Doctoral Thesis

Interactions between green apple aphids (Aphis pomi De Geer) and apple plants (Malus domestica Borkh.) subjected to water stress

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Publication Date: 1994

Permanent Link: https://doi.org/10.3929/ethz-a-000965475

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Interactions
between Green Apple Aphids
(Aphis pomi De Geer)
and Apple Plants (Malus domestica Borkh.)
Subjected to Water Stress

A dissertation submitted to the
Swiss Federal Institute of Technology Zurich
for the degree of
Doctor of Natural Sciences

presented by

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Zurich, 1994
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# Contents

Summary .................................................. I  
Zusammenfassung ...................................... V  

1. General Introduction ................................. 1  
   1.1 The Apple Orchard Ecosystem ..................... 1  
   1.2 Short Review on Water Stress .................... 3  
      1.2.1 Plant Growth .................................. 3  
      1.2.2 Plant Nutritional Quality .................... 4  
      1.2.3 Plant Water Potential ......................... 4  
      1.2.4 Plant Microenvironment ....................... 5  
      1.2.5 Plant Attractiveness ......................... 6  
   1.3 Effects of Aphids on Host Plants .............. 6  
   1.4 Materials and Methods ......................... 7  
      1.4.1 The Host Plant ................................ 7  
      1.4.2 The Aphid ..................................... 10  
      1.4.3 The Water Stress .............................. 12  

2. Effects of Water Stress on Population Dynamics  
of the Green Apple Aphid: Life Table Analysis ........ 19  
   2.1 Introduction ...................................... 19  
   2.2 Materials and Methods ......................... 20  
      2.2.1 Descriptors for the Development  
           of Aphid Populations ......................... 20  
      2.2.2 Experimental Conditions .................... 22  
      2.2.3 Data Collected ............................... 23  
      2.2.4 Data Analysis ................................ 26
2.3 Results .............................................. 27
2.3.1 Development of Water Stress in the Soil ...... 27
2.3.2 Effects of Water Stress on Plant Development .. 31
2.2.3 Effects of Water Stress on Life Table
   and Population Parameters of Aphids .......... 44
2.4 Discussion .......................................... 53
2.4.1 Effects of Water Stress on Plant Development .. 53
2.4.2 Effects of Water Stress on Aphid Life Table
   and Population Parameters of Aphids .......... 57

3. Effects of Water Stress on Population Dynamics
   of the Green Apple Aphid:
   Population Development and Growth .............. 63
3.1 Introduction ........................................ 63
3.2 Materials and Methods ............................ 65
   3.2.1 Characterization of the Development
       and Growth of Aphid Populations .......... 65
   3.2.2 Experimental Conditions .................. 66
   3.2.3 Data Collected .............................. 67
   3.2.4 Data Analysis ............................... 69
3.3 Results ............................................. 69
   3.3.1 Development of Water Stress in the Soil .... 69
   3.3.2 Effects of Water Stress on Plant Development .. 72
   3.3.3 Effects of Water Stress on
       Development and Growth of Aphid Populations ... 86
3.4 Discussion ......................................... 97
   3.4.1 Effects of Water Stress on Plant Development .. 97
   3.4.2 Effects of Aphid Populations
       on Plant Development ....................... 99
   3.4.3 Effects of Water Stress on
       the Population Development of Aphids ....... 102

4.1 **Introduction** .................................................. 107

4.2 **Materials and Methods** ......................................... 109
   4.2.1 **Collection of Phloem Sap** ............................. 109
   4.2.2 **Plant Material** ........................................ 110
   4.2.3 **Exudation Technique** .................................. 110
   4.2.4 **Analysis of Carbohydrates** .......................... 111
   4.2.5 **Analysis of Nitrogenous Substances** ............... 111
   4.2.6 **Statistical Analysis** .................................. 112

4.3 **Results** .................................................... 113
   4.3.1 **Sugars** ............................................... 113
   4.3.2 **Amino Acids** ......................................... 118

4.4 **Discussion** ................................................ 123
   4.4.1 **Sugars** ............................................... 123
   4.4.2 **Amino Acids** ......................................... 127

5. **Conclusions** .................................................. 135

5.1 **Effects of Water Stress on the Host Plant** ................... 135

5.2 **Effects of Water Stressed Plants on the Population Dynamics of Aphids** .......... 137

5.3 **Effects of an Infestation with the Green Apple Aphid on the Host Plant** .......... 141

6. **References** .................................................. 145

**Appendix** ..................................................... 159

**Abbreviations and Symbols** .................................... 161

**Curriculum Vitae**

**Acknowledgements**
Summary

Analysis of apple production in Switzerland has shown that studies on the dynamics of subsystems create the most useful base to optimize agricultural production systems. Therefore, the interactions between insect herbivores and their host, with special attention to stress factors such as water and nutrient deficiencies, are studied extensively at the Institute of Plant Sciences at the ETH, Zurich. The influence of different levels of water supply on the sub-system of apple plants (Malus domestica Borkh., cv. 'Golden Delicious') with green apple aphids (Aphis pomi De Geer)(Hemiptera: Aphididae) was examined for the first time in this study. Effects of different water regimes on plant growth and on physiological plant parameters, on aphid population dynamics, as well as on the feedback of aphids on their host were studied.

Apple plants, which were multiplied by tissue culture, were subjected to different levels of water supply ($\Psi_{\text{soil}}$: -0.05, -0.70, -1.20, -1.60 and -2.15 MPa) using the intermittent drying cycle technique. The experiments were conducted with potted plants grown in a soil mix under controlled environmental conditions (22/17°C day/night temperatures, 18:6 light/dark regime, 55-60% RH, 400-450 μmol/m²/sec PPFD). Plants reached a maximum size of 1.3 m. The effects of water stress on plant growth and on the development of aphid populations was described by relating age-specific life table and population parameters of aphids as well as density-dependent population characteristics with plant growth and plant physiological parameters such as shoot length, total leaf area, plant water relations and phloem sap quality.

Water stress decreased the plants' carrying capacity for aphid populations. The characteristics of the phloem sap were identified as the most important factors influencing the population development of aphids. The concentration and composition of the phloem sap changed with water stress, with leaf position (i.e. leaf's stage of maturity), and with infestation by aphids.
The size (per plant) and density (per leaf and per unit of shoot length) of aphid populations decreased significantly with an increase in water stress. Although the concentration of amino acids in the phloem sap increased with water stress, the amino acid to carbohydrate ratio decreased up to 10-fold, rendering the quality of the phloem sap less favorable for aphid feeding. Asparagine, the major amino acid detected in the phloem sap of apple plants (68-77% relative content in apical and growing leaves), was identified as a factor determining population development of *A. pomi*. The relative content of asparagine in the phloem significantly decreased with water stress.

A higher amino acid content in the phloem of the apex and young, growing leaves and a lower carbohydrate to nitrogen ratio, as well as higher relative contents of asparagine compared with the phloem of mature leaves, explain the preference of the green apple aphid for young tissue and the faster development of aphids on young leaves. Therefore, young apple trees and actively growing plant parts are most threatened by green apple aphid infestation.

The study presented here confirmed that aphids can exert an appreciable sink effect on plants by increasing transport of solutes to feeding sites, and that this sink effect is correlated with the size of an aphid colony. It could be demonstrated that the phloem sap of plants which were previously infested with aphids contained significantly higher asparagine and much lower sorbitol compared with phloem collected from uninfested plants, proving that aphids altered the composition of the phloem sap to favor their nutrition.

Plants grown under conditions of water stress were shown to adjust osmotically by actively accumulating solutes. The critical moisture level for a significant reduction in plant growth developed below ψ_{soil} = -0.69 MPa for plants which were not infested with aphids. An infestation with aphids, however, was demonstrated to affect the plants' ability to adjust osmotically by the drain of assimilates and solutes needed for active osmotic adjustment, shifting the critical moisture level for plants infested with aphids to ψ_{soil} > -0.69 MPa. In this critical moisture range plants were shown to be particularly sensitive to an infestation with aphids. Under unlimited water supply, plants were able to compensate for the losses in assimilates withdrawn by aphids, possibly due to a positive feedback on photosynthesis caused by an additional sink and/or due to degeneration and mobilization of metabolites.
Population parameters (mean generation time, intrinsic rate of natural increase, and days for populations to double), the components of the intrinsic rate of increase (development and pre-reproductive times, percent immature mortality, and fecundity) and population characteristics (size and density) of A. pomi were assessed using two approaches: the age-specific life table and the density-dependent population approach. Under conditions of water stress, development and pre-reproductive time of aphids as well as generation and doubling time of populations were shorter than in the well-watered treatment. Net reproduction rate (i.e. cumulative fecundity) significantly increased with water stress, whereas the intrinsic rate of natural increase was not significantly affected by drought stress. The size and density of aphid populations, however, was significantly reduced on water stressed plants. The interrelationship of various factors regulating aphid population, therefore, may not necessarily all apply at different population densities.
Leer - Vide - Empty
Zusammenfassung

Erfahrungen bei der Untersuchung der Apfelproduktionssysteme in der Schweiz haben gezeigt, dass die Analyse der Dynamik von Subsystemen den erfolgversprechendsten Weg zur Optimierung der Bewirtschaftungsformen für landwirtschaftliche Kulturen darstellt. Daher werden am Institut für Pflanzenwissenschaften der ETH Zürich seit bald 15 Jahren die Wechselwirkungen zwischen Kulturpflanzen und Pflanzenschädlingen untersucht, wobei Stressfaktoren besonders berücksichtigt werden.


Geklonte, in Gewebekultur vermehrte Apfelbäumchen wurden unter kontrollierten Bedingungen in Klimakammern bis zu einer Größe von maximal 125 cm in Töpfen gezogen. Die Pflanzen wurden jeweils bewässert, wenn die Saugspannung im Boden bestimmte Werte erreichte (\(\Psi_{\text{Boden}}\): -0.05, -0.70, -1.20, -1.60 und -2.15 MPa); dadurch konnten mehrere Versuchsvarianten mit einer unterschiedlichen kontrollierten Wasserversorgung festgelegt werden. Die Auswirkungen der verschiedenen Bewässerungsregime auf das Pflanzenwachstum und die Entwicklung von Blattlauspopulationen wurden untersucht, indem Lebenstafel- und Populationsparameter wie auch Populationsgröße und -dichte mit dem Pflanzenwachstum und pflanzenphysiologischen Parametern wie Sprosslänge und Blattfläche beziehungsweise dem Wasserstatus der Pflanze sowie der Phloemsaftqualität in Beziehung gebracht wurden.


Die vorliegende Arbeit bestätigt, dass Blattläuse einen erheblichen Sink-Effekt auf die Pflanzen ausüben können, indem sie den Transport von im Phloem gelösten Stoffen zu Orten der Nahrungsaufnahme erhöhen; das Ausmass dieses Sink-Effekts ist von der Größe der Blattlauspopulation abhängig. Es konnte bestätigt werden, dass durch einen Blattlausbefall die Zusammensetzung des Phloemsafes zugunsten der Aphiden verändert wird. Es konnte gezeigt werden, dass durch den Blattlausbefall der relative Gehalt an Asparagin im Phloemsaf erhöht und der Sorbitolgehalt verringert wird.

Pflanzen, die unter Wassermangel litten, zeigten eine osmotische Anpassung an den Wasserstress, indem sie gelöste Stoffe akkumulierten. Das Wachstum mit Blattläusen befallener Pflanzen wurde bei einer geringeren Intensität des Wasserstresses ($\Psi_{\text{Boden}} > -0.69$ MPa) beeinträchtigt als dasjenige unbefallener Pflanzen ($\Psi_{\text{Boden}} < -0.69$ MPa). Ein Blattlausbefall verringerte das
Vermögen der Pflanze, sich osmotisch an einen Wassermangel anzupassen, indem die Aphiden der Pflanze gelöste Stoffe, die zur osmotischen Anpassung benötigt werden, entzogen.

General Introduction

1.1 The Apple Orchard Ecosystem

Integrated pest management (IPM) in orchards rapidly progressed during the past decade. Key factors attributing for the rapid implementation were the profound scientific knowledge concerning the usefulness of predatory arthropods and the introduction of new selective plant protectants (Dorn, 1993a). The number of IPM compatible methods to keep populations of aphids low is, however, limited (Pflanzenschutzmittel-Verzeichnis 1987/1988, Schweiz; Dorn, 1992). Many relevant interactions in the orchard ecosystem, between insect populations and between insects and plants are not yet sufficiently known (Dorn, 1993b). This is reflected by the control parameters used in the present European Guidelines for Integrated Production (IP) of fruit (Sessler, 1993). A comprehensive system management requires more scientific knowledge.

As suggested by Baumgärtner and Delucchi (1988), complicated agro-ecosystems can be divided into subsystems and investigated with a hierarchal approach. The whole agroecosystem can then be analyzed in terms of all the variables involved in this ecosystem, and their interactions determined from a study of the dynamics of the subsystems. Some components often interact more strongly than others, allowing identification of subsystems that can conveniently be studied in separate research projects (Baumgärtner et al., 1990). The plant and its organs are always part of these subsystems because they are the basic production units to be considered, irrespective of the presence of associated organisms. Plant, plant parts,
arthropods, weeds and diseases are treated as populations which are affected by uncontrollable factors such as the climate and controllable factors such as cultural practices.

Since 1980, the apple ecosystem is one of the main fields of interest in the Entomology Group at the Institute of Plant Sciences, ETH Zürich, Switzerland. The long term goal of analyses and basic research of the apple orchard system was to create a scientific base for the development of system management programs. Experience made during the analysis of apple production systems in Switzerland has shown that the study on the dynamics of subsystems creates the most useful base to optimize agricultural production systems (Baumgärtner et al., 1990). Work with these subsystems needs profound knowledge and investigations of the processes forming crop yield, the dynamics of pests, limiting environmental factors and various cultural practices. The subsystems addressed were the 'Golden Delicious' apple tree (Baumgärtner et al. 1984, 1990), the apple scab (Baumgärtner et al., 1990), the mite (Panonychus ulmi Koch and Tetranychus urticae Koch; Baumgärtner and Zahner, 1983) and the apple aphids (Rhopalosiphum insertum Walk., Dysaphis plantaginea Pass., Aphis pomi De Geer; Graf, 1984; Graf et al., 1985). The insights obtained have most notably permitted the evaluation of cultural, chemical, and biological control of spider mites. At the same time, important gaps were shown in knowledge, requiring further research work and hypotheses relevant to practical pest management and ecological theory.

The physiological state of the host plant, which varies under different cultural practices, was little examined and therefore studied in cooperation with the Plant Nutrition Group. The effect of the nutritional state of small, potted apple plants and the effect of water stress on the development of the two-spotted spider mite (T. urticae) were investigated by Wermelinger (1985) and Schnider (1989). The effect of different levels of nitrogen fertilization of apple plants on the green apple aphid A. pomi was studied by Hugentobler (1990) and Rutz (1993).

The objective of the present study was to investigate the interrelationship between the green apple aphid and its host, the apple plant, as affected by different water regimes. For this purpose, the effect of differential water supply on plant growth and on physiological plant parameters, on the development of aphid populations, as well as the feedback effects of aphids on well-watered and water stressed host plants were examined.
simultaneously. Based on these examinations, causes for the differential
development of aphid populations feeding on apple plants grown under
different water regimes were evaluated.

1.2 Short Review on Water Stress

The aim of this study is to describe and quantify the possible effects of water
stress on the interaction between the apple plant and the green apple aphid.
Stress is any biotic or abiotic factor of the environment that affects plant
physiology, chemistry, growth, and/or development in a measurable negative
way (Heinrichs, 1988; Louda and Collinge, 1992). Water stress is the
induction of a turgor pressure below the maximal potential pressure. (Osmond
et al., 1987 cf. Louda and Collinge, 1992). It may be caused both by a
deficiency of water available to roots, or by loss of roots due to rotting as a
result of water excess (flooding). For the purposes of this study, water stress
indicates a deficiency in water and not an excess due to flooding. Water is an
essential resource for plant metabolism. All aspects of growth, development,
and reproduction are directly or indirectly dependent on an adequate water
supply. Since such stress often has strong effects on plant physiology it may
also affect the interaction between the plant and its herbivores. Both
increases and decreases in the size of aphid populations have been
attributed to water stress (Waring and Cobb, 1992). Unfortunately, the causal
relationships leading from water stress to the insect response are very difficult
to disentangle and frequently remain obscure.

The effects of water stress on plant traits and their potential relevance to
insect performance, especially of phloem sucking insects, will be briefly
reviewed:

1.2.1 Plant Growth

Many processes involved in plant growth, including cell enlargement, cell wall
synthesis and protein synthesis are extremely sensitive to water deficit. A
reduction in plant size is the most common result of water stress, because
cell enlargement is the process most sensitive to water deficit. Significantly
less tissue will be produced and thus made available to insect herbivores if
water stress occurs during the active growth phase of a plant (Hsiao, 1973;
Gershenzon, 1984; Mattson and Haack, 1987; Holtzer et al., 1988). Total leaf
area decreases appreciably with an increase in water deficiency, thereby decreasing photosynthetically active surfaces (Mattson and Haack, 1987).

1.2.2 Plant Nutritional Quality

The availability of organic nitrogen (amino acids, amides and related compounds) has been shown to be a critical, if not limiting, factor in the population growth of many insect herbivores and in particular of phloem feeders such as aphids. Nitrogen metabolism is among the processes most sensitive to water deficit (Hsiao et al., 1976). In general, water stress disturbs nitrogen metabolism in such a way that protein contents decrease while amino acids increase in concentration. The observed increase in amino acid content results from protein hydrolysis as well as from reduced plant growth, i.e. reduced protein synthesis and hence utilization of amino acids. This may lead to a qualitative improvement of the nutritional base of aphids. Photosynthesis, translocation of photosynthates, partitioning of photosynthate between sugars and starch, rate of hydrolysis of starch, and respiration are all affected by water stress and lead to changes in carbohydrate metabolism (Kramer, 1983 cf. Mattson and Haack, 1987). There is little evidence, however, that the effects of water stress on carbohydrate availability play a direct role in the growth of insect populations (e.g. Holtzer et al., 1988).

Not just the presence and absolute concentration of a specific nutritional element can improve the aphids diet; also the ratios of these nutrients may be just as important (nutritional-balance) (Mattson and Haack, 1987). A wide variety of plant secondary metabolites, also found in the phloem of plants (e.g. phenols and flavanoids), have been shown to function as chemical defenses in plants against insect herbivores. Gershenzon (1984) concluded, that water stress significantly alters the amounts of defensive compounds such as cyanogenic glycosides, glucosinolates, alkaloids, and terpenoids present in plants.

