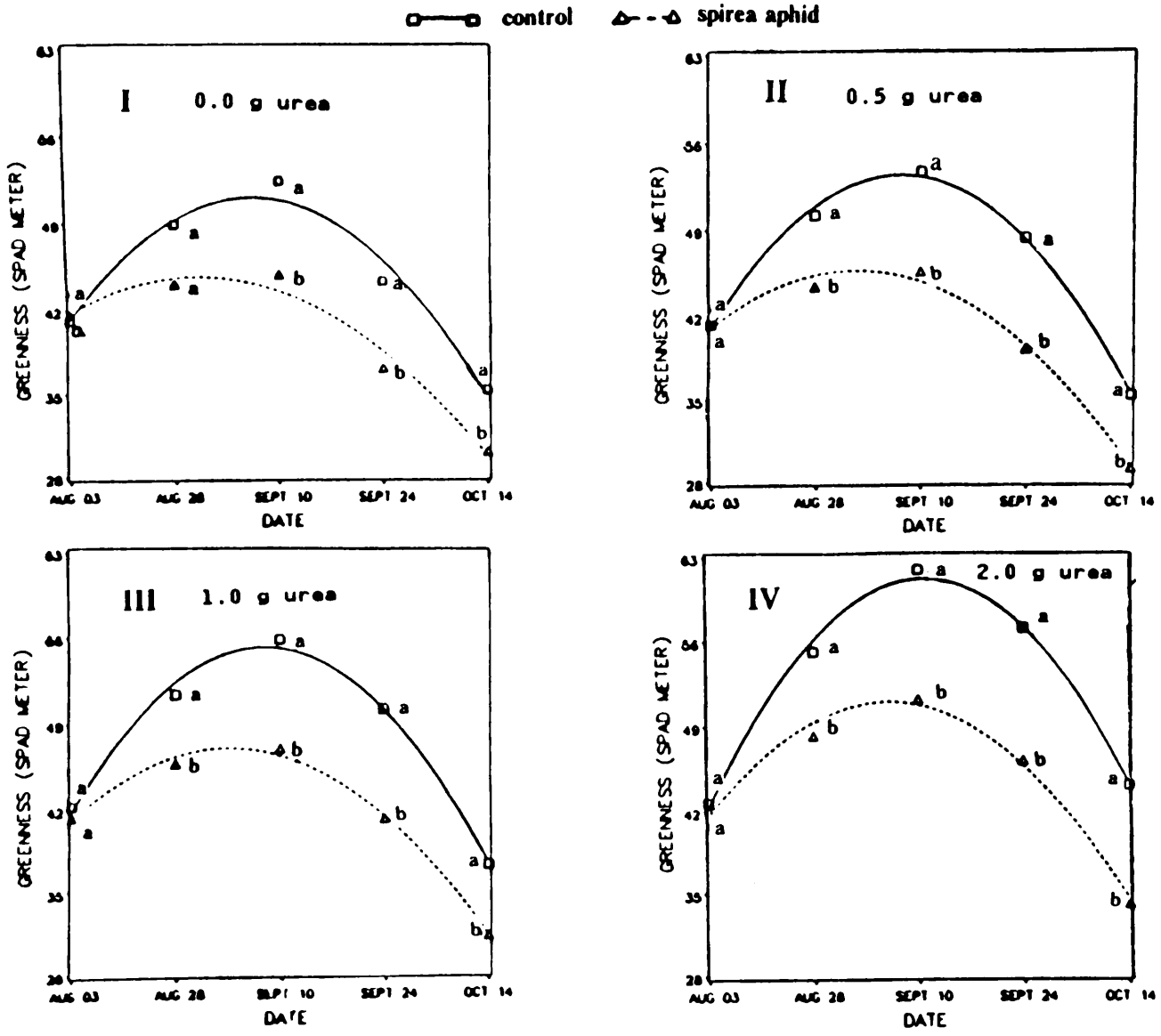

control
spirea aphid
I 0.0 g urea
II 0.5 g urea
III 1.0 g urea
IV 2.0 g urea
GREENNESS (SPAD METER)
DATE
AUG 03
AUG 28
SEPT 10
SEPT 24
OCT 14

Fig. 35. The relationship of leaf greenness to cummulative spirea aphid-days per leaf with four different nitrogen rates during the 1987 growing season. A,B,C, and D represent urea application rates of 0.0, 0.5, 1.0, and 2.0g urea/tree respectively. Greenness expressed as a relative measure. Broken lines represent 95% confidence limits about the regression line. The quadratic regression equations and r^2 values in the same order are:

A---	$Y = 42.8 + 0.003x - 9.1E\text{-}6x^2$	$r^2 = 0.84$
B---	$Y = 42.1 + 0.013x - 1.0E\text{-}5x^2$	$r^2 = 0.84$
C---	$Y = 42.3 + 0.014x - 9.6E\text{-}6x^2$	$r^2 = 0.83$
D---	$Y = 45.7 + 0.014x - 7.9E\text{-}6x^2$	$r^2 = 0.71$

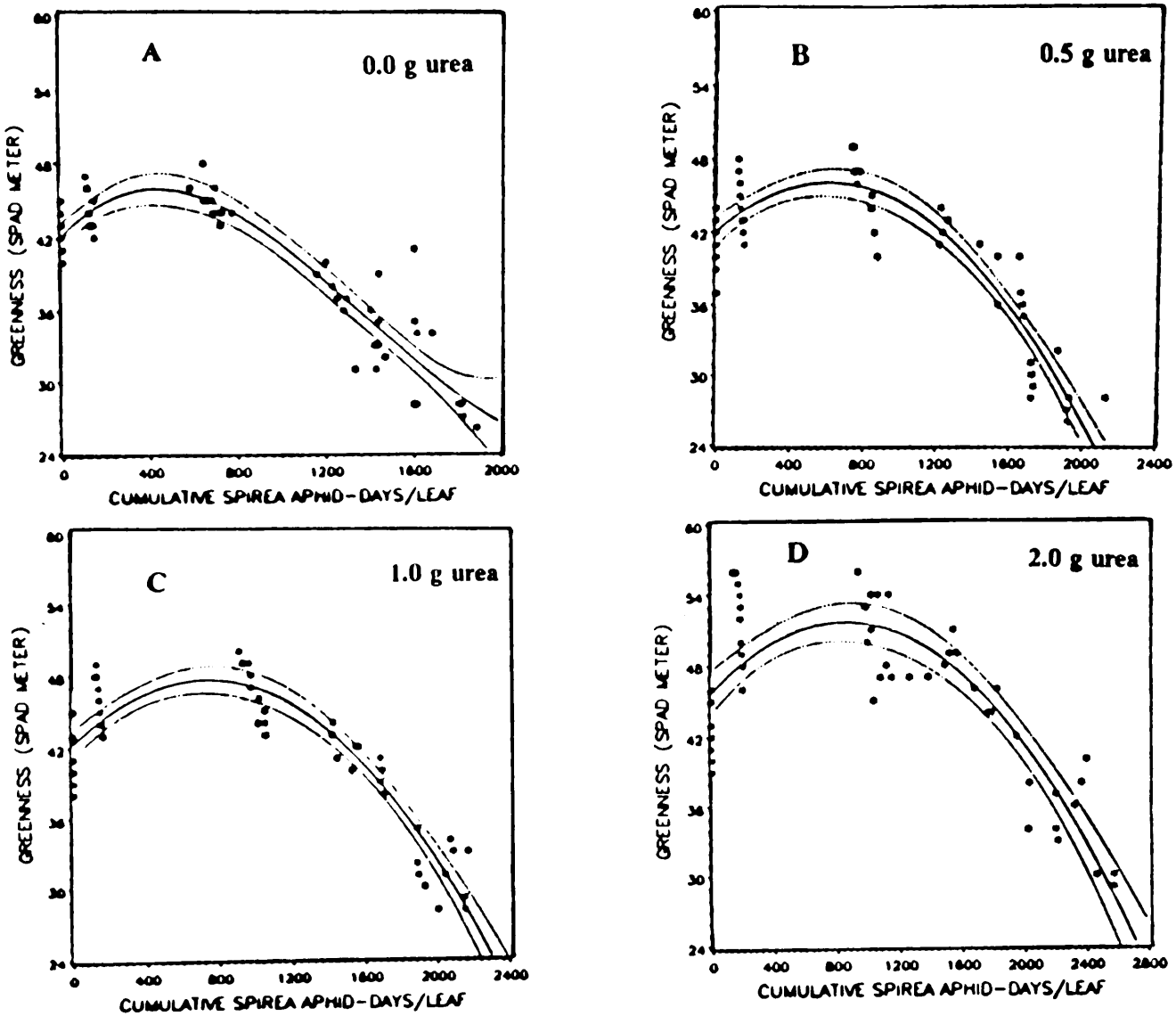
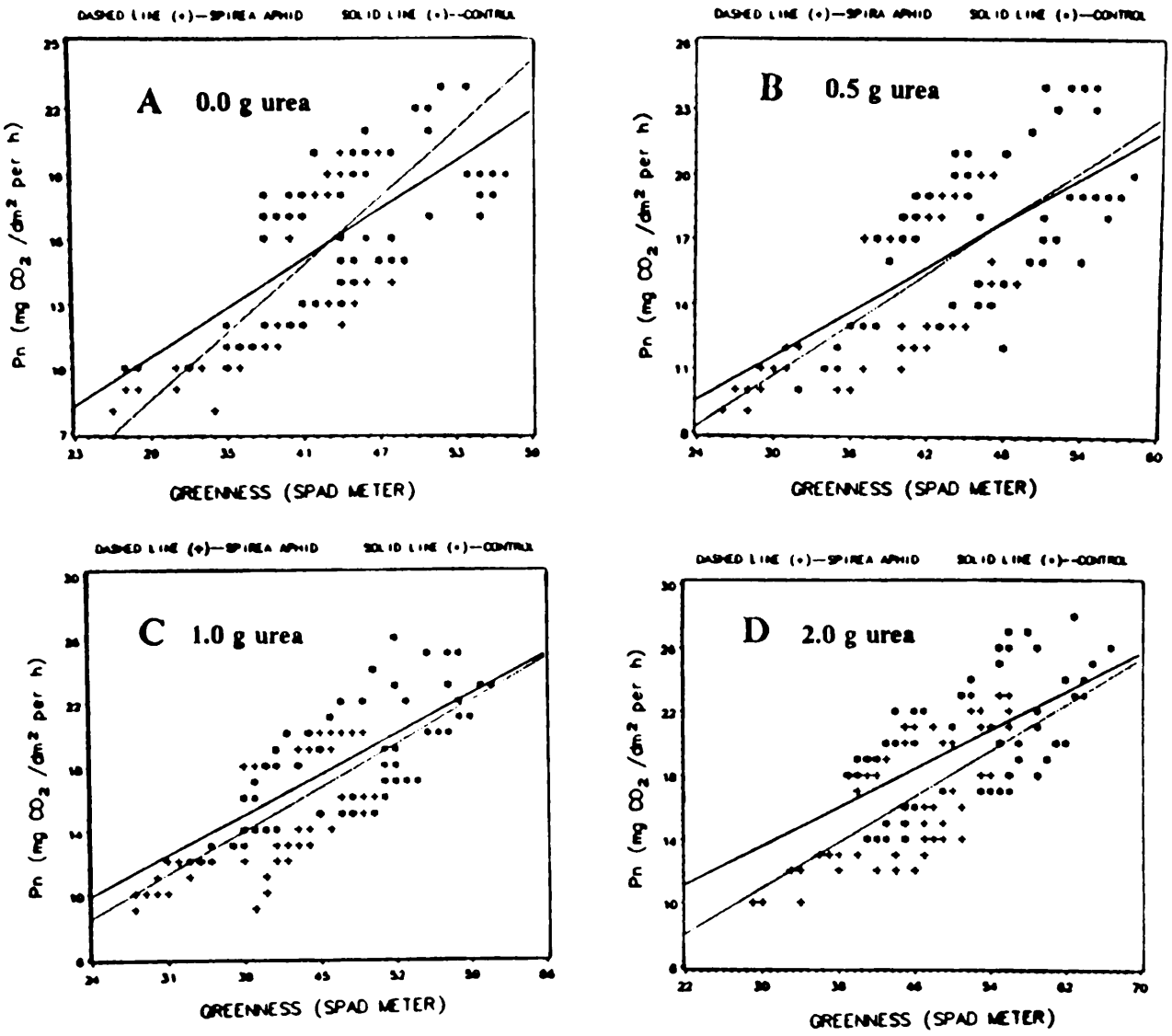

A
0.0 g urea
B
0.5 g urea
C
1.0 g urea
D
2.0 g urea
GREENNESS (SPAD METER)
CUMULATIVE SPIREA APHID-DAYS/LEAF

Fig. 36. Relationship between greenness and rate of photosynthesis (Pn) in apple leaves with four different urea application. A,B,C, and D represent relationships at 0.0, 0.5, 1.0, and 2.0g urea/tree respectively. Greenness expressed as a relative measure. Dashed line (+) and solid line (*) represent spirea aphid (SA) and control (CO) leaves respectively. Models (below) for each comparison are not significantly different from each other. The general linear regression equations (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 values in the same order are:

A/---	SA---	Y = -6.62 + 0.52x (2.26) (0.056)	r^2 = 0.59
	CO---	Y = -0.52 + 0.38x (2.19) (0.048)	r^2 = 0.51
B/---	SA---	Y = -1.20 + 0.40x (2.18) (0.054)	r^2 = 0.48
	CO---	Y = 1.27 + 0.34x (2.32) (0.050)	r^2 = 0.45
C/---	SA---	Y = -0.80 + 0.38x (2.47) (0.058)	r^2 = 0.42
	CO---	Y = 1.23 + 0.36x (2.15) (0.045)	r^2 = 0.52
D/---	SA---	Y = 0.16 + 0.36x (2.27) (0.049)	r^2 = 0.47
	CO---	Y = 4.51 + 0.30x (2.62) (0.049)	r^2 = 0.39


DASHED LINE (•)—SPIREA APHID SOLID LINE (•)—CONTROL
A 0.0 g urea
B 0.5 g urea
C 1.0 g urea
D 2.0 g urea
Pn (mg CO2 /dm2 per h)
GREENNESS (SPAD METER)

