EFFECT OF NITROGEN FERTILIZATION ON THE PHYSIOLOGICAL DAMAGE AND SUBSEQUENT RECOVERY OF MITE-INFESTED APPLE LEAVES

by

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(ABSTRACT)

Two experiments were conducted with field- and container-grown 'Delicious' apple trees to evaluate the effect of nitrogen fertilization on the physiology of apple leaves subjected to European red mite feeding, and the subsequent recovery of leaf function following mite removal.

Nitrogen (N) fertilization affected the visual damage, and the photosynthetic response of 'Imperial Delicious' apple leaves to mite feeding. Visual damage occurred first, and remained greatest on the low N treatment throughout the experiment. High N trees exhibited a linear decline in Pn with increasing MD, in contrast to the quadratic decline found under low N treatment. With equal mite populations, high N trees retained higher Pn rates. Transpiration, dark respiration, relative water content, leaf nitrogen, and total chlorophyll were negatively related to MD, but

specific leaf weight was positively related to MD. No interaction was found between N treatment and mite feeding for these parameters.

Following removal of ERM populations, 'Redchief Delicious' leaves subjected to greater than 4000 MD exhibited recovery in Pn by 28 days after mite removal. Leaves receiving less MD (1500-3000) demonstrated an inconsistent response over time. Transpiration and diffusive resistance showed no recovery. Relative water content, specific leaf weight, leaf nitrogen, and total chlorophyll linearly declined with mite feeding, and were unaffected by foliar urea treatment.

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Introduction

The European red mite, Panonychus ulmi (Koch), is a major foliage-feeding pest of pome and stone fruits throughout the temperate zone. Due to the destruction of natural predators, and resistance to many commercial acaricides, the European red mite (ERM) often attains large populations in the field. Such outbreaks of mites result in characteristic bronzing of the foliage, and are considered economically damaging to the crop.

Research conducted to date has not clearly quantified the damage caused by mite feeding. Greenhouse experiments have demonstrated the damage potential of mite infestations on leaf function and tree growth, but these results may not be applicable to field trees. Results from field experiments on growth and yield are extremely variable. Tree species, condition, vigor, and time of mite infestation all contribute to the variability.

Previous research has suggested that young, vigorous trees may be more tolerant of mite feeding. Therefore, the first objective of this study was to determine if different levels of nitrogen fertilization affected the physiological response of 'Delicious' apple leaves to ERM feeding. Photosynthesis, transpiration, dark respiration, dark transpiration, relative water content, specific leaf weight, leaf nitrogen, and total chlorophyll of mite-damaged leaves were monitored. A second objective was to determine if mite

damaged leaves recover physiological function following mite removal, and to evaluate the effectiveness of foliar urea application as a tool to induce recovery.

Literature Review

General. This review deals with tetranychid mite damage, including research conducted with species other than ERM. Most notable among these is the twospotted spider mite, *Tetranychus urticae* Koch. Twospotted spider mite (TSM) is often found in association with ERM in the field. TSM offers two distinct advantages to the researcher; a rapid population growth rate, and year-round experimentation in the greenhouse. Differences between these species will be addressed.

Mite populations are quantified in the literature in several ways: mites per plant, mites per unit leaf area, mites per leaf, and mite days. Comparison of mite populations among experiments is difficult due to the different methods of expressing population density. Mite days quantify both mite number and duration of feeding, and is therefore the preferred method of population expression. Mite days are calculated by averaging the number of mites counted on a leaf on two dates, and multiplying by the number of days between the counts. Where possible, mite populations will be expressed on a mite day basis.

European Red Mite life cycle. The first generation of ERM appears in the spring at approximately bloom time of apple. Mites pass through five developmental stages: egg, larva, protonymph, deutonymph, and adult. All non-egg stages feed, and each has a distinct color and morphology. The

developmental time for each stage is highly dependent on average temperature; higher temperatures lead to faster development. In Blacksburg, Va., developmental time from larva to adult ranged from 4 to 20 days (Cagle 1946).

Summer eggs are laid predominantly on the lower leaf surface, with number and incubation time again highly temperature dependent. Cagle (1946) reported nine generations under average temperature conditions in Blacksburg, Va. Hot, dry weather during the summer months results in large populations in the field. As fall approaches, adult ERM begin laying winter eggs on the plant stems. Winter eggs are deep red in color and are the overwintering stage of the mite. These eggs will not hatch prior to spring, even if provided with warm temperatures in the greenhouse. This largely excludes ERM from study during the winter.

Tetranychid feeding. Tetranychid mites feed by removing cellular contents through direct penetration of mesophyll cells with piercing mouthparts (Sances et al. 1979, Storms 1971). Working with McDaniel spider mite, *Tetranychus mcdanieli* McGregor, on apple, Tanigoshi and Browne (1981) established that such feeding destroyed or removed cellular organelles in the penetrated cell and altered chloroplasts in adjacent cells. Removal of cellular components in the palisade mesophyll cells of apple caused cell wall collapse, and resulted in the characteristic bronzing associated with ERM feeding (Avery and Briggs 1968b). Epidermal cells were

undamaged by this feeding, and feeding occurred on both leaf surfaces. Feeding on the upper leaf surface mostly damaged the palisade mesophyll; however, lower leaf surface feeding damaged the spongy mesophyll.

The balance of gibberellin-like and auxin-like substances was altered in 'Brompton' plum infested with ERM (Avery and Lacey 1968). The mechanism was not identified. Saliva injection by TSM on bean (Phaseolus vulgaris) affected the phosphate metabolism and the balance of growth regulators in infested plants (Storms 1971).

Substrate. Wermelinger et al. (1985) found that TSM developed faster on bean than apple. ERM populations developed faster, and were twice as numerous, on 'Delicious' as on 'McIntosh' grown under identical conditions (Johnson 1983). Chlorophyll content was greater in 'Delicious' leaves, and possibly contributed to the differences. Ferree et al. (1986) found a weak negative relationship between leaf hairs and mite oviposition rate among several apple cultivars.

Nitrogen concentration of the substrate affected total fecundity, weight, and oviposition rate of mites (Jackson and Hunter 1983, Wermelinger et al. 1985). The higher the nitrogen concentration, the higher the mite population; however, a threshold level existed above which mite populations actually decreased (Rodriguez 1958). This threshold was higher for TSM than for ERM. Van de Vrie and Delver (1979)

also reported increased mite numbers following nitrogen applications, and they suggested manipulation of the fertilizer program as a means of managing mites.

Mite influences on leaf physiology. Net photosynthesis and mite feeding were negatively related in apple and plum (Avery 1964, Avery and Briggs 1968a, Ferree et al. 1986, Schaffer et al. 1986). In greenhouse studies using TSM on 'Franklin' apple, Hall and Ferree (1975) measured net photosynthetic reductions of 26, 30, and 43% below the control with 135, 270, and 540 mite days, respectively. These data suggest an immense damage capacity for TSM; however, this study was conducted under greenhouse conditions in the winter, and direct comparisons to summer-grown trees (or field trees) may not be appropriate. Net photosynthesis of apple was reduced before visual damage from TSM feeding (Hall and Ferree 1975), however, Avery (1964) observed visual damage (bronzing) from ERM feeding before measuring reductions in net photosynthesis of plum and apple.

Nitrogen status of host plant material influenced the response to mite feeding (Jackson and Hunter 1983). Net photosynthesis was not reduced by mite feeding in pecan seedlings treated with 0 $mg \cdot liter^{-1}$ nitrogen, whereas net photosynthesis was reduced in the 200 $mg \cdot liter^{-1}$ nitrogen treatment. At 600 $mg \cdot liter^{-1}$ nitrogen, mite feeding increased photosynthesis. All seedlings receiving the high nitrogen rate had low photosynthetic rates (<10 mg CO_2 dm^{-2}

hr^{-1}), and poor growth. The 600 $mg \cdot liter^{-1}$ treatment resulted in leaf nitrogen concentrations of 4% or greater, well above the toxicity level of 3.4% measured by Sparks and Baker (1975) for pecan leaves.

Ferree et al. (1986) measured no clear transpiration effect of TSM on 'Delicious'. However, in another study using 'Golden Delicious', transpiration was reduced after 135 TSM mite days, but not later in the study (Ferree and Hall 1981). TSM feeding reduced transpiration in strawberry (Fragaria X ananassa Duch.) and peppermint (Mentha piperita L.) (DeAngelis et al. 1982, Sances et al. 1981). No experiments have been conducted to investigate the effect of ERM on transpiration; however, Schaffer et al. (1986) found reduced transpiration in avocado (Persea americana Mill.) leaves subjected to avocado red mite (Oligonychus yothersi McGregor). DeAngelis et al. (1982) measured increased dark transpiration in peppermint plants infested with TSM. Feeding damage to the cuticle was suspected as the cause of increased nighttime water loss.

Leaf chlorophyll content has been negatively correlated with mite feeding (Johnson 1983, Sances et al. 1979). Chapman et al. (1952) measured 30% reductions in chlorophyll content of 'Delicious' leaves infested with ERM. Zwick et al. (1976) found similar chlorophyll reductions following ERM infestations on 'Newtown' and 'Golden Delicious' apple.

Leaf nitrogen concentration (%) was severely reduced by mite feeding (Herbert and Butler 1973, Klopfenstein and Holdsworth 1978). Leaf nitrogen levels below 1.9% dry weight were measured following 5000 ERM mite days on 'Delicious', with control leaves remaining above 2% dry weight. DeAngelis et al. (1983) reported reduced leaf fresh weight and specific leaf weight (on a fresh weight basis), and increased leaf carbohydrate concentration in peppermint due to TSM feeding. Premature defoliation has been reported for peach infested with TSM (Bailey 1979), and 'Brompton' plum, 'Golden Delicious', and 'Delicious' apple infested with ERM (Avery and Briggs 1968a, Beers and Hull 1987).

Strawberry plants regained physiological function following TSM removal (Sances et al. 1981). Following initial reductions, whole plant net photosynthesis and transpiration recovered upon removal of high, but not low TSM populations on strawberry. Chapman et al. (1952) observed recovery of green color in 'Delicious' leaves following removal of ERM populations; however, chlorophyll extractions did not confirm these observations.

Mite effects on plant growth. Following ERM infestations on 'Brompton' plum and apple rootstocks, Briggs and Avery (1968) found reductions of 7-17% for shoot extension, 21-32% for new shoot dry weight, 12-30% for old shoot dry weight, and 7-50% for root dry weights. However, similar populations of ERM did not affect growth of container-grown 'Lord

Lambourne' apple trees. Avery and Briggs (1968a) obtained different results depending on mite density. High mite densities (1-2 mites cm^{-2}) suppressed the growth of container-grown 'Brompton' plum, but lower densities (0.5 mites cm^{-2}) increased growth in relation to mite-free controls.

Low TSM populations stimulated growth in greenhouse-grown bean plants (Storms 1971). Radioactive labelling indicated that growth stimulation resulted from an alteration of growth-regulating substances in the plant. The growth of bean plants was reduced by TSM infestations during the winter; in the summer, however, mite populations stimulated growth (Storms 1971). Unfortunately, these data were expressed as the percentage of plants with increased or decreased growth; actual measured differences, or statistical separations were not presented.

In the field, trunk growth and shoot extension of spur-type 'Delicious' was unaffected by three years of heavy ERM feeding (Klopfenstein and Holdsworth 1978). Terminal shoot growth of several apple cultivars was unaffected by ERM populations of 2000 mite days (Beers and Hull 1987). McClernan and Marini (1986) measured no reduction in terminal shoot growth, number of lateral shoots, or trunk enlargement on peach trees subjected to 8900 ERM mite days. Vigorous, non-stressed 'Newtown' and 'Golden Delicious' apple trees had no growth reductions over four years of heavy ERM infestations (Zwick et al. 1976).

Early season infestations of McDaniel spider mite were more damaging to 'Delicious' apple trees than late season infestations (Tanigoshi and Browne 1981). ERM-infested 'Cortland', but not 'Rome' or 'Delicious', trees exhibited less growth than control trees (Chapman et al. 1952).

Mite influences on bloom and fruit set. In general, bloom was reduced the year following mite infestations (Barnes and Moffit 1978, Klopfenstein and Holdsworth 1978). Lienk et al. (1956) reported bloom reductions of 75% and 34% for 'Cortland' and 'Delicious', respectively, the year following heavy early-summer ERM damage. Mite infestations coincided with flower bud formation. Mite accumulations for 'Cortland' were approximately 1500 mite days, and 'Delicious' accumulated 3500 mite days. Damage was not visible on infested trees.

'Stayman' and 'Golden Delicious', but not 'Delicious', had reduced bloom following three years of ERM populations of up to 2000 mite days (Beers and Hull 1987). Maximum mite densities were achieved in late July and August in all three years. In a similar study, Beers et al. (1987) investigated the effect of three levels of ERM on 'York' and 'Rome Beauty' with different leaf:fruit ratios (LFR). The results indicated that high mite populations (1500 mite days) reduced return bloom of both cultivars, with a negligible effect of the different LFR's. The extreme variation encountered in this experiment may have masked the effects of the

LFR treatments. Bloom was unaffected for vigorous 'Newtown' and 'Golden Delicious' trees with four years of ERM populations in excess of 1200 mite days, but this report was based solely on visual observation (Zwick et al. 1976).