1.2.3 Plant Water Potential

Osmotic adjustment is a mechanism by which water stressed plants lower the osmotic potential by dehydrating (passive) and/or accumulating solutes in cells (active) and thereby maintaining turgor. Some of the solutes responsible for osmotic adjustment are soluble carbohydrates, sugar alcohols, amino acids, organic acids, and inorganic ions (Morgan, 1984). Many of the solutes
that increase in concentration in response to water stress serve as feeding stimulants and/or primary nutrients to insects. Water stressed plants may thus be more attractive and/or nutritious to insects than nonstressed plants. Despite the positive effects of osmotic adjustment, turgor pressures in plants may decrease to zero under severe stress (Hsiao et al., 1976). The direct effect of turgor is likely to be most consequential on sucking insects. For them, turgor pressure must be considered a key variable determining the physical effort necessary to obtain food. Feeding has been attributed to capillarity, assisted by a decrease in sap surface tension due to saliva, turgor pressure of the plant sap and to active sucking with the ciberial pharyngeal pump (Pollard, 1973; Klinglauf, 1987). On the host plant, the normal turgor pressure is adequate but may become inadequate during water stress with different aphid species reacting in different ways (Pollard, 1973). It seems almost certain that aphid feeding is assisted by the pressure and persistence of supply of phloem sap (Wearing, 1968).

1.2.4 Plant Microenvironment

The microenvironment adjacent to individual organisms (temperature and humidity) is most relevant for its development. Small insect herbivores that feed on leaves can expect a microenvironment dominated by the leaf. Water stress experienced by a host plant can have considerable impact on the microenvironment experienced by insect herbivores. Leaf temperatures increase when plants suffering from water deficiency close their stomata in order to lower transpiration, which is a cooling process. Because water stressed leaves are warmer than well-watered leaves, insects feeding on stressed plants will probably maintain higher body temperatures. Temperature-dependent organisms exhibit significantly different metabolic rates under different temperature regimes. Temperature differences between well-watered and water stressed plants are commonly 2 to 4°C, but they can be as great as 10 to 15°C depending on size, shape and orientation of leaves as well as on plant species (Bucks et al., 1984 cf. Mattson and Haack, 1987; Willmer, 1986). Unfortunately, the microenvironment of many insect herbivores can be difficult to measure, not least because of the strong gradients between leaf surface and ambient atmosphere and its variable effects on insects of different size and hence stage of growth (P.W. Miles, University of Adelaide, South Australia, pers. comm.).
1.2.5 Plant Attractiveness

For insect growth, survival and reproduction, plants need to be nutritionally adequate and must also meet requirements of smell and taste to which the herbivores are genetically and conditionally prepared to respond (secondary plant metabolites). Water stress may alter behavioral responses of aphids such as the attraction to plants. Accumulation of the amino acid proline is a widely observed response of plants exposed to water stress. An increased attractiveness of water stressed plants was reported to be related to elevated levels of proline (Miles et al., 1982). Increases in soluble sugars (e.g. sucrose) at early stages of water stress were associated with phagostimulatory or perhaps nutritional effects (Bernays and Chapman, 1978 cf. Holtzer et al., 1988). Leaf texture, hairiness, leaf color, leaf thickness or other morphological leaf characteristics are also likely to change under water stress and, therefore, can have an influence on insect behavior.

1.3 Effects of Aphids on Host Plants

An aphid infestation, aside from any role it may play in the transmission of virus diseases, can negatively and/or positively affect plant development (e.g. Oatman and Legner, 1961; Hamilton et al., 1986; Kaakeh et al., 1992) depending on the insect density, time of infestation and on the host plant itself. The damage and possible yield losses caused by aphid feeding may be negligible, even at relatively high population densities. Some aphid species are important agriculturally only because they occur in such numbers that they impose a serious nutrient and fluid drain on the plants they attack eventually resulting in an apparent reduction of overall plant growth (Miles, 1989b). Aphids may increase the well-being of their host plants by stimulation of the plants' nutrient uptake (Miles, 1989a). Further, aphids have the potential to affect the physiological relations in the host plant (Pollard, 1973; Miles, 1989a). The penetration of aphids was shown to have some effect on water uptake and transpiration of their host plants (Kloft, 1954 and 1960 cf. Miles, 1989a) as well as on photosynthesis, leading to leaf senescence-like symptoms. More obvious damage caused by aphids are discoloration and deformation of plant organs caused by salivary toxins, the plant's hormonal reactions, and/or viruses transmitted by the insects (Pollard, 1973), as well as by the effects of sooty mold growing on aphid's excreta.
The high diversity of plant reactions to water stress implies that it is not easy to predict the reaction of aphids to water stress. Most probably some reactions of the plant to stress will support and promote aphid development and others will impede aphid development. As both population increases and decreases have been attributed to water stress (Waring and Cobb, 1992), the factors deciding the effect on herbivores may well be the intensity and duration of the stress.

1.4 Materials and Methods

The components of the apple ecosystem under study were: the apple plant, the green apple aphid and water stress.

1.4.1 The Host Plant

Apple tree, *Malus domestica* Borkh., cv. 'Golden Delicious'

**Production.** The bulk area (71%) of Switzerland's pome and stone fruit production is attributed to the cultivation of apples (4 943 ha). A high diversity among apple varieties exists but the variety 'Golden Delicious' is still dominant. Sixty five percent of the apple production is under intensive cultivation systems with planting densities between 300-3500 trees/ha (Pezzatti, 1992).

**Host micropropagation.** The apple plants (maximum 1 year old) used in all experiments throughout the study were produced by tissue culture. As in previous studies that have been part of the overall apple ecosystem project conducted in vitro by the Entomology and Plant Nutrition Group, a high number of genetically and physiologically similar plants were needed. Micropropagation (multiplication and rooting) of the 'Golden Delicious' plants was carried out according to Wermelinger (1985). Proliferation and rooting media were modified according to Hugentobler (1990; see Appendix Table 1). The explantate of the 'Golden Delicious' clone was a seedling. The same mother clone and its successors were micropropagated and used throughout the study presented here.

After rooting of seedlings multiplied under sterile conditions, plants were adapted to a non-sterile and water unsaturated environment. This step took place in a diluted Hoagland nutrient solution (1/8 N; see Appendix Table
modified according to Hugentobler (1990) in a growth cabinet (20°C temperature, 16/8 h day/night period, 35 μmol/m²/sec PPFD measured with a LI 185 Quantum/ Radiometer/Photometer, Lambda Instr. Corp.). The relative humidity inside the growth cabinet was reduced stepwise from near saturation to about 75% relative humidity over a period of 7-10 days depending on the progress in root development and state of the plants. During this step an average of about 25% of the plants were lost mainly due to rotting. For further adaptation, the plants were transferred to a 1/4 N modified Hoagland solution and grown under experimental environmental conditions in a walk-in growth chamber. The plants had to adapt to lower relative humidities (about 55%) and to higher light intensities (about 400 μmol/m²/sec PPFD).

**Environmental conditions.** The ambient maximum day temperature in the growth chamber was set to 22°C, and the minimum night temperature was set to 17°C. The temperature followed a sinusoid day/night curve with minimum temperature between 2\(^0\) to 6\(^0\)am and maximum temperature between 12\(^0\) to 2\(^0\)pm. The mean daily temperature under such fluctuating conditions should have been 19°C. It was found, however, that temperatures inside the growth chamber were up to 2.5°C warmer than the set temperature, and the mean daily temperature was calculated to be 19.8°C. The deviation of actual from set temperatures were highest at the maximum temperature and light intensity and zero during the night. The mean daily temperature under fluctuating conditions was calculated using the following method: the day was divided into 15 minutes segments yielding a total of 96. Temperature settings corresponding to each segment were added and then the mean daily temperature was calculated by dividing this sum by 96. This procedure is based on the assumptions that temperature effects on plants and aphids per day are additive and that temperature fluctuations do not affect aphid population development. There are a few studies that suggest that fluctuating temperatures either have no effect, or have a stimulating effect on aphid development, enhancing fecundity and longevity, compared with aphids reared at constant temperatures (Kindler and Staples, 1970 cf. Benedict and Hatfield, 1988; Michels, Jr. and Behle, 1989).

A 16:8 light/dark regime was maintained in all experiments. Lights went on and off in three intensity steps. Relative humidity in the growth chamber was set to 70%, but the effective RH was 55-60%. The humidity was kept constant during a day, however was not surveyed constantly. Maximum light
intensity measured at high noon (at the setting of maximum temperature and light intensity) amounted to 400-450 \( \mu \text{mol/m}^2/\text{sec} \) PPFD.

**Growth substrate.** When the plants reached a length of approximately 20 cm they were transferred into a soil mix and allowed to adapt to the environmental conditions described above. The soil mix used for the experiments and aphid rearing consisted of mineral soil : composted soil : quartz sand (1 to 1.5 mm diameter) : peat moss at a ratio of 5:5:1:1, respectively. Pot sizes will be described below under "experimental procedure" (see page 15).

The micropropagated plants expressed a high morphological variability mainly in plant heights. A possible reason for this phenomenon was that the clone which was started a few years ago (1986), and has undergone multiple micropropagations over the years, may have mutated. Another possibility is that the uniformity of a clone may not be expressed until 3 to 4 years after redifferentiation of the plant (cf. Hugentobler, 1990). Whatever the validity of these explanations, it was found that over the adaptation period the effect of very small differences in the system resulted in a variation of height between individuals.

After transplantation and before starting an experiment, pots were watered several times with 1/2 N modified Hoagland solution. During experiments and depending on the length of an experiment, plants were further fertilized several times with 1/2 N modified Hoagland solution.

**Plant protection.** To prevent and to control plant infestations in the growth chambers by pests other than the green apple aphid, such as powdery mildew, two-spotted spider mite, and thrips, several chemical sprays were applied. Funginex (Triforine 20%; Maag), Karathane (Dinocap 25%; Maag) and Sulfur (Thiovit 80% S, Sandoz) were used against powdery mildew. Ambush (Permethrine 25%; Maag), Apollo SC (Clofentezine 42%; Maag), and Arafos (Oxamyl 7.5%, Methanol 12%; Maag) were used against two-spotted spider mites and thrips. Two weeks before the start of an experiment, spraying was stopped and in case of further mite or thrips infestations, mechanical control and/or biological control with predatory mites (Phytoseiulus persimilis against mites and Amblyseius cucumeris against thrips) was used.

On an average, three months passed from seedling multiplication under sterile conditions until the plants were ready for the experiments.
1.4.2 The Aphid

The green apple aphid, *Aphis pomi* De Geer

**Damage.** The green apple aphid is of secondary importance on apple trees in Switzerland (Ammon et al., 1986). It can become a serious problem on small apple trees leading to shoot deformation and stunting as well as leaf malformation and curling, both resulting in a reduction of photosynthetically active leaf area. Watersprout meristems and shoot tips often die under severe aphid population densities. Older trees can compensate for the loss and are therefore not considered to be sensitive (Baker and Turner, 1916; Oatman and Legner, 1961).

**Systematics.** *Aphis pomi* De Geer: Order of Hemiptera, Suborder of Sternorrhyncha, Superfamily of Aphidoidea, Family of Aphididae and Subfamily of Aphidinae (Carver et al., 1991).

**Life cycle.** *A. pomi* is monoecious (sexual and parthenogenetic phases occur on the same host) and holocyclic in Central Europe (propagates sexually and parthenogenetically) (Tomiuk and Wöhrman, 1982). It passes the winter in the egg stage on one year old shoots. In spring the eggs hatch and the fundatrix (stem-mother) starts to reproduce parthenogenetically. Reproduction in spring and summer is entirely parthenogenetical. These generations of virgins, which are mainly apterous, feed on the succulent foliage of apple throughout summer. This aspect of the green apple aphid life cycle, beside others, makes it easy to rear the insect under controlled environmental conditions. Alatae produced are merely for dispersal when the population density on the host plant becomes too high. In fall, males and oviparous females (sexuales) are produced by the sexuparae, mate and the over-wintering eggs are deposited preferentially on watersprouts (Baker and Turner, 1916; Lathrop, 1923). The life cycle of a parthenogenetically reproduced aphid consists of 4 larval instars and an adult stage. The aphid develops from one stage to another by molting (ecdysis). The instars and in particular the fourth larval instar can be distinguished from the adult aphid by the form of its cauda. The instars among each other can be distinguished by body size and form.

**Nutrition.** The majority of aphids are phloem feeders. In general, nitrogen is the basic nutrient which first becomes limiting for aphid development. The phloem sap is rich in carbohydrates and poor in amino acids but has the advantage that nutrients are in soluble and renewable form (Risebrow and Dixon, 1987). As a result, vast quantities of soluble carbohydrates (e.g.
sucrose) are ingested in excess of dietary requirements, and this explains the high sugar content of the excreted honeydew. Melezitose is the main sugar component found in aphid honeydew. It is not found in phloem sap, however, and it appears that sucrose of the phloem is transformed to melezitose in the aphids' dietary tract.

The question of the degree to which aphids ingest their food passively and/or actively is still not entirely certain. Phloem sap is under pressure (reported turgor pressures in phloem range from 0.5 to 2.5 MPa depending on the author) (Wright and Fisher, 1980; Weatherley, 1982 cf. Schulze, 1991) and is expected to flow by capillarity (passively) into the aphid's stylet, whenever one of the sieve tube elements is pierced. Aphids have a ciberial pharyngeal pump which could be used to control passive uptake but it undoubtedly enables aphids to suck their food actively (Pollard, 1973; Klinglauf, 1987; Hugentobler, 1990), since aphids can survive on artificial diets which are not under pressure. The fact that aphids do not grow to maximum rates on such diets, even when they contain much higher concentrations of limiting nutrients than phloem sap, has been considered by some authors (e.g. Wearing et al., 1968) to indicate that on their normal food plants aphids must feed passively to some extent at least.

**Developmental biology.** The developmental threshold \( T_0 \) of *A. pomi* was reported by Lathrop (1923) to be 5°C. Experiments by Graf et al. (1985) to determine *A. pomi* life table statistics showed that \( T_0 \) was 5.9°C. Furthermore, the authors specified the highest developmental rate of *A. pomi* to be at 25.8°C, and the maximum mean fecundity to be at 19.7 - 20°C. The average daily temperature (19.8°C) approximated over a day in the present study was optimal for maximum fecundity per female.

In the study presented here, the temperature of 19.8°C was the weighted mean over the whole growth chamber. In fact, ambient temperatures inside the growth chamber varied depending on the distance from the source of light, the ventilation, the distance from chamber walls etc. These variations could amount to 5°C. Leaf surface temperatures further increase with water stress and in growth chambers can even exceed the ambient temperature. Generally, the temperature of an insect is close to air temperature or to the temperature of the microenvironment. Hence, the body temperatures of aphids are highly affected by leaf surface temperatures.

**Aphid rearing.** Aphids were reared on young apple plants grown in soil under cages in the greenhouse. Reproduction was exclusively parthenogenetic. Temperatures in the greenhouse ranged between 23°C
during the day and 18°C during the night. Daylength was 17 h. Every two to three weeks, when aphid population densities on the plants became too high and alatae were produced, uninfested plants were placed in the cages. A few adults were transferred to the uninfested plants and the damaged plants were removed and discarded.

For Exp. 1 and Exp. 2 fourth larval instars (L4) were carefully collected in the greenhouse and transferred to the experimental plants in the growth chamber. The insects were released on the tenth leaf counted from the plant apex (referred to as leaf A). These larvae were allowed to reach adulthood and start reproduction. After four to six L1 were born the mother was removed. Subsequent handling of L1 depended on the objectives set for each experiment and will be discussed in the respective section. The beginning of an experiment (day 1) was marked by the birth of the L1. Aphid transfer from the greenhouse to the growth chamber and the start of withholding water to induce water stress were counted backwards from day 0. In Exp. 3 and Exp. 4, the transferred aphids (specified in the respective sections) were allowed to reproduce and build up a population. Aphid transfer in these experiments was signified by day 0.

1.4.3 The Water Stress

**Preliminary experiments.** Three preliminary experiments were conducted to find the most appropriate method to induce water stress and to test the effect on aphid development (e.g. Sumner et al., 1983 and 1986; Krizek, 1985; Snow and Tingey, 1985). The methods tested were: (1) water stress induced in soil culture by controlled watering and (2) water stress induced in a nutrient solution using polyethylene glycol (PEG) as an osmoticum. In summary, soil culture had the advantage of approaching field patterns of water stress development and had the disadvantage of not maintaining the stress at a constant level. The nutrient solution culture had the advantage of allowing a constant and reproducible water stress as well as nutrient supply and the disadvantage of PEG being a potential toxicant for plants and aphids and of not reflecting patterns of water stress in the field. Results of the preliminary experiments also showed that water stress in the soil and the nutrient solution cultures produced different effects. Most striking were the visual differences. Plants, cultivated in nutrient solution expressed very little decrease in plant growth and development: there was no reduction in number of leaves per plant and in total leaf area, a slight reduction in shoot length and increased
leaf succulence with water stress. None of the expected typical reactions to water stress (decreased leaf water potentials, increased stomatal resistance) were observed.

**Theory.** Water in soil and plants is subject to several force fields caused by the presence of the solid phase, dissolved substances, hydrostatic or tension pressure (external gas pressure), and the gravitational field. These effects are quantitatively expressed in terms of the potential energy of water. The common unit of water potential is the megapascal (MPa). A commonly used alternative unit is bar (1 MPa = 10 bars). Water potential in any system (soil or plant) can be expressed as (Begg and Turner, 1976):

\[
\Psi = \Psi_o + \Psi_p + \Psi_m + \Psi_g
\]

- \(\Psi\) = water potential or total potential
- \(\Psi_o\) = osmotic potential due to the presence of dissolved substances.
- \(\Psi_p\) = pressure potential. In soils it is related to the hydrostatic pressure found under saturated conditions. In plants it is the turgor pressure acting outward on cell walls and membranes.
- \(\Psi_m\) = matric potential due to the forces of capillarity and molecular imbibitional forces.
- \(\Psi_g\) = gravitational potential due to the gravitational forces of water.

The \(\Psi_g\) in soils can be of importance for drainage in very wet soils but otherwise, it is considered to be negligible (Nobel, 1991). In most crop situations, the \(\Psi_g\) is considered insignificant (Begg and Turner, 1976).

Water deficit in soils is usually dominated by the \(\Psi_m\) component, which can be as low as -2 MPa or even lower. The other two components (\(\Psi_o\) and \(\Psi_p\)) in comparison are frequently negligible.