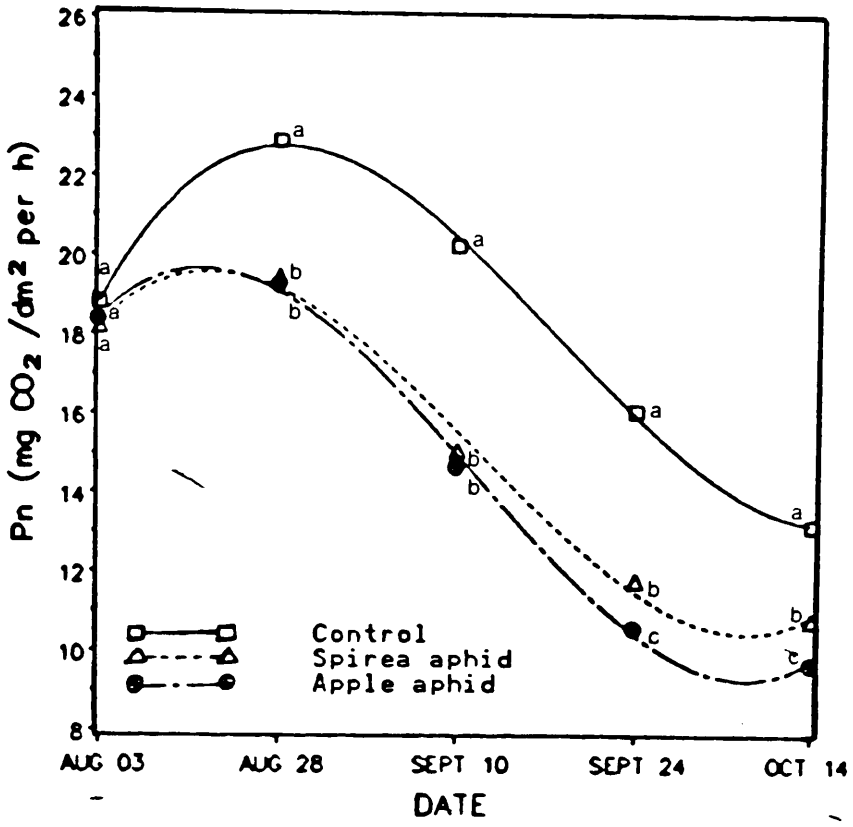
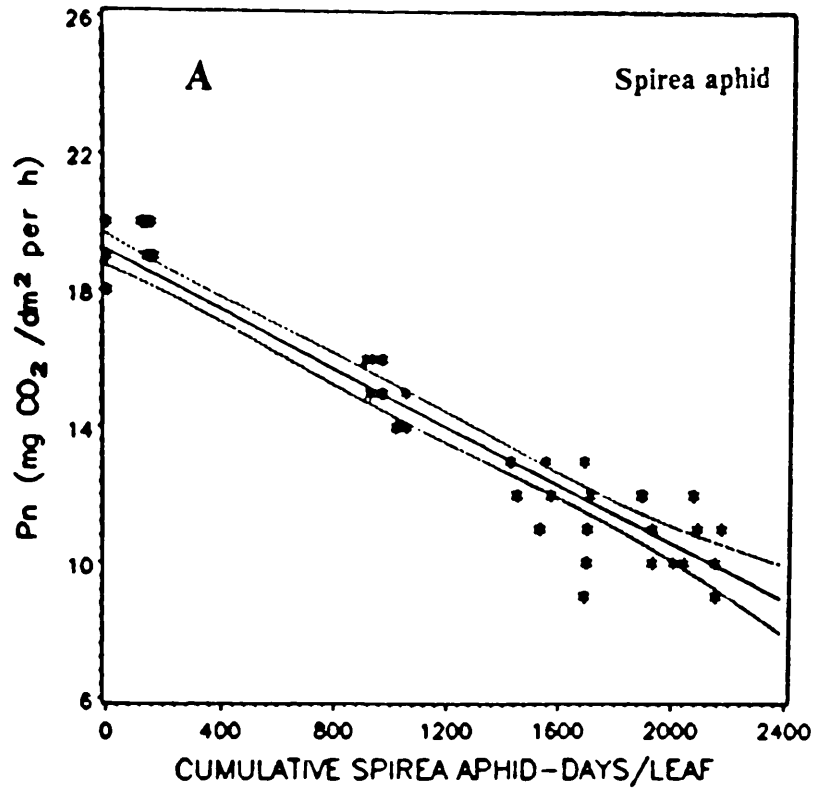


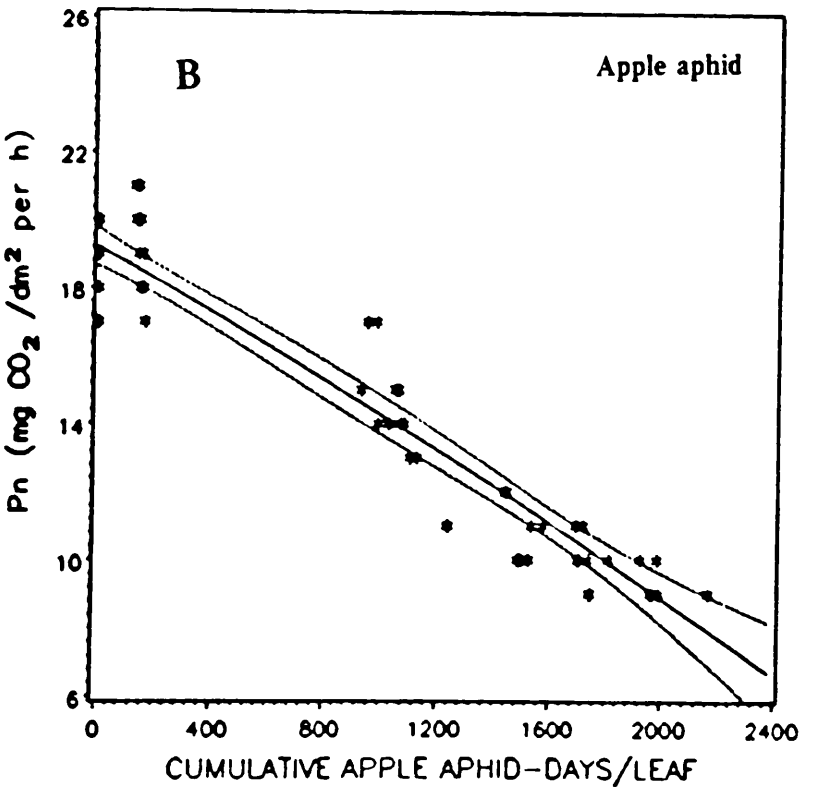
Fig. 37. Late seasonal changes in net photosynthesis (Pn) of apple leaves infested with spirea aphid vs apple aphid during 1987 growing season. Each point represents the average of 12 measurements taken from four trees. Letters represent differences within measurement dates at the 0.05 level with Tukey's studentized range test. The cubic regression equations ('x' represent days after aphid infestation) and r^2 values in the same order are:

Control	$Y = 16.9 + 0.671x - 0.020x^2 - 0.00014\ x^3$	$r^2 = 0.99$
Spirea aphid	$Y = 16.8 + 0.521x - 0.022x^2 - 0.00016\ x^3$	$r^2 = 0.99$
Apple aphid	$Y = 16.9 + 0.558x - 0.022x^2 - 0.00018\ x^3$	$r^2 = 0.99$

Fig. 38 . The relationship of net photosynthesis (Pn) to cumulative spirea aphid-days (A) and apple aphid-days (B) per leaf during the 1987 growing season. Broken lines represent 95% confidence limits about the regression line. The quadratic regression equations and r^2 values in the same order are:

A--- $Y = 19.2 - 0.0044x + 2.3E\text{-}8\ x^2$ $r^2 = 0.92$
B--- $Y = 19.2 - 0.0049x - 2.4E\text{-}7\ x^2$ $r^2 = 0.91$


A
Spirea aphid
Pn (mg CO_2 /dm^2 per h)
26
22
18
14
10
6
0
400
800
1200
1600
2000
2400
CUMULATIVE SPIREA APHID-DAYS/LEAF


B
Apple aphid
Pn (mg CO_2 /dm^2 per h)
26
22
18
14
10
6
0
400
800
1200
1600
2000
2400
CUMULATIVE APPLE APHID-DAYS/LEAF

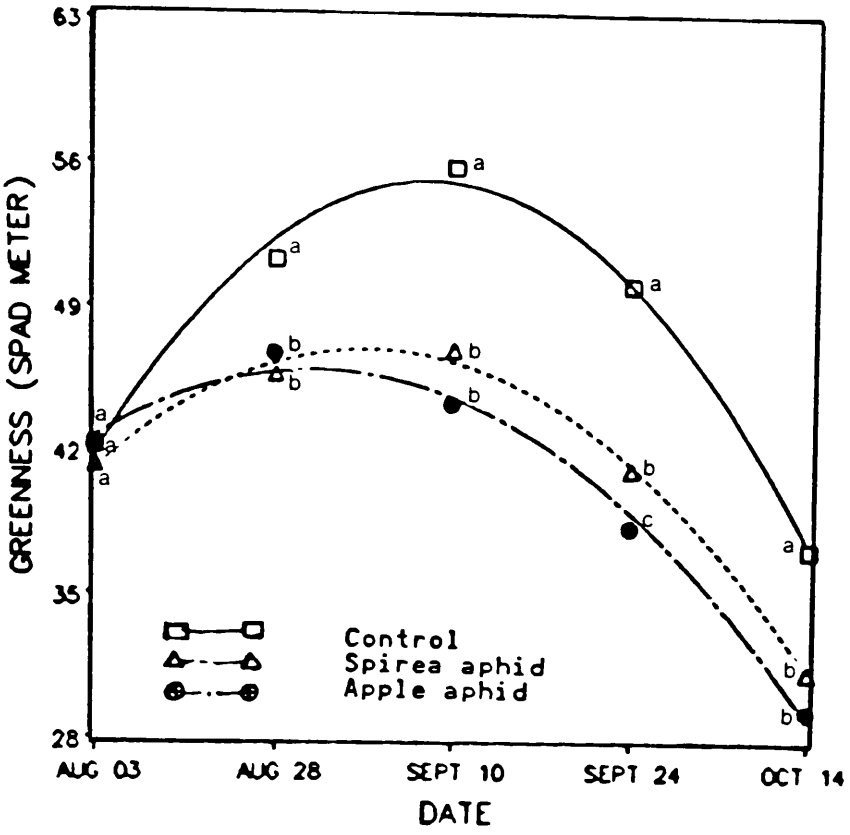
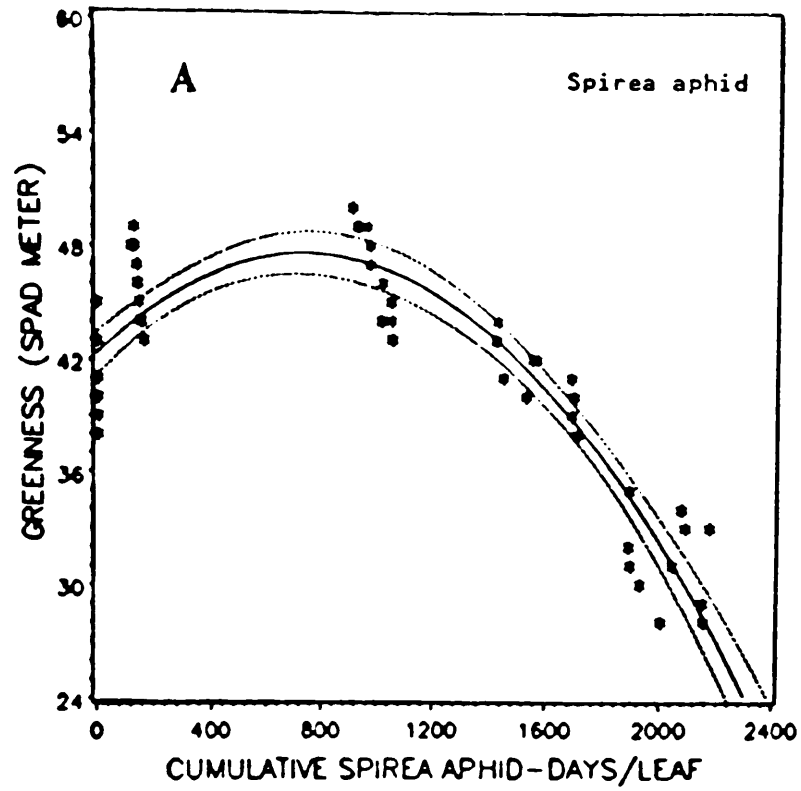


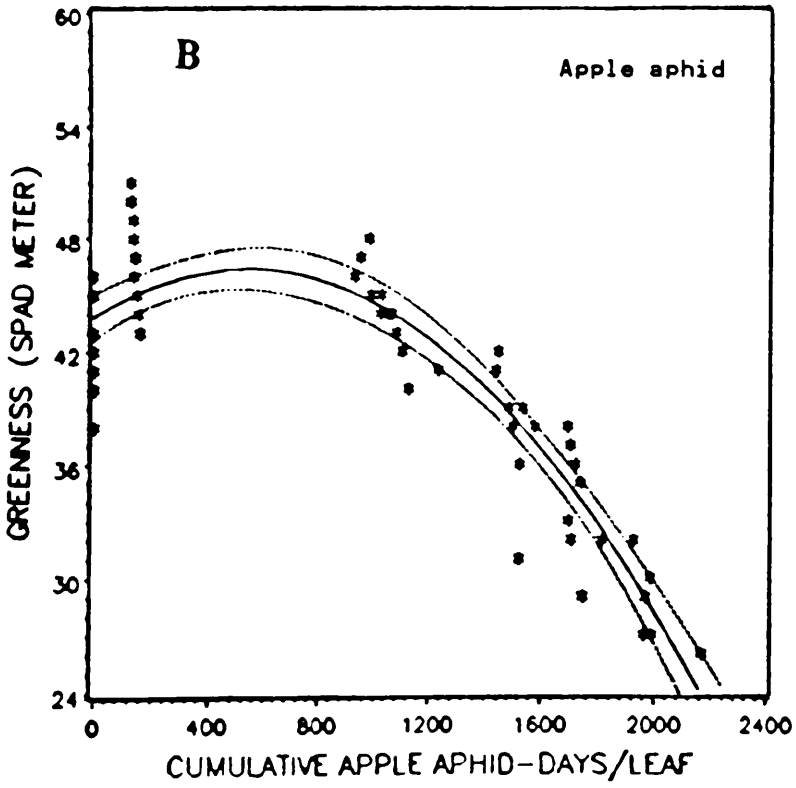
Fig. 39. Late seasonal changes in the greenness of apple leaves infested with spirea aphid vs apple aphid during 1987 growing season. Greenness was measured by SPAD meter (relative measure). Each point represents the average of 12 measurements taken from four trees. Letters represent differences within measurement dates at the 0.05 level with Tukey's studentized range test. The quadratic regression equations ('x' represents days after aphid infestation) and r^2 values in the same order are:

Control	$Y = 39.4 + 0.808x - 0.011x^2$	$r^2 = 0.96$
Spirea aphid	$Y = 39.9 + 0.448x - 0.008x^2$	$r^2 = 0.98$
Apple aphid	$Y = 41.6 + 0.340x - 0.007x^2$	$r^2 = 0.98$

Fig. 40. The relationship of leaf greenness to cumulative spirea aphid-days (A) and apple aphid-days (B) per leaf under a 1.0 g urea/tree during the 1987 growing season. Greenness expressed as relative measure. Broken lines represent 95% confidence limits about the regression line. The quadratic regression equations and r^2 values in the same order are:

A---	$Y = 42.3 + 0.014x - 9.6E\text{-}6x^2$	$r^2 = 0.83$
B---	$Y = 43.8 + 0.009x - 8.6E\text{-}6x^2$	$r^2 = 0.84$


A
Spirea aphid
GREENNESS (SPAD METER)
60
54
48
42
36
30
24
0
400
800
1200
1600
2000
2400
CUMULATIVE SPIREA APHID-DAYS/LEAF