Apple fruit set was negatively affected by ERM feeding (Ames et al. 1984, Hardman et al. 1985); however, the effect was inconsistent from year to year. Decreased set resulted from greater fruit losses at "June drop". Zwick et al. (1976) found no effect of ERM feeding on fruit set in the first year following mite feeding, but later years were not studied.

Mite influences on yield and fruit quality. Yield of 'Cresthaven' peach was not affected in the current year by ERM populations of 3600 mite days (McClernan and Marini 1986). Bailey (1979) found no influence of TSM on peach yield at moderate populations (40-50 mites per leaf), whereas high populations (greater than 100 mites per leaf) reduced yield in the current year. Mite day accumulations were not presented. Unfortunately, the yield reduction was based on a regression line dependent on two extreme points.

In the years following moderate mite infestation, yields of walnut and apple were reduced (Barnes and Moffit 1978, Klopfenstein and Holdsworth 1978). Yields of 'Cortland' and 'Delicious' trees were reduced 65% and 36%, respectively, in the year following heavy ERM infestations (Lienk et al. 1956). Light and Ludlam (1972) found yield reductions in

'Cox' due to ERM infestations in two of five years, and early season infestations were more damaging than late season infestations.

McClernan and Marini (1986) reported no effect of 3600 ERM mite days on peach quality (color, flesh firmness, soluble solids, and size) in the current year. ERM infestations actually improved red color on 'Cortland' apple due to increased light penetration into the infested tree resulting from premature leaf drop (Chapman et al. 1952). Fruit size was reduced by ERM feeding on apple for three years (Beers et al. 1987, Hoyt et al. 1979). Ames et al. (1984) found reduced red pigmentation, soluble solids, phosphorous, and calcium in 'Miller Sturdeespur Delicious' trees infested with ERM. These effects were found on trees with a heavy crop load, but trees with a light load were unaffected.

Presently, action thresholds (a level at which measures should be taken to prevent mites from causing economic damage) for ERM vary according to state, time of year, environmental conditions, mite/predator ratio, and crop load. In Pennsylvania, for example, the recommended action threshold for trees with a cropping level of 500 bushels per acre is 250 MD. Trees producing less than 300 bushels per acre have a recommended action threshold of 500 to 750 MD (Crassweller 1986)

In conclusion, mite feeding influences leaf physiology, plant growth, bloom, yield, and fruit quality. These effects

are influenced by many factors, including mite species, plant species and cultivar, nutritional status of the plant, timing of infestation, crop load, and the plant environment (greenhouse versus field). This study was designed to investigate the influence of one such factor: the interaction of nitrogen fertilization and mite feeding on apple leaf physiology. Only after evaluating such relationships is it possible to develop meaningful action thresholds for mite control in the field.

Literature Cited

Ames, G.K., D.T. Johnson, and R.C. Rom. 1984. The effect of European red mite feeding on the fruit quality of `Miller Sturdeespur' apple. J. Amer. Soc. Hort. Sci. 109:834-837.

Avery, D.J. 1964. Carbon dioxide exchange by plum and apple leaves damaged by fruit tree red spider mite. Rpt. E. Malling Res. Sta., Maidenstone, Engl. 1963:94-97.

Avery, D.J. and J.B. Briggs. 1968a. The aetiology and development of damage in young fruit trees infested with fruit tree red spider mite, Panonychus ulmi (Koch). Ann. Applied Biol. 61:277-288.

Avery, D.J. and J.B. Briggs. 1968b. Damage to leaves caused by fruit tree red spider mite, Panonychus ulmi (Koch). J. Hort. Sci. 43:463-473.

Avery, D.J. and H.J. Lacey. 1968. Changes in the growth-regulator content of plum infested with the fruit tree red spider mite Panonychus ulmi (Koch). J. Expt. Bot. 19:760-769.

Bailey, P. 1979. Effect of late season populations of two-spotted mite on yield of peach trees. J. Econ. Ent. 72:8-10.

Barnes, M.M. and H.R. Moffit. 1978. A five-year study of the effects of the walnut aphid and the European red mite on Persian walnut productivity in coastal orchards. J. Econ. Ent. 71:71-74.

Beers, E.H. and L.A. Hull. 1987. Effect of European red mite (Acari: Tetranychidae) injury on vegetative growth and flowering of four cultivars of apples. Env. Ent. 16:569-574.

Beers, E.H., L.A. Hull, and J.W. Grimm. 1987. Relationships between leaf:fruit ratio and varying levels of European red mite stress on fruit size and return bloom of apple. J. Amer. Soc. Hort. Sci. 112:608-612.

Briggs, J.B. and D.J. Avery. 1968. Effects of infestation with fruit tree red spider mite, Panonychus ulmi (Koch), on the growth and cropping of young fruit trees. Ann. Applied Biol. 61:269-276.

Cagle, L.R. 1946. Life history of the European red mite. Virginia Agric. Expt. Sta. Tech. Bull. 98.

Chapman, P.J., S.E. Lienk, and O.F. Curtis, Jr. 1952. Responses of apple trees to mite infestations: I. J. Econ. Ent. 45:815-821.

Crassweller, R.M. 1986. Tree Fruit Production Guide 1986. Cooperative Extension Service, The Pennsylvania State University, University Park, Pa.

DeAngelis, J.D., R.E. Berry, and G.W. Krantz. 1983. Evidence for spider mite (Acari: Tetranychidae) injury-induced leaf water deficits and osmotic adjustment in peppermint. Env. Ent. 12:336-339.

DeAngelis, J.D., K.C. Larson, R.E. Berry, and G.W. Krantz. 1982. Effects of spider mite injury on transpiration and leaf water status in peppermint. Env. Ent. 11:975-978.

Ferree, D.C. and F.R. Hall. 1981. Influence of physical stress on photosynthesis and transpiration of apple leaves. J. Amer. Soc. Hort. Sci. 106:348-351.

Ferree, D.C., F.R. Hall, and M.A. Ellis. 1986. Influence of mites and diseases on net photosynthesis of apple leaves, p. 56-62. In: A.N. Lakso and F. Lenz (eds.). The regulation of photosynthesis in fruit trees. 1986. Symp. Proc. Pub., NY State Agr. Expt. Sta., Geneva, NY.

Hall, F.R. and D.C. Ferree. 1975. Influence of twospotted spider mite populations on photosynthesis of apple leaves. J. Econ. Ent. 68:517-520.

Hardman, J.M., H.J. Herbert, and K.H. Sanford. 1985. Effect of populations of the European red mite, Panonychus ulmi, on the apple variety 'Red Delicious' in Nova Scotia. Can. Ent. 117:1257-1265.

Herbert, H.J. and K.P. Butler. 1973. The effect of European red mite, Panonychus ulmi (Acarina: Tetranychidae), infestations on N, P, and K concentrations in apple foliage throughout the season. Can. Ent. 105:263-269.

Hoyt, S.C., L.K. Tanigoshi, and R.W. Browne. 1979. Economic injury level studies in relation to mites on apple, p. 3-11. In: J.C. Rodrigues (ed.) Recent Advances in Acarology, Vol. 1, Academic Press, New York.

Jackson, P.R. and P.E. Hunter. 1983. Effects of nitrogen fertilizer level on development and populations of pecan leaf scorch mite (Acari: Tetranychidae). J. Econ. Ent. 76:432-435.

Johnson, D.L. 1983. On the relationship between the European red mite and apple leaf chlorophyll. J. Ent. Soc. Brit. Columbia 80:42-45.

Klopfenstein, W.G. and R.P. Holdsworth. 1978. The effects of European red mite feeding on the growth and yield of spur-type Delicious apple. Res. Circ. Ohio Agr. Res. Dev. Ctr. 239:35-37.

Lienk, S.E., P.J. Chapman, and O.F. Curtis, Jr. 1956. Responses of apple trees to mite infestations: II. J. Econ. Ent. 49:350-353.

Light, W.I.St.G. and F.A.B. Ludlam. 1972. The effects of fruit tree red spider mite (Panonychus ulmi) on yield of apple trees in Kent. Plant Pathol. 21:175-181.

McClernan, W.A. and R.P. Marini. 1986. European red mite on yield, fruit quality, and growth of peach trees. Hortscience. 21:244-246.

Rodriguez, J.G. 1958. The comparative NPK nutrition of Panonychus ulmi (Koch) and Tetranychus telarius (L.) on apple trees. J. Econ. Ent. 51:369-373.

Sances, F.V., J.A. Wyman, and I.P. Ting. 1979. Morphological responses of strawberry leaves to infestations of twospotted spider mite. J. Econ. Ent. 72:710-713.

Sances, F.V., J.A. Wyman, I.P. Ting, R.A. Van Steenwyk, and E.R. Oatman. 1981. Spider mite interactions with photosynthesis, transpiration and productivity of strawberry. Env. Ent. 10:442-448.

Schaffer, B., J. Pena, S.P. Lara, and D. Buisson. 1986. Net photosynthesis, transpiration, and stomatal conductance of avocado leaves infested by avocado red mites. Proc. Interamer. Soc. Trop. Hort. 30:73-82.

Sparks, D. and D.H. Baker. 1975. Growth and nutrient response of pecan seedlings, Carya illinoensis, to nitrogen levels in sand culture. J. Amer. Soc. Hort. Sci. 100:392-399.

Storms, J.J.H. 1971. Some physiological effects of spider mite infestation on bean plants. Neth. J. Plant Pathol. 77:154-167.

Tanigoshi, L.K. and R.W. Browne. 1981. Coupling the cytological aspects of spider mite feeding to economic injury levels on apple. Protection Ecol. 3:29-40.

van de Vrie, M. and P. Delver. 1979. Nitrogen fertilization of fruit trees and its consequences for the development of Panonychus ulmi populations and the growth of fruit trees, p. 23-30. In: J.G. Rodrigues (ed.) Recent Advances in Acarology, Vol. 1, Academic Press, New York.

Wermelinger, B., J.J. Oertli, and V. Delucchi. 1985. Effect of host plant nitrogen fertilization on the biology of the two-spotted spider mite, Tetranychus urticae. Ent. exp. appl. 38:23-28.

Zwick, R.W., G.J. Fields, and W.M. Mellenthin. 1976. Effects of mite population density on 'Newtown' and 'Golden Delicious' apple tree performance. J. Amer. Soc. Hort. Sci. 101:123-125.

Chapter I

Nitrogen Fertilization Influences the Physiology of Apple Leaves Subjected to European Red Mite Feeding

Abstract. Nitrogen (N) fertilization was evaluated for an effect on the physiology of greenhouse-grown 'Imperial Delicious' apple leaves damaged by European red mite (ERM), *Panonychus ulmi* (Koch). A factorial arrangement of three N levels and three mite levels was used in a completely randomized design. Net photosynthesis (Pn), transpiration (Tr), and visual damage ratings were measured on 0, 5, 12, 21, and 26 days after mite placement (DAMP). Dark respiration (Rd), dark transpiration (dark Tr), relative water content (RWC), specific leaf weight (SLW), leaf nitrogen (LFN), and total chlorophyll (TCHL) were measured at the end of the experiment. Visual damage was observed with 75 mite days (MD), and was consistently greater on the low N treatment. Pn was negatively affected by mite feeding in all three N treatments. However, with equal MD, the high N treatment retained higher Pn than the low or medium N treatment. No mite by nitrogen interactions were found for the remaining physiological measurements. Tr, Rd, LFN, and TCHL increased with N, and were reduced by mite feeding. Mite feeding increased dark Tr at all N levels. RWC was unaffected by N and was reduced by mite feeding. SLW increased with N and MD.

Introduction

The European red mite (ERM), Panonychus ulmi (Koch), is an important foliage-feeding pest on apple. ERM remove cellular contents by direct penetration of mesophyll cells with piercing-sucking mouthparts (Avery and Briggs 1968b). Mite feeding leads to cellular collapse and results in the characteristic bronzing of the foliage. Mite feeding negatively influences photosynthesis (Avery 1964, Avery and Briggs 1968a, Hall and Ferree 1975), transpiration (Ferree and Hall 1981), leaf chlorophyll content (Chapman et al. 1952, Johnson 1983), and leaf nitrogen (Herbert and Butler 1973, Klopfenstein and Holdsworth 1978). The balance of growth-regulating substances was also altered by mite feeding (Avery and Lacey 1968).

Plant response to mite feeding was influenced by experimental conditions (Storms 1971) and plant vigor (Zwick et al. 1976). Summer-grown bean plants were more tolerant of twospotted spider mite (TSM), Tetranychus urticae Koch, feeding than winter-grown plants (Storms 1971). Field experiments measured variable responses to mite feeding, and often mite feeding had no effect on growth of peach (McClernan and Marini 1986) or apple trees (Beers and Hull 1987). Zwick et al. (1976) attributed the lack of a mite effect on the growth and cropping of 'Newtown' and 'Golden Delicious' apple trees to the extreme vigor of the trees studied. Nitrogen (N) treatment influenced the effect of pecan leaf

scorch mite, Eotetranychus hicoriae (McGregor), on photosynthesis of pecan seedlings (Jackson and Hunter 1983). Photosynthetic rates were reduced by mite feeding on low N seedlings, but high N seedlings exhibited increased photosynthetic rates following mite feeding.