In the plant, \(\Psi\) had gained wide acceptance as a fundamental measure of plant water status (indicator of physiological water stress). Leaf water potential in the plant (\(\Psi\)) is the algebraic sum of \(\Psi_o\), \(\Psi_p\) and \(\Psi_m\). However, in most cases the potential components of concern are the \(\Psi_p\) and \(\Psi_o\), unless the tissue is badly dehydrated (Hsiao, 1973; Begg and Turner, 1976; Holtzer et al., 1988; Schnider, 1989). On the other hand, as was reviewed by Jones and Corlett (1992), the notion of Hsiao (1973) and Oertli (1976), that \(\Psi_p\) rather than \(\Psi\) was the crucial factor controlling physiological responses of plants to drought has been supported by later investigations (Turner and
Jones, 1980; Sinclair and Ludlow, 1985). Furthermore, Sinclair and Ludlow (1985) suggested that changes in cell volume act as the transducer of turgor pressure, i.e. of water deficit. Turgor pressure ($\Psi_p$) is thought to influence cellular volume through elastic and plastic dimensional changes of cell walls (cf. Sinclair and Ludlow, 1985; Turner, 1986). Cell elasticity and extensibility vary with species and may be affected by environmental conditions such as nutrient and/or water availability (Studer, 1993). In addition, several investigations suggested that roots act as a sensor of water deficit via root-produced plant growth regulators (e.g. ABA and cytokinins) (Davies, 1987; Tardieu and Davies, 1993). According to Turner (1986), the identification of the root as the site of "sensing" soil water deficits does not eliminate the role of turgor pressure as the transducer of water deficits, but moves the emphasis from leaves to roots.

The plant water deficit that develops in any particular situation is the result of a complex combination of soil, plant, and atmospheric factors (Begg and Turner, 1976; Schnider, 1989), all of which interact to control the rate of water absorption and water loss. It is the result of both reduction in soil matric potential ($\Psi_{soil}$) which occurs, usually progressively, over a period of time, and fluctuations in evaporation rate which occur with daily changes in net radiation and humidity. Typically, plants experience diurnal cycles in which water deficits develop in the late morning and afternoon and may be relieved during the evening when transpirational loss decreases and at night when transpiration may become zero. Characteristically $\Psi$ in the leaves is lower than $\Psi_{soil}$ during the day but recovers to $\Psi_{soil}$ at night (Holtzer et al., 1988; Schulze, 1991). Components of plant water potential in phloem and xylem cells can be much different from bulk tissue. In xylem, $\Psi_o$ is very small and $\Psi_p$ is mostly negative. On the other hand, $\Psi_o$ for phloem can be very strongly negative, and $\Psi_p$ is positive (Holtzer et al., 1988; Nobel, 1991).

**Adaptation.** Many plants developed morphological and physiological adaptations to water stress. They range from drought escape to dehydration tolerance on a cellular basis. The mechanisms of adaptation act either in maintaining turgor or in maintaining volume by increased elasticity. Some examples of ways to maintain turgor are the maintenance of water uptake (increased root density and depth), reduction of water loss (increase in stomatal as well as cuticular resistance and reduction of leaf area), and osmotic adjustment. For a review of morphological and physiological adaptations to water stress in apple trees see Lakso (1983).
Osmotic adjustment is the ability of higher plants to accumulate solutes in response to water deficit (active osmotic adjustment), thereby maintaining turgor, cell enlargement and growth (probably at a reduced rate), stomatal opening and photosynthesis, rather than the mere accumulation of solutes as a consequence of decreasing water contents in cells (passive osmotic adjustment) (Turner and Jones, 1980; Morgan, 1984; Turner, 1986). Some of the solutes responsible for osmotic adjustment are soluble carbohydrates (fructose, glucose), sugar alcohols (sorbitol), amino acids (proline), organic acids, and inorganic ions (Morgan, 1984). Osmotic adjustment of leaves and other organs varies with plant species and even among cultivars. The degree of osmotic adjustment is affected by both the rate of stress and stress preconditioning of the plant (Turner and Jones, 1980; Morgan, 1984). Apple leaves are very drought tolerant (Davies and Lakso, 1979a; Sritharan and Lenz, 1989) and were observed to adjust osmotically in response to drought (Lakso, et al. 1984). Sorbitol was found to be a primary carbohydrate in the cell sap of apple leaves (Wang and Stutte, 1992) as well as of many woody rosaceous species and to contribute to total osmotic adjustment (cf. Ranney et al., 1991). Davies and Lakso (1979a) proposed that, apart from any diurnal changes, the tissue elasticity of apple changes but not \( \Psi_0 \) throughout the season in response to water deficiency. It seems that a combination of a number of morphological and physiological adaptations contribute to drought tolerance in apple trees.

**Experimental procedure.** The level of water stress in the soil was measured as soil matric potential (\( \Psi_{\text{soil}} \)) by means of tensiometers and gypsum blocks (Soil Moisture Equipment Corp., CA). Tensiometers are suitable for measurement of low levels of soil water stress (i.e. high \( \Psi_{\text{soil}} \)). Below \( \Psi_{\text{soil}} \) of -0.08 MPa, tensiometers give incorrect readings. Gypsum blocks, on the other hand, are too insensitive when there is little water stress (\( \Psi_{\text{soil}} > -0.10 \) MPa). Throughout this study, therefore, \( \Psi_{\text{soil}} \) of control (well-watered) treatments were measured by means of tensiometers and that of the water stressed treatments by means of gypsum blocks.

One tensiometer was placed approximately in the center of the control pot (at 10 cm soil depth). In the water stressed pots, 1 to 2 gypsum blocks were placed in 14 cm soil depth and 1 to 2 gypsum blocks in 7 cm soil depth diagonally opposite. Measuring \( \Psi_{\text{soil}} \) by gypsum blocks or/and tensiometers does not give an exact measure of the water status in the rhizosphere of the plant’s roots. Soil matric potential at the root surface is expected to be lower than at the point of measurement, especially when the soil dries out and soil
water conductance decreases. Measurements of soil matric potentials ($\Psi_{\text{soil}}$) are point measurements in time and space. Therefore, the $\Psi_{\text{soil}}$-values measured and the water stress intensities extrapolated from them had to be considered to represent a range rather than an absolute value. Soil matric potential was measured daily and in the afternoon (after the period of maximum temperature and light intensities). The readings of the gypsum blocks placed in 14 cm soil depth indicated when watering became necessary.

Water stress was induced by withholding water from the plants for a period of time and allowing the soil to dry out gradually until a desired intensity of stress was reached; the plants were then rewatered. All plants experienced several drying cycles, a pattern called Intermittent drying cycles. Since the length of an experiment was at least one month, the intermittent drying cycle technique was inevitable.

To allow the soil to dry as gradually and uniformly as possible under the given conditions, care had to be taken in choosing the soil mix and the pot size. To reduce evaporation from the soil surface, a 1 cm layer of quartz sand (2 - 3.5 mm in diameter) was spread to cover the soil surface. Water stress in the soil is a dynamic process and cannot be held uniform in a pot. The top soil and the soil around the roots will dry more quickly than the soil at the bottom of the pot. The quartz sand cover was an attempt to reduce the rate at which the top soil dried out due to environmental conditions (light irradiation, temperature and ventilation). The pot sizes used were 20 L square pots (30*30*26 cm) for two plants and 10 L round pots (30 cm in diameter) for one plant.

To quantify the effect of water stress on plants, physiological reactions of the plant to water stress had to be measurable. Those changes which directly or indirectly could affect or influence aphid development were of special interest:

Leaf water potentials ($\Psi$) were measured in general at predawn using the Scholander bomb method (PMS Instrument Co. Model 1002) (Scholander et al., 1965). A leaf was cut with a sharp razor blade at the base of its petiole and directly measured. The same leaf was then immediately frozen to -70°C (to interrupt metabolism and destroy cell membranes), its sap squeezed out, and osmotic potential $\Psi_0$ measured by a vapor pressure osmometer (Wescor 5100 C). The readings in milliosmol/kg water (mOs/kg) were transformed to pressure units (MPa) according to the conversion factor: 1000 mOs/kg = -2.43 MPa at 20°C (based on the equation of van’t Hoff, refer to
Schnider, 1989; Wermelinger et al., 1990). Turgor pressure ($\Psi_p$) was calculated according to the equation: $\Psi_p = \Psi - \Psi_0$.

Water content of a plant can also indicate water stress. Water content was calculated on a fresh weight basis. Fresh (FW) and dry weight (DW) were determined for the harvested shoots (leaves and stem) as well as for leaves used to measure water potentials during the experiments. Plant tissues were dried for 48 h at 65°C.

Individual leaf (also during the experiment) and total leaf area at harvest (LA) were measured by means of a portable leaf area meter (LI 3000 A). The parameters described above that were used to quantify water stress in plants, were all destructive in nature.

In addition, non-destructive parameters such as the increase in shoot length and number of leaves, rate of shoot extension and leaf appearance per day, and leaf surface temperatures were assessed. Shoot length and total number of leaves were measured and counted at constant time intervals throughout the experiments. Leaf surface temperatures were measured using different techniques and will be discussed in the relevant section. This applies also to other parameters which were assessed only in a certain experiment.
Leer - Vide - Empty
2

Effects of Water Stress on Population Dynamics of the Green Apple Aphid: Life Table Analysis

2.1 Introduction

The range of effects of water stress on plant sucking insects, in particular on aphids, is broad. Literature on the action of water stressed host plants on aphid species has accumulated since the nineteen fifties and continues to report diversely on the effects on the insects. For example, Kennedy and Booth (1959 cf. Sumner et al., 1983) reported that drought reduced survival and fecundity of Aphis fabae (Scopoli) on spindle and on sugarbeet. Wearing and van Emden (1967) found A. fabae reproduction unaffected by drought stressed Vicia faba (F.) and marigolds. Pea aphid (Acyrthosiphon pisum Harris) fecundity increased on drought stressed garden peas (Baker and Tauber, 1954 cf. Sumner et al., 1983). Brevicoryne brassicae (Linnaeus) reproductive rates declined with increasing water stress in brussel sprouts (Wearing and Van Emden, 1967), whereas Myzus persicae (Sulzer) exhibited reduced reproduction only at high stress levels on brussels sprouts and highest reproduction at intermediate stress levels. For additional literature on this aspect see Waring and Cobb (1992).

Despite the wealth of literature, however, the development of the green apple aphid (Aphis pomi De Geer) on hosts suffering from water stress does not seem to have been studied previously.
The objective of the present section was to study the effect of water stress on apple plants and on the population dynamics of resident green apple aphid. For this purpose the effect of water stress on the host plant itself had to be characterized and quantified first in order to delineate adequate descriptors for the effect of water stressed hosts on population dynamics of aphids. The best single measure of the population growth of insect species under defined conditions is regarded to be the intrinsic rate of natural increase $r_m$ (Southwood, 1978; Hulting et al., 1990; Kaakeh and Dutcher, 1992). The intrinsic rate of natural increase can be calculated by parameters derived from age-specific or cohort life tables.

### 2.2 Materials and Methods

#### 2.2.1 Descriptors for the Development of Aphid Populations

The rate of increase in a population with a stable age distribution in an unlimited environment is given by:

$$ \frac{dN}{dt} = r_m N $$

where $N$ indicates the number of organisms, $t$ is time and $r_m$ is the birth rate minus death rate, and thus a measure for the populations' growth potential. If the number of organisms at time $t_0$ is $N_0$ then, by integration, the population at time $t$ is given by:

$$ N_t = N_0 e^{r_m t} $$

Under constant conditions, age-specific fecundity ($m_x$), survival rate ($l_x$) and development time ($x$) has often been estimated (Dixon, 1987; Chi, 1988). The data of these life table parameters can be used to iteratively approximate $r_m$ using the equation:

$$ \sum_x e^{(-r_m x)} l_x m_x = 1 $$
Since $r_m$ is an estimate, it is important to recognize the uncertainty associated with this value. Meyer et al. (1986) introduced a Jackknife estimate of the variance of $r_m$ which can be used to assess this uncertainty. Some programs to perform the required computations were made available lately (Abou-Setta et al., 1986 (in Basic); Chi, 1988 (in Basic); Hulting, et al., 1990 (in Pascal); Wermelinger et al., 1991 (in Fortran)).

Other useful measures (descriptors) are the net reproductive rate ($R_0$), the mean generation time ($G$) and the doubling time ($DT$). The net reproductive rate is the multiplication rate per generation and is obtained by multiplying $l_x$ and $m_x$:

$$R_0 = \sum_x l_x m_x$$

Mean generation time is defined as the mean development time of a female from birth until half of its progeny had been born and is calculated by:

$$G = \frac{\ln R_0}{r_m}$$

and mean doubling time is defined as the mean development time needed until half of the progeny in a population was born and is calculated by:

$$DT = \frac{\ln 2}{r_m}$$

For a detailed description of these parameters and their use in ecological studies the reader is referred to Messenger (1964), Krebs (1972), and Southwood (1978).

Since leaf surface temperatures, may increase significantly under water stress, a concurrent increase in the aphid's body temperature is assumed. Since aphids are poikilotherm and react rather sensitively to temperature this increase in the body temperature may affect the rate of aphid development. The temperature effect can be considered by expressing the aphid's development in terms of physiological rather than chronological time (Gilbert et al., 1976 cf. Asante et al., 1991).

Chronological time expressed in days is converted to physiological time using the equation:

$$t' = t (T - T_0)$$
where: $t'$ = physiological time (day-degrees)  
$t$ = chronological time (days)  
$T$ = estimate of aphid body temperature ($^\circ$C)  
$T_0$ = developmental temperature threshold ($^\circ$C)

### 2.2.2 Experimental Conditions

Two experiments, Exp. 1 and Exp. 2, were conducted to study plant and life table parameters of apple plants (cv. 'Golden Delicious') and the green apple aphid under conditions of water stress.

In both experiments, two apple plants were grown diagonally opposite in 20 L square containers. Soil mix, environmental conditions, experimental preparations, as well as induction and measurement of water stress (four gypsum blocks per container in water stressed treatments) were as described on pages 8, 9, 15, 16 and 17. The treatments (i.e. water regimes) applied were:

1. control (C): $\Psi_{soil} > -0.05$ MPa (well-watered plants)
2. moderately stressed (M): $\Psi_{soil} > -0.60$ MPa
3. severely stressed (S): $\Psi_{soil} > -1.80$ MPa

If not otherwise mentioned, $\Psi_{soil}$ of the water stressed treatments was calculated from the average reading of all four gypsum blocks. Experiment 1 comprised 8 replicates (8 containers) per water stress treatment. In each container one of the two plants was infested with aphids and the other was not. The uninfested plant was used to measure leaf water potentials throughout the experiment. In Exp. 2, both plants of a container were infested with aphids resulting in 16 replicates per water stress treatment. In both experiments, two fourth larval instars (L4) were transferred to the 8th, 9th or 10th leaf counted from the plant apex at the beginning of the experiments (referred to as leaf A) and were allowed to develop into adults and reproduce. Any L4 that were missing or had died due to the transfer were replaced the following day. In Exp. 1 and Exp. 2, four and six respectively of the newly born L1 were kept per plant in all treatments, and the remaining aphids were removed, resulting in a theoretical cohort size of 32 aphids per treatment in Exp. 1 and 96 aphids in Exp. 2. Experiments 1 and 2 lasted for 40 and 55 days, respectively.
2.2.3 Data Collected

Plant Parameters

Plant growth. In both experiments, plant size (i.e. shoot length and total number of leaves) was measured weekly. Mean total plant size, mean increase in plant size (final - initial measurement), mean extension rate per day and mean rate of leaf appearance per day were weighted over the whole experimental period. This procedure made it possible to disregard fluctuations in plant growth due to intermittent drying, particularly in water stressed treatments. Plant growth was observed to decrease with the build up of water stress in the soil and to increase again after watering. Mean internodal length of the growth which took place during the experimental period was calculated.

Plant water relations. During Exp. 1, leaf water and osmotic potentials of the first fully expanded leaf (the 10th leaf from the apex) of aphid uninfested plants were measured predawn at the peak of a drying cycle and on the directly consecutive leaf 1 to 2 days after watering (start of a new drying cycle). This was repeated several times during Exp. 1 (maximum 4 times). At harvest, leaf water and osmotic potentials of the first fully expanded leaf of uninfested plants as well as of plants infested with aphids were measured at predawn. During Exp. 2, predawn leaf water and osmotic potentials of the first fully expanded leaf at the peak of a drying cycle were measured only once, in order not to disturb the aphids feeding on the plants. In Exp. 2 at harvest, predawn leaf water and osmotic potentials of two apical leaves, of two just fully expanded leaves and of two mature leaves (in the lower parts of the plant) were assessed. Turgor pressures and water contents of these leaves were calculated.

In addition, one plant of each treatment in Exp. 2 was chosen to measure leaf water potential components on every second leaf from top to bottom of the plant.

Area of Individual leaves. In Exp. 2, the area of each leaf sampled at harvest to measure leaf water potentials (see plant water relations above) was assessed.
Leaf surface temperature. Leaf surface temperatures were measured in the growth chamber at maximum light intensities and ambient temperature (ca 23°C). In Exp. 1, temperatures were measured on the lower leaf surface of the seventh leaf from the apex by means of an infrared thermometer (Telatemp Corp., Fullerton, CA). The disadvantage, however, was that leaves had to be turned over to measure lower leaf surfaces, and that the 30 cm operating distance, which was required for accurate measurements, could often not be held free of other objects. Leaf surface temperatures were measured at the beginning (day 5), in the middle (day 27) and at the end (day 40) of the experiment. In Exp. 2, lower leaf surface temperatures were assessed by means of a portable thermocouple device (chromel/alumel, 0.02 mm in diameter). Leaf surface temperatures were measured 9 times during Exp. 2. The tenth leaf from the apex, counted on the day of measurement, and leaf A, which was defined at the beginning of the experiment were measured. The temperature sensor was held next to the midrib one third the distance from the leaf petiole.

Plant biomass production. A "harvest" time was defined in Exp. 1 as directly after all aphids of a cohort had terminated their reproduction, irrespective of the level of water supply in the containers at that point. In Exp. 2, harvest was after all aphids of a cohort had died and water stress reached the peak of the current drying cycle. At harvest shoot length, total number of leaves, fresh and dry weight as well as water content of the shoots were assessed.

Life Table Parameters

The duration of each larval stage, the pre-reproductive time (time from birth until birth of first offspring), reproductive time (time from birth of first to last offspring) as well as adult longevity, survival rates and fecundity were recorded daily. Larvae and adults that had died during the study were recorded and larval as well as adult mortality was calculated. In Exp. 1, the different instar stages were recorded to determine the length of their development time. This was done on the basis of relative body size, ecdysis, cauda form and color. During adulthood offspring were counted daily and removed with a small brush. Age-specific fecundity ($m_x$) and mortality ($l_x$) as well as development time (x) of adult aphids within cohorts feeding on differentially water stressed plants were assigned to a life table and summary statistics were then calculated.
For the conversion of the chronological time (t) to physiological time (t'), aphids' body temperatures under the different water regimes were estimated. For this purpose leaf surface temperatures measured in each plant in Exp. 1 and Exp. 2 were averaged over a day according to the method described on page 8. In Exp. 1, mean daily body temperature of aphids was calculated to be 20.9, 21.4 and 21.7°C in the well-watered, moderately and severely stressed treatment, respectively. In Exp. 2, mean daily body temperature of aphids feeding on the tenth leaf from the apex was 21.2, 21.4 and 21.3°C in the well-watered, moderately and severely stressed treatment, respectively. On leaf A in Exp. 2 (see page 23), mean daily temperature was 19.5, 19.6 and 19.8°C in the well-watered, moderately and severely stressed treatment, respectively. This approach assumed that:
- Temperature effects (fluctuating daily temperatures, e.g. day/night temperatures) on the development of aphids were additive.
- The body temperatures of aphids in the three water supply treatments remained constant throughout the experiments (no effect of intermittent watering on leaf temperatures).
- All leaves within a plant and a treatment had the same temperature (beside the rough separation between the tenth leaf from the apex on the day of measurement, and the tenth leaf from the apex at the beginning of Exp. 2, i.e. leaf A).