B
Apple aphid
GREENNESS (SPAD METER)
60
54
48
42
36
30
24
0
400
800
1200
1600
2000
2400
CUMULATIVE APPLE APHID-DAYS/LEAF

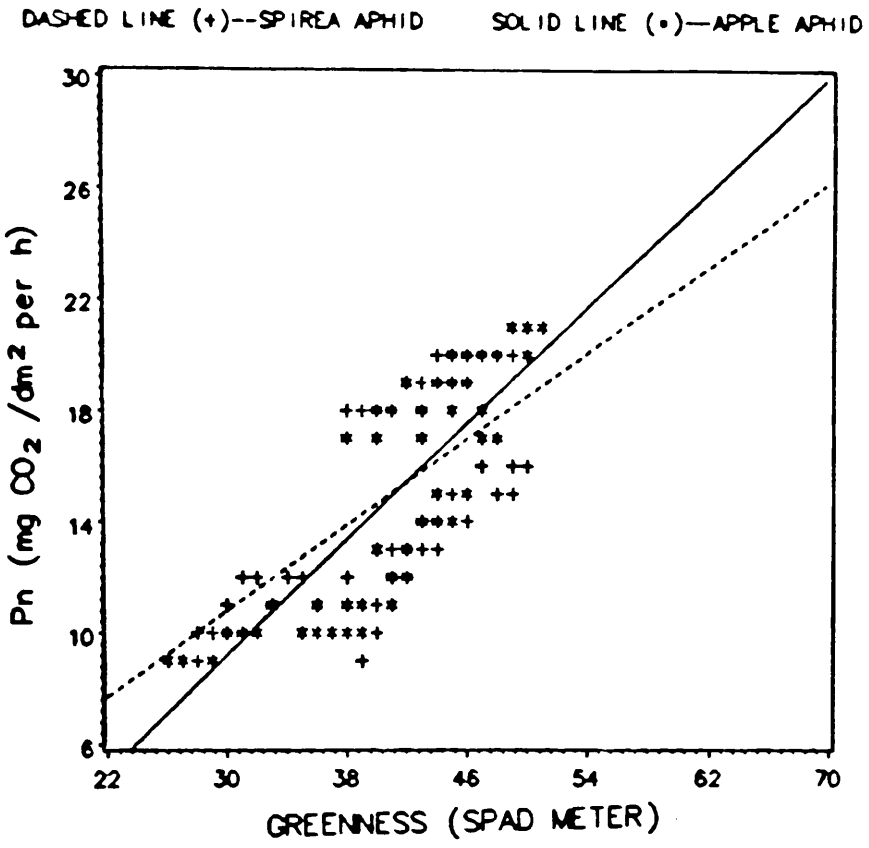


Fig. 41. Relationship between greenness and rate of photosynthesis (Pn) in apple leaves infested with spirea and apple aphids. Greenness expressed as a relative measure. Dashed line (+) and solid line (*) represent the relationship for spirea aphid (SA) and apple aphid (AA) infestation, respectively. The slopes of the regression equations are not significantly different from each other ($P < 0.05$). The general linear regression equations (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 values in the same order are:

SA--- $Y = -0.80 + 0.38x$ $r^2 = 0.42$
(2.47) (0.058)

AA-- $Y = -6.44 + 0.52x$ $r^2 = 0.70$
(1.83) (0.046)

Discussion

Effects of aphid infestation and SM mold accumulation on Pn rates gave similar results. Pn was reduced by the presence of both aphids and SM. Wood et al. (1988) recently indicated that the heavy levels of SM present on pecan foliage in the autumn reduced Pn of pecan leaves by 70%. Reduced Pn was due to the blockage of photosynthetically active radiation and an increase in abaxial leaf surface temperature. Washing SM from pecan leaves allowed them to recover indicating that SM blocks the light and does not directly damage leaves (Wood et al. 1988). In this study, Pn was reduced even when leaves were washed as compared with controls. A possible explanation for this reduction is that the period between measurements (30 minutes) was too short to allow the leaves to acclimate to their normal condition. SM accumulation prevents the normal function of leaves and its control can be accomplished by controlling aphid populations in young apple trees. The effect in this greenhouse study may have been more severe than in a field situation, where honeydew deposits are subject to rainfall and other factors.

Photosynthesis, leaf chlorophyll content, and leaf greenness were negatively related in a quadratic manner to cumulative aphid-days. Pn and greenness were positively correlated to nitrogen treatment. A similar relationship between Pn and chlorophyll was obtained on citrus leaves (Syvertsen 1987). The relationship between leaf chlorophyll content of leaves and Pn has been studied by many researchers with some conflicting opinions. Haberlandt (1914) showed a close relationship between photosynthetic activity and number of chloroplasts per unit of leaf area. Hesketh (1963) showed that Pn varied with species, but not chlorophyll content. He also stated that the critical differences among species must be in mesophyll diffusion and the kinetics of the dark reaction. Buttery & Buzzell (1977) reported a quadratic relationship between Pn and

chlorophyll content in soybean leaves. Marini & Marini (1983) indicated that Pn of peach leaves was not related linearly to chlorophyll concentration. In this study, the linear relationship between chlorophyll and Pn was based on a small range of measurements. Using a broad range of data, the relationship may not be linear. Regression statistics for relating Pn to chlorophyll, Pn to greenness, and chlorophyll to greenness indicated that there was a significant linear relationships in different sets of leaves (i.e. infested with aphids, infected with sooty mold, and control). Comparing between treatments for each relationship, the slopes and intercepts of the regression equations were significantly different; and this may indicate that there is no consistent relationship between methods under different conditions (i.e. the relationship is unique for each set of leaves). Chlorophyll content may be related to Pn, but plant age, nutritional status, and other parameters may influence the results. Substances injected by aphids (Dixon 1975) may also affect apple leaf structure and function.

Phloem functions in translocation of photosynthates (Zimmermann 1971). Callose accumulation at the sieve plates in response to aphid feeding may affect translocation of photosynthates from leaves. Aphid feeding disrupted phloem continuity (Pollard 1977, Wood et al. 1985, Varn et al. in press). Wood et al. (1985) stated that the blackmargined aphid, yellow pecan aphid, and black pecan aphid damage several leaf cell types and also induce phloem injury. The midrib phloem of apple leaves infested with rosy apple aphid had more sieve plates with accumulations of callose than did uninfested leaves (Varn et al. in press). In this study, callose accumulation in response to spirea aphid feeding occured but to a lesser degree than from rosy apple aphid feeding (Varn et al. in press). The reduced amount of callose may result from stylet penetration not reaching the phloem as extensively as might be expected with some other aphid species. Another possible reason might be that callose accumulation is not the only clogging component in phloem injured by aphids. Wood et al. (1985) reported that slime

plugging along with callose clogging occur when phloem is injured by pecan aphid. Slime (phloem protein) is a degradation product of protoplasts produced upon loss of turgor in sieve tubes for any reason (Zimmerman 1971).

Photosynthesis reduction in this study may be caused by disruption of the phloem as well as a collapse of cells in various tissues (intervienal tissues). The stylets of most aphid species are believed to terminate in the sieve tubes with few exceptions (Davidson 1923, McLean & Kinsey 1967). Other phloem cells (parenchyma, companion cells) might be used as food sources. Aphis fabae Scopoli has been reported to feed in the epidermis, cortex, pith, mesophyll and xylem as well as phloem of the broad bean, Vicia faba (Davidson 1923). Several studies (Monzette 1934, Mittler 1953, Miles 1964, 65) reported that aphid injury may be caused by an excretion of toxic saliva into the leaf. A variety of substances such as pectinases, cellulases, auxin, and tryptophan are reported to cause phloem injury after injection (Pollard 1977). More histological research using apple leaves is needed to determine the reactions of leaves to spirea aphid feeding and also to determine the major compounds responsible for the phloem injury.

Leaves are important interceptors of light for manufacturing of photosynthates and are necessary for maximum growth. The results of the present study indicate that spirea aphid is capable of restricting leaf function. Preventing aphid feeding must be achieved to increase Pn rates and chlorophyll content of apple leaves and this in turn will increase leaf efficiency and the accumulation of photosynthates in various tissues.

The ADC CO_2 analyzer and the SPAD meter can be used for direct and rapid measurements on many leaves in the laboratory as well as in the field. They can be used as an alternative for the cumbersome and expensive systems to determine Pn wherever trees are grown.

THE EFFECT OF SPIREA APHID AND NITROGEN FERTILIZATION ON GROWTH, DRY MATTER ACCUMULATION, AND CARBOHYDRATE CONCENTRATION IN YOUNG APPLE TREES, WITH COMPARISONS TO APPLE APHID

Introduction

Carbohydrates are the primary source of reserve energy stored in the vegetative organs of the plant (Smith 1969). Dry matter production of plants depends on the amount of photosynthetic surface that they display and the rate of CO_2 fixation (photosynthesis per unit of leaf area) (Ramirez et al. 1988), and the amount of light and water available to the leaf.

Apple grain aphid, Rhopalosiphum fitchii (Sanderson), apple aphid, Aphis pomi DeGeer, and rosy apple aphid, Dysaphis plantaginea (Passerini) are the most common aphid species using apple as primary host (Brunner & Howitt 1981). However, the spirea aphid, Aphis spiraecola Patch, has also been observed on apple (Parella et al. 1981, Blackman & Eastop 1985). The spirea aphid greatly outnumbers apple aphid in most of the apple orchards sampled in Virginia, West Virginia, and Maryland (Pfeiffer et al. 1989); the spirea aphid can also use apple as primary host.

Aphids feed on leaf phloem sap and it has been assumed that when they are present in large numbers they remove a great deal of photosynthate and nutrients (Dixon 1971a). Research on the effects of apple aphid on wood formation by mature apple trees has usually been restricted to the measurement of terminal shoot length (Oatman & Legner 1961, Hamilton et al. 1986). Drepanosiphum platanoides (Schrank) reduced leaf size in sycamore (Acer pseudoplatanus (L.)) by 40 % and the production of stem wood by 62 % (Dixon 1971a). European linden saplings (Tilia x vulgaris Hayne) infested with Eucallipterus tiliae L. increased less in weight because of poor root growth (Dixon 1971b).

Pecan, Carya illinoensis Wangenheim C. Koch, is infested by three species of aphids (Monelliopsis nigropunctata (Granovsky), Monellia caryella (Fitch), Melanocallis caryaefoliae (Davis)) which significantly reduced dry matter accumulation in stems and roots of potted pecan seedlings grown in a greenhouse (Tedders et al. 1982). Dry weight of roots (approximately 7-10%), but not aerial portions, of apple trees was reduced by Panonychus ulmi (Koch) feeding for three seasons (Hull et al. 1986).

Varn & Pfeiffer (1989) indicated that rosy apple aphid significantly reduced dry weight accumulation of all portions of apple trees during the first season's growth. At the ten-leaf stage of the second season, the dry weight of trees infested with the rosy apple aphid during the previous year were still significantly lower than those of control trees. Spirea aphid, at an initial population density of 65 aphids per leaf at the peak stage, did not reduce dry weight accumulation by the young trees.

The nutritional state of the host plant affects growth and carbohydrates (Smith 1966, Radin & Parker 1979, DeJong & Phillips 1981, Ramamurthy & Ludders 1982). Supplementary nitrogen caused large changes in total amount and distribution of dry matter increment (Priestley 1972). The objective of this study is to determine the effect of spirea aphid and nitrogen fertilization on growth, dry matter accumulation, and carbohydrate concentration in young apple trees.

Materials and Methods

A- 1986-1987 Growing Season Experiments:

Twenty one-year-old 'Redchief Delicious' apple trees were used. The trees were weighed, pruned, and planted at a uniform depth in 5-liter pots. Trees were fertilized, grown, and infested with aphids (chapter 1). Trees were randomly assigned to two

groups (spirea aphid-infested and control). Ten trees from each group were selected for uniformity of size and arranged as single-tree-replicates in a randomized complete block design. At the end of the first growing season, after leaf fall (December 4), the trees were harvested. To study the effect of aphids on the tree growth the season following infestation, another two groups of trees (infested n = 10 and control n = 7) were overwintered in a cold room and the trees were hereafter called second-season trees. Trees were removed from cold storage (May 5, 1987) and placed in the greenhouse for normal budbreak and resumption of growth after dormancy. Trees were harvested in the second season at the ten-leaf stage, when three of the four shoots produce at least ten unfolded leaves. Trees were harvested in the two seasons were used to determine dry matter accumulation and nonstructural carbohydrates (NSC = sugars, starches, and fructosans).

Fresh weights of leaves, central shoot, lateral shoots, rootstock (above and below the ground level), and roots were immediately taken at each harvest. All tissues were dried in a forced air oven at 70 ^{o}C for 3-4 days, for dry weight measurements. Tissues were ground and percent NSC in each tissue were determined using the 0.5% takadiastase solution method for extraction as described by Smith (1969) (Appendix B). In the takadiastase method, the enzyme preparation hydrolyzes disaccharides and starch to monomers and, since it contains invertase, maltase, and amylase enzymes, fructosans extracted in the solution are hydrolyzed to monomers with weak acid. Total NSC (g) were calculated by multiplying the percentages by the dry weights of the appropriate tissues.

Data to determine the effect of changes in aphid density on growth of apple shoots were collected. Total length of the four shoots/tree was recorded several times during the growing season (August 3 and 28, September 18, October 11, and November 2). The

first measurement was three days after aphid inoculation. Shoot growth rate was calculated as the change in total length of all shoots/tree between two consecutive dates divided by the number of days elapsed. Root/shoot ratio was calculated as the ratio of root dry weight to the shoot dry weight. Shoot length for trees harvested at the ten-leaf stage was also measured during 1986 growing season as well as at time of harvest.

B- 1987-88 Growing Season Experiments:

Sixty-four one-year-old 'Redchief Delicious' apple trees were planted and grown in the greenhouse as in the previous year and were assigned to two groups (n = 32 each). The first group was infested with spirea aphids and the second group was kept free of aphids. Three weeks after planting, four treatments consisting of four nitrogen rates (0.0, 0.5, 1.0, and 2.0 g urea per tree) were applied to infested and control trees every three weeks. The experimental design was a randomized complete block design with a 2x4 factorial arrangement of treatments (eight treatments) with eight-single-tree replications each. Trees were ranked by weight and were sequentially assigned to treatments, resulting in uniform distribution of weight. Trees were infested with spirea aphids as in the previous year. Aphids were counted twice a week, from the top six unfolded leaves of four shoots/tree. Density and duration of infestation were recorded adapting the procedure of Sances et al. (1981). Half of the trees from each treatment (n = 4) were harvested at the end of the first growing season and the remaining trees (n = 4) were harvested at the ten-leaf stage in the second season. Trees were divided into five parts (leaves, lateral shoots, central shoot, rootstock, and roots). The relationships between nitrogen rate and the growth, dry matter accumulation, and carbohydrate concentration in all tree partitions were investigated. Also, the relationship between dry matter accumulation and net photosynthesis (Pn) was determined.

The effect of spirea aphid vs apple aphid populations on the growth of young apple trees:

Twenty-four one-year-old apple trees were planted and grown in spring, 1987. Trees were assigned to three groups (spirea aphid-infested n = 8, apple aphid-infested n = 8, and non-infested control n = 8). One g urea/tree was applied at three-week intervals. Half of the trees from each group were harvested at the end of the season and the remaining trees were kept in cold storage to resume growth in the spring and were harvested at the ten-leaf stage. Total nitrogen content was evaluated as before.

Statistical Analysis:

Data in 1986 were analyzed using analysis of variance (ANOVA). In 1987, effects of nitrogen and aphid infestation were discussed as main effects, since interactions were not significant in most of the results. Tukey's studentized range test ($P < 0.05$) was used to separate treatment means within measurement dates. Regression analyses using GLM and REG procedures of the Statistical Analysis System (SAS Institute 1985) were then performed to test for significant correlation between variables. The highest significant regression equation (linear, quadratic, or cubic) was used to describe the relationship between variables.

Results

A- 1986-1987 Growing Season:

1- First Season Trees : Trees harvested at the end of the growing season had similar weights at planting (Table 12). Population trends of spirea aphid on trees treated with

Table 12. The effect of feeding by spirea aphid on the total accumulation of fresh and dry matter by young apple trees as compared with the fresh weight of the trees at planting (trees harvested at the end of first growing season in 1986).