If vigorous trees are indeed more tolerant of mite feeding, action thresholds for mite control could be modified, and control costs reduced. This study was designed to evaluate the influence of different N treatments on the physiology of 'Delicious' leaves subjected to ERM feeding.

Materials and Methods

'Imperial Delicious'/MM.111 trees were planted in 3.7 liter containers on 13 April 1988, and grown in a greenhouse under natural light. The medium consisted of equal parts of peat, perlite, and vermiculite. Trees were headed approximately 5 cm above the bud union at planting, and one vigorous shoot per plant was allowed to develop. Endosulfan (Thiodan 50W) and methomyl (Lannate 50W) were used as needed at 1.2 and 2.4 $g \cdot liter^{-1}$ formulation, respectively, to control aphid and whitefly. Carbaryl (Sevin 50W) was applied at 2.4 $g \cdot liter^{-1}$ formulation before mite placement to control mite predators. Micronutrients were applied to saturation at planting as a soluble trace element mix (S.T.E.M.) at 600 $mg \cdot liter^{-1}$. $MgSO_4$, at 12.5 $g \cdot liter^{-1}$, was applied foliarly 4 weeks after planting.

Eighteen uniform trees were chosen for the study. The experimental design was completely randomized with a factorial arrangement of three N levels and three mite levels. Each tree was randomly assigned either a low, medium, or high N treatment to provide six trees per N treatment. Nitrogen treatments were supplied by adding 400 ml per plant of ammonium nitrate solutions at 0 (low), 150 (medium), or 300 (high) $mg \cdot liter^{-1}$. These solutions were applied weekly, for a total of five weeks. Therefore, each tree received 0, 20, or 40 mg N/tree/week, respectively. Phosphoric acid (H_3PO_4) and potassium chloride (KCL), at 50 and 120

$mg \cdot liter^{-1}$ respectively, were applied in the solutions with the N treatments.

On 1 June, three uniform, fully-expanded leaves per tree were randomly assigned either a control, low, or high mite treatment providing six single-leaf replicates of each mite level per nitrogen treatment. Initial mite treatments consisted of 0, 15, and 50 mites per leaf. Mites were allowed to develop naturally, and were counted every 3 days. Mite populations were recorded as mite days. Mite days (MD) were calculated by averaging the number of mites counted on two consecutive dates, and multiplying by the days between the counts. Due to variation in leaf size between N treatments, mite populations were periodically adjusted to keep mites per unit leaf area equal among N treatments.

At 0, 5, 12, 21, and 26 days after mite placement (DAMP), net photosynthesis (Pn) of each leaf was measured in the laboratory with an Anarad model Ar-600 infrared gas analyzer. The chamber was a modification of the chamber described by Syvertsen and Smith (1983), without a heat exchanger. Fans were used to negate boundary layer resistance. Light levels were maintained at 1050 $\mu mols\ m^{-2}s^{-1}$, and the air flow rate into the chamber was 0.05 liters s^{-1}. Air temperature in the chamber was 28 ± 1C and relative humidity was 60 ± 5%. Transpiration (Tr) was measured simultaneously with a General Eastern model 1100 dew point hygrometer. Dark respiration (Rd) and dark transpiration

(dark Tr) were measured 26 DAMP with the same system in the dark. Rd and dark Tr were measured in the afternoon, and the trees were placed in darkness for two hours before measurement. Each leaf was rated for visual damage of mite feeding before each photosynthetic measurement. Ratings were based on a scale of 1 to 8, where 1 represented no damage and 8 represented severe bronzing.

On 28 June (26 DAMP), leaves were harvested, weighed, and area was measured with a LI-COR (LI-3000) portable leaf area meter. Two leaf discs (1.2 cm^2 total) were sampled from each side of the midrib, and leaf chlorophyll content (TCHL) was determined by acetone extraction following the method of Arnon (1949), as modified by Marini (1986). The leaves were dried at 60C for 72 hr, and dry weights were recorded. Leaf N content (LFN) was determined by modified Kjeldahl analysis as described by Bremner and Breitenbeck (1983). Relative water content (RWC) of the leaf was determined by subtracting the dry weight from the fresh weight and dividing by the fresh weight.

Results and Discussion

Cumulative mite days (MD) for the low and high mite treatments had a sigmoidal increase with time (Fig. 1). Control leaves accumulated less than 50 MD by the completion of the experiment. Variation about the means increased on the later measurement dates due to mite population differences among the N treatments. Expression of mite population density on an area basis (MD cm^{-2} leaf area) did not improve data analysis; therefore, all data analysis was performed on a MD per leaf basis. On day 26, the low and high populations equaled approximately 980 and 2200 MD, respectively.

Net photosynthesis was reduced by mite feeding in all three N treatments. Since there was a significant mite by N interaction, Pn was regressed on cumulative MD per leaf for each N level. Pn declined in a quadratic manner with increasing MD in the low and medium N treatments (Fig. 2). Mite day accumulations above 2000 for the low and medium N treatments reduced Pn 50 and 34%, respectively. Additional MD accumulations caused no further reduction in Pn (Fig. 2). A negative linear relationship was found between Pn and MD in the high N treatment (Fig. 2). Accumulation of 2200 MD resulted in a 34% reduction in Pn. Following equal MD, high N leaves maintained greater Pn rates than low N leaves (Fig. 2). This resulted from a higher initial Pn rate and less of a decline in Pn following mite damage.

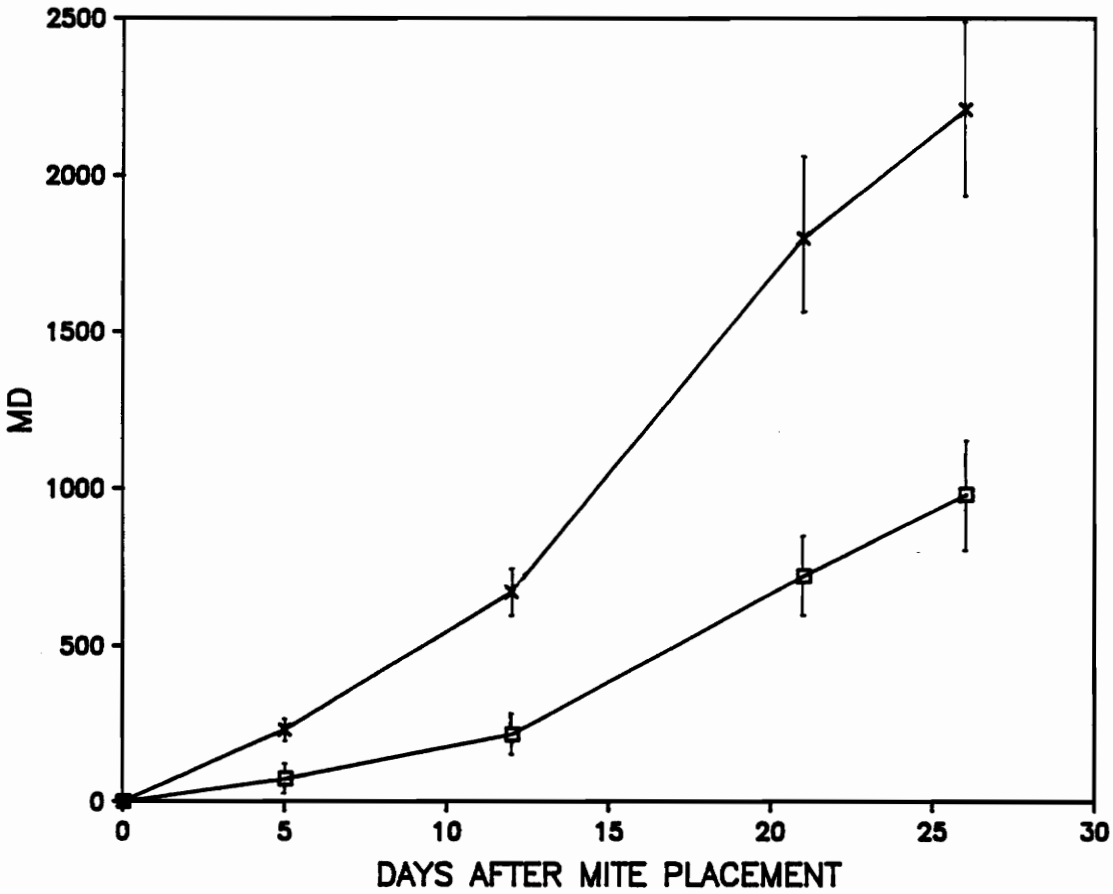
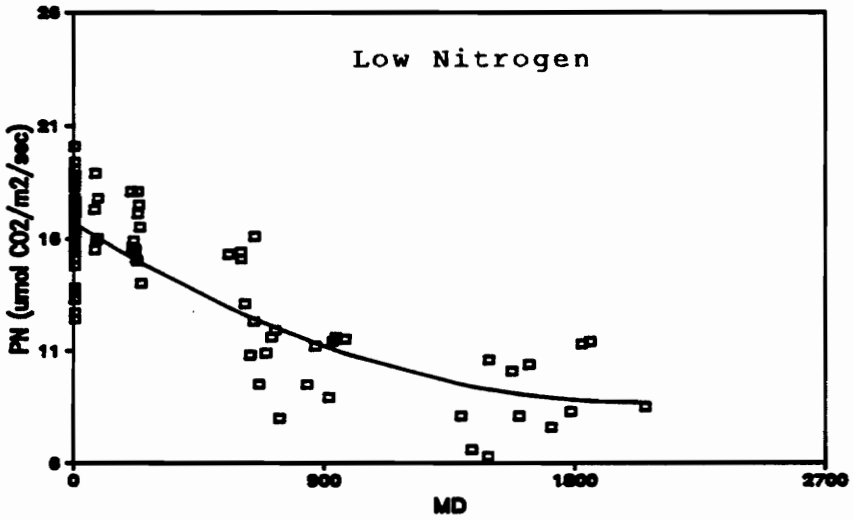
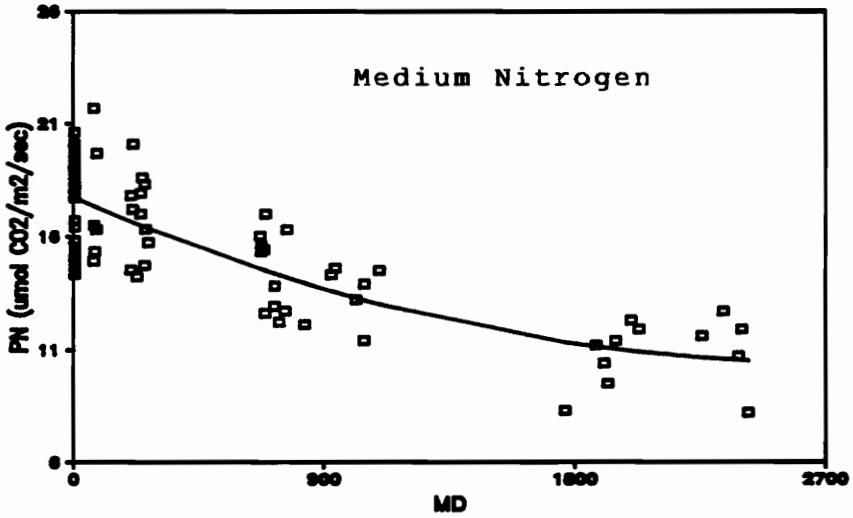
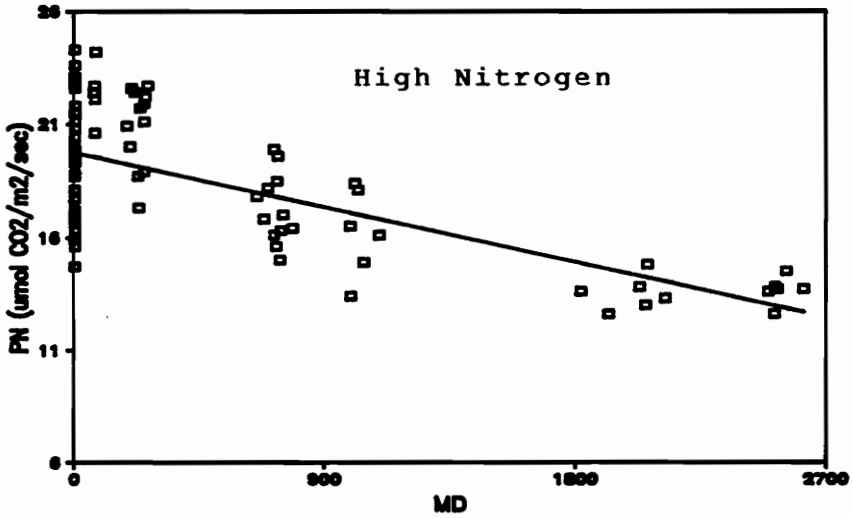


Fig. 1. Cumulative mite days per leaf (MD) for the low (□) and high (x) mite treatments. Each point represents the average of the 3 nitrogen treatments on each measurement date, and the bars represent the standard deviation of the mean.

Fig. 2. The relationship between net photosynthesis (Pn) and cumulative mite days per leaf (MD) for 'Imperial Delicious' apple leaves grown at 3 nitrogen levels. Low nitrogen: $Pn = 16.7 - 0.008\ MD + 1.9x10^{-6}\ MD^2$, $R^2 = 0.68$, Medium nitrogen: $Pn = 17.7 - 0.005\ MD + 1.0x10^{-6}\ MD^2$, $R^2 = 0.62$, High nitrogen: $Pn = 19.7 - 0.003\ MD$, $R^2 = 0.44$.