It was observed that leaves which were nearer to the source of light in the growth chamber had higher surface temperatures than more distant leaves, irrespective of water stress treatment. Surface temperatures of uppermost leaves at noon were even higher than the ambient temperature. The distance of leaves to the source of light was an artefact encountered throughout the experiments which influenced aphid development. During Exp. 2 it was noticed that aphids which settled on the upper part of the plant (growing leaves) behaved and developed quite differently from aphids that had settled on the lower part of the plant (mature leaves). After termination of the experiment, therefore, the plants were divided into upper and lower parts and data analysis was done on the parts separately. The apex and the upper 15 leaves were assigned to the upper and the rest to the lower part of the plant.

Plants of Exp. 1 were cut down at harvest to the fourth node above soil surface. These plants were allowed to regrow to be used in Exp. 2. Only one shoot was allowed to develop. This technique was applied to obtain a high root to shoot ratio which was expected to induce a more gradual and uniform
water stress on the plant. After the harvest of Exp. 2 plants a soil sample of each container was taken to determine soil water content at the time of harvest when water stress was at the peak of the current drying cycle.

2.2.4 Data Analysis

To approximate $r_m$, $R_0$, $G$, and $DT$ a computer program kindly made available by B. Wermelinger (WSL/FNP, CH-8903 Birmensdorf) and described in Wermelinger et al. (1991) was used. To test the effect of water stress on plant, life table and population parameters, differences among means were compared by standard analysis of variance (ANOVA) followed by a test of least significance difference (LSD) at a 5% level of significance if not otherwise mentioned (Statgraphics 5.0, STSC Inc., Rockville, MA, USA). Simple linear regression was applied to describe the relationship between $\Psi_{soil}$ and $\Psi$, $\Psi_o$ and $\Psi_p$ as well as among potential components within a plant. Correlation coefficients were computed.
2.3 Results

2.3.1 Development of Water Stress in the Soil

The water stress that developed in the well-watered, moderately and severely stressed treatments and among the 8 replicates of any one treatment proved to be highly variable. Figures 1 and 2 show the development of water stress in one well-watered, one moderately and two severely stressed examples of Exp. 1 and Exp. 2, respectively. The examples shown are of representative single pots.

At the beginning of an experiment, particularly in Exp. 1, $\Psi_{\text{soil}}$ was very high because plants were still relatively small in size and were very well watered. Watering frequency decreased with an increase in intensities of water stress. Until plant harvest in Exp. 1, well-watered plants experienced 5 to 8, moderately stressed 3 to 5 and severely water stressed plants 2 to 3 drying cycles. In Exp. 2, well-watered plants experienced 9 to 12, moderately stressed 6 to 9 and severely stressed plants 5 to 7 drying cycles until plant harvest. As expected, the chosen $\Psi_{\text{soil}}$ values of -0.05, -0.60 and -1.80 MPa for the well-watered, moderately and severely stressed treatments, respectively, could not be maintained exactly. The $\Psi_{\text{soil}}$ ranges measured at the peak of the drying cycles in both experiments are given in Table 1.

Table 1. Range of soil matric potentials ($\Psi_{\text{soil}}$) in replicates of a treatment at maximum stress under well-watered (control), moderate and severe conditions of water stress in experiments 1 and 2 $^a$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Range of soil matric potentials (MPa)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment 1</td>
<td>Experiment 2</td>
</tr>
<tr>
<td>Control</td>
<td>-0.02 to -0.05</td>
<td>-0.03 to -0.06</td>
</tr>
<tr>
<td>Moderate</td>
<td>-0.42 to -1.56</td>
<td>-0.63 to -4.21</td>
</tr>
<tr>
<td>Severe</td>
<td>-1.21 to -2.44</td>
<td>-1.56 to -8.22</td>
</tr>
</tbody>
</table>

$^a$ $\Psi_{\text{soil}}$ was calculated from the average reading of the two gypsum blocks placed at 14 cm soil depth.
Fig. 1. Soil matric potentials ($\Psi_{\text{soil}}$) in representative, single pots of well-watered, moderately and severely water stressed treatments during experiment 1.
Soil matric potentials ($\Psi_{\text{soil}}$) in representative, single pots of well-watered, moderately and severely water stressed treatments during experiment 2.

Fig. 2.
The variability in $\Psi_{\text{soil}}$ at the peak of drying cycles was rather high within and between the different treatments. Additionally, the drying cycles within a treatment did not develop synchronically; therefore, it was not always possible to consider pooled data for comparisons of certain parameters (e.g. components of leaf water potentials) at a specific sampling date. The two last plots in Figs. 1 and 2 visualize this problem of data pooling. The differences in timing between the drying cycles in each comparison was due to procedure: when drying in one container was at its peak, watering of the other had just taken place and a new drying cycle had been initiated.

In Exp. 1, soil water potentials measured at 7 cm soil depth were lower compared with water potentials measured at 14 cm. In Exp. 2, the gradient within a container was smaller. A higher root to shoot ratio, particularly at the beginning of the experiment, and a more uniform root distribution over the container resulted in a more uniform moisture distribution in a container.

In Exp. 1, the first drying cycle was initiated at day 0 of the experiment. The peak of the first drying cycle was reached ca 15 days after the experiment had started. At the beginning plants were rather small and had enough soil volume to buffer water loss and to postpone the onset of stress. In Exp. 2, the first drying cycle was initiated before the experiment had started and reached its peak between day 5 and day 10 of the experiment.

In Fig. 3, a soil water retention curve specific for the soil mix used in this study is presented. Soil water retention was determined using a pressure plate apparatus (for pressures down to -1.0 MPa) and by measuring soil water content and soil matric potentials in Exp. 2 at harvest.

The soil mix used was characterized by an average water holding capacity (25 g water per 100 g soil at field capacity). There was a relatively large portion of coarse soil pores, probably due to the sand mixed in. Because the soil also contained considerable amounts of clay, plant unavailable water made up quite a large amount of the soil water fraction (ca 8.5%, see Fig. 3).
2.3.2 Effects of Water Stress on Plant Development

Plant growth. In Table 2, the weighted plant growth over the experimental period of both experiments is shown. Initial shoot length and number of leaves (at day -6) ranged between 20 - 71 cm (mean = 42.2 cm) and 21 - 38 leaves (mean = 28.3 leaves), respectively in Exp. 1 and between 35.5 - 87.5 cm (mean = 67.0 cm) and 27 - 42 leaves (mean = 36.0 leaves) in Exp. 2. In both experiments, mean initial plant size among the three treatments was similar. Final growth was measured at day 40 for Exp. 1 and day 50 for Exp. 2.

In Exp. 1, plant growth was significantly reduced with increasing intensities of water stress. During Exp. 2, shoot length and extension rate per day were significantly reduced with increasing water stress, whereas leaf number and rate of leaf appearance per day decreased significantly only between the moderately and severely stressed treatment.
Table 2. Total shoot length and total leaf number at harvest as well as the increase in shoot length, increase in number of leaves, extension rate per day and rate of leaf appearance per day averaged over the experimental period, of well-watered (control), moderately and severely water stressed apple plants in Exp. 1 and Exp. 2 (Exp. 1, measured at day 40; Exp. 2, measured at day 50). Means ±SE (in parenthesis) are shown. \(^a, b\); \(l. = \) length, app. = appearance.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>total shoot l. (cm)</th>
<th>total leaf number</th>
<th>increase shoot l. (cm)</th>
<th>increase in leaf number</th>
<th>extension rate (cm/d)</th>
<th>leaf app. rate (leaves/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>120.3 ± 2.2</td>
<td>63.5 ± 1.2</td>
<td>74.9 ± 2.1</td>
<td>34.5 ± 0.5</td>
<td>1.59 ± 0.03</td>
<td>0.73 ± 0.01</td>
</tr>
<tr>
<td>Moderate</td>
<td>101.4 ± 2.6</td>
<td>57.5 ± 1.3</td>
<td>61.8 ± 1.9</td>
<td>30.8 ± 0.5</td>
<td>1.32 ± 0.04</td>
<td>0.65 ± 0.01</td>
</tr>
<tr>
<td>Severe</td>
<td>86.1 ± 2.0</td>
<td>52.0 ± 1.0</td>
<td>44.3 ± 2.3</td>
<td>24.4 ± 0.8</td>
<td>0.94 ± 0.05</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>125.0 ± 2.8</td>
<td>70.3 ± 1.3</td>
<td>64.3 ± 2.9</td>
<td>34.8 ± 1.4</td>
<td>1.43 ± 0.07</td>
<td>0.77 ± 0.03</td>
</tr>
<tr>
<td>Moderate</td>
<td>115.8 ± 2.2</td>
<td>68.0 ± 1.4</td>
<td>56.9 ± 2.4</td>
<td>31.4 ± 1.5</td>
<td>1.26 ± 0.05</td>
<td>0.70 ± 0.03</td>
</tr>
<tr>
<td>Severe</td>
<td>108.9 ± 3.4</td>
<td>61.9 ± 2.4</td>
<td>49.4 ± 1.7</td>
<td>26.5 ± 2.0</td>
<td>1.10 ± 0.04</td>
<td>0.59 ± 0.05</td>
</tr>
</tbody>
</table>

\(^a\) mean of 16 replicates (aphid infested and uninfested plants showed no difference in growth).

\(^b\) means in a column followed by the same letter are not significantly different (\(P \leq 0.05\)).

Mean rate of extension per day was lower in Exp. 2 for well-watered and moderately stressed plants and higher for severely stressed plants compared with Exp. 1. Mean rate of leaf appearance per day was slightly lower in Exp. 1 compared with Exp. 2. Plants of Exp. 2 were larger at day 0 and grew for a longer time compared with those in Exp. 1 (difference \(\Delta\) in shoot length was about 25 cm; \(\Delta\) time about 10 days). Thus, plants in Exp. 2 suffered more from limited soil volume and space compared with the plants in Exp. 1, particularly the fast growing well-watered and moderately stressed plants.
Internodal length. In Exp. 1, the internodal length decreased significantly with increasing intensity of water stress (control: 2.17 cm; moderate: 2.01 cm; severe: 1.80 cm; P ≤ 0.05). Hence, leaf production was not affected by water stress to the same extent as shoot elongation. In Exp. 2, the internodal length did not change significantly with water stress, although there appeared to be a trend towards longer internodes (2.31 cm) in severely stressed plants compared with well-watered and moderately stressed plants (1.85 cm). Here, also, leaf production was impaired by severe water stress.

Individual leaf area. In Exp. 2, the area of fully expanded leaves of well-watered plants was significantly larger compared with those of severely water stressed plants (P ≤ 0.05). The difference in single leaf area between well-watered and severely stressed plants was about 10 cm². The extent of the reduction in leaf area due to water stress was not yet apparent in immature growing leaves which had not reached their maximum size.

Visual differences. Clear visual differences among plants grown under different water regimes were observed particularly in Exp. 1. The more intense the water stress became, the smaller plants and leaves were. The shape of the plant differed under the three water regimes: well-watered plants took the shape of a triangle pointing downwards, moderately stressed plants that of a rectangle and severely stressed plants that of a triangle pointing upwards. Leaf color became lighter and leaf toughness as well as hairiness increased with increasing intensities of water stress. Throughout the experiments, wilting was not observed in any treatment.

Leaf surface temperature. Leaf surface temperatures at maximum light and temperature intensities in the growth chamber did not differ significantly among treatments. The example of leaf surface temperatures illustrated the problem of pooling data of treatments measured at the same time (while water stress development among containers was variable). In Exp. 2, however, significant differences in leaf surface temperature between the tenth uppermost leaf on measuring day and leaf A (tenth leaf from the apex on day 0) of the plant were apparent (P ≤ 0.05). Figure 4 shows mean leaf surface temperatures measured at several dates during Exp. 1 and Exp. 2. In Exp. 2, surface temperatures of the tenth uppermost leaf on measuring day and that of leaf A (tenth leaf from the apex at day 0) are plotted separately.
Leaf surface temperatures measured in apple plants grown under different water regimes and at maximum intensities of light and temperature at several dates during Exp. 1 and Exp. 2. Means (Exp. 1: 8 replicates and Exp. 2: 16 replicates) of one date labelled by the same letter are not significantly different ($P \leq 0.05$).
At the beginning of the experiment, leaf surface temperatures, particularly in Exp. 1, did not differ among treatments. Withholding water had just started and plants were not yet suffering from water stress. Later in the experiments, a general increase in leaf surface temperature could be observed which was due to the plants growing towards the source of light. In Exp. 2, this increase in leaf surface temperature was restricted to the upper parts of the plant, whereas in the lower parts a general decrease in leaf surface temperatures was observed. This decrease can be explained by the shading of the lower parts of the plant by the increasing number of leaves in the upper parts. The differences in leaf surface temperatures between the tenth uppermost leaf on measuring day and leaf A (tenth leaf from the apex at day 0) reached 5°C. Leaf surface temperature in Exp. 1 and that of leaf A in Exp. 2, tended to increase with increasing intensities of water stress. In Exp. 2, surface temperatures of leaves positioned in the upper parts of the plant were affected, in addition to the water stress, by the distance of the measured leaves from the source of light (effect also observed by Schnider, 1989).

**Plant biomass production.** Table 3 shows fresh and dry weight as well as water contents of shoots of individual plants. Fresh and dry weight of plant shoots in both experiments decreased with an increase in the intensity of water stress. This decrease was significant between the well-watered and both water stressed treatments in Exp. 1 and between the well-watered and the severely stressed treatment in Exp. 2.

**Table 3.** Fresh and dry weight as well as water content of shoots of well-watered (control), moderately and severely water stressed apple plants in Exps. 1 and 2. Means ±SE (in parenthesis) are shown.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. 1</td>
<td>Exp. 2</td>
<td>Exp. 1</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>70.80 a</td>
<td>99.84 x</td>
<td>25.56 a</td>
</tr>
<tr>
<td></td>
<td>(± 6.68)</td>
<td>(± 4.93)</td>
<td>(± 2.61)</td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>46.78 b</td>
<td>90.82 xy</td>
<td>17.62 b</td>
</tr>
<tr>
<td></td>
<td>(± 4.88)</td>
<td>(± 4.64)</td>
<td>(± 1.98)</td>
</tr>
<tr>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>33.37 b</td>
<td>76.34 y</td>
<td>13.23 b</td>
</tr>
<tr>
<td></td>
<td>(± 2.95)</td>
<td>(± 5.70)</td>
<td>(± 1.25)</td>
</tr>
</tbody>
</table>

* Exp. 1: 8 replicates; Exp. 2: 16 replicates (except moderate: 14 replicates) means in a column followed by the same letter are not significantly different (P ≤ 0.05).
The reduction in fresh as well as dry weight between well-watered and both water stressed plants was much higher in Exp. 1 compared with Exp. 2. As mentioned above (see page 31), beside water stress additional stresses eventually impaired overall plant growth in Exp. 2, particularly that of the faster growing well-watered plants. Another difference between Exp. 1 and Exp. 2 was that the plants in Exp. 1 were harvested on the same day (irrespective of water stress level), whereas plants in Exp. 2 were harvested at the peak of the current drying cycle: well-watered plants first, then moderately stressed, then severely stressed plants.

In Exp. 1, plant water content decreased significantly with an increase in stress intensity. In Exp. 2, the decrease in water content was only significant between well-watered and stressed plants, irrespective of stress intensity. The water content of plants in Exp. 1 was generally higher than in Exp. 2, supporting the assumption that an additional stress had acted on plants in Exp. 2. As presented in Fig. 5, leaves and stems of single plants in Exp. 2 were affected differently by water stress.

![Graph](image)

**Fig. 5.**

Fresh and dry weight as well as water content (on FW basis) of leaves and stem of single apple plants grown under well-watered (control), moderately and severely water stressed conditions in Exp. 2. Means (16 replicates, except moderate: 14 replicates) between treatments labelled by the same letter are not significantly different (P ≤ 0.05).
In Exp. 2, fresh and dry weight of stems gradually decreased with increasing intensities of water stress. The decrease between well-watered and severely stressed plants was significant. Fresh and dry weight of leaves decreased significantly only between moderately and severely stressed plants. The water content of stems decreased significantly between well-watered and water stressed treatments. No significant difference among treatments was observed in water contents of leaves.

In Exp. 2, mean leaf loss due to senescence (bottom leaves) at harvest among treatments was not significantly different and reached 27.1, 23.6 and 22.7% of the total leaf number for well-watered, moderately and severely water stressed plants, respectively.

**Plant water relations.** As demonstrated in Fig. 6, the course of predawn $\Psi$, $\Psi_0$ and $\Psi_p$ development in apple leaves of a plant in Exp. 1 over the experimental period reflected the course of water stress development in the soil. Examples shown are of representative single plants.

A consistent trend between the components of plant water potential and soil matric potentials was observed. In severely stressed plants, predawn $\Psi$ and to a lesser extent $\Psi_0$ decreased with decreasing $\Psi_{soil}$ and increased again when the stress was relieved by watering (increase in $\Psi_{soil}$). Hence, $\Psi_p$ fluctuated during the experiment (e.g. between 0.50 and 1.50 MPa; Fig. 6).

In Exp. 1, $\Psi$ ranged between -0.40 and -1.40 MPa in well-watered plants, between -0.40 and -1.78 MPa in moderately stressed and between -0.50 and -2.85 MPa in severely stressed plants. The osmotic potential in leaves ($\Psi_o$) ranged between -1.33 and -2.10 MPa in well-watered plants, between -1.55 and -2.22 MPa in moderately stressed and between -1.71 and -3.20 MPa in severely stressed plants. Turgor ($\Psi_p$) in the leaves of well-watered plants ranged between 0.38 and 1.40 MPa, in moderately stressed plants between 0.33 and 1.50 MPa and in severely stressed plants between 0.21 and 1.85 MPa. In Exp. 2, $\Psi_p$ ranged between 0.53 and 2.35 MPa in well-watered plants, between 0.00 and 1.73 MPa in moderately stressed plants and between 0.00 and 2.00 MPa in severely stressed plants (see also Table 4).

The effect of water stress on leaves of different age (position within the plant) is briefly discussed. Leaves at different stages of development in well-watered and moderately stressed plants were similarly affected by water stress (data not shown). Apical leaves (5 youngest leaves) tended to maintain the lowest $\Psi_p$ (particularly in moderately stressed plants) within a plant. With an increase in leaf age, $\Psi_p$ increased until leaves were fully expanded.
Predawn leaf water ($\Psi$), osmotic ($\Psi_o$) and turgor potential ($\Psi_p$) in relation to soil matric potential ($\Psi_{\text{soil}}$) development of one well-watered, one moderately and one severely stressed apple plant in Exp. 1. Points indicate one leaf measurement. Dotted lines indicate periods with no measurements.