Treatment[1]	Fresh Weight at Planting	Fresh Weight at Harvest[2]	Dry Weight at Harvest[2]	Net Gain (fresh)[2]
Control	261.0 (4.27) a	478.0 (12.7) a	258.6 (7.28) a	217.0 (12.7) a
Spirea Aphid	248.0 (5.22) a	397.3 (11.3) b	215.5 (6.76) b	149.3 (13.6) b

[1] Means (SEM in parentheses) in the same column followed by the same letter are not significantly different ($P < 0.05$) using ANOVA. $n = 10$ for each treatment.
[2] Total weight without leaves.

different nitrogen rates are illustrated in chapter 1. Total fresh and dry weights of infested trees at harvest were reduced significantly ($P < 0.05$) as compared with the controls (Table 12). Figure 42 shows different tree portions of control and infested trees analyzed for carbohydrates. Fresh and dry weight reductions were evident in all tree partitions except trunk (Table 13). Percent dry weight reductions were: lateral shoots 32, rootstock 16, and roots 17%. Percent fresh weight reductions were: lateral shoots 33, rootstock 15, and roots 19%. Rootstock accounted for about 45% of the total dry weight in both infested and control trees

Total fresh and dry weights of top and bottom parts of the trees were also reduced (Table 14). Shoot/root ratios of infested trees tended to be similar to that of controls. A comparison of the root growth at the end of the season in both groups is illustrated in Figure 43, showing a greater root volume in the control relative to the aphid-infested treatment.

Shoot lengths were measured during the 1986 growing season to assess the effect of spirea aphids on shoot growth. Shoot length increased more for control trees than for infested trees (Table 15). In infested trees, a 28.2% increase in shoot length was obtained compared with 52.9% in the control. Shoot length changes during the infestation period is indicated in Figure 44. Shoot length was highly correlated with aphid-days accumulation ($r^2 = 0.77$) (Fig. 45). Shoot elongation started to slow when shoots were exposed to ca. 6000 aphid-days, but this was largely because of normal slowing of growth over season (Fig. 44). Shoot length and shoot dry weight were related in a positive linear manner for both infested and control trees (Fig. 46). Since infested shoots had a reduced slope, the shoots must have been thicker.

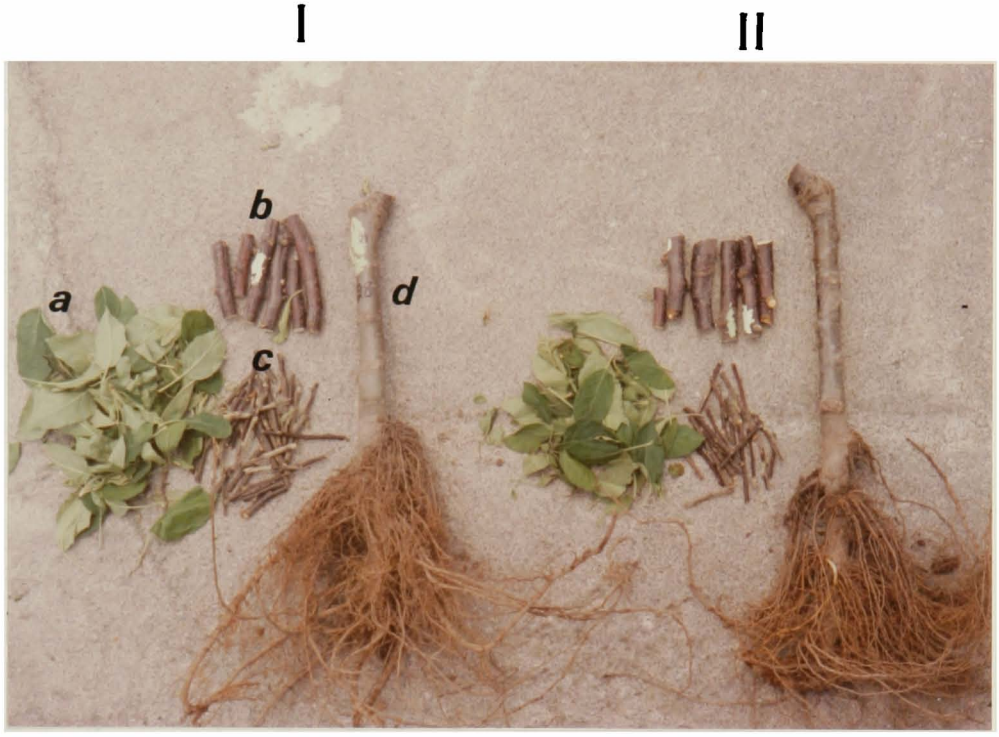


Fig. 42. Apple tree parts of representative trees from the two treatments after harvest at the end of first growing season of 1987. (I) control and (II) spirea aphid-infested: (a) leaves, (b) central shoot, (c) lateral shoots, and (d) rootstock (above and below the ground) and roots.

Table 13. The effect of feeding by spirea aphid on the accumulation of fresh and dry matter by young apple tree parts (trees harvested at the end of the end of first growing season in 1986).

	Dry Weight (g)			
Treatment *	Shoots	Trunk	Rootstock	Roots
Control	25.7 (2.13) a	52.1 (1.48) a	115.8 (5.24) a	65.0 (3.61) a
Spirea Aphid	17.4 (0.87) b	47.3 (2.35) a	97.1 (4.31) b	53.7 (1.80) b
	Fresh Weight (g)			
Control	48.7 (3.84) a	86.7 (2.80) a	196.8 (8.88) a	145.8 (9.28) a
Spirea Aphid	32.5 (1.28) b	79.7 (3.77) a	166.9 (6.35) b	118.2 (3.41) b

* Means (SEM in parentheses) in the same column followed by the same letter are not significantly different ($P < 0.05$) using ANOVA. $n = 10$ for each treatment.

Table 14. The effect of feeding by spirea aphid on fresh /dry root, shoot/root, and top and bottom parts of young apple trees harvested at the end of first growing season in 1986. Top = shoot + trunk, Bottom = rootstock + roots.

Variable *	Control	Spirea Aphid
Root (fresh/dry)	2.25 (0.12) a	2.21 (0.06) a
Shoot/Root (fresh)	0.35 (0.05) a	0.27 (0.01) a
Shoot/Root (dry)	0.42 (0.05) a	0.35 (0.02) a
Top (fresh)	135.4 (3.79) a	112.2 (4.17) b
Top (dry)	77.8 (2.16) a	64.7 (2.81) b
Bottom (fresh)	342.6 (11.9) a	285.1 (8.21) b
Bottom (dry)	180.8 (7.40) a	150.8 (4.64) b

* Means (SEM in parentheses) in the same row followed by the same letter are not significantly different ($P < 0.05$) using ANOVA.

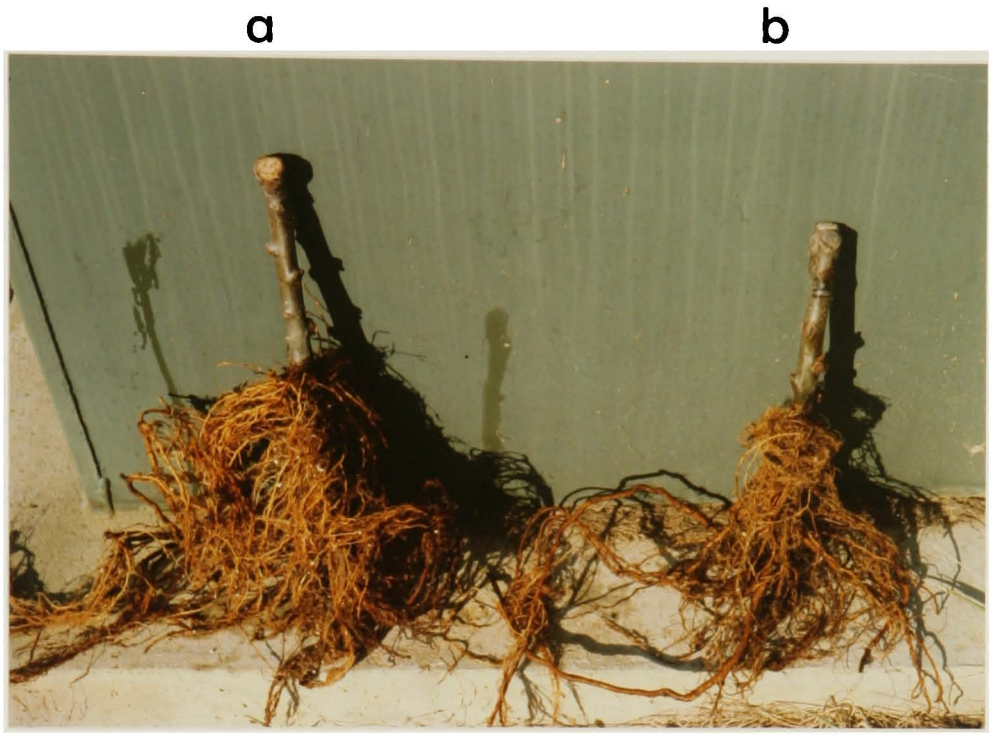


Fig. 43. Roots and rootstocks of representative trees from the two treatments after harvest at the end of the first growing season, 1986. (a) control and (b) spirea aphid-infested.

Table 15. The effect of feeding by spirea aphid on shoot growth of young apple trees harvested at the end of the 1st growing season in 1986.

Variable[1]	Control	Spirea Aphid
Shoot length incease (cm)	37.7 (4.44) a	17.6 (0.82) b
Shoot growth increase (%)[2]	52.9 (4.33) a	28.2 (1.56) b
Shoot growth rate (cm/day[3])	0.43 (0.05) a	0.20 (0.01) b

[1] Means (SEM in parentheses) in the same row followed by the same letter are not significantly different ($P < 0.05$) using ANOVA.

[2] The difference (%) of shoot length at aphid inoculation to the shoot length at the end of the growing season divided by the shoot length at aphid inoculation.

[3] Changes in total length of all shoots/tree on two consecutive dates divided by the number of days elapsed.

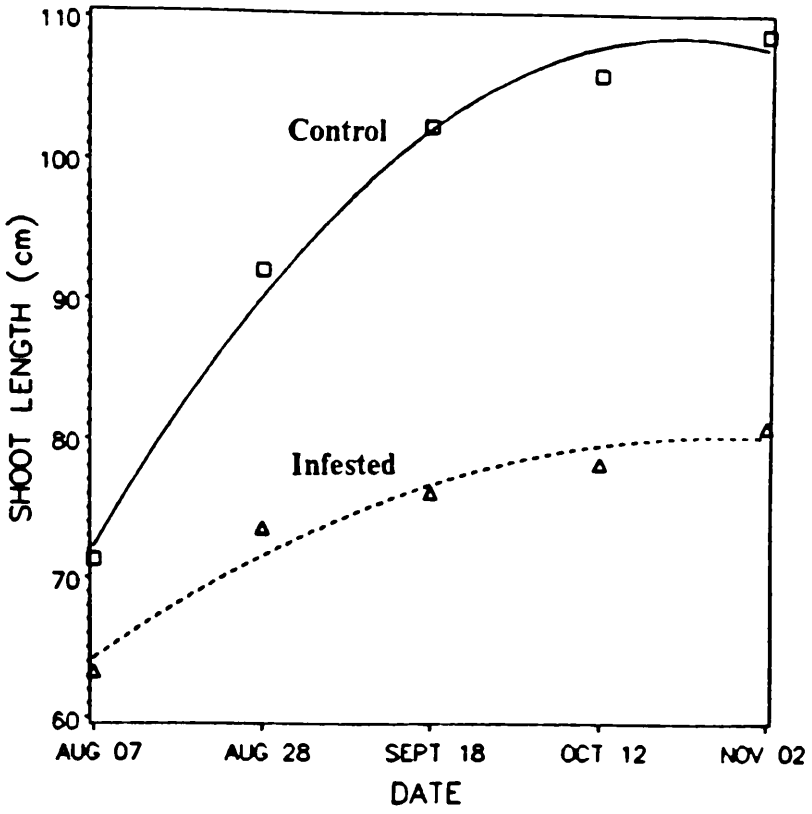


Fig. 44. Changes in shoot length during 1986 growing season for trees harvested at the end of the season. A = control and B = spirea aphid-infested. The quadratic regression equations ('x' represents days after aphid infestation) and r^2 values in the same order are:

A--- $Y = 65.1 + 1.085x - 0.007x^2$ $r^2 = 0.99$
B--- $Y = 61.3 + 0.437x - 0.003x^2$ $r^2 = 0.96$

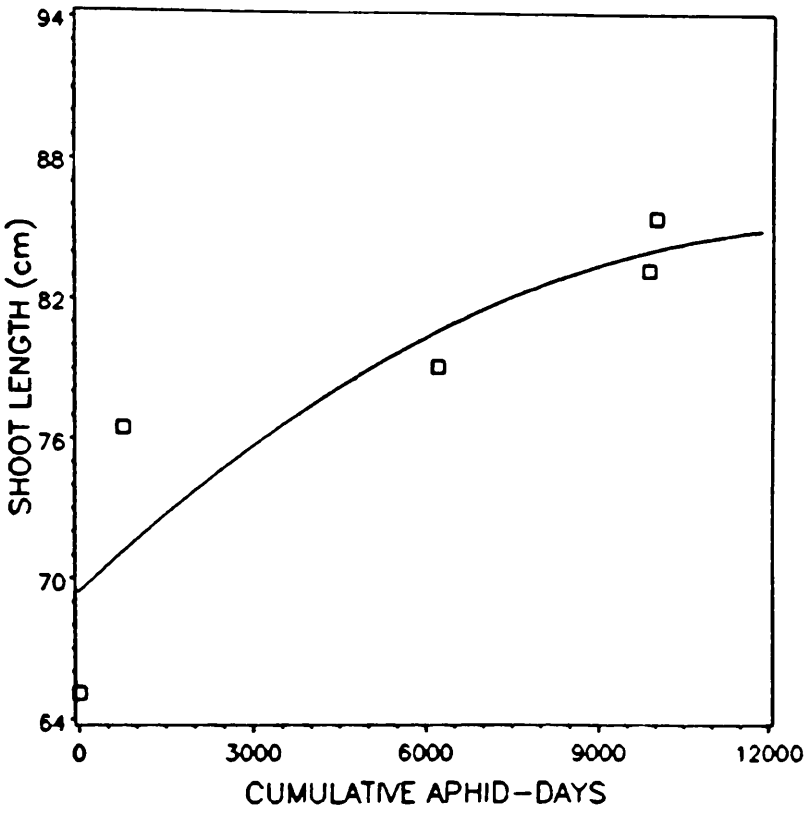
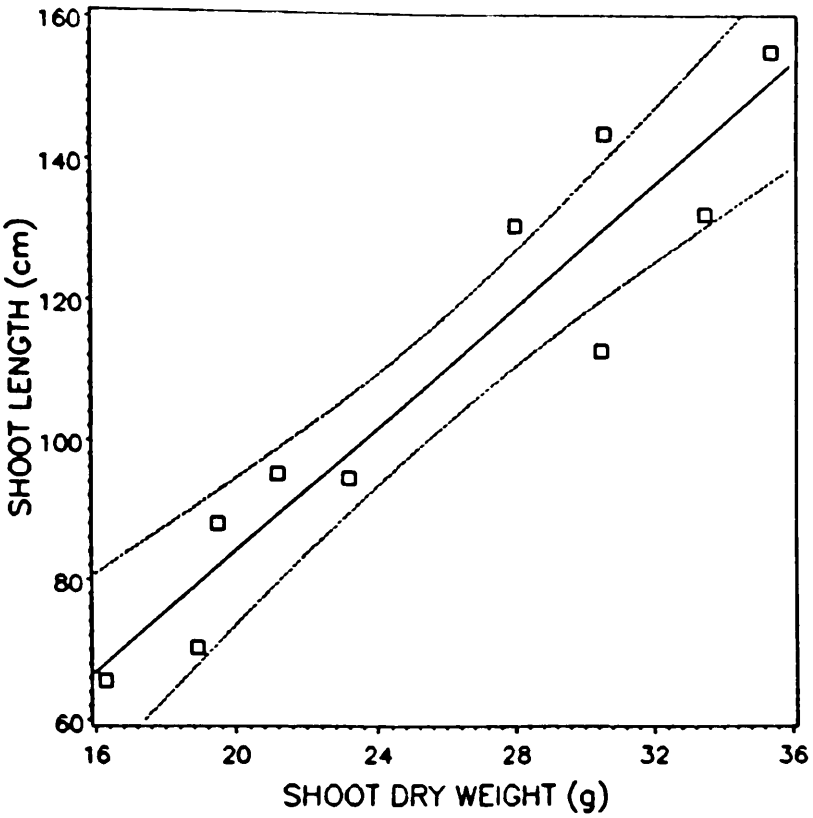