Low Nitrogen
PN (umol CO2/m2/sec)
26
21
16
11
6
0
900
1800
2700
MD


Medium Nitrogen
PN (umol CO2/m2/sec)
26
21
16
11
6
0
900
1800
2700
MD


High Nitrogen
PN (umol CO2/m2/sec)
26
21
16
11
6
0
900
1800
2700
MD

Net photosynthesis measurements were terminated 26 days after mite placement because low N trees were unable to support large mite populations, while populations continued to increase on the medium and high N trees. Mite population differences were probably related to leaf N content. Previous researchers reported a positive relationship between leaf N content and mite populations in pecan (Jackson and Hunter 1983).

Fluctuations in Pn from one measurement date to another, and aging of the test leaves contributed to the variation in the data, and resulted in relatively poor coefficients of determination. To explain this variation, multiple regression was performed with first- and second-order components of days after mite placement and MD included as independent variables. This technique resulted in improved models for all N treatments (Table 1).

Net photosynthesis and ERM feeding were negatively related for apple and plum (Avery 1964, Avery and Briggs 1968a). Hall and Ferree (1975) measured a 26% reduction in Pn following 135 MD of TSM on container-grown 'Franklin' apple trees in the winter. Due to differences in experimental design and data analysis, direct comparison of our data with those of Hall and Ferree (1975) is difficult; however, in the high N treatment 1800 MD were required to achieve a 26% reduction in Pn. The two experiments differed in the mite species studied (TSM versus ERM), and the time of year

Table 1. Multiple regression equations of net photosynthesis (Pn) on days after mite placement (DAMP) and mite days (MD) for 'Imperial Delicious' apple trees grown at 3 nitrogen levels.

Nitrogen Level (mg N/tree/week)	Regression equation[z]	R^2
0	$Pn = 17.4 - .01\ DAMP^2 - .006\ MD + 1.8x10^{-6}\ MD^2$	.78
20	$Pn = 18.8 - .14\ DAMP - .003\ MD + 5.0x10^{-7}\ MD^2$	.76
40	$Pn = 20.0 + .19\ DAMP - .01\ DAMP^2 - .002\ MD$	.63

[z]Regression equations were significant at $P < .0001$.

the experiment was conducted (winter versus spring).

Following pecan leaf scorch mite feeding, Pn was reduced in pecan seedlings fertilized with 200 $mg \cdot liter^{-1}$ N, but seedlings receiving 600 $mg \cdot liter^{-1}$ N responded positively to mite feeding (Jackson and Hunter 1983). The positive response to mite feeding was attributed to the removal of "excess" N by the mites. Direct comparison with our results is difficult due to the lack of methods presented by Jackson and Hunter, however, we found reductions in Pn at all three N levels (Fig. 2). The 600 $mg \cdot liter^{-1}$ N level used by Jackson and Hunter (1983) resulted in leaf N concentrations of 4% or greater. These seedlings had low Pn rates (<10 mg CO_2 dm^{-2} hr^{-1}), and reduced growth compared to the other N treatments. Sparks and Baker (1975) found N toxicity symptoms in pecan seedlings with leaf N concentrations above 3.4%.

Visual damage ratings were negatively related to N level (Table 2). Visual damage was first observed on the low N treatment, and this treatment had consistently higher ratings (more damage) throughout the experiment. Visual damage ratings were positively related to mite level (Table 3). Visual damage was detected five days after mite placement, corresponding to 75 MD. These results agree with the findings of Avery (1964) for ERM on apple. Hall and Ferree (1975), in contrast, were unable to detect visual damage at 540 MD with TSM on apple. Differences in feeding patterns

Table 2. The influence of nitrogen fertilization on European red mite damage ratings (Rating) and transpiration (Tr) of 'Imperial Delicious' apple leaves.

Nitrogen Level (mg N/tree/week)	Days after mite placement				
	0	5	12	21	26
			Rating[z]		
0	1.0 a[y]	2.4 b	3.3 b	4.6 c	4.9 c
20	1.0 a	2.2 a	3.0 a	4.2 b	4.6 b
40	1.0 a	2.0 a	2.9 a	3.9 a	4.3 a
			Tr (mmol $H_2O/m^2/s$)		
0	3.6 a	2.9 a	2.3 a	2.6 a	2.3 a
20	3.6 a	2.9 a	2.4 a	2.6 a	2.4 a
40	3.9 b	3.5 b	2.7 b	3.0 b	2.8 b

[y]Separation of main effect means within column and parameter by Duncan's multiple range test, 5% level.

[z]Leaves rated as 1 = undamaged to 8 = severely bronzed.

between mite species used in these experiments probably contributed to the conflicting results.

No interaction was found between N and mite level for Tr; therefore, only main effects are presented. Tr was greatest in the high N treatment on all measurement dates (Table 2). Transpiration was reduced 10 and 12% by 1800 and 2200 MD, respectively, on the final two measurement dates (Table 3). Previous studies measured an inconsistent mite effect on Tr of apple (Ferree et al. 1986, Ferree and Hall 1981), but these studies were conducted with TSM. The effect of ERM feeding on Tr of apple has not previously been investigated.

Since there were no interactions between N level and mite level for the parameters measured at the conclusion of the experiment, main effect means for several variables are presented in Table 4. Dark respiration increased with N, and decreased by 33% following 2200 MD (Table 4). We are unaware of other studies relating mite feeding and Rd. Reductions in Rd probably resulted from removal of cellular contents through mite feeding; directly removing the enzymes, and substrate necessary for Rd function.

Transpiration in the dark (dark Tr) was unaffected by N treatment, but there was a 4 to 5-fold increase in water loss over the control in both the low and high mite treatments (Table 4). Leaf water potential was not measured, but relative water content (RWC) of the leaf was reduced 4 and

Table 3. The influence of European red mite population level on visual damage rating and transpiration (Tr) of 'Imperial Delicious' apple leaves at 0, 5, 12, 21, and 26 days after mite placement (DAMP).

Mite Level	Mite Days	Rating[z]	Tr (mmol $H_2O/m^2/s$)
		0 DAMP	
Control	0 a[y]	1.0 a	3.8 a
Low	0 a	1.0 a	3.7 a
High	0 a	1.0 a	3.6 a
		5 DAMP	
Control	0 a	1.0 a	3.3 a
Low	74 b	2.0 b	3.1 a
High	244 c	3.5 c	3.0 a
		12 DAMP	
Control	0 a	1.0 a	2.6 a
Low	215 b	3.0 b	2.4 a
High	670 c	5.2 c	2.4 a
		21 DAMP	
Control	0 a	1.0 a	2.9 b
Low	720 b	4.7 b	2.8 b
High	1800 c	6.9 c	2.6 a
		26 DAMP	
Control	0 a	1.0 a	2.6 b
Low	980 b	5.4 b	2.6 b
High	2200 c	7.4 c	2.3 a

[y]Separation of main effect means within column and date by Duncan's multiple range test, 5% level.

[z]Leaves rated as 1 = undamaged to 8 = severely bronzed.

6% for the low and high mite treatments, respectively (Table 4).

DeAngelis et al. (1982) measured increased water loss in the dark (dark Tr) on peppermint leaves infested with TSM. Infested leaves lost up to 3.5 times more water than control leaves. Their data suggested that increased water loss was not a result of altered stomatal function in the dark. Instead, water loss was attributed to increased astomatal, or cuticular transpiration rate resulting from mechanical damage to the cuticle, and disruption of epidermal cells. Histological studies demonstrated that ERM feeding did not damage epidermal cells (Avery and Briggs 1968b). Therefore, the increased dark Tr measured in our study was probably due to cuticular damage alone.

Specific leaf weight (SLW) increased with increasing N and MD (Table 4). The increased SLW associated with N was probably due to an influence on the leaf morphology (eg. increase in cell number, cell size, or structural carbohydrates), or an increase in soluble sugars or starch. We have no explanation for the increased SLW following mite feeding except as an increase in dry weight resulting from a wound response to mite feeding.

Previous research demonstrated a highly significant relationship between SLW and Pn for young apple leaves (Marini and Barden 1981). However, following mite feeding, we found no correlation ($P>0.05$) between SLW and Pn. The use of

Table 4. The effect of nitrogen and European red mite treatments on dark respiration (Rd), dark transpiration (dark Tr), relative water content (RWC), specific leaf weight (SLW), total chlorophyll (TCHL), and leaf nitrogen (LFN) of 'Imperial Delicious' apple leaves.

Nitrogen level		Rd	dark Tr	RWC	SLW	TCHL	LFN	
(mg N/tree/wk)		(umol/m²/s)	(mmol/m²/s)	(%)	(mg/cm²)	(ug/cm²)	(mg N/cm²)	(%)
0		.25 a[z]	.10 a	67.7 a	5.6 a	21 a	.10 a	1.8 a
20		.26 a	.09 a	67.7 a	6.0 b	23 a	.13 b	2.3 b
40		.33 b	.12 a	68.1 b	6.1 b	30 b	.16 c	2.7 c
Mite Level	MD							
Control	0	.32 b	.03 a	70.2 c	5.6 a	28 c	.14 b	2.5 c
Low	980	.30 b	.13 b	67.4 b	6.0 b	25 b	.13 b	2.2 b
High	2200	.21 a	.15 b	65.9 a	6.1 b	22 a	.12 a	2.0 a

[z]Separation of main effect means within column and variable by Duncan's multiple range test, 5% level.

SLW to characterize previous light conditions or to estimate Pn potential may be complicated by mite feeding.

Total chlorophyll was positively related to N, but mite levels of 980 and 2200 MD reduced TCHL by 11 and 21%, respectively, compared to the controls (Table 4). Chapman et al. (1952) measured 30% reductions in TCHL of 'Delicious' leaves following ERM feeding. TCHL has often been used as the only measure of physiological damage in apple leaves due to mite feeding (Chapman et al. 1952, Zwick et al. 1976), however, we found poor relationships between TCHL and gas exchange characteristics of the leaf (data not shown).

Johnson (1983) found no correlation between ERM population and TCHL ($mg \cdot g^{-1}$ dry weight) of field-grown 'Delicious' and 'McIntosh' apple trees. Yet, we found significant ($P<0.05$) negative linear relationships between ERM populations and TCHL, on a $mg \cdot g^{-1}$ dry weight basis (data not shown). The contrasting results may be due to morphological differences between field and greenhouse-grown leaves. Increased leaf thickness in the field could limit exploitation of the leaf due to physical limitations of the mite mouthparts (Avery and Briggs 1968b), thus resulting in a weak relationship between mites and chlorophyll.

Leaf nitrogen content ($mg \cdot cm^{-2}$) increased with N treatment, but was reduced 14% by 2200 MD (Table 4). LFN (%) followed the same trend with a 20% reduction by 2200 MD (Table 4). Reductions in LFN (%) of up to 25% were reported for

'Delicious' apple leaves subjected to ERM feeding (Herbert and Butler 1973, Klopfenstein and Holdsworth 1978).

Mite feeding clearly has a negative influence on Pn of apple leaves, yet the mechanism of Pn reduction remains unclear. A multiple regression was performed for Pn, with first- and second-order terms of LFN (area basis), RWC, Tr, and TCHL included as independent variables. The analysis indicated that the linear model including LFN, and RWC was superior to all other variable combinations for Pn determination (R^2=0.61). TCHL and Tr did not add significant information to the model in the presence of LFN and RWC. Therefore, for our study, LFN and RWC were the best criteria for evaluation of Pn.

Our results indicate that the existing action threshold levels for ERM control in apple should be modified to account for nitrogen concentration of the tree. An increase in leaf nitrogen concentration from 2% to 3% resulted in a 40% higher Pn rate following equal MD. Action thresholds vary according to state, time of year, mite/predator ratios, and environmental conditions; however, thresholds should be increased on high N trees. These results are based on container-grown trees, and comparisons to the field situation are made with caution; however, preliminary studies indicate that field-grown trees may be more tolerant of mite feeding than greenhouse-grown trees.

Literature Cited

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol. 24:1-15.

Avery, D.J. 1964. Carbon dioxide exchange by plum and apple leaves damaged by fruit tree red spider mite. Rpt. E. Malling Res. Sta., Maidenstone, Engl. 1963:94-97.

Avery, D.J. and J.B. Briggs. 1968a. The aetiology and development of damage in young fruit trees infested with fruit tree red spider mite, Panonychus ulmi (Koch). Ann. Applied Biol. 61:277-288.

Avery, D.J. and J.B. Briggs. 1968b. Damage to leaves caused by fruit tree red spider mite, Panonychus ulmi (Koch). J. Hort. Sci. 43:463-473.

Avery, D.J. and H.J. Lacey. 1968. Changes in the growth-regulator content of plum infested with the fruit tree red spider mite Panonychus ulmi (Koch). J. Expt. Bot. 19:760-769.

Beers, E.H. and L.A. Hull. 1987. Effect of European red mite (Acari: Tetranychidae) injury on vegetative growth and flowering of four cultivars of apples. Env. Ent. 16:569-574.