- △ leaf water potential
- ○ osmotic potential
- ◇ turgor pressure
- ■ soil matric potential
Thereafter, $\Psi_p$ remained almost constant until leaves started senescence, at which point leaf $\Psi_p$ decreased again. Leaf water potentials ($\Psi$) tended to respond similarly as $\Psi_p$. Osmotic potentials, however, were highest (least negative) in leaves of the apex. Similar trends were reported by Steinberg et al. (1989). These authors reported a "gradation" in $\Psi$, $\Psi_o$ and $\Psi_p$ that reflects the maturity of the leaves along 1-year old branches of peach ($\Psi$ apex < $\Psi$ mature leaves; $\Psi_o$ apex > $\Psi_o$ mature and growing leaves; $\Psi_p$ apex < $\Psi_p$ mature and growing leaves).

In Table 4, mean $\Psi$, $\Psi_o$ and $\Psi_p$ and their corresponding $\Psi_{soil}$ at the peak of drying cycles in Exp. 1. (assessed predawn during the experiment) and in Exp. 2 (assessed predawn at harvest) are shown.

Table 4. Predawn leaf water ($\Psi$), osmotic ($\Psi_o$) and turgor potentials ($\Psi_p$) of apple plants as well as their corresponding soil matric potentials ($\Psi_{soil}$) measured at the peak of drying cycles under well-watered (control), moderately and severely water stressed conditions in Exp. 1 (during the experiment) and in Exp. 2 (at harvest); n indicates number of observations. Means ±SE (in parenthesis) are shown.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>$\Psi$ (MPa)</th>
<th>$\Psi_o$ (MPa)</th>
<th>$\Psi_p$ (MPa)</th>
<th>$\Psi_{soil}$ (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>46</td>
<td>-0.72 a</td>
<td>-1.70 a</td>
<td>0.98 a</td>
<td>-0.01 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+ 0.04)</td>
<td>(+ 0.02)</td>
<td>(+ 0.04)</td>
<td>(+ 0.00)</td>
</tr>
<tr>
<td>Moderate</td>
<td>26</td>
<td>-1.03 b</td>
<td>-1.96 b</td>
<td>0.93 ab</td>
<td>-0.88 b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+ 0.05)</td>
<td>(+ 0.03)</td>
<td>(+ 0.05)</td>
<td>(+ 0.04)</td>
</tr>
<tr>
<td>Severe</td>
<td>25</td>
<td>-1.69 c</td>
<td>-2.50 c</td>
<td>0.81 b</td>
<td>-2.19 c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+ 0.10)</td>
<td>(+ 0.06)</td>
<td>(+ 0.08)</td>
<td>(+ 0.15)</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>114</td>
<td>-0.66 x</td>
<td>-1.82 x</td>
<td>1.17 x</td>
<td>-0.01 x</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+ 0.01)</td>
<td>(+ 0.03)</td>
<td>(+ 0.03)</td>
<td>(+ 0.001)</td>
</tr>
<tr>
<td>Moderate</td>
<td>77</td>
<td>-1.20 y</td>
<td>-2.17 y</td>
<td>0.97 y</td>
<td>-0.98 y</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+ 0.05)</td>
<td>(+ 0.03)</td>
<td>(+ 0.06)</td>
<td>(+ 0.07)</td>
</tr>
<tr>
<td>Severe</td>
<td>78</td>
<td>-1.74 z</td>
<td>-2.13 y</td>
<td>0.39 z</td>
<td>-1.84 z</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+ 0.07)</td>
<td>(+ 0.04)</td>
<td>(+ 0.06)</td>
<td>(+ 0.17)</td>
</tr>
</tbody>
</table>

* means in a column followed by the same letter are not significantly different ($P \leq 0.05$).
In both experiments, $\Psi_{\text{soil}}$ was distinctly different at the peak of drying cycles in the three treatments (well-watered, moderate and severe water stress). In Exp. 1, $\Psi$, $\Psi_0$ and to a lesser extent $\Psi_p$ decreased significantly with increasing intensities of water stress. In Exp. 2, $\Psi$ and $\Psi_p$ decreased significantly with increasing water stress, whereas $\Psi_0$ significantly decreased between the well-watered and both water stressed treatments. Leaf turgor pressures in the severely water stressed plants of Exp. 2 were much lower than in Exp. 1.

The data presented in Table 4 show a relationship between water supply in the soil and water relations in the plant. Thus, when all readings of $\Psi$, $\Psi_0$ and $\Psi_p$ in Exp. 1 as well as in Exp. 2 were plotted against corresponding $\Psi_{\text{soil}}$, regression lines could be fitted for each component of plant water potential and $R^2$-values calculated (Fig. 7).

In both experiments, $\Psi$, $\Psi_0$ and $\Psi_p$ decreased to various degrees with a decrease in $\Psi_{\text{soil}}$. Predawn $\Psi$ correlated best with $\Psi_{\text{soil}}$ (Exp. 1: $r=0.75$; Exp. 2: $r=0.83$; see Fig. 7). The extent of decrease in $\Psi$ due to a decrease in $\Psi_{\text{soil}}$ was similar to that expressed by the slope of the regression lines (b of the equation). In Exp. 1, $\Psi_0$ decreased with an increase in the intensity of water stress, whereas in Exp. 2 almost no change in $\Psi_0$ was observed. This indicated that osmotic adjustment had occurred in Exp. 1 but not in Exp. 2. In addition, the correlation between $\Psi_0$ and $\Psi_{\text{soil}}$ was higher in Exp. 1 ($r=0.75$) compared with Exp. 2 ($r=0.34$), again indicating a trend to osmotic adjustment in Exp. 1 but not in Exp. 2. Consequently, $\Psi_p$ in Exp. 2 dropped with increasing intensities of water stress, whereas, $\Psi_p$ could be maintained in Exp. 1 to a certain degree with an increase in water stress.

In conclusion, $\Psi_p$ in leaves tended to decrease with an increase in soil water stress, the extent of which depended on the capability of the plant to adjust osmotically, i.e. to accumulate solutes.

Relating $\Psi_0$ and $\Psi_p$ with their corresponding $\Psi$ also indicated the extent to which leaves of plants grown under different water regimes could adjust osmotically. In Fig. 8, $\Psi_0$ and $\Psi_p$ were plotted against $\Psi$, regression lines for each treatment fitted and $R^2$-values calculated.
Fig. 7. Predawn leaf water ($\Psi$), osmotic ($\Psi_o$) and turgor potentials ($\Psi_p$) of apple plants in relation to soil matric potentials ($\Psi_{soil}$) in Exp. 1 and Exp. 2. Regression equations, $R^2$- and P-values are shown.
Comparison of predawn leaf osmotic ($\Psi_o$) and turgor ($\Psi_p$) potentials as well as leaf water content with predawn leaf water potentials ($\Psi$) of well-watered (control), moderately and severely stressed apple plants in Exp. 1. Regression equations for each treatment and the corresponding $R^2$- and P-values are shown.
In Exp. 1, $\Psi_o$ of moderately and particularly of severely stressed plants decreased with a decrease in $\Psi$, whereas $\Psi_o$ of well-watered plants did not change with decreasing $\Psi$. Since leaf water contents did not decrease significantly with decreasing $\Psi$, the decrease in $\Psi_o$ in moderately and severely stressed plants could primarily be attributed to active osmotic adjustment to water stress. In Exp. 2, $\Psi_o$ of well-watered and severely stressed plants also decreased with an increase in $\Psi$ (data not shown). The relationship between the two components was, however, not very close (low $R^2$- and high $P$-values). Leaf turgor pressure of well-watered and water stressed plants in both experiments decreased to various degrees with a decrease in $\Psi$. The relationship between $\Psi_p$ and $\Psi$ was relatively close as was indicated by high $R^2$-values; this was certainly due to a collinearity between $\Psi$ and $\Psi_p$, since $\Psi_p$ was calculated as $\Psi_o + \Psi$.

The comparison between the regression lines indicated a disparate ability of plants grown under different water regimes to adjust osmotically: whereas moderately and particularly severely stressed plants of Exp. 1 showed distinct osmotic adjustment, well-watered plants could not adjust $\Psi_o$ when $\Psi$ decreased. At $\Psi = -1.0$ MPa, $\Psi_o$ in severely water stressed plants was lower than in moderately water stressed plants (difference $\Delta$ in $\Psi_o = 0.21$ MPa), and $\Psi_o$ in moderately water stressed plants was lower than in well-watered plants ($\Delta$ in $\Psi_o = 0.25$ MPa). With respect to $\Psi_p$ the opposite was true: at $\Psi = -1.0$ MPa, $\Psi_p$ in severely stressed plants was higher compared with moderately stressed plants ($\Delta$ in $\Psi_p = 0.20$ MPa), which in turn was higher than in well-watered plants ($\Delta$ in $\Psi_p = 0.24$ MPa; see Fig. 8).

In Exp. 2, $\Psi_o$ and $\Psi_p$ at a given $\Psi$ did not differ significantly in plants grown under the three water regimes. This indicated that plants under conditions of water stress did not show any significant osmotic adjustment.
2.3.3 Effects of Water Stress on Life Table and Population Parameters of Aphids

Mortality of Immatures. In aphid cohorts of Exp. 1, mortality of immatures was 3.7, 8 and 8% in the well-watered, moderately and severely water stressed treatments, respectively. In Exp. 2 on the other hand, it was 19.6, 16.1 and 10% on the upper parts and 17, 25 and 6.5% on the lower parts of plants grown under well-watered, moderately and severely water stressed conditions, respectively. The upper parts of the plant consisted of the apex and the upper 15 leaves counted from the apex and the lower parts comprised the rest of the plant. Accidental aphid losses ranged between 20-25% in both experiments.

Figures 9 and 10 show the survivorship and cumulative fecundity of *A. pomi* cohorts feeding on apple plants grown in different water regimes.

![Fig. 9](image.png)

**Fig. 9.** Survival rates and mean cumulative fecundity in cohorts of *A. pomi* feeding on apple plants grown under well-watered (control), moderately and severely water stressed conditions in experiment 1.

Development time of Immatures. In Exp. 1, the development time of immature aphids (from birth to adulthood) in the three water supply treatments was not significantly different and lasted on an average 8.6 days.
as can be seen in Table 5. In Exp. 2, the time of development of immature aphids feeding on the lower parts of the plant was about the same length as in Exp. 1 but about one day longer than on the upper parts (estimated from Fig. 10).

However in Exp. 1, a difference in the development time between the larval instars within a treatment was observed. The development time for L4 was significantly shorter than for the previous three larval instars, the durations of which were similar (Table 5). The fourth larval instar comprised a mean of 21.5% of the total premature development time in all treatments.
Table 5. Observed larval instar (L1, L2, L3, L4) and total immature time of development of *A. pomi* reared on apple plants growing under well-watered, moderately and severely water stressed conditions in Exp. 1. Ranges, means and ± SE (in parenthesis) are given a.

<table>
<thead>
<tr>
<th>larval instar</th>
<th>well-watered</th>
<th>time of development (days)</th>
<th>moderately stressed</th>
<th>severely stressed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>range</td>
<td>mean</td>
<td>range</td>
<td>mean</td>
</tr>
<tr>
<td>L1</td>
<td>1-3</td>
<td>2.31 a</td>
<td>1-3</td>
<td>2.00 bc</td>
</tr>
<tr>
<td>L2</td>
<td>1-4</td>
<td>2.35 a</td>
<td>1-4</td>
<td>2.39 a</td>
</tr>
<tr>
<td>L3</td>
<td>2-4</td>
<td>2.31 a</td>
<td>2-4</td>
<td>2.35 ab</td>
</tr>
<tr>
<td>L4</td>
<td>1-3</td>
<td>1.81 b</td>
<td>1-3</td>
<td>1.91 c</td>
</tr>
<tr>
<td>L1 - L4 b</td>
<td>7-10</td>
<td>8.77 x (± 0.20)</td>
<td>7-11</td>
<td>8.65 x (± 0.23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7-10</td>
<td>8.35 x (± 0.35)</td>
</tr>
</tbody>
</table>

a means in a column followed by the same letter are not significantly different (P ≤ 0.05).
b means in this row followed by the same letter are not significantly different (P ≤ 0.05).

Mortality of adults. In Exp. 1, the mortality of adults until reproduction had ended, was 23, 30.4 and 13 % for aphid cohorts feeding on plants grown under well-watered, moderately and severely stressed conditions, respectively (see Fig. 9). In Exp. 2, adult mortality was 22, 4 and 8 % on the upper parts and 10, 12 and 0 % on the lower parts of well-watered, moderately and severely water stressed plants, respectively (see Fig. 10).

In Exp. 1 and particularly on the lower parts of plants in Exp. 2, age-specific survivorship (*l*ₜ) of aphid cohorts feeding on moderately water stressed plants decreased faster compared with the other two water supply treatments (Figs. 9 and 10). On the lower parts of plants in Exp. 2, all adults of a cohort were dead between day 53 (severe stress) and 55 (moderate stress) of aphid age (Fig. 10). On the upper parts of plants in Exp. 2, all adults of a cohort were dead between day 42 (moderate stress) and day 55 (severe stress; see Fig. 10).

Development time of aphids. In Exp. 1, pre-reproductive time of aphids (time from birth to birth of first offspring) decreased significantly between well-
watered and severely water stressed plants (Table 6). About one third of the
virginoparae on well-watered and moderately stressed plants and nearly 90%
on severely stressed plants began to reproduce on the first day after molting.
The bulk of the offspring was born during the first 20 days of aphid lifespan
(see Figs. 9 and 11).
On the upper parts of plants in Exp. 2 (i.e. apex and the uppermost 15
leaves), no significant difference in pre-reproductive time among treatments
was found and pre-reproductive time was in general shorter than on the lower
parts (i.e. the rest of the plant). On the lower parts of the plants, however, the
pre-reproductive time of aphids decreased significantly between the well-
watered and water stressed treatments.

Table 6. Pre-reproductive and reproductive time (days) of A. pomi feeding on
well-watered (control), moderately and severely water stressed apple
plants in Exp. 1 as well as on the upper (apex and upper 15 leaves
counted from the apex) and lower parts (rest of the plant) of plants in
Exp. 2; n indicates cohort size. Ranges and means ± SE (in
parenthesis) are shown.a

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pre-reproductive time (d)</td>
<td>reproductive time (d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>range</td>
<td>mean</td>
<td>range</td>
<td>mean</td>
</tr>
<tr>
<td>Control</td>
<td>26</td>
<td>8-11</td>
<td>9.15 a (± 0.23)</td>
<td>6-27</td>
<td>19.16 ab (± 1.23)</td>
</tr>
<tr>
<td>Moderate</td>
<td>23</td>
<td>7-11</td>
<td>9.04 ab (± 0.24)</td>
<td>4-26</td>
<td>15.59 a (± 1.31)</td>
</tr>
<tr>
<td>Severe</td>
<td>23</td>
<td>7-11</td>
<td>8.48 b (± 0.20)</td>
<td>2-31</td>
<td>20.13 b (± 1.74)</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td>Experiment 2 upper parts of the plant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>37</td>
<td>6-11</td>
<td>8.14 a (± 0.21)</td>
<td>4-27</td>
<td>15.16 a (± 0.73)</td>
</tr>
<tr>
<td>Moderate</td>
<td>26</td>
<td>6-11</td>
<td>8.19 a (± 0.27)</td>
<td>11-27</td>
<td>17.31 a (± 0.74)</td>
</tr>
<tr>
<td>Severe</td>
<td>36</td>
<td>6-11</td>
<td>8.03 a (± 0.21)</td>
<td>5-42</td>
<td>17.31 a (± 1.11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 2 lower parts of the plant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>40</td>
<td>7-11</td>
<td>9.38 x (± 0.18)</td>
<td>8-33</td>
<td>21.03 x (± 0.89)</td>
</tr>
<tr>
<td>Moderate</td>
<td>33</td>
<td>6-13</td>
<td>8.85 y (± 0.24)</td>
<td>2-37</td>
<td>18.85 x (± 1.25)</td>
</tr>
<tr>
<td>Severe</td>
<td>43</td>
<td>6-10</td>
<td>8.67 y (± 0.14)</td>
<td>11-42</td>
<td>24.81 y (± 1.16)</td>
</tr>
</tbody>
</table>

a means in a column followed by the same letter are not significantly different
(P ≤ 0.05).
In Exp. 1 and on the lower parts of plants in Exp. 2, mean reproductive time (time between first born and last born larvae) was significantly longest on severely stressed plants compared with that in the other two treatments. On the upper parts, however, no difference in the reproductive time of aphids among treatments was observed. In both experiments, the actual reproductive time of some individual aphids in a cohort was longest on severely stressed plants. The reproductive time of aphids feeding on the lower parts of the plant was significantly longer ($P \leq 0.05$) compared with the upper parts.

Age-specific fecundity. As is shown in Figs. 11 and 12, mean age-specific fecundity ($m_x$) fluctuated in both experiments.

![Graph](image)

**Fig. 11.** Age-specific fecundity of *A. pomi* feeding on well-watered (control), moderately and severely water stressed apple plants in Exp. 1. Means of a cohort are shown.

In Exp. 1, age-specific fecundity in cohorts of all three treatments was highest between day 12 and 20 after birth (Fig. 11). In Exp. 2, age-specific fecundity was highest between day 11 and day 15 in both upper and lower parts of the plants (Fig. 12). Mean age-specific fecundity in a cohort was higher on the uppermost 15 leaves compared with lower parts in a plant (by an average of two larvae per female).
Fig. 12. Age-specific fecundity of *A. pomi* feeding on upper (apex and upper 15 leaves counted from the apex) and lower parts (rest of the plant) of well-watered (control), moderately and severely water stressed apple plants in Exp. 2. Means of a cohort are shown.

In Exp. 1 and in Exp. 2, maximum fecundity per day in a lifespan of a virginopara was not significantly affected by water stress (data not shown). However, this maximum fecundity within a treatment was significantly lower (P < 0.05) in the lower parts compared with that in the upper parts of the plants.

**Total fecundity.** Figure 9 (see page 43) shows that the differences in mean cumulative fecundity between aphids feeding on well-watered and on water
stressed plants in Exp. 1 were not significant (48.5 to 53.5 larvae per female). On the upper parts of plants in Exp. 2, mean cumulative fecundity was higher on water stressed plants (ca 50 larvae per female) than on well-watered plants (38.5 larvae per female; see Fig. 10, page 44). This difference in fecundity was significant between the well-watered and the moderately stressed treatments. On the lower parts, mean cumulative fecundity was highest on severely (35.7 larvae per female) and lowest on moderately stressed plants (23.5 larvae per female). The cumulative fecundity of aphids was significantly higher on the upper parts of the plants (i.e. apex and the upper 15 leaves from the apex) compared with the lower parts (i.e. the rest of the plant; P ≤ 0.05).