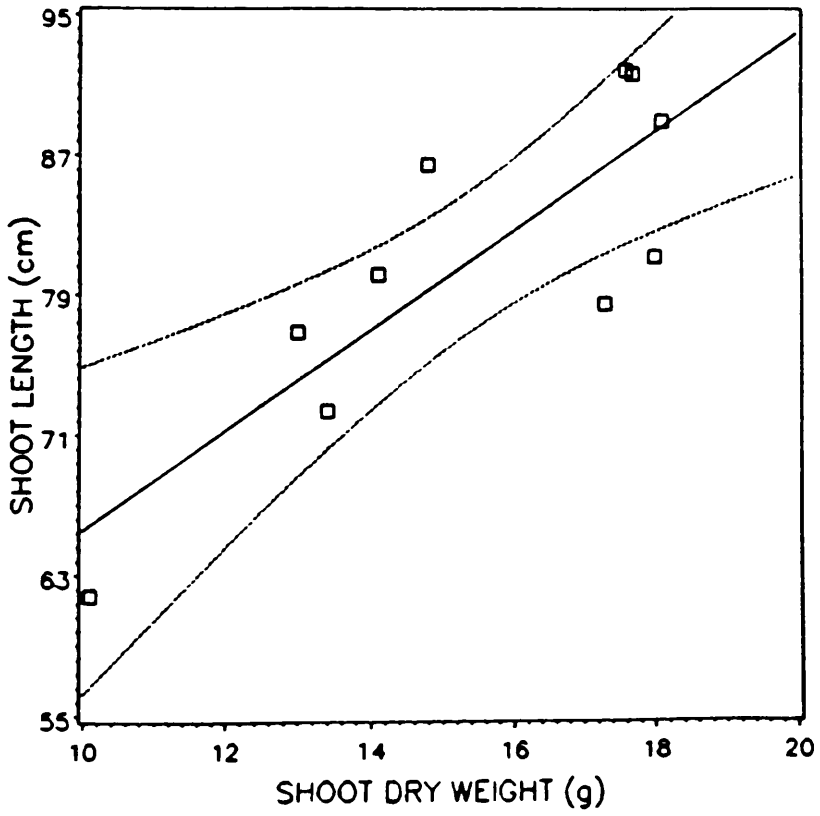
Fig. 45. Relation of shoot length to cumulative aphid-days per shoot during 1986 growing season, for trees harvested at the end of growing season. The quadratic regression equation and r^2 value are:

$$Y = 67.2 + 0.0024x - 1.2E\text{-}7\ x^2 \qquad r^2 = 0.77$$

Fig. 46. Relationship between shoot length and shoot dry weight during 1986 growing season. A = control trees and B = spirea aphis-infested. The slopes of the regression equations are significantly different from each other ($P < 0.05$). The general linear regression equations (values in parentheses are the SE of the intercept and the slope, respectively) and r^2 values in the same order are:

A---	$Y = -3.24 + 4.36x$	$r^2 = 0.89$
	(14.0) (0.529)	
B---	$Y = 37.22 + 2.84x$	$r^2 = 0.69$
	(10.5) (0.673)	


SHOOT LENGTH (cm)
160
140
120
100
80
60
16
20
24
28
32
36
SHOOT DRY WEIGHT (g)


SHOOT LENGTH (cm)
95
87
79
71
63
55
10
12
14
16
18
20
SHOOT DRY WEIGHT (g)

Spirea aphid reduced the percentage of NSC in lateral shoots and roots by 22 and 7% respectively as compared with the control, but there was no significant effect in central shoot (trunk) and rootstock (Table 16). The highest concentrations were found in the roots in both groups.

Total amount of NSC accumulated was also significantly different in all tree partitions due to the reduction in dry weights in these parts except that of trunk (Table 16). Significant percent reductions were: lateral shoot 47, rootstock 22, and roots 23%.

2- Second Season Trees : Trees allocated to the two treatments and harvested at the ten-leaf stage had similar weights at planting (Table 17). Population trends of aphids on trees harvested at the ten-leaf stage are illustrated in chapter 1. Weights of trees fed upon by spirea aphid were still lower than the control trees when trees were harvested at the ten-leaf stage in 1987; previous infestation of spirea aphids caused a reduction in both fresh and dry weight of all tree partitions except trunk (Table 18). When growth begins in the spring, carbohydrate reserves in the storage organs are being depleted by accelerated respiration and used in growth of new shoots and leaves. Percent dry weight reductions were: lateral shoots 39, rootstock 14, roots 30, and leaves 31%. Percent fresh weight reduction were: lateral shoots 26, roots 29, and leaves 37%. Total fresh and dry weights of top and bottom parts of the tree were also reduced.

Effects of spirea aphid on leaf number, fresh/dry leaf, fresh/dry root, shoot/root and top and bottom parts in young apple trees in the spring following infestation are presented in Table 19. The total number of leaves was reduced to 27% due to the spirea aphid feeding in the previous season. Ratio of fresh and dry weights of leaves and roots, and the shoot/root ratio were not affected by treatments.

Table 16. The effect of feeding by spirea aphid on nonstructural carbohydrate content of young apple trees harvested at the end of first growing season in 1986.

	Percent nonstructural carbohydrate (dry wt. basis)			
Treatment *	Shoots	Trunk	Rootstock	Roots
Control	4.87 (0.100) a	3.95 (0.122) a	4.90 (0.167) a	9.02 (0.195) a
Spirea Aphid	3.80 (0.122) b	3.75 (0.134) a	4.62 (0.077) a	8.42 (0.167) b
	Total amount nonstructural carbohydrate (g)			
Control	1.26 (0.122) a	2.05 (0.064) a	5.68 (0.361) a	5.84 (0.300) a
Spirea Aphid	0.67 (0.052) b	1.79 (0.139) a	4.47 (0.164) b	4.53 (0.182) b

* Means (SEM in parentheses) in the same column followed by the same letter are not significantly different ($P < 0.05$) using ANOVA. $n = 10$ for each treatment.

Table 17. The effect of feeding by spirea aphid on the total accumulation of fresh and dry matter by young apple trees as compared with the fresh weight of the trees at planting (trees harvested in the second growing season at the ten-leaf stage in 1987).

	Fresh Wt. at Planting	Fresh Wt. at Harvest		Dry Wt. at Harvest		Net Gain (fresh wt.)	
Treatment*	no leaves	leaves	no leaves	leaves	no leaves	leaves	no leaves
Control	259.0 a (6.4)	463.1 a (13.1)	429.6 a (13.4)	247.4 a (8.2)	231.9 a (7.7)	204.1 a (9.1)	170.6 a (9.4)
Spirea Aphid	244.0 a (4.5)	384.8 b (12.2)	363.6 b (11.8)	200.1 b (3.9)	189.3 b (4.0)	140.8 b (9.5)	119.6 b (9.3)

* Means (SEM in parentheses) in the same column followed by the same letter are not significantly different ($P < 0.05$) using ANOVA. $n = 7$ (control), $n = 10$ (infested).

Table 18. The effect of feeding by spirea aphid on the accumulation of fresh and dry matter by young apple trees harvested in the second growing season at the ten-leaf stage in 1987. Top = shoot, trunk, and leaves. Bottom = rootstock and roots.

	Dry Weight (g)						
Treatment *	Leaves	Shoots	Trunk	Total Top	Rootstock	Roots	Total Bottom
Control	15.5 a (0.67)	19.7 a (1.06)	45.1 a (2.40)	80.3 a (3.11)	115.1 a (5.25)	52.0 a (1.61)	167.1 a (6.05)
Spirea Aphid	10.7 b (0.73)	12.1 b (0.25)	41.5 a (0.68)	64.3 b (0.58)	99.3 b (3.22)	36.5 b (1.06)	135.8 b (3.74)
			Fresh Weight (g)				
Control	33.6 a (1.06)	35.1 a (1.56)	78.0 a (3.02)	146.7 a (3.34)	179.4 a (5.75)	137.0 a (8.63)	316.4 a (11.8)
Spirea Aphid	21.2 b (1.22)	25.9 b (0.64)	70.1 a (2.38)	117.2 b (2.77)	169.0 a (5.73)	98.6 b (5.99)	267.6 b (10.3)

* Means (SEM in parentheses) in the same column followed by the same letter are not significantly different ($P < 0.05$) using ANOVA. $n = 7$ (control), $n = 10$ (infested).

Table 19. The effect of spirea aphid on leaf number, fresh /dry weight ratio of leaves, fresh/dry weight ratio of roots, shoot/root ratio (fresh and dry wts) of young apple trees harvested in the second growing season at the ten-leaf stage in 1987.

Treatment *	Control	Spirea Aphid
No.leaves(expanded)	45.0 (2.42) a	33.0 (1.79) b
Total No. leaves	63.0 (2.41) a	49.0 (1.81) b
Fresh/Dry leaves	2.18 (0.11) a	1.99 (0.04) a
Fresh/Dry roots	2.70 (0.16) a	2.64 (0.14) a
Shoot/Root (fresh)	0.26 (0.01) a	0.27 (0.02) a
Shoot/Root (dry)	0.38 (0.03) a	0.33 (0.01) a

* Means (SEM in parentheses) in the same row followed by the same letter are not significantly different ($P < 0.05$) using ANOVA. $n = 7$ (control), $n = 10$ (infested).

Seasonal shoot length changes, during the infestation period of the previous year, are illustrated in Figure 47. Shoot length and aphid-days during 1986 were correlated for those trees to be harvested in 1987 ($r^2 = 0.80$) (Fig. 48). Feeding by spirea aphid during the previous season significantly reduced shoot length at the ten-leaf stage in 1987 (control 122.3 cm, infested 98,6 cm).

When trees were harvested at the ten-leaf stage, percent NSC in lateral shoots, roots, and leaves were reduced by aphids (Table 20). Percent NSC in central shoot and rootstock were not significantly reduced. Total amount of NSC accumulated was affected significantly in all tree partitions. Percent reductions were: lateral shoots 50, trunk 15, rootstock 16, roots 41, and leaves 55%.

B- <u>1987-1988 Growing Season:</u>

1- First Season Trees : The effects of spirea aphid feeding and nitrogen application rate on the fresh and dry matter accumulation and carbohydrate concentrations during the growing season containing non-significant interaction terms are presented in Tables 21-24. When the interaction term in a factorial analysis is not significant, the main effects may be compared directly. Fresh weights of trees treated with four nitrogen rates were similar at planting. Total fresh and dry weights at planting as well as the net gain (fresh wt.) of infested trees were significantly less than controls (Table 21). Weight of trees tended to be positively related to nitrogen rate.

Aphids reduced both fresh and dry matter accumulation (Table 22). Dry and fresh weights of all tree parts tended to increase with increasing rates of nitrogen. Fresh and dry weight of trunk did not differ between treatments following nitrogen application. No differences were noted between the low nitrogen levels (0.5 and 1.0 g) for both fresh

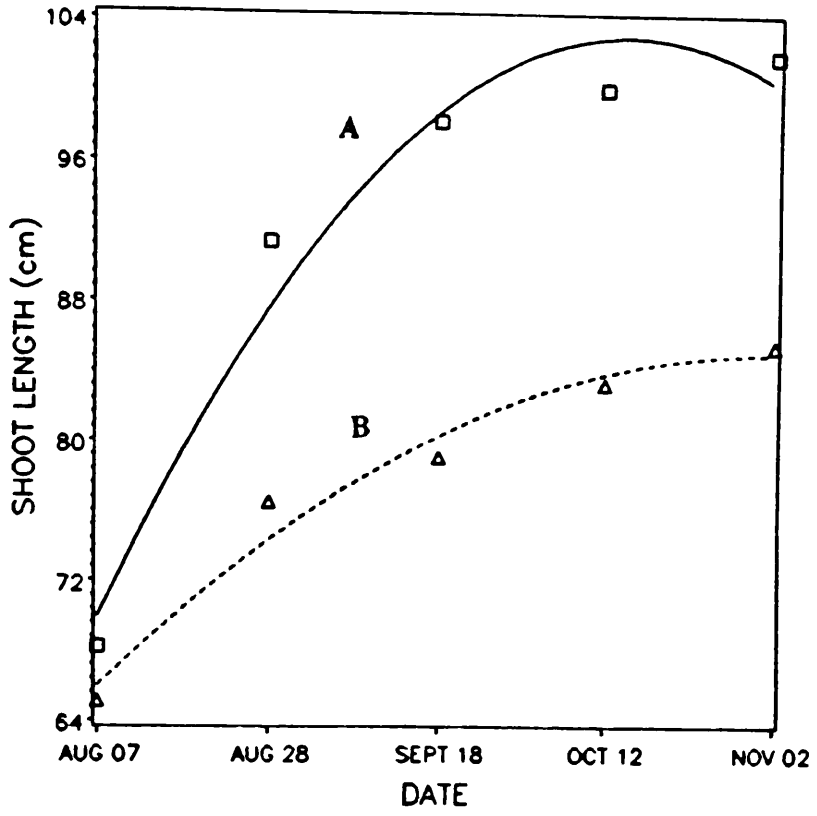


Fig. 47. Changes in shoot length during 1986 growing season for trees harvested in the second growing season at the ten-leaf stage in 1987. A = control and B = spirea aphid infestation. The quadratic regression equations ('x' represent days after aphid infestation) and r^2 values in the same order are:

A---	$Y = 62.6 + 1.107x - 0.008x^2$	$r^2 = 0.96$
B---	$Y = 62.8 + 0.486x - 0.003x^2$	$r^2 = 0.97$

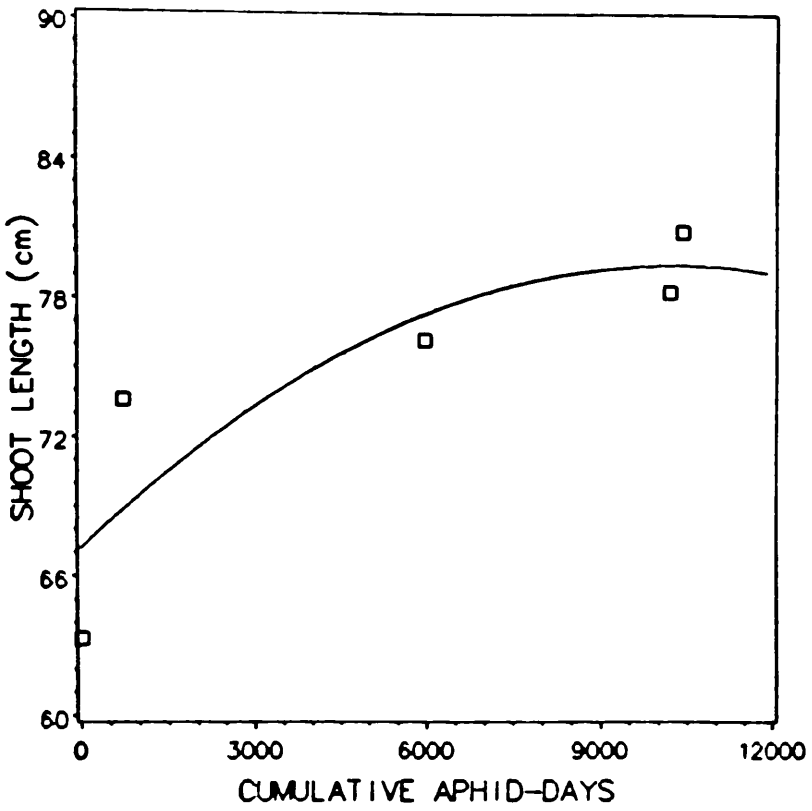


Fig. 48. Relation of shoot length to cumulative aphid-days per shoot during 1986 growing season for trees harvested at the ten-leaf stage in 1987. The quadratic regression equation and r^2 value are:

$$Y = 69.4 + 0.0023x - 8.7E\text{-}8\ x^2 \qquad r^2 = 0.80$$

Table 20. The effect of spirea aphid on nonstructural carbohydrate content of young apple trees harvested in the second growing season at the ten-leaf stage in 1987.