Bremner, J.M. and G.A. Breitenbeck. 1983. A simple method for determination of ammonium in semimicro-Kjeldahl analysis of soils and plant materials using a block digester. Commun. In Soil Sci. Plant Anal. 14(10):905-913.

Chapman, P.J., S.E. Lienk, and O.F. Curtis, Jr. 1952. Responses of apple trees to mite infestations: I. J. Econ. Ent. 45:815-821.

DeAngelis, J.D., K.C. Larson, R.E. Berry, and G.W. Krantz. 1982. Effects of spider mite injury of transpiration and leaf water status in peppermint. Env. Ent. 11(4):975-978.

Ferree, D.C. and F.R. Hall. 1981. Influence of physical stress on photosynthesis and transpiration of apple leaves. J. Amer. Soc. Hort. Sci. 106(3):348-351.

Ferree, D.C., F.R. Hall, and M.A. Ellis. 1986. Influence of mites and diseases on net photosynthesis of apple leaves, p56-62. In: A.N. Lakso and F. Lenz (eds.), The regulation of photosynthesis in fruit trees. 1986. Symp. Proc. Pub. NY State Agr. Expt. Sta., Geneva, NY.

Hall, F.R. and D.C. Ferree. 1975. Influence of twospotted spider mite populations on photosynthesis of apple leaves. J. Econ. Ent. 68(4):517-520.

Herbert, H.J. and K.P. Butler. 1973. The effect of European red mite, Panonychus ulmi (Acarina: Tetranychidae), infestations on N, P, and K concentrations in apple foliage throughout the season. Can. Ent. 105:263-269.

Jackson, P.R. and P.E. Hunter. 1983. Effects of nitrogen fertilizer level on development and populations of pecan leaf scorch mite (Acari:Tetranychidae). J. Econ. Ent. 76:432-435.

Johnson, D.L. 1983. On the relationship between the European red mite and apple leaf chlorophyll. J. Ent. Soc. Brit. Columbia 80:42-45.

Klopfenstein, W.G. and R.P. Holdsworth. 1978. The effects of European red mite feeding on the growth and yield of spur-type Delicious apple. Res. Circ. Ohio Agr. Res. Dev. Ctr. 239:35-37.

Marini, R.P. 1986. Do net gas exchange rates of green and red peach leaves differ? Hortscience 21(1):118-120.

Marini, R.P. and J.A. Barden. 1981. Seasonal correlations of specific leaf weight to net photosynthesis and dark respiration of apple leaves. Photosynthesis Res. 2:251-258.

McClernan, W.A. and R.P. Marini. 1986. European red mite on yield, fruit quality, and growth of peach trees. Hortscience. 21(2):244-246.

Sparks, D. and D.H. Baker. 1975. Growth and nutrient response of pecan seedlings, Carya illinoiensis, to nitrogen levels in sand culture. J. Amer. Soc. Hort. Sci. 100:392-399.

Storms, J.J.H. 1971. Some physiological effects of spider mite infestation on bean plants. Neth. J. Plant Pathol. 77:154-167.

Syvertsen, J.P. and M.L. Smith. 1983. An inexpensive leaf chamber for measuring net gas exchange. HortScience 18:700-701.

Zwick, R.W., G.J. Fields, and W.M. Mellenthin. 1976. Effects of mite population density on 'Newtown' and 'Golden Delicious' apple tree performance. J. Amer. Soc. Hort. Sci. 101:123-125.

Chapter II

Physiological Recovery of Apple Leaves Following European Red Mite Damage: Evaluation of Foliar Urea Application As a Means of Inducing Recovery

Abstract. Forty two-year-old 'Redchief Delicious'/MM.111 apple trees growing on the Virginia Tech Horticulture farm were used to evaluate physiological recovery of leaves following mite injury. A factorial combination of two mite levels (mites/no mites) and two urea levels (urea/no urea) were used in a randomized complete block design. Net photosynthesis (Pn), transpiration (Tr), and diffusive resistance (Rs) were measured periodically until 28 days after mite removal (DAMR). Relative water content (RWC), specific leaf weight (SLW), leaf nitrogen (LFN), and total chlorophyll (TCHL), were measured at 28 DAMR. Foliar urea application did not induce recovery of any measured parameters. Pn declined quadratically with mite feeding, and leaves subjected to greater than 4000 MD demonstrated recovery. Tr and Rs were negatively and positively related to mite feeding, respectively, and there was no evidence of recovery. SLW, LFN, TCHL, and RWC declined linearly with mite feeding.

Introduction

Previous research has demonstrated the negative effect of mite feeding on apple leaves, including reductions in net photosynthesis (Pn) (Avery 1964, Avery and Briggs 1968a, Hall and Ferree 1975), transpiration (Tr) (Ferree and Hall 1981), total chlorophyll content (TCHL) (Chapman et al. 1952, Zwick et al. 1976), and nitrogen content (LFN) (Herbert and Butler 1973, Klopfenstein and Holdsworth 1978). However, recovery of physiological function in apple leaves (following mite removal) has not been investigated adequately. Net gas exchange of citrus leaves recovered upon removal of damaging populations of citrus red mite, Panonychus citri (McGregor) (Syvertsen et al. 1986). Sances et al. (1981) reported increased Pn and Tr in strawberry plants following reductions in high populations of twospotted spider mite (TSM), Tetranychus urticae Koch. Chapman et al. (1952) reported visual recovery of green color in 'Delicious' apple leaves following removal of high populations of European red mite (ERM), Panonychus ulmi (Koch).

Severe feeding damage often occurs in apple orchards before mites are controlled. Management decisions could be impacted if mite damaged leaves were capable of physiological recovery. The objective of this study was to evaluate the recovery potential of Pn, Tr, TCHL, and LFN in 'Delicious' apple leaves following removal of high densities of ERM. Also, because nitrogen is reduced by mite feeding,

foliar urea application was evaluated as a practical method to induce or advance recovery.

Materials and Methods

Two-year-old 'Redchief Delicious'/MM.111 trees, growing on the Virginia Tech Horticulture Farm, Blacksburg, Va., were selected. Trees were spaced 2 X 3 m in a solid block (4 rows of 10 trees each). On 25 February 1988, the trees were headed to approximately 30 cm above the bud union to encourage vigorous, uniform growth. Two uniform shoots were selected, and allowed to develop; other shoots were removed.

Endosulfan (Thiodan 50W) was applied as needed at 1.2 $g \cdot liter^{-1}$ formulation to control aphids. Carbaryl (Sevin 50W) was applied as needed at 2.4 $g \cdot liter^{-1}$ formulation to control Japanese beetle and eliminate mite predators. On 23 May, cyhexatin (Plictran 50W) was applied at 450 $mg \cdot liter^{-1}$ formulation to all of the trees to assure they remained mite-free until mite treatments commenced.

The experimental design was a randomized complete block with a factorial arrangement of treatments consisting of two urea treatments (urea/no urea) and two mite levels (mites/no mites). Each tree within a block was randomly assigned to a treatment combination to provide 10 replicates per combination.

On 3 July, 'Delicious' apple spurs infested with ERM were placed in the mite treatment trees. Control trees received biweekly cyhexatin applications at 450 $mg \cdot liter^{-1}$ to protect against contaminant mites. Mite populations were allowed to develop naturally, and were counted weekly on one

test leaf per tree. Mite populations were recorded as mite days per leaf. Mite days (MD) were calculated by averaging the number of mites counted on consecutive dates, and multiplying by the days between the counts. All mites were killed (cyhexatin resistance was not observed) with a cyhexatin application (450 $mg \cdot liter^{-1}$) on 18 August. Urea treatment consisted of two foliar applications of urea at 6 g/liter (2.7 g N $liter^{-1}$), applied on 19, and 27 August.

Net photosynthesis was measured 0, 7, 21, and 28 days after mite removal (DAMR) with an ADC Model LCA-2 portable infrared gas analyzer (Analytical Development Corp., Hoddesdon, U.K.). Transpiration and Rs were measured 7, 21, and 28 DAMR with a LICOR Model LI-1600 steady state porometer (LI-COR, Lincoln, Nebraska).

At dawn on 16 September (29 DAMR), leaves were harvested, weighed, and the areas were measured with a LI-COR Model LI-3000 portable leaf area meter (LI-COR, Lincoln, Nebraska). Two leaf discs (1.2 cm^2 total area) were sampled from each side of the midrib, and TCHL was determined by acetone extraction following the method of Arnon (1949), as modified by Marini (1986). The leaves were dried at 60C for 72 hr, and dry weights were recorded. Leaf nitrogen content was determined by modified Kjeldahl analysis as described by Bremner and Breitenbeck (1983). Relative water content was determined by subtracting the dry weight from the fresh weight and dividing by the fresh weight.

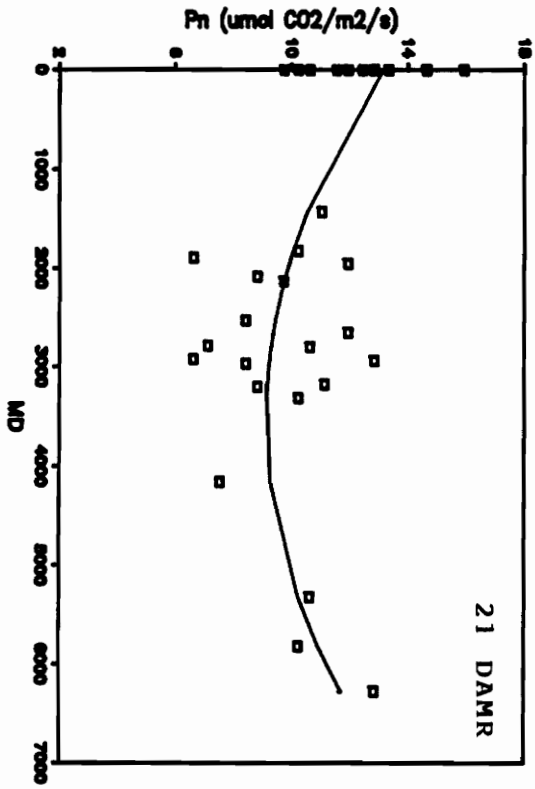
Analysis of variance (ANOVA) was performed to test the significance ($P<0.05$) of main effects and interactions on each measurement date. Regression analysis was performed for each variable, using MD as the independent variable according to the ANOVA results.

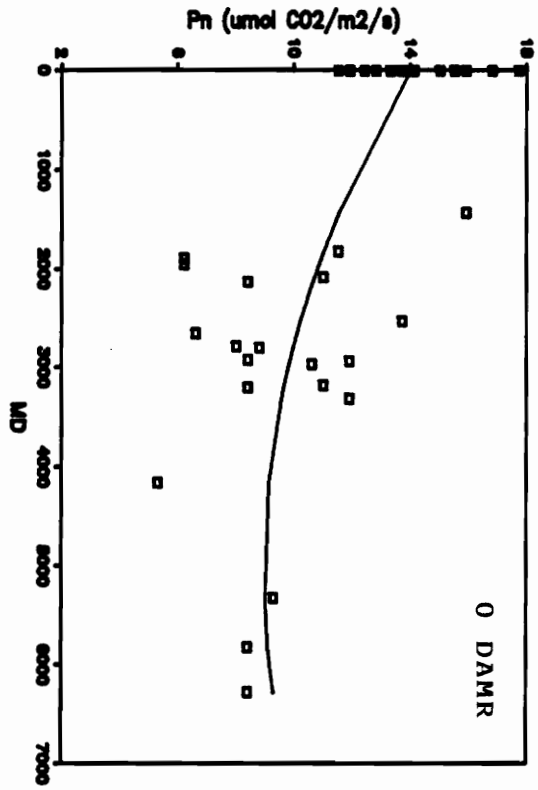
Results and Discussion

Analysis of variance indicated no urea effect or mite by urea interaction for Pn, Tr, or Rs; therefore, the data were pooled and regression analysis performed on these parameters by measurement date. Negative quadratic relationships were found between Pn and MD on all four measurement dates (Fig. 1), but the relationship changed with time. Pn of leaves with greater than 4000 MD increased on the later measurement dates (Fig. 1). Leaves subjected to appreciably fewer MD (1500-3000 MD) demonstrated considerable variation, but had no apparent recovery in Pn. We have no explanation for this phenomenon.

The negative effect of mite feeding on Pn of apple leaves is well documented (Avery 1964, Hall and Ferree 1975). We are unaware, however, of any investigation of photosynthetic recovery in apple leaves following mite removal. Youngman and Barnes (1986) were unable to detect recovery in gas exchange rates following removal of several mite species on almond leaves. Citrus leaves, however, demonstrated recovery in gas exchange rates upon removal of citrus red mite populations (Syvertsen et al. 1986). The discrepancy between the two studies probably relates to physiological differences between citrus and almond leaves. Sances et al. (1981) measured photosynthetic recovery in strawberry plants in the field approximately 45 days after drastic reductions in TSM population densities. Severely damaged plants rapidly

Fig. 1. The relationship between net photosynthesis (Pn) and cumulative mite days per leaf (MD) for 'Redchief Delicious' apple leaves at 0, 7, 21, and 28 days after mite removal (DAMR). 0 DAMR: $Pn = 13.97 - 0.002\ MD + 2.3x10^{-7}\ MD^2$, $R^2 = 0.55$, 7 DAMR: $Pn = 12.8 - 0.003\ MD + 3.3x10^{-7}\ MD^2$, $R^2 = 0.50$, 21 DAMR: $Pn = 13.1 - 0.0023\ MD + 3.3x10^{-7}\ MD^2$, $R^2 = 0.50$, 28 DAMR: $Pn = 13.0 - 0.0022\ MD + 3.8x10^{-7}\ MD^2$, $R^2 = 0.31$.