Total fecundities observed in this study were in general lower than those reported by Graf (1984; 75 larvae/female at 20°C) and Rutz et al. (1990; 75 larvae/female at 20/16°C fluctuating day/night temperatures).

Population parameters (summary statistics). In both experiments, the experimentally determined life table parameters (x, mx and lx) were tabulated to calculate net reproduction rate R0, intrinsic rate of natural increase rm, mean generation time G and doubling time DT. These results are presented in Table 7 for Exp. 1 and Exp. 2. In Exp. 2, summary statistics were calculated separately for the upper and the lower parts of the plants.

In Exp. 1 and on the upper parts of plants in Exp. 2, the intrinsic rate of natural increase was not significantly affected by water stress. On the lower parts of plants in Exp. 2, rm increased significantly for cohorts feeding on severely stressed plants compared with well-watered plants. In both experiments, however, rm tended to increase with an increase in stress intensity. Furthermore, on the upper parts of plants in Exp. 2, rm was significantly higher (P ≤ 0.05) compared with rm of the lower parts.

Intrinsic rates of natural increase presented in this study were within the range of rm estimates reported for A. pomi and a range of other aphid species (Graf et al., 1985; Sumner et al., 1986; Kaakeh and Dutcher, 1992; Zhou and Carter, 1992). Gaston (1988) stated that most aphid species have maximum rm values in the range of 0.11-0.50 surviving offspring per female per day. Intrinsic rates of natural increase for the green apple aphid reported by Rutz et al. (1990) and by Graf et al. (1985) were in general higher than rm-values reported in the present study probably because of higher fecundity rates per female.
Table 7. Intrinsic rate of natural increase ($r_m$), net reproduction rate ($R_0$), mean generation time (G, days), and mean doubling time (DT, days) of *A. pomi* cohorts reared on apple plants grown under well-watered (control), moderately and severely water stressed conditions in Exp.1 as well as on the upper (apex and the uppermost 15 leaves) and the lower (the rest of the plant) parts of plants in Exp. 2. ±SD are shown; n indicates cohort size a.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$R_0$ ± SD</td>
<td>$r_m$ ± SD</td>
<td>G  ± SD</td>
<td>DT ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>26</td>
<td>48.52 a ± 17.58</td>
<td>0.2644 a ± 0.0436</td>
<td>14.7 a ± 2.1</td>
<td>2.6 a ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>23</td>
<td>45.00 a ± 22.96</td>
<td>0.2707 a ± 0.0505</td>
<td>14.1 a ± 1.7</td>
<td>2.6 a ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>23</td>
<td>53.48 a ± 21.82</td>
<td>0.2813 a ± 0.0421</td>
<td>14.2 a ± 1.7</td>
<td>2.5 a ± 0.4</td>
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<tr>
<td></td>
<td></td>
<td>Experiment 2 upper parts of the plant</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>37</td>
<td>38.50 a ± 13.01</td>
<td>0.2943 a ± 0.0448</td>
<td>12.4 a ± 1.6</td>
<td>2.4 a ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>26</td>
<td>51.06 b ± 12.48</td>
<td>0.3051 a ± 0.0443</td>
<td>12.9 a ± 1.5</td>
<td>2.3 a ± 0.3</td>
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<td></td>
</tr>
<tr>
<td>Severe</td>
<td>36</td>
<td>48.50 b ± 17.24</td>
<td>0.3087 a ± 0.0509</td>
<td>12.6 a ± 1.8</td>
<td>2.2 a ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Experiment 2 lower parts of the plant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>28.81 y ± 9.66</td>
<td>0.2287 x ± 0.0478</td>
<td>14.7 x ± 2.0</td>
<td>3.0 x ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>33</td>
<td>23.48 x ± 11.59</td>
<td>0.2335 xy ± 0.0552</td>
<td>13.5 y ± 1.6</td>
<td>2.9 xy ± 0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>43</td>
<td>35.70 z ± 11.47</td>
<td>0.2527 y ± 0.0367</td>
<td>14.2 xy ± 1.5</td>
<td>2.7 y ± 0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* means in a column followed by the same letter are not significantly different ($P \leq 0.05$).

In both experiments, the net reproduction rate of aphid cohorts ($R_0$) corresponded to the cumulative fecundity of cohorts which was discussed previously (see page 49). In Exp. 1 and upper plant parts of Exp. 2, generation time (G) and cohort doubling time (DT) were not significantly affected by water stress. On the lower parts of plants in Exp. 2, G of aphids reared on well-watered plants was significantly longer than on moderately stressed plants and DT decreased with an increase in stress intensity. The difference between DT of cohorts feeding on well-watered and severely stressed plants was significant. Generation and doubling time correlated negatively with $r_m$ (G in Exp. 1: $r = -0.75$, $P<0.001$; G in Exp. 2: $r = -0.84$; $P<0.001$; DT in Exp. 1: $r = -0.999$, $P<0.001$; DT in Exp. 2: $r = -0.978$; $P<0.001$).
The intrinsic rate of natural increase was also found to relate negatively to aphid pre-reproductive time. Calculating $r'_m$ using physiological instead of chronological time altered the summary statistic to some extent and also resulted in no significant difference between treatments. The values presented in Table 8 refer to the case study in Exp. 1 and Exp. 2 (see page 24).

In Exp. 1, $r'_m$ did not differ significantly among treatments. Moreover, the increasing trend of $r_m$ with increasing intensities of water stress (see Table 7) was not confirmed by the $r'_m$ values (Table 8).

In Exp. 2, for cohorts feeding on the upper and lower parts of the plants no significant difference in $r'_m$ was detected. However, $r'_m$ in the lower parts of the plant tended to be higher in the severely stressed compared with the well-watered treatment. The increasing trend of $r_m$ with increasing intensities of water stress (see Table 7) was not as clear with the observed $r'_m$ (Table 8). The intrinsic rate of natural increase, however, was significantly higher for cohorts feeding on the upper parts compared with those on the lower parts of the plants ($P \leq 0.05$).

Table 8. Intrinsic rate of natural increase ($r_m$) of *A. pomi* cohorts reared on apple plants grown under well-watered (control), moderately and severely water stressed conditions in Exp. 1 and on the upper (apex and the uppermost 15 leaves) as well as the lower (the rest of the plant) parts of plants in Exp. 2. ±SD are shown; n indicates cohort size.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 1</th>
<th>Treatment</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r'_m$ ± SD</td>
<td></td>
<td>$r'_m$ ± SD</td>
</tr>
<tr>
<td>Control</td>
<td>0.0170 a ± 0.0027</td>
<td>Control</td>
<td>0.0184 a ± 0.0027</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.0168 a ± 0.0031</td>
<td>Moderate</td>
<td>0.0189 a ± 0.0027</td>
</tr>
<tr>
<td>Severe</td>
<td>0.0171 a ± 0.0025</td>
<td>Severe</td>
<td>0.0192 a ± 0.0031</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r'_m$ ± SD</td>
</tr>
<tr>
<td>Control</td>
<td>0.0162 x ± 0.0033</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.0164 x ± 0.0038</td>
</tr>
<tr>
<td>Severe</td>
<td>0.0181 x ± 0.0048</td>
</tr>
</tbody>
</table>

*a* means in a column followed by the same letter are not significantly different ($P \leq 0.05$).
2.4 Discussion

2.4.1 Effects of Water Stress on Plant Development

In the present study, apple plants were clearly affected by water stress: stressed plants were smaller in size, had lower growth rates, had lower shoot fresh and dry weight, and lower individual leaf area (Tables 2 and 3, Fig. 5). Although plant growth decreased significantly with water stress, overall growth did not stop even in the severely stressed treatments, which reached very low leaf water potentials ($\Psi < -2.85$ MPa measured predawn; see Table 4 and Fig. 7). The plants continued to suffer from severe intensities of water stress for only a short period, however, before they were relieved by watering (as a result of the intermittent drying cycles).

Apple trees are drought tolerant, having the capability to grow and carry on photosynthesis even at low water potentials (Davies and Lakso, 1979a; Sritharan and Lenz, 1989). Reductions in plant growth rate, particularly in terms of leaf area expansion are commonly observed in water stressed apple trees and are considered an important water stress response. Leaf area expansion of apple was strongly influenced by turgor pressure in potted trees (Lakso, 1979; Lakso et al., 1984): as turgor decreased, leaf expansion also decreased linearly until growth finally ceased as turgor fell below 0.25 MPa.

The rate of stress development, stress intensity and stress duration experienced by the plants determines, among other factors, the extent of plant suffering (Lakso et al., 1984; Wang and Stutte, 1992). Rapid stress development may not give sufficient time for adaptation mechanisms such as osmotic adjustment. Hence, methods used to induce water stress can greatly affect the above mentioned factors, and should therefore be taken into consideration when different studies on plant responses to water stress are compared. For example in studies with aphids, water stress was induced by the intermittent drying cycle technique (Wearing, 1972), by just letting the soil dry (Miles et al., 1982), or hydroponically using an osmoticum (Sumner et al., 1983 and 1986). The rate of water stress development also strongly depends on the ratio between plant size and/or plant growth rate and the soil volume available. Wang and Stutte (1992) mention that water stress develops much more rapidly in potted plants grown under controlled environmental conditions (growth chambers, greenhouse) than in field-grown trees: in experiments with potted plants, water stress can develop even within 1 to 2 days, due to limited soil volume. According to Miles (P.W. Miles, University of Adelaide, South
Australia, pers. comm.) and Louda and Collinge (1992), all plants in pots may be moderately stressed compared with the levels measured in the field.

An effect of limited soil volume on the development of water stress and on plant growth could clearly be observed in Exp. 2. Plants were already at the beginning of the experiment about 20 cm larger than in Exp. 1. Very soon the soil volume became limited (small pots) and the plants began to suffer from a rapid development of water stress and probably of nutrient deficiencies. This was reflected in generally slower plant growth in Exp. 2, particularly of the well-watered and moderately stressed plants, compared with Exp. 1 (see Table 2) and probably was the cause for the inability of the plants to adjust osmotically (to be discussed below).

In a very few studies, reactions of plant sucking insects to water stress have been determined in relation to actual changes in plant water relations (e.g. Schnider, 1989 and English-Loeb, 1990 for mites; Dorschner et al., 1986 for aphids). Predawn measurements of leaf water potentials (Ψ) in Exp. 1 and 2 reflected to a certain extent the three water supply levels in the soil (well-watered, moderately and severely stressed; see Table 4 and Fig. 6). Hsiao (1973) proposed for general purposes three functional levels for water stress in leaves: mild stress entails a lowering of Ψ by several bars below corresponding values in well-watered plants; moderate stress refers to a lowering of Ψ by more than a few bars but less than 12 or 15 bars (1.2 or 1.5 MPa); and severe stress refers to a lowering of Ψ by more than 15 bars (1.5 MPa). The level of water stress in the soil applied in the present study and the resulting Ψ in plants (at the peak of drying cycles) corresponded quite well to the levels proposed by Hsiao (1973) (see Table 4).

Measurements of predawn Ψ may be indicative of the range of water supply in the soil on the plant level but does not necessarily describe the physiological state of a plant or its adaptation to water stress. Turgor pressure is generally thought to be more relevant for many plant processes than Ψ per se. Hsiao et al. (1976) explained that a lowering of Ψ₀ by more than 0.2 or 0.3 MPa through dehydration normally reduces Ψᵃ to nearly zero in many crop species. In contrast, a similar drop in Ψ₀ through solute accumulation may maintain cell turgor and cell volume as well as turgor mediated processes. It is, however, difficult to distinguish between active and passive increase in solute concentration when Ψ₀ of extracted tissue sap is measured. Nevertheless, Studer (1993) has demonstrated that predawn measurements of Ψ₀ of tissue sap can provide a good basis for comparison of the degree of "active" osmotic adjustment in water stressed plants.
In Exp. 1, osmotic adjustment in response to water stress occurred (see Fig. 7). Stressed plants had higher $\Psi_p$ than control plants at any $\Psi$ and were capable of maintaining positive $\Psi_p$ at lower $\Psi$ (see Fig. 8). Furthermore, severely stressed plants could maintain turgor better than moderately stressed plants at low $\Psi$: under moderate stress conditions, zero turgor was reached at $\Psi = -2.0$ MPa and under severe stress, at $\Psi < -3.0$ MPa (see Fig. 8). Since $\Psi$ was measured predawn and leaf water content did not vary significantly with $\Psi$ within a treatment, the observed decreases in $\Psi_0$ can be attributed to active solute accumulation. In addition, $\Psi_0$-values corresponded well to ranges reported in other studies on apples and other woody plants (compare below). The higher $\Psi_p$ observed in rewatered, stressed compared with always well-watered plants (see Fig. 8) can be explained by the accumulation of solutes (lower $\Psi_0$) during the water stress period. Osmotic adjustment in water stressed plants (even if it is not sufficient to maintain turgor during stress) can lead to higher turgor values after rewatering than in well watered plants (Studer, 1993).

In Exp. 2, $\Psi_0$ did not decrease enough to maintain turgor at low $\Psi$ (see Fig. 7). Turgor reached zero at $\Psi_{soil}$ about -3.5 MPa (corresponding to predawn $\Psi$ about -2.3 MPa). The capacity for osmotic adjustment in Exp. 2 was probably limited by a too rapid development of high levels of water stress (limited soil volume) and by additional stresses (nutrient deficiencies, mite and powdery mildew infestation). This lack of osmotic adjustment was reflected in generally slower plant growth in Exp. 2 compared with Exp. 1 (see Table 2).

Wang and Stutte (1992) cited studies in which decreases in $\Psi_0$ in apple varying from 0.5 to 3.0 MPa have been found. Only 0.2 to 0.5 MPa of these decreases, however, could be attributed to active adjustment; the remainder was due to passive dehydration. Goode and Higgs (1973) observed osmotic adjustment in apple trees of as much as 0.7 MPa during the day (diurnal) and of up to 2.0 MPa over a growing season. Davies and Lakso (1979a) noticed $\Psi_0$ to decrease by as much as 1.65 MPa diurnally in field grown apple trees, which is a far greater decrease than generally observed in other mesophytes. Results from Fanjul and Rosher (1984) showed that osmotic adjustment occurred in both field and pot grown trees and that the extent of adjustment was similar (0.3 MPa) for both potted and orchard trees despite differences in the rate and intensities of stress imposed. On the other hand, Wang and Stutte (1992) noticed that $\Psi_p$ could not be maintained through osmotic adjustment when $\Psi$ dropped below -1.6 MPa in greenhouse grown 2-year old potted Jonathan apple trees.
It has been noted that osmotic adjustment does not represent the only mechanism for maintaining $\Psi_p$ in apple trees under water stress. High tissue elasticity allows apple leaves to decrease relative water content (RWC) to levels that would cause irreparable damage to most herbaceous plants, thereby buffering turgor against changes in $\Psi$ (Davies and Lakso, 1979a). In addition, particularly in big trees growing under field conditions, stored water from leaf and trunk tissues (capacitors) can be released to the transpiration stream, which buffers $\Psi$ under conditions of high transpirational demand (Davies and Lakso, 1979a and 1979b). This was also confirmed by the results of the study presented here: whereas no significant changes in water contents of leaves occurred with decreasing supply of water (see Figs 5 and 8), water content of stems significantly decreased with increasing water stress (Fig. 5). The ability of apple trees to continue growth over a wide turgor pressure range is in itself an important adaptation to water stress.

The distance of leaves from the source of light in the growth chamber was a main artefact in measuring leaf surface temperatures. The nearer the leaves were to the source of light, the higher their temperatures. This artefact confounded differences in leaf surface temperature between treatments due to water stress, particularly on the upper parts of the plants. Leaf surface temperatures of the upper parts of well-watered plants were higher than the ambient air temperature which was approximately 23°C (see Fig. 4) and, despite the transpirational cooling effects, higher than temperatures measured on the upper leaves of severely stressed plants.

In plants which have access to unlimited water supplies (well-watered), leaves can transpire freely (Burrage, 1976 cf. Willmer, 1986), and may be cooler than ambient temperature when air temperatures are high (e.g. 30°C). These differences between foliage temperatures and ambient air temperature are dependent on both net radiation and soil water availability. Plants respond to water stress by closing their stomata to reduce water loss by transpiration, i.e. leaf temperatures increase. Under field conditions, net radiation received by a leaf is a function of the leaf size, leaf shape, reflectance of the leaf surface, texture of the leaf, the site of the leaf (height above ground and orientation; Willmer, 1986). In growth chambers, the distance from the source of light, however, can become the dominating factor influencing leaf surface temperature, as was observed in the present study.

In both experiments, temperature measurements were only point measurements in time and space (location). Temperatures of only one leaf
were measured, and at maximum light intensities and growth chamber temperatures. Willmer (1986) cited that temperature variation over a single large leaf (upper leaf surface) could be up to 7°C with the higher temperature being in the center of the leaf. It follows that the leaf surface temperatures measured in Exp. 1 and Exp. 2, only crudely described the situation under water stress in the growth chamber. Nevertheless, leaf surface temperature in this study tended to increase with an increase in stress intensity (see Fig. 4). The average difference in temperature between well-watered and water stressed plants was about 2°C. This difference among treatments disappeared during the night and when plants were rewatered, rendering data pooling difficult.

In conclusion, plant growth, fresh and dry weights, leaf water potential components decreased to various degrees, whereas leaf surface temperatures tended to increase with an increase in intensity of water stress. Osmotic adjustment was observed in Exp. 1 and to a lesser extent in Exp. 2. Clear differences between treatments were probably underestimated, however, by several unfavorable "conditions" in these experiments, such as intermittent drying, limited pot size and growth under artificial light. Water stress caused changes in plant growth, plant water relations and leaf surface temperatures, which in one way or another could have affected aphid population development and will be discussed in the following.

2.4.2 Effects of Water Stress on Life Table and Population Parameters of Aphids

The development time of immature aphids is variable and dependent on extrinsic factors such as temperature and food quality (Baker and Turner, 1916; Dixon, 1987). In Exp. 1 of the present study, the overall development time of immatures in the three water supply treatments did not decrease significantly with water stress (see Table 5). At the beginning of the experiment water stress was just beginning to build up, hence the expected increase in leaf surface temperatures mediated by water stress had not yet occurred in the appropriately treated plants. Graf (1984) reported a decrease in larval development time of *A. pomi* from 7.2 days at 20°C to 5.3 days at 25.8°C.

Baker and Turner (1916) reported an average larval development time for *A. pomi* of 7 to 8 days, the time being equally divided between the four stages.
The present study, however, showed that the mean total development time of larvae of 8.6 days was unevenly distributed among the instars, L4 being approximately 20% shorter than the previous larval instars. Gilbert et al. (1976 cf. Asante et al., 1991) also considered that the first three instar periods of many aphid species are similar in duration. Graf (1984) in his study reported that the development time for the first and the last larval instar of *A. pomi* tended to be longer than the other two immature stages. Graf (1984) conducted his experiments under constant temperatures, whereas in this study temperatures fluctuated rhythmically between 22°C and 17°C.