	Percent nonstructural carbohydrate (dry wt basis)				
Treatment*	Leaves	Shoots	Trunk	Rootstock	Roots
Control	3.32 (0.05) a	3.75 (0.08) a	3.07 (0.07) a	3.18 (0.09) a	6.71 (0.01) a
Spirea Aphid	2.55 (0.10) b	3.05 (0.01) b	2.85 (0.09) a	3.08 (0.13) a	5.63 (0.17) b
	Total amount nonstructural carbohydrate (g)				
Control	0.51 (0.02) a	0.74 (0.05) a	1.39 (0.09) a	3 .67 (0.23) a	3.49 (0.10) a
Spirea Aphid	0.28 (0.03) b	0.37 (0.02) b	1.18 (0.03) b	3.06 (0.17) b	2.06 (0.10) b

* Means (SEM in parentheses) in the same column followed by the same letter are not significantly different ($P < 0.05$) using ANOVA. $n = 7$ (control), $n = 10$ (infested).

Table 21. The effect of spirea aphid and nitrogen application rate on the accumulation of fresh and dry matter by young apple trees (at harvest) as compared with fresh weight at planting. Trees harvested at the end of first growing season in 1987 (factorial analyses containing non-significant interaction terms). SA = spirea aphid infestation, Co = control.

	Infestation Status[2]		Urea application Rate (*g*)[3]			
Variable[1]	SA	Co	0.0 g	0.5 g	1.0 g	2.0 g
Fresh Weight at Planting	288.8 a (4.47)	301.0 a (5.56)	291.0 a (6.67)	294.5 a (8.65)	294.5 a (7.87)	299.5 a (7.27)
Fresh Weight at Harvest	489.5 b (6.30)	594.4 a (9.99)	508.0 c (17.0)	530.3 bc (21.0)	547.0 b (20.1)	582.5 a (24.7)
Dry Weight at Harvest	250.5 b (3.93)	309.6 a (6.22)	260.8 c (9.88)	271.2 bc (10.8)	281.4 b (11.9)	306.8 a (14.4)
Net Gain (fresh weight)	200.8 b (6.70)	293.4 a (8.47)	217.0 c (17.8)	235.8 bc (17.7)	252.5 b (18.8)	283.0 a (20.3)

[1] Means (SEM in parentheses) in the same row (within infestation status and urea application rate) followed by the same letter are not significantly different ($P < 0.05$).
[2] ANOVA test for mean comparison in the main effects. n = 16.
[3] Tukey's studentized range test for mean comparison in the main effects. n = 8.

Table 22. The effect of spirea aphid and nitrogen application rate on the accumulation of the fresh and dry matter of tree partitions. Trees harvested at the end of 1st growing season in 1987 (factorial analyses containing non-significant interaction terms). SA = spirea aphid infestation, Co = control.

Variable[1]	Infestation Status[2]		Urea application Rate (g)[3]			
	SA	Co	0.0 g	0.5 g	1.0 g	2.0 g
DRY WEIGHT						
Shoots	16.9 b (0.63)	27.8 a (1.06)	19.6 b (1.78)	21.6 b (2.41)	22.6 ab (2.31)	26.2 a (2.51)
Trunk	45.4 b (1.06)	53.6 a (1.94)	46.5 a (1.91)	48.0 a (2.59)	49.3 a (2.74)	54.1 a (2.85)
Rootstock	114.1 b (2.01)	129.1 a (2.44)	116.4 b (3.33)	118.7 ab (3.52)	122.9 ab (4.53)	129.5 a (4.38)
Roots	59.5 b (1.24)	77.6 a (1.91)	64.8 b (3.84)	66.2 b (3.42)	68.3 ab (3.61)	75.0 a (4.55)
FRESH WEIGHT						
Shoots	34.0 b (1.12)	56.9 a (1.86)	40.5 b (4.01)	43.0 b (4.54)	45.9 b (4.48)	52.4 a (5.20)
Trunk	89.3 b (1.45)	95.1 a (1.89)	88.4 a (1.94)	91.6 a (3.02)	92.8 a (2.71)	96.0 a (2.16)
Rootstock	197.9 b (2.31)	222.4 a (2.76)	203.9 b (4.86)	206.9 ab (5.48)	211.5 ab (5.48)	218.4 a (6.40)
Roots	137.3 b (2.39)	172.3 a (3.03)	146.3 b (6.48)	151.3 b (7.09)	154.4 b (7.07)	167.3 a (7.84)

[1] Means (SEM in parentheses) in the same row (within infestation status and urea application rate) followed by the same letter are not significantly different ($P < 0.05$).
[2] ANOVA test for mean comparison in the main effects. n = 16.
[3] Tukey's studentized range test for mean comparison in the main effects. n = 8. main effects.

and dry weights in all tree partitions. The decrease in weights of the tree parts is evidence of the sensitivity of young apple trees to aphid feeding.

The effects of aphid feeding and nitrogen rates on trunk diameter, fresh/dry roots, shoot/roots, and fresh and dry weights of top and bottom parts of the trees are presented in Table 23. Trunk diameter was related to nitrogen, but was not reduced by aphid feeding (Table 23). The dynamic growth relationships between shoots and roots, as reflected in growth ratios, were affected by both aphids and nitrogen. Shoot/root weight (fresh and dry basis) increased with nitrogen rate. Top, and bottom parts, either fresh or dry weights, were significantly reduced by aphids.

The percentage and the amount of NSC in all tree partitions were reduced by spirea aphid feeding and were positively related to nitrogen rate (Table 24). No significant differences were obtained between the low level (0.5 g) and the unfertilized controls.

Table 25 summarizes results of the analysis of variance to test effects of nitrogen fertilization and aphid infestation with significant interaction terms (aphid infestation and nitrogen rate are statistically dependent). Aphids reduced leaf number/tree, fresh and dry weights of leaves, and leaf NSC at all nitrogen levels. The fresh/dry weight ratio for leaves was reduced by aphids at the intermediate nitrogen levels. All variables except the fresh/dry weight ratio of leaves were positively related to nitrogen level. Greater leaf surface for photosynthesis during the growing season would most likely increase dry matter production. In this study, leaf weights of control trees were significantly greater at all nitrogen rates than infested trees.

2- Second Season Trees : Effects of aphid feeding and nitrogen rates on the growth of trees harvested at the ten-leaf stage containing non-significant interaction terms in

Table 23. The effect of spirea aphid and nitrogen application rate on trunk diameter, fresh/dry weight ratio of roots, shoot/root weight ratios, and fresh and dry weights of top and bottom parts of young apple trees harvested at the end of the first growing season in 1987 (factorial analyses containing non-significant interaction terms). SA = spirea aphid infestation, Co = control, Top = leaves, shoots, and trunk. Bottom = rootstock and roots.

	Infestation Status[2]		Urea application Rate (*g*)[3]			
Variable[1]	SA	Co	0.0 g	0.5 g	1.0 g	2.0 g
Trunk Diameter[4]	2.50 a (2.31)	2.68 a (2.22)	2.37 b (0.03)	2.58 ab (0.03)	2.62 ab (0.02)	2.79 a (0.04)
Root (Fresh/Dry)	2.22 a (0.019)	2.31 a (0.022)	2.27 a (0.038)	2.29 a (0.017)	2.27 a (0.038)	2.24 a (0.038)
Shoots/Roots (fresh)	0.25 b (0.005)	0.33a (0.053)	0.27 b (0.016)	0.28 b (0.017)	0.29 ab (0.016)	0.31 a (0.017)
Shoots/Roots (dry)	0.28 b (0.008)	0.36 a (0.009)	0.30 b (0.017)	0.32 ab (0.021)	0.33 ab (0.019)	0.35 a (0.047)
Top (fresh)	154.3 b (3.07)	199.7 a (5.53)	157.9 c (6.71)	172.1 bc (9.57)	181.1 b (9.39)	196.9 a (11.2)
Top (dry)	76.8 b (1.78)	102.4 a (3.62)	79.5 b (3.93)	86.4 b (5.50)	90.2 b (5.76)	102.3 a (6.69)
Bottom (fresh)	335.2 b (3.99)	394.7 a (4.89)	350.1 b (10.6)	358.1 b (11.9)	365.9 b (11.6)	385.6 a (13.8)
Bottom (dry)	173.7 b (2.85)	207.2 a (3.25)	181.2 b (6.41)	184.9 b (6.06)	191.2 b (7.30)	204.5 a (8.08)

[1] Means (SEM in parentheses) in the same row (within infestation status and urea application rate) followed by the same letter are not significantly different ($P < 0.05$).
[2] ANOVA test for mean comparison in the main effects. n = 16.
[3] Tukey's studentized range test for mean comparison in the main effects. n = 8.
[4] trunk diameter was measured in July 24 and November 7 of 1987 growing season. Numbers indicate the net gain (mm) between measurements.

Table 24. The effect of spirea aphid and nitrogen application rate on nonstructural carbohydrate content of young apple trees harvested at the end of the first growing season in 1987 (factorial analyses containing non-significant interaction terms). SA = spirea aphid infestation, Co = control, and NSC = nonstructural carbohydrate.

Variable[1]	Infestation Status[2]		Urea application Rate (g)[3]			
	SA	Co	0.0 g	0.5 g	1.0 g	2.0 g
Percent NSC						
Shoots	3.72 b (0.081)	4.97 a (0.104)	4.16 b (0.258)	4.22 b (0.242)	4.31 ab (0.230)	4.69 a (0.309)
Trunk	4.02 b (0.089)	4.36 a (0.104)	3.84 b (0.124)	4.09 ab (0.133)	4.31 a (0.132)	4.50 a (0.115)
Rootstock	4.63 b (0.082)	5.03 a (0.093)	4.66 b (0.105)	4.69 ab (0.139)	4.84 ab (0.124)	5.13 a (0.147)
Roots	8.47 b (0.169)	9.13 a (0.088)	8.50 b (0.177)	8.63 b (0.149)	8.84 ab (0.169)	9.22 a (0.145)
Leaves	3.55 b (0.076)	4.51 a (0.070)	3.84 b (0.200)	3.94 ab (0.204)	4.06 ab (0.215)	4.28 a (0.191)
Total NSC (g)						
Shoots	0.63 b (0.031)	1.40 a (0.078)	0.84 b (0.117)	0.94 b (0.147)	1.01 b (0.152)	1.28 a (0.201)
Trunk	1.83 b (0.074)	2.34 a (0.104)	1.80 b (0.103)	1.97 b (0.135)	2.12 ab (0.130)	2.45 a (0.169)
Rootstock	5.28 b (0.148)	6.53 a (0.187)	5.42 b (0.203)	5.59 b (0.304)	5.98 ab (0.337)	6.64 a (0.305)
Roots	5.05 b (0.151)	7.09 a (0.222)	5.54 b (0.414)	5.73 b (0.372)	6.06 b (0.297)	6.95 a (0.518)

[1] Means (SEM in parentheses) in the same row (within infestation status and urea application rate) followed by the same letter are not significantly different ($P < 0.05$).
[2] ANOVA test for mean comparison in the main effects. n = 16.
[3] Tukey's studentized range test for mean comparison in the main effects. n = 8.

Table 25. The effect of spirea aphid and nitrogen application rate on leaf number, accumulation of leaf fresh and dry matter, fresh/dry weight of leaf, leaf number, and amount of leaf nonstructural carbohydrate (factorial analyses containing significant interaction terms, 1987-1988). SA = spirea aphid infestation, Co = control, NSC = nonstructural carbohydrate.[1]

Variable	Infestation Status	Urea Application Rate 0.0 g	0.5 g	1.0 g	2.0 g
First Growing Season (1987)					
Leaf Number	Co	80 (3.16) d *	98 (2.78) c *	117 (3.11) b *	133 (3.09) a *
	SA	65 (2.58) c	69 (4.39) bc	77 (2.50) ab	86 (3.09) a
Leaf Fresh Weight	Co	33.0 (1.47) c *	46.1 (1.83) b *	51.0 (1.22) b *	61.0 (1.29) a *
	SA	25.0 (1.47) c	29.0 (0.91) bc	34.0 (1.58) ab	36.0 (2.12) a
Leaf Dry Weight	Co	15.2 (0.71) c *	19.3 (0.83) b *	21.2 (0.82) b *	27.4 (0.39) a *
	SA	11.7 (0.48) b	14.2 (0.41) ab	15.5 (0.91) a	16.5 (0.61) a
Leaf (Fresh/Dry wt. ratio)	Co	2.17 (0.047) b	2.38 (0.031) a *	2.40 (0.036) a *	2.23 (0.049)ab
	SA	2.13 (0.046) a	2.04 (0.039) a	2.20 (0.034) a	2.16 (0.058) a
Leaf NSC (g)	Co	0.66 (0.034) c *	0.86 (0.041) b *	0.97(0.038) b *	1.30 (0.034) a *
	SA	0.40 (0.033) c	0.49 (0.024) bc	0.55 (0.038) ab	0.63 (0.026) a
Second Growing Season (1988)					
Leaf (Fresh/Dry wt. ratio)	Co	1.94 (0.025) a	2.06 (0.021) a *	1.93 (0.044) a *	1.98 (0.056) a
	SA	1.92 (0.024) b	1.97 (0.003) ab	2.08 (0.038) ab	1.98 (0.035) b

[1] Means (SEM in parentheses) in the same row followed by the same letter are not significantly different ($P < 0.05$, using Tukey's studentized range test test). Asterisk indicates a significant difference between treatments at each application rate.

factorial analyses are presented in Tables 26-29. Fresh and dry weights of trees were reduced by aphids and were positively related to nitrogen levels (Table 26). The effects of aphid and nitrogen rate on fresh and dry matter accumulation and carbohydrate concentrations by young apple trees were similar to those obtained from the first season trees (Table 27-29).