Pn (umol CO2/m2/s)
0 DAMR
MD
0
1000
2000
3000
4000
5000
6000
7000
2
6
10
14
18


Pn (umol CO2/m2/s)
21 DAMR
MD
0
1000
2000
3000
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7000
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6
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14
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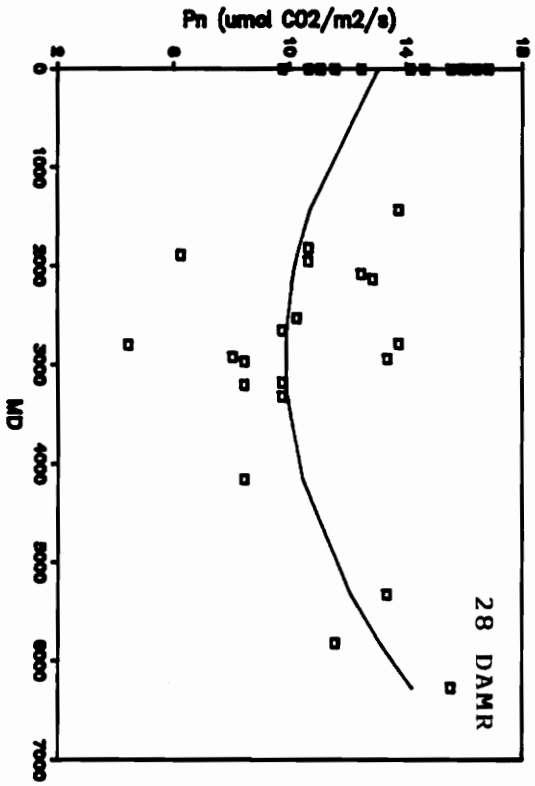
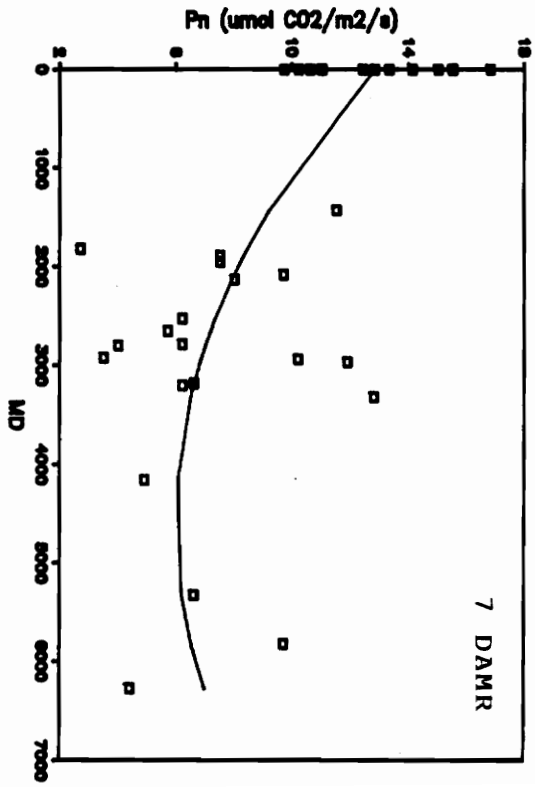

Pn (umol CO2/m2/s)
7 DAMR
MD
0
1000
2000
3000
4000
5000
6000
7000
2
6
10
14
18

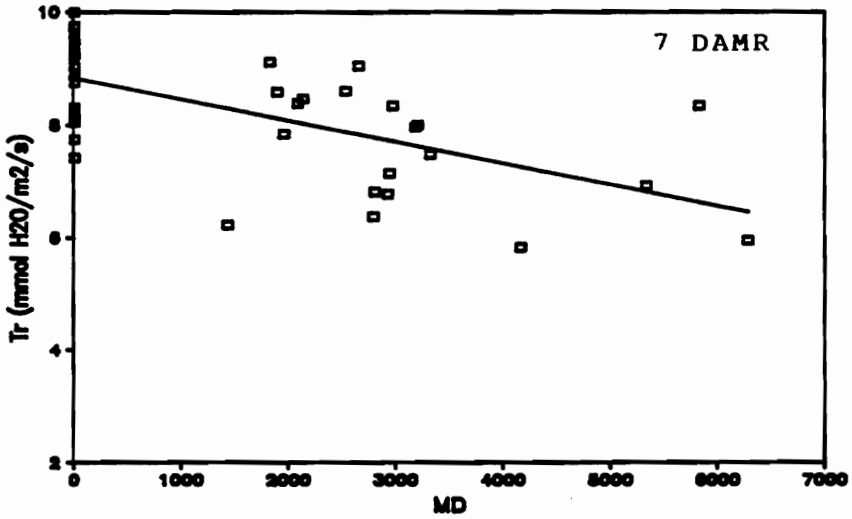

Pn (umol CO2/m2/s)
28 DAMR
MD
0
1000
2000
3000
4000
5000
6000
7000
2
6
10
14
18

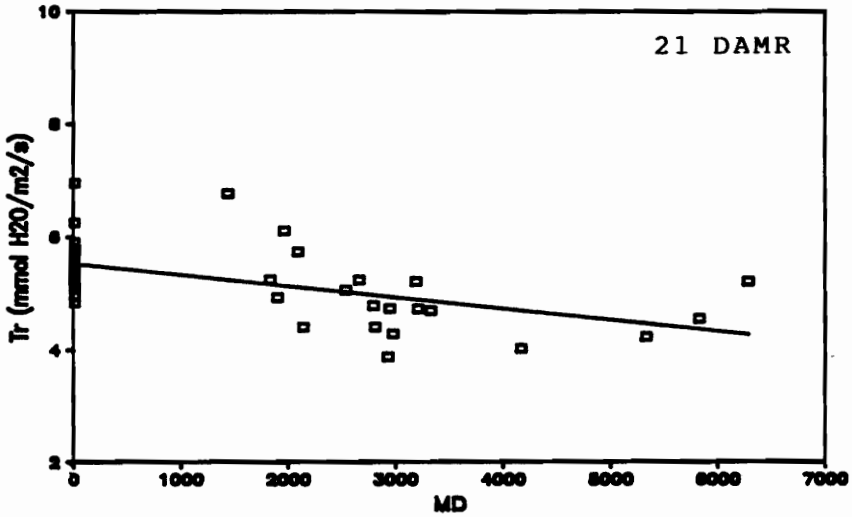
produced new leaves when TSM populations declined, and because Pn was measured on the whole plant, recovery could have resulted from the contribution of the new leaves. Individual leaf recovery was not evaluated. Plants subjected to lower densities of mites did not rapidly produce new foliage when mite populations declined, and no recovery was measured. Therefore, actual photosynthetic recovery on individual leaves probably did not occur.

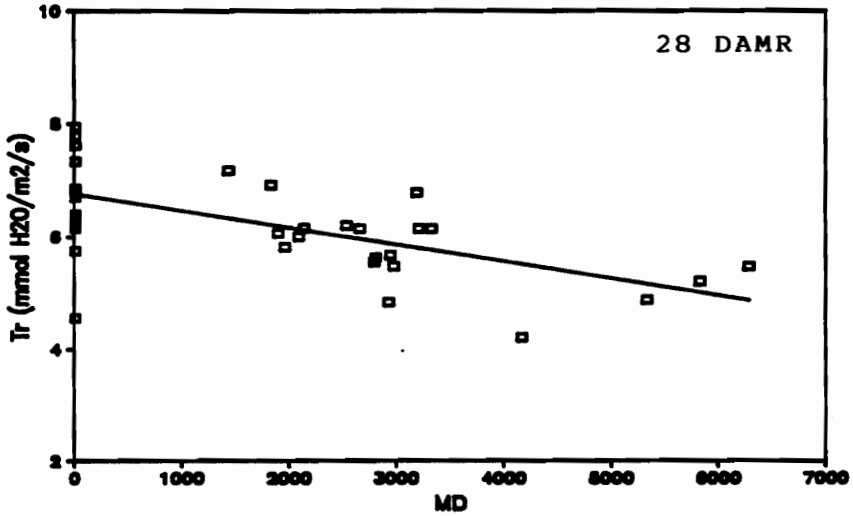
Negative linear relationships were found between Tr and MD on 7, 21, and 28 DAMR (Fig. 2). Tr of both control and mite injured leaves was greatest 7 DAMR, resulting in a significantly ($P<0.05$) greater intercept on this date. This difference can be attributed to daily variation in Tr. The Tr response to mite feeding was quite variable on all three measurement dates (Fig. 2), yielding poor coefficients of determination. The slopes of the regression equations were statistically equivalent on successive measurement dates, indicating no recovery in Tr up to 28 DAMR.

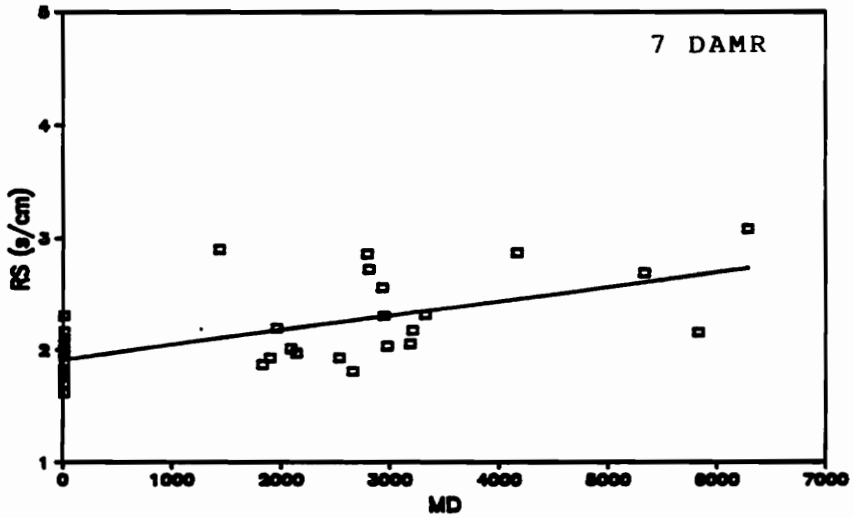
Positive linear relationships were found between Rs and MD on 7, 21, and 28 DAMR (Fig. 3). The regression line intercept was significantly ($P<0.05$) lower 7 DAMR, which concurred with the Tr response on this date. As with Tr, there was large variation in the Rs response to mite feeding on each measurement date. Regression line slopes were equivalent on successive dates, indicating no recovery in Rs by 28 DAMR.

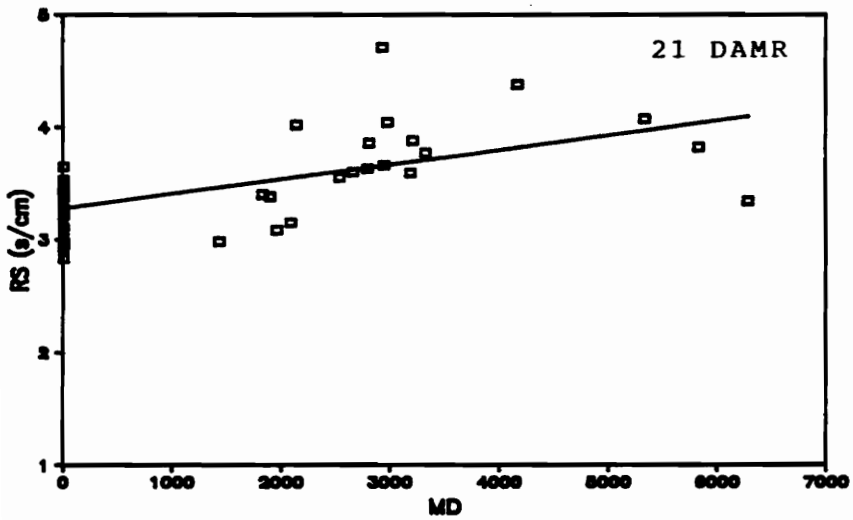
Fig. 2. The relationship between transpiration (Tr) and cumulative mite days per leaf (MD) for 'Redchief Delicious' apple leaves at 7, 21, and 28 days after mite removal (DAMR). 7 DAMR: Tr = 8.82 - 0.0004 MD, R^2 = 0.39, 21 DAMR: Tr = 5.52 - 0.0002 MD, R^2 = 0.30, 28 DAMR: Tr = 6.75 - 0.0003 MD, R^2 = 0.40