Another parameter contributing to the generation time of aphid populations is the **pre-reproductive time**. This period is of variable duration and dependent on food quality and temperature (Dixon, 1987). High food quality will favor a more rapid aphid development, while an increase in temperature increases overall developmental rate. In Exp. 1 and on the lower parts of plants in Exp. 2, a significant decrease in the pre-reproductive time was observed with an increase in intensities of water stress. This decrease could well have been induced by a slight increase in leaf surface temperatures mediated by water stress (see Fig. 4). On the upper parts of plants in Exp. 2 (i.e. apex and the uppermost 15 leaves), however, no change in the pre-reproductive time was measured. The shorter distances of the upper leaves of well-watered plants to the light source heated the leaf surfaces up more than the upper leaves of the slower growing, severely stressed plants (see Fig. 4). This probably "masked" any significant effect of water stress on leaf surface temperature and hence on the aphids' pre-reproductive time as well as longevity and **fecundity** on the uppermost parts of the plants.

In Exp. 2, the **reproductive time** period of aphids feeding on severely water stressed plants as well as on leaves positioned in the lower parts of the plant was longer compared with well-watered plants and the uppermost parts of the plants (see Table 6). The rate of population growth, however, is determined to a much greater extent by the rate of reproduction in early life than by the total number of larvae born in the entire lifespan (Dixon, 1987).

The lower surface temperatures as well as the less favorable nutritional quality of leaves in the lower parts of the plants probably accounted for the significantly longer **development times** and the significantly lower **fecundities** observed in aphid cohorts feeding on those leaves compared with leaves of the upper parts (see Table 6 and Fig. 10).
Graf (1984) reported a maximum fecundity per virginopara of *A. pomi* of 75 larvae/female when reared at constant 20°C. At 25.8°C fecundity decreased to 68.5 larvae/female. Aphids usually achieve their highest reproductive rates in early adult life. The size and timing of the peak in reproductive activity is important in determining *r*ₘ-values (Dixon, 1987). Aphids in this study reached their reproductive peaks between 12 and 20 days after birth in Exp. 1 and between 11 and 15 days in Exp. 2 (see Figs. 11 and 12). Beside genetic variability, intermittent drying of the soil and its effect on the plants probably resulted in the fluctuations in age-specific fecundity observed in these experiments. For example, some aphids completely stopped reproducing for 1 or 2 days then resumed reproduction in lower numbers.

Aphids which develop when host quality is poor devoted less resources to reproduction and thus had larger fat reserves and lived longer than aphids that develop when host quality is high (Dixon, 1975; Dixon and Wellings, 1982; Leather et al., 1983 cf. Dixon, 1987). In the present study, it was found that aphids feeding on the lower parts of plants (probably of inferior food quality), beside having a longer development time, were smaller in body size, darker in color and had lower body water content than aphids reared on the upper parts. The report that several aphid species give birth to small offspring when conditions are favorable and to large offspring when conditions are harsh, was not confirmed in the study presented here; on the contrary, the opposite trend was observed.

In the present study, the intrinsic rate of natural increase (*r*ₘ) increased in tendency (Exp. 1 and on the upper parts of plants in Exp. 2) or significantly (on the lower parts of plants in Exp. 2) with an increase in intensities of water stress (Table 7). This increase in *r*ₘ may well be due to the increase in leaf surface temperatures mediated by water stress. Graf (1984) reported an increase in *r*ₘ from 0.117 (1/day) at 10.8°C to 0.427 (1/day) at 25.8°C. Furthermore, the significantly higher *r*ₘ on leaves of the upper parts of the plant (i.e. apex and the uppermost 15 leaves) compared with those of the lower parts (i.e. the rest of the plant) was due partly to differences in leaf surface temperatures (see Fig. 4) but mainly to the poorer food quality (reflected in *R*ₒ) of leaves in the lower parts of the plants.

When this temperature effect was taken into consideration by expressing *r*ₘ on a physiological time basis (*r*ₘ', Table 8) no effect of water stress on *r*ₘ' was revealed, although the relativity of the *r*ₘ'-values between treatments tended to be different from the relativities of the *r*ₘ-values. When Graf (1984)
and Graf et al. (1985) expressed $r_m$ per day-degree, then a decrease in $r_m$ of *A. pomi* between 14.8°C and 25.8°C was observed (at 14.8°C $r_m = 0.0245$ and at 25.8°C $r_m = 0.0214$). It follows that any changes in the aphids' habitat temperatures could be expected to affect $r_m$.

Since the $r_m$-value combines information on the development time, survival rates and the age-specific fecundity (Lewontin, 1965) it is affected by the same factors, or a combination of factors, as its component variables. The intrinsic rate of natural increase as well as G and DT under conditions of water stress and the experimental conditions in Exp. 1 and Exp. 2 were probably defined by the mortality of immatures and early adults, by the pre-reproductive time and/or by fecundity, particularly in early stages of adulthood.

Changes in $r_m$, due to water stress were reported to result mainly from changes in larval mortality (Birch and Wratten, 1984), pre-reproductive and early reproductive time (Lewontin, 1965; Reed et al., 1992; Wennergren and Landin, 1993) and generation time (Gaston, 1988).

Several circumstances may be responsible for the results obtained. The importance of the high variability in leaf surface temperatures (other than changes due to water stress) were probably underestimated. Surface temperatures of a leaf may even vary up to several degrees depending on leaf size, shape, architecture etc. (Willmer, 1986). In this study, temperature differences in the growth chamber at the same distance from the light source already fluctuated by 1-3°C (data not shown) due to irregular light intensities and uneven ventilation. Differences in leaf surface temperatures between the treatments, on the other hand, were small (< 3°C). Therefore, differences in habitat temperatures of aphids (i.e. body temperatures) between the different treatments and their effects on the development of aphids were most probably overshadowed by the much higher variability in temperature due to other factors than water stress. It can further be noted that the effect of small changes in the habitat temperature of aphids on aphid development can be variable. Benedict and Hatfield (1988) mention that even subtle temperature changes (less than 3°C) can be expected to have an impact on insect population dynamics. On the other hand, Hoffmann and Hogg (1991) concluded in their study that the higher temperatures found in water stressed fields were insufficient to compensate for the change in $r_m$ of the potato leaf hopper in their treatments. Another important factor to be considered is the high variability among data within a treatment. Aphids although originating
parthenogenetically from the same "mother" are not necessarily biologically similar (Chi, 1988). In addition, under unfavorable conditions the genetic heterogeneity of a population in terms of individual fitness of the females seems to become more evident than in an optimum environment (Wermelinger et al., 1991). In the present study, aphids in a cohort were reared on different plants. Samples for life table construction were then pooled so that the effect of plant variation could be disregarded. Although the apple plants used originated from the same clone, their growth and development under the given conditions, and particularly under intermittent drying, nevertheless varied to a great extent.

Last but not least, the green apple aphids had a wide choice of microclimates (temperature and humidity) and feeding sites within their host plant, simply by moving short distances around the host plant. Therefore they could be subject to different temperatures, levels of relative humidity and nutrition through their behavioral actions, to some extent independent of experimental design and intention. Although relative humidity was held constant (55-60% RH) in the growth chambers, and was therefore not surveyed, it was expected to vary in the near proximity of the leaf surface. Changes in leaf surface temperatures due to the distance from the source of light, to day and night fluctuations and/or water stress may affect relative humidity considerably. In addition upper and lower leaf surfaces can provide disparate environments for the insect. The mobility of aphids may be an additional factor in the explanation why $r_m$ did not differ significantly among the treatments in the present study.

The influence of water stress on the population dynamics of aphids depends on the method (soil vs nutrient solution) and patterns (intermittent vs constant stress) used to induce water stress. Wearing's (1972) results showed that although intermittent water stress was largely beneficial and continuous water stress largely detrimental to the reproduction and survival of M. persicae and B. brassicae feeding on potted brussels sprouts, there were differences between species on different leaf ages. Sumner et al. (1986) found that hosts constantly stressed by drought (water stress induced hydroponically by the addition of the osmolyte PEG) decreased the $r_m$ of Schizaphis graminum (Rondani). Miles et al. (1982) reported that water stress (complete stop of watering) increases the rate of development of B. brassicae born on rape plants, but as stress becomes pronounced insects became restless and when plants became severely wilted they were rejected by the insects. Thus, aphid behavior was markedly affected by water stress causing an increase in the
movement of aphids which in turn can influence $r_m$-values determined in the present study on the different parts of the plants as outlined above.

Different hosts (woody vs herbaceous) and aphid species can react differently to water stress. Stress effect will be manifested more slowly in trees versus herbaceous plants (Waring and Cobb, 1992). Herbaceous plants may be less tolerant of water stress than other plant types, rendering poorer hosts when water stressed.

The results of experiments under controlled environmental conditions do not always correspond well to results obtained under field conditions (e.g. Price et al., 1989; Waring and Cobb, 1992). Nevertheless, there do exist reports which prove that experiments conducted in growth chambers and/or in greenhouses are comparable to field trials (Fanjul and Rosher, 1984; Hoffman and Hogg, 1991).

An important consideration in studying the effect of water stress on the population dynamics of aphids is which approach was used: a density-independent life table approach or a density-dependent population approach. The average rate of increase assigned to a given aphid species existing at low density may not be representative of the rate of increase of the same aphid existing at a moderate to high density level (Kaakeh and Dutcher, 1992). Since in this section the method of constructing and analyzing life tables did not provide a satisfactory understanding of the effect of water stressed hosts on the development of the green apple aphid, in the following section the density-dependent population approach is applied with the objective to shed more light on the effect of water stress on the population dynamics of aphids.

In conclusion, counter to expectations, the combined effects of changes in aphid habitat temperatures encountered in this study and imposition of water stress could not be shown to significantly affect aphid development and population rate of growth. The water stress applied to the plants, nevertheless, appeared to have had some "beneficial" effects on the insects: a slight decrease in the pre-reproductive time, especially on leaves positioned in the lower parts of the plant, and a slight increase in fecundity ($R_0$) and $r_m$ were observed.
3


3.1 Introduction

In the previous section, the effect of water stress on aphid population development was described by relating life table parameters and summary statistics of aphid cohorts with plant growth and the physiological parameters of plants. The intrinsic rate of natural increase, an adequate descriptor for population development under unlimited conditions, was calculated for aphid cohorts feeding on plants grown under various levels of water supply. In contrast to much published work the results have shown that the intrinsic rate of natural increase \( r_m \) was little affected by water stress, although water stress had a significant effect on shoot growth, biomass accumulation and water relations of the plant. This result is, from a theoretical and pest management point of view, important enough to be interpreted in detail. The experimental method used in the previous section did not provide an explanation for the results obtained. Age-specific life tables are summary statements on the life of a typical individual of a cohort (Price et al., 1989), and \( r_m \) is regarded as the best available single description of the population growth of insect species under defined conditions (Southwood, 1978; Hulting et al., 1990; Kaakeh and Dutcher, 1992). Price et al. (1989) pointed out that
many researchers were led into thinking that life table construction and analysis was sufficient to explain insect population dynamics. Life table analysis, often applied as a purely correlative approach, does not reveal causation and the need for further studies to explain causes and effects is recognized.

In the following, an attempt is made to understand the effects of water stress on plant growth and on the development of aphid populations from biophysical and biochemical relationships. The aim is to create a base for better understanding of the results obtained using the method of the previous section.

Already in 1923, Lathrop stated that plant growth frequently constituted a factor limiting the rate of development of *Aphis pomi* De Geer feeding on slowly growing foliage. The positive correlation of long shoots and vigorously growing plants and insect herbivore development has often been emphasized (Price et al., 1989; Waring and Cobb, 1992; P.W. Miles, University of Adelaide, South Australia, pers. comm.).

Resources, however, are often abundant and not limiting until insect herbivore densities become very high and/or the resource is modified by water and nutrient stress (Price et al., 1989) and/or by the capacity of the plant to master allelochemical responses (Miles and Oertli, 1993). Resource quantity and quality are often not considered in the construction of age-specific life tables. By removing the progeny of the individuals used for constructing life tables, the carrying capacity of a plant, its ability to defend against mass attack and the abundance of an insect are neglected. The rate of development of aphid populations is probably determined by the balance between assimilate demand of the aphids and the carrying capacity of the plant in terms of assimilates produced and its ability to synthesize defensive chemicals under given environmental conditions.

Individual aphids, which do not transmit viruses, have few if any effects on their host plant (Pollard, 1973; references cf. Miles, 1989b; Miles 1990) and are important agriculturally only because they can occur in high numbers. Aphids are considered to cause less damage than other insects because they can feed on the products of photosynthesis without destroying the photosynthetic machinery (Llewellyn, 1972 cf. Meyer, 1993; cf. Pollard, 1973; cf. Miles, 1989a). Even mass feeding by aphids may only damage the plants by an overall reduction of growth due to a massive drain of plants' assimilates towards the insects (sink-effect) (Pollard, 1973; Mallot and Davy, 1978 cf.
Wellings et al., 1989; for other references see Miles, 1989a and 1989b; Wellings et al., 1989). An apparent reduction of overall growth results when the drain of assimilates exceeds the capacities of the plant for compensatory growth (aphid density threshold for significant reduction in yield)(Miles, 1989a).

To explain causal relationships between modified host resources (quantity and quality) and aphid population development and, conversely, between high aphid population densities and host development, aphid populations were allowed to build up on apple trees grown under different water regimes. Population characteristics were assessed and were related to plant growth and plant physiological parameters.

3.2 Materials and Methods

Two experiments (Exp. 3 and Exp. 4) were conducted to study the development and growth of aphid populations on apple plants grown under different water regimes.

3.2.1 Characterization of the Development and Growth of Aphid Populations

The size and density of aphid populations (according to Krebs, 1972 primary characteristics of populations) as well as age distribution and alatae production (secondary characteristics) were assessed for populations feeding on apple plants grown under different water regimes. Measurements were taken after a specific time of development in Exp. 3 and after host plants had stopped growing and were apparently suffering from high aphid population densities in Exp. 4. Further, this approach weighted the "oscillating" effects of intermittent drying and of the corresponding changes in plant growth and in physiological plant parameters on aphid development, since population size is a longer term effect.
3.2.2 Experimental Conditions

In both experiments, one apple plant was grown per 10 L round plastic pot. Soil mix, environmental conditions, experimental preparations as well as water stress induction and measurement (2 gypsum blocks per pot in water stressed treatments) were as described on pages 8, 9, 15, 16 and 17. The water regimes, i.e. treatments imposed in Exp. 3 were similar to Exp. 1 and Exp. 2 (control: $\Psi_{soil} = -0.05$ MPa, moderate: $\Psi_{soil} = -0.60$ MPa, severe: $\Psi_{soil} = -1.80$ MPa). In Exp. 4, the treatments applied were:

1. control (C): $\Psi_{soil} > -0.05$ MPa (well-watered)
2. mildly stressed (MD): $\Psi_{soil} > -0.20$ MPa
3. moderately 1 stressed (Mod. 1): $\Psi_{soil} > -0.40$ MPa
4. moderately 2 stressed (Mod. 2): $\Psi_{soil} > -0.60$ MPa

Treatments in Exp. 4 were chosen to be intermediate in stress intensity between the well-watered and the moderately water stressed treatments in Exp. 3. If not otherwise mentioned, $\Psi_{soil}$ of the water stressed treatments was calculated from the average of both gypsum block readings.

In both experiments, half of the plants of a treatment were infested with aphids (+A) while the other half was not (-A). In Exp. 3, the number of replicates of +A and -A plants in each treatment was 6 and 5, respectively. In Exp. 4, the number of replicates of +A and -A plants in each treatment was 3.

Similarly to Exp. 1, plants of Exp. 3 were cut down to the fourth node above soil surface. These plants were allowed to regrow to be used in Exp. 4. Only one branch was kept.

In Exp. 3, two fourth larval instars (L4) of *A. pomi* were transferred to the upper as well as the lower parts of the plant and were separated by a cardboard barrier. The barrier was placed below the first completely expanded leaf (tenth leaf counted from plant apex) at the beginning of the experiment (referred to as leaf A). Aphids were collected after 22 days of feeding on the host. This period was enough to have a rather dense aphid population on the upper part of the plants, particularly on well-watered plants. Based on the age distribution of a population after 22 days in the different treatments in Exp. 3, a certain number of aphids of each age category was transferred to the plants of Exp. 4 (well-watered: 3 apterous adults, 4 L3-L4, 17 L1-L2; mildly, Mod. 1 and Mod. 2 water stressed: 3 apterous adults, 6 L3-
L4, L1-L2). The objective was to reach as quickly as possible a stable age structure in a population; age structures were expected to be rather constant and specific under a certain set of growth conditions. According to Wennergren and Landin (1993), variation in environmental conditions change not only population growth but also the structure of the population which after initial oscillations, will then become almost fixed.

Plants in Exp. 4 were not divided into upper and lower parts. Aphids were transferred to the first completely expanded leaf (10th leaf counted from the apex; referred to as leaf A). When a plant infested with aphids stopped growing, suffering from a high density pressure, aphid populations were collected.

3.2.3 Data Collected

Plant Parameters

Plant water relations. In Exp. 3, predawn and midday leaf water (Ψ), osmotic (Ψ₀) and turgor (Ψₚ) potentials of two consecutive leaves (10th and 11th leaf from the apex) in +A and -A plants were determined at harvest. In Exp. 4, predawn Ψ, Ψ₀ and Ψₚ were assessed for every tenth leaf from the apex of -A plants only. Leaves of +A plants were covered with honeydew which affected measurements. Washing the honeydew off the leaves was found to falsify measurements of Ψ₀. In both experiments, fresh weight, dry weight, and water content of these leaves were assessed.

Plant growth. In Exp. 3 and Exp. 4, shoot length and number of leaves (i.e. plant size) were measured and counted every second to fourth day until the trees were harvested. Mean total plant size, mean increase in plant size (final - initial measurement), mean extension rate per day and mean rate of leaf appearance per day were weighted over the whole experimental period. Comparisons in growth among treatments were made at day 27 in Exp. 3 and day 24 in Exp. 4. Mean internodal length of the growth which took place during the experimental period was calculated.

Plant biomass production. In Exp. 3, the fresh and dry weight of total shoot, of leaf and stem as well as the total leaf area of +A and -A plants were determined at harvest. After all aphid populations were collected, +A and -A plants were harvested at the peak of the current drying cycle; the date of harvest varied among plants depending on treatment. In Exp. 4, the fresh and dry weight of shoot, leaf and stem of the additional growth of +A and -A
plants were assessed at harvest. Plants infested with aphids were harvested after aphid populations were collected and when the peak of the current drying cycle was reached. Corresponding -A plants were harvested parallel to +A plants when the peak of the current drying cycle was reached. Leaf area was measured in -A plants only. For both experiments, tissue water content was determined.