The effect of spirea aphid vs apple aphid populations on the growth, dry matter accumulation, and carbohydrate concentration of young apple trees:

1- First Season Trees : Fresh weight of trees infested with either species did not differ at planting (289.0 g and 305.0 g for spirea aphid and apple aphid, respectively) and fresh weight, dry weight, and fresh weight gain did not differ at harvest (Table 30). Aphid species did not affect fresh and dry weight of any tree partitions (Table 30). Table 31 illustrates nonsignificant differences for leaf numbers, trunk diameter, fresh/dry roots and leaves, shoot/roots, and top and bottom parts of young apple trees. Table 32 shows that the percent of NSC in all tree partitions from trees infested with apple aphid was slightly lower than from those trees infested with spirea aphid but was not significantly different ($P < 0.05$). The effects of both species on the amount of NSC were not significant in any tree partition. Research is currently underway to investigate the interaction of these species in apple orchard (Brown et al. 1988, Hogmire et al. 1988).

2- Second Season Trees : The fresh and dry weights of trees harvested at the ten-leaf stage were not significantly influenced by aphid species (Table 33). Similar results to those of the first season trees were obtained. Aphid species did not significantly affect the the fresh and dry matter accumulation and percent and total NSC in any tree partition (Tables 34-36).

Table 26. The effect of spirea aphid and nitrogen application rate on the accumulation of the fresh and dry matter by young apple trees (at harvest) as compared with fresh weight at planting. Trees harvested in the second growing season at the ten-leaf stage in 1988 (factorial analyses containing non-significant interaction terms). SA = spirea aphid infestation, Co = control.

	Infestation Status[2]		Urea application Rate (g)[3]			
Variable[1]	SA	Co	0.0 g	0.5 g	1.0 g	2.0 g
Fresh Weight at Planting	272.8 a	294.8 a	279.5 a	283.0 a	285.0 a	287.5 a
	(4.53)	(5.21)	(8.52)	(9.38)	(6.01)	(8.49)
Fresh Weight at Harvest	413.2 b	515.1 a	435.1 c	453.9 bc	466.3 b	501.2 a
	(6.60)	(7.96)	(19.0)	(19.1)	(19.2)	(22.7)
Dry Weight at Harvest	208.7 b	268.2 a	220.6 c	230.5 bc	239.4 b	263.3 a
	(4.83)	(5.26)	(11.9)	(10.6)	(12.0)	(13.5)
Net Gain (fresh weight)	140.5 b	220.3 a	155.6 b	170.9 ab	181.3 ab	213.7 a
	(6.66)	(9.29)	(17.1)	(15.8)	(17.1)	(19.4)

[1] Means (SEM in parentheses) in the same row (within infestation status and urea application rate) followed by the same letter are not significantly different ($P < 0.05$).
[2] ANOVA test for mean comparison in the main effects. n = 16.
[3] Tukey's studentized range test for mean comparison in the main effects. n = 8.

Table 27. The effect of spirea aphid and nitrogen application rate on the accumulation of the fresh and dry matter of tree partitions. Trees harvested in the second growing season at the ten-leaf stage in 1988 (factorial analyses containing non-significant interaction terms). SA = spirea aphid infestation, Co = control.

	Infestation Status[2]		Urea application Rate (g)[3]			
Variable[1]	SA	Co	0.0 g	0.5 g	1.0 g	2.0 g
DRY WEIGHT						
Shoots	12.6 b (0.67)	21.6 a (1.05)	14.3 b (1.76)	16.3 b (1.79)	16.9 b (1.96)	21.0 a (2.14)
Trunk	41.3 b (1.31)	45.7 a (1.26)	40.1 b (1.70)	42.0 b (1.66)	43.6 ab (1.06)	48.3 a (2.21)
Rootstock	100.4 b (2.31)	120.5 a (2.65)	104.7 b (4.49)	107.4 ab (4.73)	111.3 ab (4.88)	118.3 a (5.41)
Roots	38.8 b (1.34)	62.1 a (1.98)	46.7 b (4.54)	48.6 ab (4.61)	50.1 ab (4.48)	56.3 a (5.73)
Leaves	15.7 b (0.54)	18.3 a (0.52)	14.9 c (0.81)	16.2 bc (0.58)	17.6 ab (0.72)	19.3 a (0.57)
FRESH WEIGHT						
Shoots	24.6 b (1.30)	41.6 a (1.50)	29.0 b (3.17)	31.1 b (3.21)	33.0 b (3.50)	39.3 a (4.11)
Trunk	76.1 b (1.54)	80.3 a (1.43)	73.8 b (1.37)	77.4 ab (2.75)	78.4 ab (1.64)	83.1 a (1.71)
Rootstock	174.7 b (3.81)	205.7 a (3.26)	183.0 a (7.31)	186.4 a (7.34)	191.1 a (6.70)	200.3 a (8.28)
Roots	107.1 b (3.33)	151.4 a (4.06)	120.8 b (8.73)	126.4 ab (9.43)	128.6 ab (9.79)	141.1 a (10.3)
Leaves	30.8 b (1.03)	36.2 a (1.08)	28.6 c (1.54)	32.7 b (1.48)	35.2 ab (1.12)	37.4 a (1.34)

[1] Means (SEM in parentheses) in the same row (within infestation status and urea application rate) followed by the same letter are not significantly different ($P < 0.05$).
[2] ANOVA test for mean comparison in the main effects. n = 16.
[3] Tukey's studentized range test for mean comparison in the main effects. n = 8.

Table 28. The effect of spirea aphid and nitrogen application rate on leaf number, trunk diameter, fresh/dry roots, shoot/root and top/bottom part ratio of young apple trees harvested in the second growing season at the ten-leaf stage in 1988 (factorial analyses containing non-significant interaction terms). SA = spirea aphid infestation, Co = control, Top = leaves, shoots, and trunk, and Bottom = rootstock and roots.

	Infestation Status[2]		Urea application Rate (g)[3]			
Variable[1]	SA	Co	0.0 g	0.5 g	1.0 g	2.0 g
Leaf Number	51.5 a (1.06)	60.0 a (0.97)	52.0 c (2.18)	55.0 bc (1.76)	56.5 ab (1.70)	59.5 a (2.04)
Trunk Diameter[4]	2.56 a (0.049)	2.67 a (0.052)	2.40 b (0.039)	2.55 ab (0.053)	2.72 a (0.037)	2.79 a (0.046)
Root (Fresh/Dry)	2.81 b (0.122)	2.41 a (0.074)	2.65 a (0.126)	2.69 a (0.220)	2.63 a (0.153)	2.57 a (0.124)
Shoots/Roots (fresh)	0.23 b (0.009)	0.28 a (0.008)	0.24 a (0.014)	0.24 a (0.012)	0.25 a (0.014)	0.28 a (0.017)
Shoots/Roots (dry)	0.33 a (0.016)	0.35 a (0.017)	0.31 a (0.024)	0.36 a (0.022)	0.34 a (0.025)	0.38 a (0.020)
Top (fresh)	131.5 b (3.06)	158.0 a (3.30)	131.4 c (5.55)	141.2 bc (5.51)	146.6 b (5.05)	159.8 a (6.38)
Top (dry)	69.6 b (1.95)	85.6 a (2.45)	69.3 c (3.77)	74.5 bc (3.05)	78.2 b (3.34)	88.6 a (3.91)
Bottom (fresh)	281.8 b (4.44)	357.1 a (4.99)	303.8 b (13.7)	312.8 b (14.3)	319.8 b (14.6)	341.8 a (16.9)
Bottom (dry)	139.1 b (3.32)	182.4 a (3.75)	151.4 b (8.45)	156.0 b (8.21)	161.3 b (9.21)	174.6 a (10.5)

[1] Means (SEM in parentheses) in the same row (within infestation status and urea application rate) followed by the same letter are not significantly different ($P < 0.05$).
[2] ANOVA test for mean comparison in the main effects. n = 16.
[3] Tukey's studentized range test for mean comparison in the main effects. n = 8.
[4] Trunk diameter was measured in July 24 and November 7 of 1987 growing season. Numbers indicate the net gain (mm) between measurements.

Table 29. The effect of spirea aphid and nitrogen application rate on nonstructural carbohydrate content of young apple trees harvested in the second growing season at the ten-leaf stage in 1988 (factorial analyses containing non-significant interaction terms). SA = spirea aphid infestation, Co = control, and NSC = nonstructural carbohydrate.

	Infestation Status[2]		Urea application Rate (*g*)[3]			
Variable[1]	SA	Co	0.0 g	0.5 g	1.0 g	2.0 g
Percent NSC						
Shoots	2.84 b (0.07)	3.78 a (0.09)	3.09 b (0.19)	3.19 b (0.18)	3.28 b (0.18)	3.69 a (0.23)
Trunk	3.02 b (0.08)	3.36 a (0.09)	2.88 c (0.09)	3.09 bc (0.12)	3.28 ab (0.12)	3.50 a (0.11)
Rootstock	3.19 b (0.07)	3.56 a (0.10)	3.09 c (0.08)	3.28 bc (0.12)	3.47 ab (0.14)	3.66 a (0.12)
Roots	5.58 b (0.10)	6.92 a (0.09)	5.97 b (0.29)	6.09 b (0.27)	6.25 b (0.25)	6.69 a (0.27)
Leaves	2.89 b (0.08)	3.64 a (0.06)	3.06 b (0.16)	3.19 ab (0.17)	3.31 ab (0.18)	3.51 a (0.16)
Total NSC (g)						
Shoots	0.36 b (0.02)	0.83 a (0.06)	0.46 b (0.08)	0.54 b (0.08)	0.58 b (0.09)	0.80 a (0.12)
Trunk	1.25 b (0.06)	1.54 a (0.07)	1.53 c (0.07)	1.30 bc (0.09)	1.43 b (0.07)	1.69 a (0.09)
Rootstock	3.20 b (0.11)	4.30 a (0.15)	3.25 c (0.19)	3.53 bc (0.21)	3.89 ab (0.29)	4.33 a (0.25)
Roots	2.17 b (0.09)	4.31 a (0.17)	2.87 b (0.39)	3.03 b (0.39)	3.20 b (0.39)	3.86 a (0.52)
Leaves	0.46 b (0.02)	0.67 a (0.03)	0.46 c (0.04)	0.52 bc (0.04)	0.59 b (0.05)	0.68 a (0.04)

[1] Means (SEM in parentheses) in the same row (within infestation status and application rate) followed by the same letter are not significantly different ($P < 0.05$).
[2] ANOVA test for mean comparison in the main effects. $n = 16$.
[3] Tukey's studentized range test for mean comparison in the main effects. $n = 8$.

Table 30. The effects of spirea and apple aphid on the accumulation of the fresh and dry matter of tree partitions (trees harvested at the end of first growing season in 1987).

Variable *	Spirea Aphid	Apple Aphid
DRY WEIGHT		
Shoots	16.8 (0.98) a	15.9 (0.79) a
Trunk	45.4 (1.89) a	42.6 (3.74) a
Rootstock	113.6 (4.03) a	112.1 (4.62) a
Roots	60.3 (2.45) a	59.3 (2.13) a
Leaves	15.5 (0.91) a	14.8 (0.50) a
Total	251.5 (4.19) a	244.6 (11.1) a
FRESH WEIGHT		
Shoots	34.5 (1.32) a	35.3 (2.02) a
Trunk	90.3 (3.33) a	91.3 (5.66) a
Rootstock	199.5 (4.13) a	193.5 (5.12) a
Roots	137.3 (4.27) a	137.5 (5.88) a
Leaves	33.6 (1.58) a	32.0 (1.41) a
Total	495.5 (7.308) a	489.5 (19.84) a
NET GAIN (fesh wt.)	206.5 (5.631) a	184.5 (11.12) a

* Means (SEM in parentheses) in the same row, for each variable, followed by the same letter are not significantly different ($P < 0.05$, using ANOVA test). $n = 4$ for each treatment.

Table 31. The effects of spirea and apple aphid on leaf number, shoot diameter, fresh/dry roots and leaves, shoot/root, and top/bottom part ratio of young apple trees (trees harvested at the end of first growing season in 1987). Top = leaves, shoots, and trunk, Bottom = rootstock and roots.

Variable *	Spirea Aphid	Apple Aphid
Leaves (no.)	77.0 (2.50) a	73.0 (2.08) a
Trunk Diameter Increase (cm)**	2.51 (0.04) a	2.54 (0.04) a
Leaves (fresh/dry)	2.20 (0.03) a	2.16 (0.030) a
Roots (fresh/dry)	2.28 (0.06) a	2.32 (0.032) a
Shoot/Root (fresh)	0.25 (0.01) a	0.26 (0.005) a
Shoot/Root (dry)	0.28 (0.02) a	0.27 (0.01) a
Top (fresh)	158.8 (4.61) a	158.5 (8.96) a
Top (dry)	77.7 (2.69) a	73.3 (4.87) a
Bottom (fresh)	336.8 (6.21) a	331.0 (10.9) a
Bottom (dry)	173.9 (5.60) a	171.3 (6.57) a

* Means (SEM in parentheses) in the same row, for each variable, followed by the same letter are not significantly different ($P < 0.05$, using ANOVA test). $n = 4$ for each treatment.

** Diameter was measured in July 24 and November 7 of 1986 growing season.

Table 32. The effects of spirea and apple aphid on the accumulation of the fresh and dry matter by young apple trees (trees harvested at the end of first growing season in 1987). NSC = nonstructural carbohydrate.

Variable *	Spirea Aphid	Apple Aphid
Percent NSC (dry wt basis)		
Shoots	3.75 (1.10) a	3.53 (0.23) a
Trunk	4.13 (0.13) a	4.23 (0.13) a
Rootstock	4.62 (0.16) a	4.37 (0.16) a
Roots	8.50 (0.18) a	8.24 (0.31) a
Leaves	3.56 (0.16) a	3.21 (0.12) a
Total NSC (g)		
Shoots	0.63 (0.03) a	0.56 (0.04) a
Trunk	1.87 (0.10) a	1.80 (0.19) a
Rootstock	5.26 (0.29) a	4.89 (0.09) a
Roots	5.13 (0.30) a	4.87 (0.13) a
Leaves	0.55 (0.39) a	0.48 (0.02) a

* Means (SEM in parentheses) in the same row, for each variable, followed by the same letter are not significantly different ($P < 0.05$, using ANOVA test). $n = 4$ for each treatment.

Table 33. The effects of spirea and apple aphid on the accumulation of the fresh and dry matter by young apple trees as compared with the fresh weight at planting (trees harvested in the second growing season at the ten-leaf stage in 1988).

Variable *	Spirea Aphid	Apple Aphid
Fresh Weight at Planting	278.0 (7.95) a	274.0 (8.33) a
Fresh Weight at Harvest	418.3 (9.30) a	401.2 (11.8) a
Dry Weight at Harvest	209.3 (7.81) a	196.3 (9.01) a
Net Gain (fresh)	140.3 (7.63) a	127.2 (6.11) a

* Means (SEM in parentheses) in the same row, for each variable, followed by the same letter are not significantly different ($P < 0.05$, using ANOVA test). $n = 4$ for each treatment.

Table 34. The effects of spirea and apple aphid on the accumulation of the fresh and dry matter by young apple trees (trees harvested in the second growing season at the ten-leaf stage in 1988).

Variable *	Spirea Aphid	Apple Aphid
DRY WEIGHT		
Shoots	12.7 (1.12) a	11.9 (0.46) a
Trunk	41.9 (1.54) a	37.3 (3.03) a
Rootstock	99.7 (3.98) a	96.4 (4.98) a
Roots	39.1 (3.30) a	36.6 (2.87) a
Leaves	16.0 (0.46) a	14.1 (0.55) a
FRESH WEIGHT		
Shoots	24.8 (2.50) a	22.4 (1.44) a
Trunk	76.8 (2.14) a	75.7 (5.54) a
Rootstock	176.5 (6.84) a	170.8 (3.64) a
Roots	107.0 (9.03) a	102.2 (2.56) a
Leaves	33.3 (0.35) a	30.1 (1.45) a

* Means (SEM in parentheses) in the same row, for each variable, followed by the same letter are not significantly different ($P < 0.05$, using ANOVA test). $n = 4$ for each treatment.