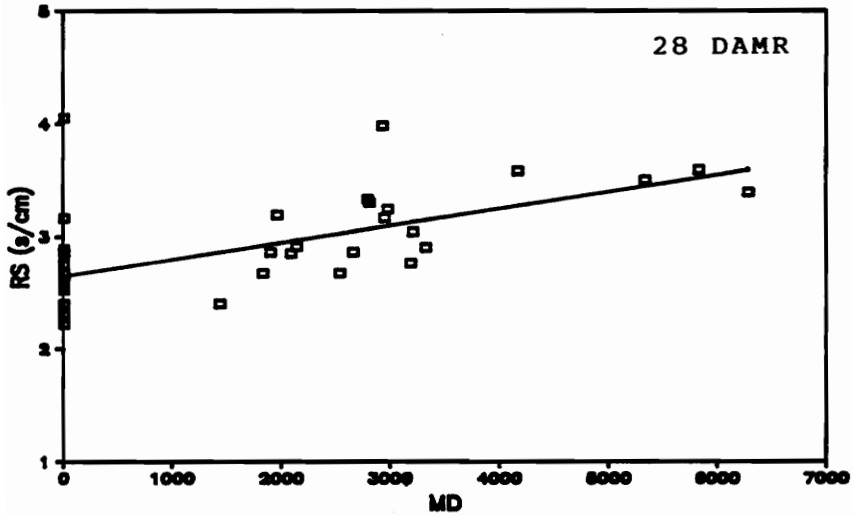

7 DAMR
Tr (mmol H2O/m2/s)
MD
10
8
6
4
2
0
1000
2000
3000
4000
5000
6000
7000


21 DAMR
Tr (mmol H2O/m2/s)
MD
10
8
6
4
2
0
1000
2000
3000
4000
5000
6000
7000


28 DAMR
Tr (mmol H2O/m2/s)
MD
10
8
6
4
2
0
1000
2000
3000
4000
5000
6000
7000


7 DAMR
RS (g/cm)
MD
0
1000
2000
3000
4000
5000
6000
7000
1
2
3
4
5


21 DAMR
RS (g/cm)
MD
0
1000
2000
3000
4000
5000
6000
7000
1
2
3
4
5


28 DAMR
RS (g/cm)
MD
0
1000
2000
3000
4000
5000
6000
7000
1
2
3
4
5

Transpiration was reduced by TSM feeding in strawberry (Sances et al. 1981), peppermint (DeAngelis et al. 1982) and apple (Ferree and Hall 1981, Ferree et al. 1986); however, the relationship was inconsistent in apple. The relationship between Tr and MD for our data was weak, as evidenced by the poor coefficients of determination, but the relationship was consistent throughout the experiment (Fig. 2). Sances et al. (1981) measured recovery in Tr on strawberry plants approximately 45 days after large declines in TSM populations. As discussed earlier, Tr was measured on a whole plant basis following the production of many new leaves by the damaged plants. Recovery was not measured on plants subjected to lesser mite densities.

No interactions were found between the urea and mite treatment for RWC, SLW, LFN, or TCHL; therefore, the data were pooled and each variable was regressed on MD. RWC declined linearly with increasing MD (Fig. 4). The data were variable, and urea treatment had no influence on the response. Histological studies demonstrated extensive cuticular damage in apple following ERM feeding (Avery and Briggs 1968b). Increased water loss in the dark was measured in peppermint leaves infested with TSM (DeAngelis et al. 1982).

Specific leaf weight declined linearly with increasing MD, and there was no apparent urea effect (Fig. 5). The data were variable, and severe mite damage resulted in small reductions in SLW. Reductions in SLW were probably due to

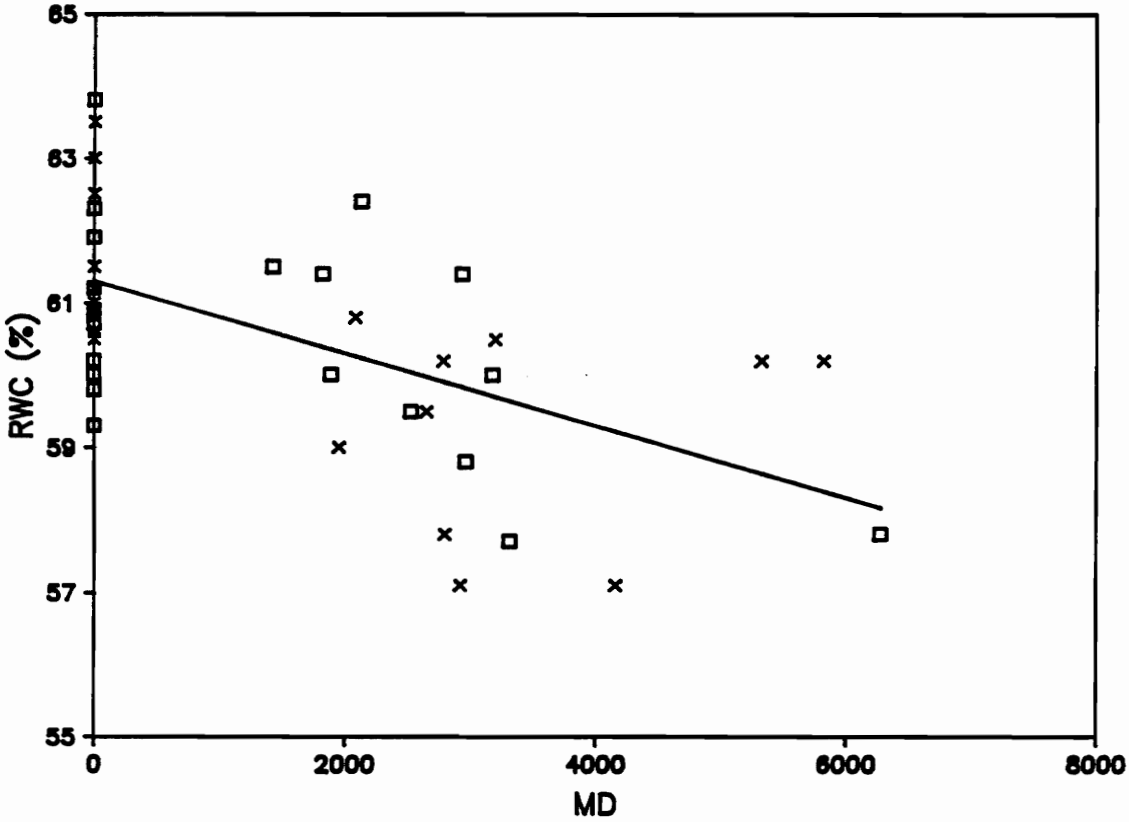


Fig. 4. The relationship between relative water content (RWC) and cumulative mite days per leaf (MD) for 'Redchief Delicious' apple leaves. RWC = 61.3 - .005 MD, R^2 = .32. Each point represents one observation of the urea (×), or no urea (□) treatment.

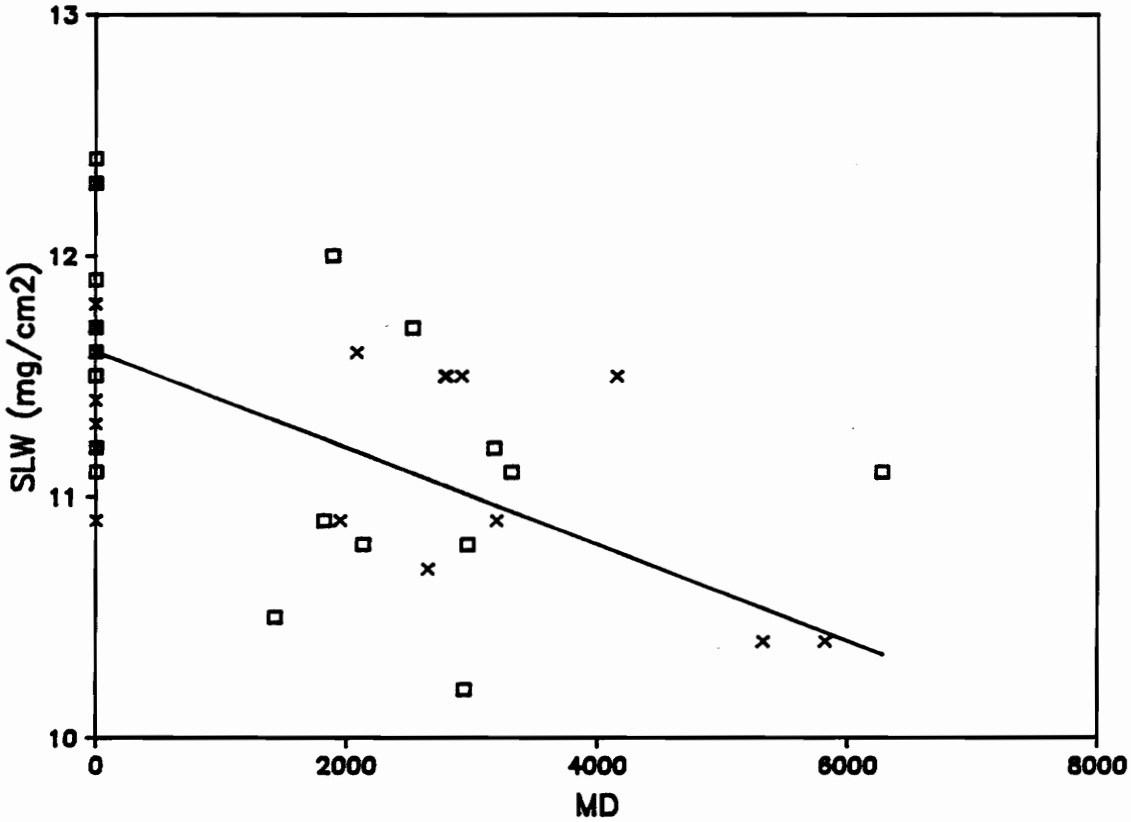


Fig. 5. The relationship between specific leaf weight (SLW) and cumulative mite days per leaf (MD) for 'Redchief Delicious' apple leaves. Each point represents one observation of the urea (□) or no urea (X) treatment. SLW = 11.6 - .0002 MD, R^2 = .29.

the removal of cellular contents by mite feeding. In a previous greenhouse study we found increased SLW with mite feeding (data not presented). We have no explanation for the discrepancy in the results. We are unaware of other studies relating SLW and mite feeding.

A negative linear relationship was found between LFN (area basis) and MD, and much of the variation in the data may have resulted from the urea treatment (Fig. 6). Urea did not significantly ($P>F = 0.13$) influence LFN according to analysis of variance. Separate regression analysis by urea treatment was not performed because the no-urea treatment may have yielded a biased relationship. There was only 1 severely damaged leaf in this treatment, and that observation could have heavily influenced the resulting regression line.

Ferree and Forshey (1988) measured a 4% increase in LFN (%) on 'Delicious' spur leaves two weeks after the application of four post-bloom urea sprays (6 g urea $liter^{-1}$). The inability to increase LFN with urea sprays in our experiment may have resulted from insufficient application frequency, or lack of leaf absorption due to time of year. ERM feeding caused a 10% reduction in LFN (%) of 'Delicious' (Klopfenstein and Holdsworth 1978). LFN (%) of less than 1.9% resulted from the ERM feeding. No relationship was found between LFN (%) and MD for our data. We measured LFN levels

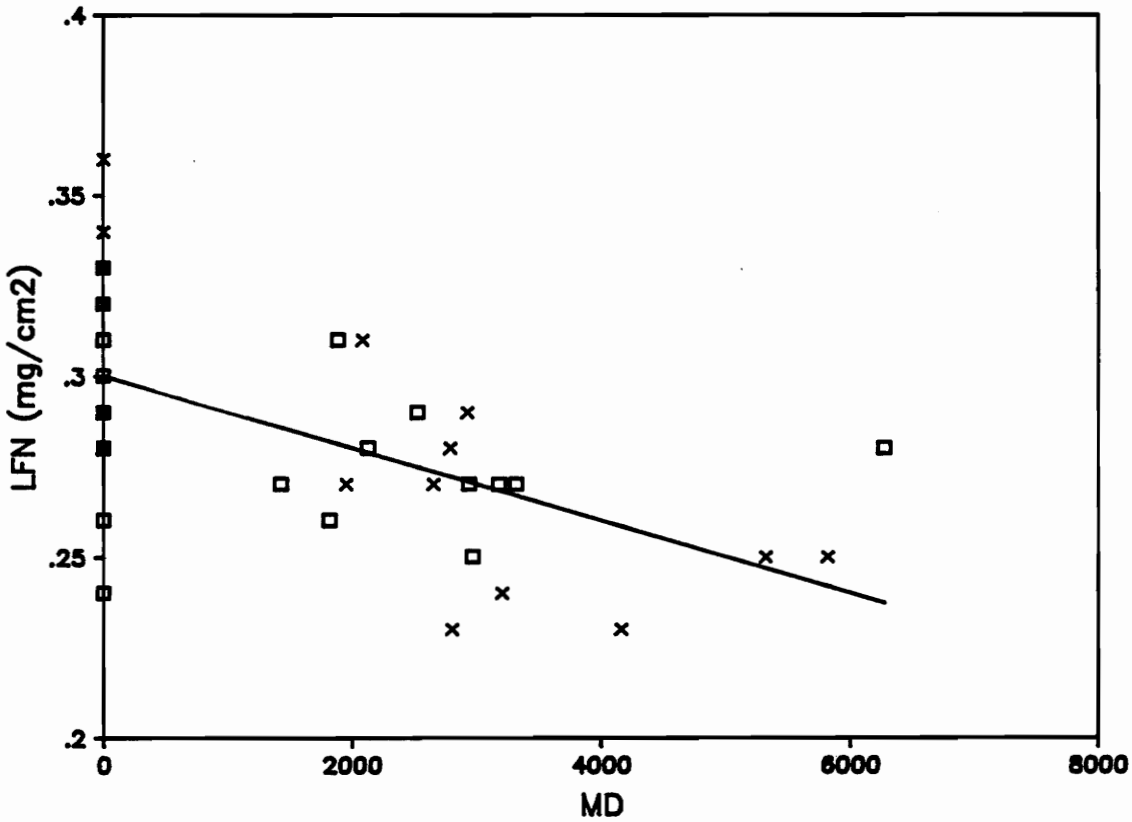


Fig. 6. The relationship between leaf nitrogen (LFN) and cumulative mite days per leaf (MD) for 'Redchief Delicious' apple leaves. Each point represents one observation of the urea (X) or no urea (□) treatment. LFN = .30 - .00001 MD, R^2 = .35.

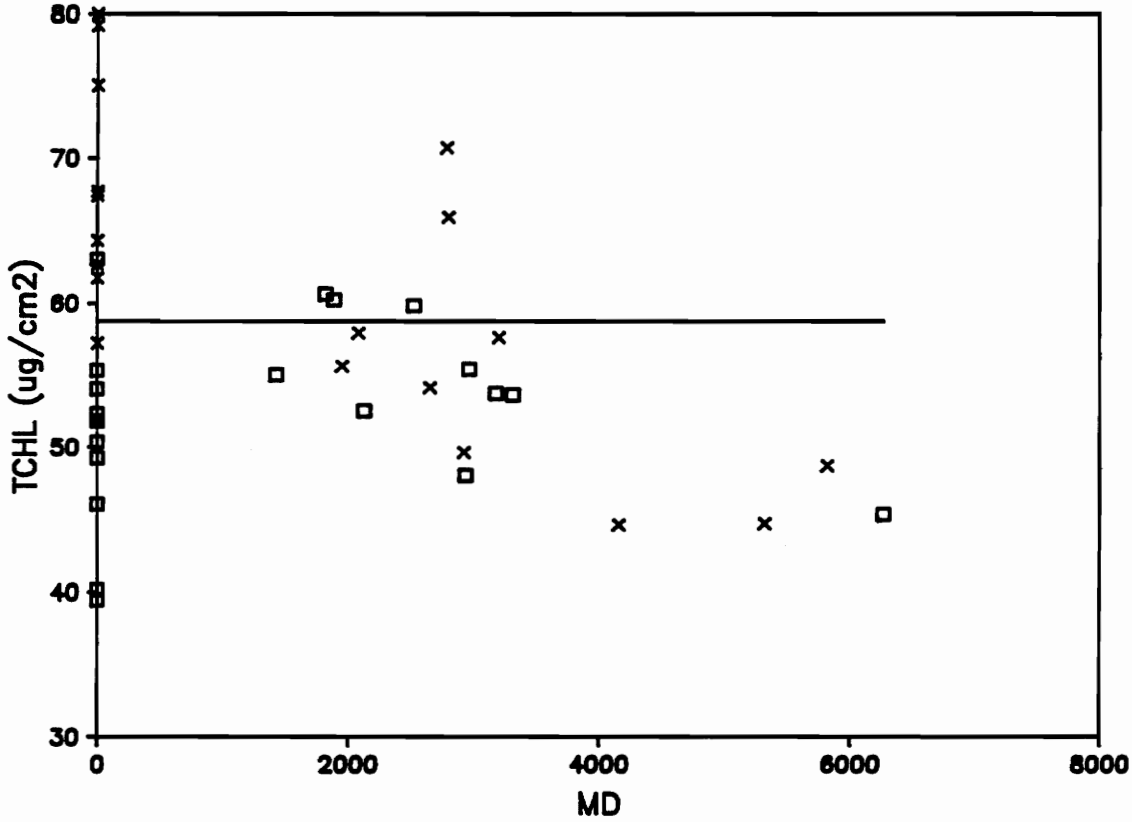


Fig. 7. The relationship between total chlorophyll (TCHL) and cumulative mite days per leaf (MD) for 'Redchief Delicious' apple leaves. Each point represents one observation of the urea (X) or no urea (□) treatment. TCHL = 58.7 - 3.7×10^{-7} MD, R^2 = .13.

well above 2% on all mite injured leaves (data not presented).

Total chlorophyll (area basis) decreased linearly with increasing MD (Fig. 7); however, due to the extreme variability in the data, the relationship was questionable. The regression line was nearly horizontal, indicating that leaves subjected to greater than 4000 MD had only slight or no reduction in TCHL. As with the LFN data, extreme variation in the control leaves contributed to the poor regression fit.

Reductions in TCHL of 30% were reported for 'Delicious' apple leaves subjected to high populations of ERM feeding (Chapman et al. 1952, Zwick et al. 1976). Chapman et al. (1952) reported visual recovery in green color following mid-summer ERM outbreaks. Chlorophyll extraction conducted on 12 October, however, provided little evidence of recovery. We observed an apparent recovery in green color by 28 days after mite removal, but successive TCHL measurements would have been required to confirm these observations.

To obtain more information regarding mite-induced Pn reductions, a multiple regression was performed for Pn with the first- and second-order terms of LFN (area basis), RWC, TCHL, and Tr included as dependent variables. The results indicated that the model containing the first- and second-order LFN and second-order RWC terms yielded the best Pn estimates (R^2 = 0.42). Thus, the best model described less

than half of the variation in the data, but LFN and RWC were superior to all other parameters in estimation of Pn.

The mite populations attained in this study were representative of ERM populations in the Blacksburg, Va. area for the 1988 season. Due to cool weather in the early spring, mite populations developed slowly, resulting in low populations for the season. A greater number of severely damaged leaves may have improved the regression relationships, and helped explain more of the variation.

In conclusion, there is little conclusive evidence from this study for recovery of physiological function following ERM removal. Severely damaged leaves (>4000 MD) did exhibit recovery in Pn, but leaves receiving less damage were variable and exhibited no clear trend. A visual recovery in leaf greenness was observed by 28 days after mite removal, but successive chlorophyll extractions were not made, and recovery could not be evaluated. The use of urea as a technique to induce or hasten recovery was unsuccessful. Sufficient absorption of urea may not have occurred. Had the experiment been conducted earlier in the year, or a surfactant included with the urea application, urea may have had more of an effect.

Literature Cited

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol. 24:1-15.

Avery, D.J. 1964. Carbon dioxide exchange by plum and apple leaves damaged by fruit tree red spider mite. Rpt. E. Malling Res. Sta., Maidenstone, Engl. 1964:94-97.

Avery, D.J. and J.B. Briggs. 1968a. The aetiology and development of damage in young fruit trees infested with the fruit tree red spider mite, Panonychus ulmi (Koch). Ann. Applied Biol. 61:277-288.

Avery, D.J. and J.B. Briggs. 1968b. Damage to leaves caused by fruit tree red spider mite, Panonychus ulmi (Koch). J. Hort Sci. 43:463-473.

Bremner, J.M. and G.A. Breitenbeck. 1983. A simple method for determination of ammonium in semimicro-Kjeldahl analysis of soils and plant materials using a block digester. Commun. In Soil Sci. Plant Anal. 14(10):905-913.

Chapman, P.J., S.E. Lienk, and O.F. Curtis, Jr. 1952. Responses of apple trees to mite infestations: I. J. Econ. Ent. 45:815-821.

DeAngelis, J.D., K.C. Larson, R.E. Berry, and G.W. Krantz. 1982. Effects of spider mite injury of transpiration and leaf water status in peppermint. Env. Ent. 11(4):975-978.

Ferree, D.C. and C.G. Forshey. 1988. Influence of pruning and urea sprays on growth and fruiting of spur-bound 'Delicious' apple trees. J. Amer. Soc. Hort. Sci. 113(5):699-703.

Ferree, D.C. and F.R. Hall. 1981. Influence of physical stress of photosynthesis and transpiration of apple leaves. J. Amer. Soc. Hort. Sci. 106(3):348-351.

Ferree, D.C., F.R. Hall, and M.A. Ellis. 1986. Influence of mites and diseases on net photosynthesis of apple leaves, p56-62. In: A.N. Lakso and F. Lenz (eds). The regulation of photosynthesis in fruit trees. 1986. Symp. Proc. Pub., NY State Agr. Expt. Sta., Geneva, NY.

Hall, F.R. and D.C. Ferree. 1975. Influence of twospotted spider mite populations on photosynthesis of apple leaves. J. Econ. Ent. 68(4):517-520.

Herbert, H.J. and K.P. Butler. 1973. The effect of European red mite, Panonychus ulmi (Acarina: Tetranychidae), infestations on N, P, and K concentrations in apple foliage throughout the season. Can. Ent. 105:263-269.

Klopfenstein, W.G. and R.P. Holdsworth. 1978. The effects of European red mite feeding on the growth and yield of spur-type Delicious apple. Res. Circ. Ohio Agr. Res. Dev. Ctr. 239:35-37.

Marini, R.P. 1986. Do net gas exchange rates of green and red peach leaves differ? Hortscience 21(1):118-120.

Sances, F.V., J.A. Wyman, I.P. Ting, R.A. Van Steenwyk, and E.R. Oatman. 1981. Spider mite interactions with photosynthesis, transpiration, and productivity of strawberry. Env. Ent. 10:442-448.

Syvertsen, J.P., L.G. Albrigo, and C.C. Childers. 1986. Photosynthesis and water use efficiency of spider mite damaged citrus leaves, p 52-56. In: A.N. Lakso and F. Lenz (eds.). The regulation of photosynthesis in fruit trees. 1986. Symp. Proc. Pub., NY State Exp. Sta., Geneva, NY.

Youngman, R.R. and M.M. Barnes. 1986. Interaction of spider mites (Acari: Tetranychidae) and water stress on gas-exchange rates and water potential of almond leaves. Env. Ent. 15:594-600.

Zwick, R.W., G.J. Fields, and W.M. Mellenthin. 1976. Effects of mite population density on 'Newtown' and 'Golden Delicious' apple tree performance. J. Amer. Soc. Hort. Sci. 101(2):123-125.

Final Discussion

This study consisted of two experiments designed to evaluate the effect of N fertilization on the physiology of 'Delicious' apple leaves subjected to European red mite (ERM) feeding. Our results indicated that the N status of the leaf influenced the physiological response to mite feeding; however, following mite damage, foliar urea applications had little effect on leaf recovery. Direct comparisons between the two studies are made with caution due to the difference in strains of 'Delicious' used (Imperial and Redchief) and experimental conditions (container- and field-grown trees).

Nitrogen fertilization affected both Pn and the visual response of container-grown 'Imperial Delicious' leaves subjected to ERM feeding. Visual damage appeared first on the low N trees, and these trees demonstrated the most severe damage throughout the experiment. Under equal mite pressures, the high N trees retained higher Pn than trees receiving less N. Tr, Rd, RWC, SLW, LFN, and TCHL were reduced by mite feeding, and dark Tr was increased, regardless of nitrogen level.

Foliar applications of urea had no appreciable effect on the physiological recovery of 'Redchief Delicious' apple leaves following mite removal. Our treatment may not have resulted in sufficient urea uptake into the leaf to measure a response. Had urea been applied with a surfactant, or

earlier in the year, there may have been a greater effect. There was, however, some evidence of leaf recovery in Pn following mite removal. Leaves subjected to greater than 4000 MD gradually increased in Pn up to the conclusion of the experiment, 28 days after mite removal. Leaves receiving less mite damage were variable, with no discernible trend. Tr and Rs exhibited no recovery over this period.

In both the field and greenhouse studies, the most important measured parameters for Pn determination were LFN (area basis) and RWC. Leaves higher in nitrogen and with a greater RWC tended to have superior Pn to other leaves. Many of the studies dealing with mite damage have centered on losses in TCHL, but our results indicate TCHL is relatively unimportant when compared to LFN (area basis) and RWC. Few studies have evaluated mite damage in terms of leaf water relations, yet our results indicate such parameters are important in mite damage. Mite damage may be best evaluated through LFN (area basis) analysis, and RWC determination; however, under field conditions, we were able to explain only half of the variation in Pn.

Currently, action thresholds for ERM control vary according to state, time of year, crop load, mite/predator ratios, and environmental conditions. In Pennsylvania, for example, the recommended action threshold is 500 to 750 MD for light-bearing mature trees. Our results indicate that an increase in LFN (%) from 2% to 3% resulted in 40% higher Pn.

Thus, the action threshold for high N light-bearing trees should be higher. Direct recommendations can not be made from these data because Pn measurements were taken on a single leaf at five points in time, and the relationship between these measurements and growth (and cropping) cannot be clearly defined.

Greenhouse experiments may not be applicable to field situations; therefore, there is a need for further research on the effect of N on mite feeding damage in the field. Additional research is also needed to evaluate more thoroughly the physiological recovery following mite damage, because this information would further impact control decisions.

Vita

Richard John Campbell was born on August 18, 1964 in Homestead, Florida. He received his primary and secondary education in the Dade County Public school system. In the Fall of 1982, he entered the University of Florida, where he received a Bachelor of Science in Fruit Crops in May 1986.

In September 1986 he entered Virginia Polytechnic Institute and State University on a Presidential Fellowship, and received a Masters of Science degree in Horticulture in December 1988.

Richard J Campbell