Table 35. The effects of spirea and apple aphid on leaf number, shoot diameter, fresh/dry roots and leaves, shoot/root, and top/bottom part ratio of young apple trees (trees harvested in the second growing season at the ten-leaf stage in 1988). Top = leaves, shoots, and trunk, Bottom = rootstock and roots.

Variable *	Spirea Aphid	Apple Aphid
Leaves (no.)	53.0 (1.78) a	49.0 (1.47) a
Trunk Diameter Increase (cm) **	2.49 (0.04) a	2.43 (0.03) a
Leaves (fresh/dry)	2.08 (0.08) a	2.12 (0.02) a
Roots (fresh/dry)	2.79 (0.28) a	2.80 (0.17) a
Shoot/Root (fresh)	0.23 (0.01) a	0.22 (0.01) a
Shoot/Root (dry)	0.34 (0.05) a	0.32 (0.04) a
Top (fresh)	134.8 (1.29) a	128.2 (6.09) a
Top (dry)	70.6 (0.99) a	63.3 (2.75) a
Bottom (fresh)	283.5 (9.57) a	273.0 (5.87) a
Bottom (dry)	138.8 (7.18) a	133.0 (7.70) a

* Means (SEM in parentheses) in the same row, for each variable, followed by the same letter are not significantly different ($P < 0.05$, using ANOVA test). $n = 4$ for each treatment.

** Diameter was measured in July 24 and November 7 of 1986 growing season.

Table 36. The effects of spirea and apple aphid on the accumulation of the fresh and dry matter by young apple trees (trees harvested in the second growing season at the ten-leaf stage in 1988). NSC = nonstructural carbohydrate.

Variable *	Spirea Aphid	Apple Aphid
Percent NSC (dry wt basis)		
Shoots	2.88 (0.13) a	2.69 (0.12) a
Trunk	3.06 (0.16) a	2.95 (0.12) a
Rootstock	3.19 (0.16) a	3.03 (0.13) a
Roots	5.63 (0.16) a	5.46 (0.12) a
Leaves	2.94 (0.21) a	2.71 (0.18) a
Total NSC (g)		
Shoots	0.37 (0.04) a	0.32 (0.01) a
Trunk	1.29 (0.08) a	1.10 (0.09) a
Rootstock	3.18 (0.20) a	2.92 (0.14) a
Roots	2.21 (0.23) a	2.00 (0.19) a
Leaves	0.47 (0.03) a	0.38 (0.02) a

* Means (SEM in parentheses) in the same row, for each variable, followed by the same letter are not significantly different ($P < 0.05$, using ANOVA test). $n = 4$ for each treatment.

Discussion

Aphids feed on leaf phloem sap and when they are present in large numbers are assumed to remove a great deal of photosynthate and nutrients (Dixon 1971b). Dry matter production depends basically on the amount of photosynthetic surface, and the rate of carbon fixation per unit of leaf surface, which is affected by leaf chlorophyll content, nutritional status, and water relations as well as duration of growth. Johnson & Lasko (1985) stated that photosynthates from the top five to eight unfolded leaves were needed to supply the growth of the shoot tip. In this study, feeding by spirea aphids significantly reduced both dry matter accumulation and percent NSC during the infestation period. This response to aphid infestation is similar to that of sycamore, in which there is a reduction of growth in all parts of the tree (Dixon 1971a). Reduced number of leaves caused by spirea aphid feeding may reduce whole-plant Pn and cause a reduction in root weight as well as its surface area. In general, these results differ from those recently reported by Varn & Pfeiffer (1989) who stated that dry matter accumulation and carbohydrate concentration were not affected by spirea aphid feeding. The infestation period (40 days) in their study may have been too short to affect the trees; also the population levels expressed as no.aphids per leaf (ca. 65 aphids per leaf on all immature leaves on all terminals) may have been too low to affect the trees. The greater effect on growth caused by spirea aphid feeding in this study may be attributed to the removal of NSC and reductions in leaf chlorophyll content and net gas exchange (chapter 2). An infestation period of approximately three months as well as a higher aphid density in all terminal leaves was enough to reduce Pn and in turn may reduce carbohydrate reserves.

Maintenance of apple trees during the winter and resumption of growth in early spring depends on accumulated carbohydrate reserves during the previous season. When

growth begins in the spring, carbohydrate reserves in the storage organs are readily depleted and can be used for respiration and growth when photosynthate is not supplied directly from photosynthetic activity. In young apple trees, less than one-fourth of the carbohydrate reserves was used in growth of new tissues in the spring and much of the remainder was used in respiration (Hansen & Grausland 1973); the greater part of the carbohydrate reserves of roots is used up during metabolism (Tromp 1983). Spirea aphid feeding during the growing season resulted in reduced photosynthetic rates (chapter 2), causing reduced NSC and subsequently decrease growth at the ten-leaf stage. In this study, percentage of NSC determined at the ten-leaf stage was less than that estimated at the end of the first season. An estimated 28% of the carbohydrate reserves was used for new growth.

Pest suppression activities are initiated on the basis of the relationship between pest population densities and sufficient reduction in plant quality to result in economic damage (economic injury level). The density at which control measures should be initiated to prevent an increasing population from reaching the economic injury level (EIL) is called the economic threshold (ET). An apple tree's response to aphid injury is an important component in establishing an EIL. Spirea aphid and apple aphid affected the growth of young apple trees similarly. Therefore the EIL will probably be the same for each species. However, the ET may differ between species, depending on pesticide selection, because specific pesticides affect the species differently (Hogmire et al. 1988).

Previous studies with aphids have stressed the importance of maintaining the trees free of aphids during the growing season (Wood et al. 1985, Varn & Pfeiffer 1989). On the basis of the data obtained in the present study, the role of aphid infestation in reducing carbohydrate reserves and their impact on the growth of young apple trees are

clearly demonstrated. This reduction indicates that spirea aphid feeding impaired the normal functioning of young apple trees.

SUMMARY AND CONCLUSIONS

This study was conducted in 1986 and 1987 to evaluate the effects of spirea aphid feeding and nitrogen fertilization on net photosynthesis (Pn), leaf chlorophyll content, growth, dry matter accumulation, and carbohydrate concentrations of young apple trees. The effects of spirea aphid was compared with that of the apple aphid.

Trees were artificially infested and grown in unheated greenhouse with screened ends. Young, actively growing, terminal leaves were more susceptible to colonization than mature, old leaves. In this study, spirea aphid populations responded differently to different nitrogen treatments. Trees fertilized with high nitrogen seemed to support greater aphid populations than trees fertilized with lower nitrogen rates. The leaf nitrogen concentration increased significantly and linearly with increasing amounts of urea applied to both infested and control trees. Aphid also reduced leaf nitrogen at all nitrogen levels. Changes in the chemical composition of leaves might affect aphid populations. Other factors such as leaf mineral nutrient composition and water content should be considered in further studies on aphid-apple interaction.

Spirea aphids significantly reduced net photosynthesis (Pn) at various levels of infestations, and this reduction persisted after aphid removal. Photosynthetic rates decreased with increasing accumulation of sooty mold (SM). Light and medium SM levels (trace-30% and 30-70% cover, respectively) did not differ significantly but were different from control.

The presence of both aphids and sooty mold interfered with leaf function. Sooty mold accumulation, which altered leaf function, can be controlled by controlling aphids in young apple trees. The effect in this greenhouse study may have been more severe than in a field situation, where honeydew deposits are subject to rainfall and other factors.

Leaf greenness and chlorophyll were significantly reduced by spirea aphid. Chlorophyll concentrations obtained by methanol extraction technique was linearly related to greenness obtained by SPAD readings, for both infested and control leaves and for sooty mold and control leaves. Phtosynthetic rates and leaf greenness were correlated with aphid-days accumulations at each nitrogen rate. Pn increased linearly with increasing greenness. Pn and leaf greenness were positively related to nitrogen rates. decrease in chlorophyll content helps to explain the reduction in net photosynthesis. Pn may be related to chlorophyll, but leaf age, nutritional status, and other parameters may influence the results.

Callose accumulation at the ploem sieve plates in response to aphid feeding was observed but to a lesser degree than for other aphids reported on apple and pecan leaves. Reduced Pn in this study may be caused by disruption of the phloem as well as a collapse of cells in various tissues (intervienal tissues). More histological research is needed to determine the reactions of apple leaves to spirea aphid feeding and also to determine the major compounds responsible for the phloem injury.

Accumulation of fresh and dry weights in all tree partitions (leaves, shoots, trunk, rootstock, and roots), and the dynamic growth relationships between shoots and roots, as reflected in growth ratios, were affected by both aphids and nitrogen fertilization. Weights of of all tree parts tended to increase with increasing rates of nitrogen. Leaf weight and shoot/root weight (fresh and dry basis) increased with increasing nitrogen. The weights of top and bottom parts, either fresh or dry, were significantly reduced by aphids. The percent and the amount of NSC in all tree partitions were reduced by spirea aphid feeding and were positively related to nitrogen rate.

At the ten-leaf stage in the year following treatment, spirea aphid feeding reduced fresh and dry weights of all tree partitions, except the trunk. Reductions of the total fresh and dry weights of top (leaves, shoots, and trunk) or bottom (rootstock and roots) parts of the tree were also evident. Spirea aphid feeding during the first growing season significantly reduced shoot length by the end of the season and at the ten-leaf stage in the succeeding year. When growth begins in the spring, carbohydrate reserves in the storage organs are depleted by accelerated respiration and growth of new shoots and leaves.

Spirea aphid feeding reduced the percentage of NSC in lateral shoots and roots. At the ten-leaf stage, percent NSC in lateral shoots, roots, and leaves were reduced by aphid feeding the previous season. Spirea aphid feeding decreased the amount of NSC in all tree partitions due to reduced dry weights of these parts. Nitrogen application (2.0 g /tree) significantly increased both percent and amount of NSC. The low level (0.5 g) did not differ from the control.

Dry matter production depends basically on leaf area and the carbon fixation per unit of leaf surface, which is influenced by leaf chlorophyll content, nutritional status, water relations, and duration of growth. In this study, spirea aphid feeding significantly reduced both dry matter accumulation and percent NSC during the infestation period. Reduced number of leaves caused by spirea aphid feeding may reduce whole-plant Pn and cause a reduction in root weight as well as its surface area. The greater effect on growth caused by spirea aphid feeding in this study may be attributed to the removal of NSC and reductions in leaf chlorophyll content and Pn (chapter 2). An infestation period of approximately three months with high aphid populations on all terminal leaves reduced Pn and carbohydrate reserves.

Development of spirea aphid and apple aphid was similar on trees fertilized with a moderate rate of nitrogen. Pn and leaf chlorophyll content declined to a similar extent with accumulated aphid-days, for both aphid species. Aphid species did not affect any of tree growth or NSC accumulations.

Previous studies with aphids have stressed the importance of maintaining the trees free of aphids during the growing season. Data from this study indicate that spirea aphid is capable of restricting leaf function (i.e. reductions of Pn and chlorophyll content) and the accumulation of carbohydrate reserves in various tissues. Spirea aphid feeding impaired the normal functioning of young apple trees.

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APPENDICES

Appendix A. Kjeldahl nitrogen determination for plant tissue.

Preparation of Plant Tissue for Analysis

1. Dry tissue at 70 C in forced air draft oven for 48 hours.
2. Grind tissue to pass a 40 mesh sieve Wiley mill.
3. Store in coin envelopes in desiccator until analysis.

Digestion

1. Weigh 50 mg. dry sample in 100 ml. Kjeldahl flask.
2. Add 50 mg. digestion mixture (200 g $K_2 SO_4$, 20 g $CuSO_4 .5H_2O$, 2 g Selenium).
3. Add 2 ml. concentrated $H_2 SO_4$ and swirl.
4. Put the samples in the digestion block and the heat at 450 C until sample is clear.
5. Cool samples and add 5-10 ml. of water immediately prior to distilling.

Distillation

1. Add 5 ml. boric acid indicator to a 50 ml. erlenmeyer flask marked at 30 ml. and place it under the condenser tube.
2. Add 7 ml., 10N, NaOH to the distillation flask.
3. Distill until 30 ml. is collected in the flask containing the boric acid indicator.

Titration

Titrate the solution in the 50 ml. erlenmeyer flask with 0.05 N $KH(IO_3)_2$. Prepare blanks by same procedure as for the samples; also run some water blanks using the same procedure as the distillation of the samples.

Calculations

% N in tissue = [(14 mg./meq.) (0.05 or N of acid used) (mls acid) (100%)] / mg. tissue.

Appendix B. Enzyme removal of nonstructural carbohydrates (NSC) from plant tissue

First Day

1. Weigh 500 mg. of tissue into a 125 ml. erlenmeyer flask.
2. Add ca. 15 ml. of distilled water.
3. Gelatinize starch by boiling 1 to 2 min. on a hot plate and then to cool to room temperature.
4. Add 10 ml. of buffer solution.
5. Add 10 ml. of 0.5% takadiastase solution (glucose-free amyloglucosidae from Aspergillus oryzae (Sigma) containing about 50 units/ml.).
6. Stopper and incubate at ca. 38 C for 44 hrs.
7. Swirl several times during incubation.

Third Day

1. Filter into suitable volumetric flask through Whatman #1 paper.
2. Wash flask and filter paper several times.
3. Add 2 ml. of 10% neutral lead acetate to volumetric (precipitates protein).
4. Complete volume with water.
5. Shake 4 or 5 times to mix thoroughly.
6. Decant ca. 30 ml. into centrifuge and centrifuge 5 min.
7. Decant into 50 ml. erlenmeyer containing ca. 100 mg. of powdered potassium-oxalate.
8. Store in a refrigerator for at least 4 hrs or overnight.

Fourth Day

1. Filter through Whatman #42 paper without washing.
2. Pipette an aliquot into a test tube. Add enough sulfuric acid solution to obtain an 0.1 N solution with an aliquot.
3. Heat in a boiling water bath.

4. Cool and neutralize with sodium hydroxide solution.
5. Analyze directly for reducing power as follow:
    * add 10 ml. of Reagent '50' to an aliquot of the unknown solution to a 25 x 200 mm. test tube. Mix thoroughly and cover with a marble.
    * test an enzyme blank (i.e. to correct for enzyme contamination), Reagent '50' blank, and a monosaccharide standard solution blank.
    * heat tubes for 15 min. in a boiling water bath and then cool to less than 30 C.
    * add 2 ml. potassium iodide-potassium oxalate solution to each tube, followed with 10 ml. 1.0 N H_2 SO_4.
    * titrate with 0.02 N sodium thiosulfate using 0.25 ml. gelatinized starch solution as the indicator.

Calculation

- Reagent '50' blank titration . . . . . .(A) ml.
- Enzyme blank titration . . . . . .(B) ml.
- 5 mg. glucose standard titration. . . . . (C) ml.
- Unknown NSC titration . . . . . (D) ml.
- Differences between Reagent '50' and Glucose standard. . . . . A-C = E
- Differences between Enzyme and unknown NSC. . . . . B-D = F
- Amount of sugar in sample aliquot. . . . . (5 mg / E) x F = G mg. glucose

* Percent NSC in tissue = [(G) mg. x dilution factor x 100] / sample weight (mg)

VITA

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The author was married to Wafa in 1984. They have three children. Two duaghters, Yaman and Rola, and one son, Anas.

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