**Characteristics of Aphid Populations**

In Exp. 3 after 22 days of rearing, aphid populations which had developed on the upper and lower parts of the plants were collected separately. For this purpose, a suction device was constructed with which a large number of aphids could be collected efficiently and alive. After collection, the different developmental stages in an aphid population (L1+L2, apterous L3+L4, apterous adults, winged L3+L4, alatae) were separated, counted (the whole population was counted) and their fresh as well as dry weight determined (Sartorius S4 supermicro balance, Sartorius GmbH, Göttingen, Germany).

In Exp. 4 when +A infested plant had stopped growing, aphids of a population were collected with the self-made suction device. Since aphid populations in Exp. 4 were rather large, and in order to maximize efficiency at work, a technique for taking representative samples out of a collected population was developed and applied. Collected aphids were mixed with 300 ml of 70% ethanol solution in a 500 ml plastic bottle, agitated and an aliquot of 50 ml poured into a 100 ml bottle. The aphids were then filtered out of the solution, washed with water and allowed to dry for a short time before being counted. Aphids were still alive after sampling which facilitated the distinction among the different stages. Two samples of each population were taken. The different aphid developmental stages (L1, L2, apterous L3 and L4, apterous adults, winged L3+L4, alatae) of a sample were separated, counted and their fresh as well as dry weight determined. The standard error between the two samples ranged from 0.01% to 5.10% for the different developmental stages. The rest of the population was weighed and the total number of aphids as well as the age structure of the population were approximated according to the samples.
3.2.4 Data Analysis

To test the significance of water stress on plant parameters, differences between means of uninfested plants were compared under different water regimes by standard analysis of variance (ANOVA) followed by a test of least significance difference (LSD) at a 5% level of significance if not otherwise mentioned (Statgraphics 5.0, STSC Inc., Rockville, MA, USA). Similarly the significance of an infestation with aphids on plant parameters was tested by comparing aphid infested and uninfested plants. To test the significance of water stress on population characteristics, differences between means, upper and lower parts of plants in Exp. 3 taken separately, were compared using ANOVA followed by LSD test at a 5% level of significance. Similarly the significance of feeding site (i.e. leaf position within plant) on the development of aphid populations was tested. Simple linear regression was applied to describe the relationship between $\Psi_{\text{soil}}$, $\Psi$, $\Psi_o$ and $\Psi_p$ as well as among potential components within a plant. Predawn and midday potential measurements were correlated.

3.3 Results

3.3.1 Development of Water Stress in the Soil

Figures 13 and 14 describe the development of soil water stress in Exp. 3 and Exp. 4 induced by the intermittent drying cycle technique. An example of each treatment in both experiments is plotted. The examples shown are of representative, single pots.

Watering frequency decreased with an increase in intensities of water stress. In Exp. 3, the well-watered treatment experienced 4 to 6, the moderately stressed 2 to 4 and the severely stressed treatment 1 to 3 drying cycles (to day 40 of the experiment). In Exp. 4, the well-watered treatment experienced 6 to 8, the mildly stressed 5 to 7, the moderately 1 stressed 4 to 6 and the moderately 2 stressed treatments 3 to 5 drying cycles (to day 40 of the experiment).

As in Exp. 1 and Exp. 2, the chosen intensities of soil water stress of the treatments could not be consistently maintained, particularly in Exp. 4. In Exp. 3, the severe stress treatment, which was supposed to reach a $\Psi_{\text{soil}}$ of
Soil matric potentials (\(\Psi_{\text{soil}}\)) in representative, single pots of well-watered, moderately and severely water stressed treatments during experiment 3.

-1.8 MPa, reached a mean \(\Psi_{\text{soil}}\) of -2.4 MPa instead (see Table 13 below). In Exp. 4, the chosen difference in \(\Psi_{\text{soil}}\) from one treatment to the other was 0.2 MPa. Since already at the beginning of the experiment the plants were rather big (mean size of 85 cm), it was impossible to maintain these \(\Psi_{\text{soil}}\) under the given experimental conditions (see Table 14 below).
Soil matric potentials ($\Psi_{\text{soil}}$) in representative, single pots of well-watered, mildly, moderately 1 and moderately 2 water stressed treatments during experiment 4.
In Table 9, the ranges of \( \Psi_{soil} \) measured at maximum stress over the entire experimental period of Exp. 3 and Exp. 4 are presented.

Table 9. Range of soil matric potentials (\( \Psi_{soil} \)) in replicates of a treatment at maximum stress under well-watered (control), moderate and severe conditions of water stress in experiment 3 and under well-watered, mild, moderate 1 and moderate 2 conditions of water stress in experiment 4. 

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Experiment 3</th>
<th>( \Psi_{soil} ) (MPa)</th>
<th>Experiment 4</th>
<th>( \Psi_{soil} ) (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>-0.00 to -0.08</td>
<td>Control</td>
<td>-0.00 to -0.05</td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
<td>-0.46 to -1.43</td>
<td>Mild</td>
<td>-0.18 to -1.56</td>
</tr>
<tr>
<td>Severe</td>
<td></td>
<td>-1.07 to -4.96</td>
<td>Moderate 1</td>
<td>-0.28 to -2.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Moderate 2</td>
<td>-0.58 to -2.18</td>
</tr>
</tbody>
</table>

\( \Psi_{soil} \) readings taken at 14 cm soil depth.

As in Exp. 1 and Exp. 2, the variability among replicates of a treatment was high. The gradient in \( \Psi_{soil} \) between the 7 and 14 cm soil depths in a pot in Exp. 4 was smaller than in Exp. 3 (Figs. 13 and 14). As already mentioned in section 2, the practice of allowing plants of the previous experiment to regrow after cutting them down permitted a more uniform soil water distribution within the pot.

In both experiments, water was first withheld at day -10. Therefore, at the beginning of an experiment (day 0 = aphid transfer to growth chamber), a drying cycle was already in progress.

### 3.3.2 Effects of Water Stress on Plant Development

**Plant growth.** In Exp. 3, initial shoot length ranged between 22.0 - 61.0 cm (mean = 44.0 cm) at day -5 and total number of leaves between 16 - 35 leaves (mean = 25.7 leaves) at day 1. In Exp. 4, initial shoot length ranged between 50.0 - 113.5 cm (mean = 84.8 cm) and leaf number between 26 - 57 leaves (mean = 43.1 leaves) at day -3. The mean initial size of plants among treatments was similar. Since plant growth expressed in number of leaves showed results similar to plant growth expressed in shoot length, only data of the development of the shoot under water stress are presented in Table 10.
Table 10. Total shoot length at harvest as well as increase in shoot length and extension rate per day averaged over the experimental period, of apple plants grown under different water regimes in Exp. 3 and Exp. 4 (Exp. 3\textsuperscript{a}, measured at day 27; Exp. 4\textsuperscript{b}, measured at day 24). Means ±SE (in parenthesis) are of aphid infested (+A) and uninfested (-A) plants\textsuperscript{c}.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total shoot length (cm)</th>
<th>Increase in shoot length (cm)</th>
<th>Extension rate (cm/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+A</td>
<td>-A</td>
<td>+A</td>
</tr>
<tr>
<td>Control</td>
<td>80.4 \textpm 4.8</td>
<td>76.8 \textpm 5.7</td>
<td>32.1 \textpm 2.2</td>
</tr>
<tr>
<td>Moderate</td>
<td>50.3 \textpm 5.8</td>
<td>49.7 \textpm 8.6</td>
<td>3.5 \textpm 1.4</td>
</tr>
<tr>
<td>Severe</td>
<td>49.8 \textpm 5.3</td>
<td>55.3 \textpm 3.1 ab</td>
<td>1.7 \textpm 0.7</td>
</tr>
</tbody>
</table>

Experiment 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total shoot length (cm)</th>
<th>Increase in shoot length (cm)</th>
<th>Extension rate (cm/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+A</td>
<td>-A</td>
<td>+A</td>
</tr>
<tr>
<td>Control</td>
<td>120.7 \textpm 7.4</td>
<td>123.3 \textpm 10.6</td>
<td>31.2 \textpm 1.9</td>
</tr>
<tr>
<td>Mild</td>
<td>113.0 \textpm 3.5</td>
<td>110.0 \textpm 8.0</td>
<td>23.3 \textpm 0.8</td>
</tr>
<tr>
<td>Mod. 1</td>
<td>106.8 \textpm 5.3</td>
<td>114.8 \textpm 1.8</td>
<td>22.8 \textpm 4.8</td>
</tr>
<tr>
<td>Mod. 2</td>
<td>114.0 \textpm 12.5</td>
<td>111.8 \textpm 8.2</td>
<td>25.5 \textpm 2.0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} +A plants: mean of 6 replicates (except moderately stressed: 5 replicates)  
\textsuperscript{b} -A plants: mean of 5 replicates (except severely stressed: 3 replicates)  
\textsuperscript{c} +A plants: mean of 2 replicates (except well-watered: 3 replicates)  
\textsuperscript{c} -A plants: mean of 3 replicates  
Means in a column followed by the same letter are not significantly different (P ≤ 0.05).
In Exp. 3, mean total and mean increase in shoot length as well as mean extension rate per day of aphid infested (+A) and uninfested (-A) plants significantly decreased with water stress. In Exp. 4, the growth of +A plants was not significantly affected by the different water stress treatments. In the -A plants, however, mean increase in shoot length and mean extension rate per day decreased significantly between the mildly and moderately 1 stressed treatments.

Although plant size at the beginning of Exp. 3 (mean of 45 cm) was similar to that of Exp. 1 (mean of 42 cm) and $\Psi_{\text{soil}}$ within treatments were more or less similar, growth parameters of the plants were different. Plant growth in Exp. 3 (see Table 10) was in general smaller than in Exp. 1 (see Table 2). Uninfested well-watered plants grew on an average 20 cm or 0.25 cm/day less in Exp. 3 compared with Exp. 1 (comparisons were made at day 40, data not shown). The average difference in plant growth between Exp. 1 and Exp. 3 for the moderate and severe stress treatments was 50 cm or 1.00 cm/day and 40 cm or 0.85 cm/day, respectively.

In both experiments, the growth of uninfested (-A) and with aphid infested (+A) plants were not significantly different ($P < 0.05$), except for those growing under conditions of mild water stress in Exp. 4. The growth of -A plants under conditions of mild stress was higher than that of +A plants (see Table 10).

The growth of individual plants decreased with the build up of water stress in the soil and increased again after watering. The reaction of the extension rate per day of shoots to intermittent drying of the soil is pictured in Fig. 15 by presenting the growth pattern of two Mod. 2 stressed plants.

The effects of soil drying and rewatering on plant growth were very obvious. A drying cycle in the Mod. 2 stressed treatment decreased the extension rate of shoots by at least 0.5 cm/day depending on the level of water stress and on plant size. More severely stressed plants showed higher reductions than less severely stressed plants (not all data shown). As time progressed, growth rates generally decreased. Plants increased in size and the limited soil volume became an additional constraint to plant growth. In Exp. 4, aphid infested plants stopped growing at the end of the experiment suffering from high aphid densities rather than from water stress (comparison between uninfested and with aphids infested single plants; Fig. 15).
Fig. 15. Extension rate per day of shoots in relation to the development of water stress in the soil ($\Psi_{\text{soil}}$). Shown are examples of one uninfested (- Aphids) and one with aphid infested plant (+ Aphids) grown under conditions of moderately 2 water stress in Exp. 4.
**Internodal length.** In Exp. 3, mean internodal length of moderately stressed plants was significantly smaller (0.98 cm ±SE = 0.17) than in well-watered (1.87 cm ±SE = 0.08) and in severely stressed plants (1.21 cm ±SE = 0.16; P ≤ 0.05). A similar trend was also observed in Exp. 4: with increasing intensities of stress, internodal lengths first decreased, but increased again from Mod. 1 to Mod. 2 stressed plants (data not shown). These results suggest that at low intensities of water stress stem elongation was restricted to a greater extent than leaf production. At high water stress, however, leaf production was strongly decreased and was reflected in an increase in internodal length.

In Exp. 3, no difference in internodal length between aphid infested (+A) and uninfested (-A) plants was measured (data not shown). In Exp. 4, however, a significant difference in internodal length between +A and -A plants under well-watered and under conditions of mild water stress was observed. Plants infested with aphids had significantly shorter internodes than -A plants (difference of 0.37 cm). With an increase in stress intensity this difference became insignificant (difference of 0.06 cm).

**Leaf area.** Means of the total leaf area of plants at harvest in Exp. 3 and of the leaf area of the additional growth in Exp. 4 are presented in Table 11.

In Exp. 3, mean total leaf area of well-watered, +A and -A plants was significantly higher than that of water stressed plants. In Exp. 4, no significant difference in leaf area was observed among -A plants of the different treatments, although a decrease in leaf area from well-watered/mildly stressed to Mod. 1 and further to Mod. 2 stressed plants was observed. The high variability observed was also probably partly due to the variable harvest dates. A difference in leaf area between the two moderately stressed treatments was registered, although plant growth (increase in shoot length; see Table 10) was slightly lower for the Mod. 1 compared with the Mod. 2 treatment. This suggested that water stress had a greater effect on leaf area expansion than leaf production and stem elongation.
Table 11. Leaf area of plants grown under well-watered (control) and water stressed conditions at harvest in Exp. 3 and Exp. 4. In Exp. 3, total leaf area of aphid infested (+A) and uninfested (-A) plants were determined separately*. In Exp. 4, leaf area of the increase in growth of uninfested (-A) plants was measured b. Means ± SE (in parenthesis) are shown c.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total leaf area (cm²)</th>
<th>Treatment</th>
<th>Leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+A</td>
<td></td>
<td>-A</td>
</tr>
<tr>
<td>Control</td>
<td>880.8 a (± 91.3)</td>
<td>1135.9 a   (± 108.9)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>544.4 b (± 59.4)</td>
<td>620.7 b    (± 105.1)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>527.6 b (± 62.2)</td>
<td>664.0 b    (± 82.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Plant biomass production.** In Exp. 3, fresh and dry weight of plants decreased significantly with water stress (Table 12). Fresh and dry weight of well-watered plants (+A and -A plants) was almost twice as high as that of water stressed plants. Water stressed plants had on average 6% lower tissue water content than well-watered plants. Reductions in water content between well-watered and water stressed plants were greater in stems than in leaves (data not shown). In general, the water content of stems was lower (4.5-12.5%) than of leaves, irrespective of treatment. Although in Exp. 3 the fresh and dry weight of aphid infested (+A) plants was lower than in uninfested (-A) plants, differences were not significant. Aphid infested plants tended to have slightly higher tissue water content compared with -A plants, but the overall difference was again insignificant.
In Exp. 4, the decrease in mean fresh and dry weight between plants grown under well-watered and water stressed conditions was significant for +A plants. Well-watered plants had twice as high fresh and dry weights compared with water stressed plants. Fresh and dry weights of -A, Mod. 2 stressed plants were significantly lower than that of well-watered plants. Plant yield parameters of aphid infested and uninfested plants in Exp. 4 were not compared due to considerable differences in the harvest date.

Table 12. Shoot fresh and dry weights as well as shoot water content of aphid infested (+A) and uninfested (-A) plants under well-watered (control) and water stressed conditions in Exp. 3a and Exp. 4b. Means ±SE (in parenthesis) are shown. In Exp. 3, values are of total shoot, whereas in Exp. 4 values are of the additional increase in shootc.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>fresh weight (g)</th>
<th>dry weight (g)</th>
<th>water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+A (±)</td>
<td>-A (±)</td>
<td>+A (±)</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>34.86 a (± 3.59)</td>
<td>41.85 a (± 5.32)</td>
<td>12.50 a (± 1.37)</td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.93 b (± 2.54)</td>
<td>21.94 b (± 4.58)</td>
<td>7.745 b (± 1.09)</td>
</tr>
<tr>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.91 b (± 2.76)</td>
<td>21.40 b (± 3.74)</td>
<td>7.88 b (± 1.80)</td>
</tr>
<tr>
<td>Experiment 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.46 x (± 1.32)</td>
<td>19.15 x (± 2.24)</td>
<td>8.55 x (± 0.57)</td>
</tr>
<tr>
<td>Mild</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.26 y (± 2.39)</td>
<td>17.80 x (± 2.66)</td>
<td>3.18 y (± 0.92)</td>
</tr>
<tr>
<td>Mod. 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.59 y (± 0.97)</td>
<td>15.13 xy (± 2.15)</td>
<td>2.53 y (± 0.43)</td>
</tr>
<tr>
<td>Mod. 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.59 y (± 1.83)</td>
<td>11.04 y (± 0.78)</td>
<td>2.99 y (± 0.72)</td>
</tr>
</tbody>
</table>

a +A plants: mean of 6 replicates (except moderately stressed: 5 replicates)

b -A plants: mean of 5 replicates (except severely stressed: 4 replicates)

c mean of 3 replicates (except Mod. 1 stressed -A plants: 2 replicates)

means in a column followed by the same letter are not significantly different (P £ 0.05).
In contrast to Exp. 3, the water content of plant tissue was significantly lower in +A than in -A plants. Higher fresh and dry weights and lower tissue water content in well-watered, +A plants as compared to -A plants were probably caused by an artefact: well-watered, +A plants were completely covered with aphid honeydew, not all of which could be wiped off before plants were weighed.

**Plant water relations.** Soil matric potentials ($\Psi_{soil}$) were positively correlated with the components of leaf water potential ($\Psi$, $\Psi_o$, $\Psi_p$). In Figs. 16 and 17, all readings of $\Psi$, $\Psi_o$ and $\Psi_p$ assessed at harvest in Exp. 3 and Exp. 4 were plotted against corresponding $\Psi_{soil}$ and regression lines were fitted.

As in Exp. 1 and Exp. 2, $\Psi$ (measured predawn and at midday) correlated best with $\Psi_{soil}$ (Exp. 3: $r=0.82$ predawn, $r=0.83$ midday; Exp. 4: $r=0.45$ predawn). In Exp. 3, the extent of decrease in $\Psi_o$ with decreasing $\Psi_{soil}$ was more or less similar to that measured in Exp. 1, although the decrease in $\Psi$ was more steep in Exp. 1 than in Exp. 3 (comparison of slopes (b) in both experiments; see Figs. 7 and 16). In Exp. 4, the decrease in $\Psi_o$ was not as distinct as in Exp. 3: a steep decrease in $\Psi_o$ to maintain $\Psi_p$ above a critical value was not necessary, since $\Psi_{soil}$ did not reach as severe stress intensities as in the other experiments.

In Exp. 3, the components of plant water potential were determined predawn and at midday. The slopes of decrease (b) in the components of leaf water potentials with decreasing soil water potential were very similar, whether measured predawn or at midday (see Fig. 16). However, the intercepts ("heights") of the regression lines were different. Leaf water potentials ($\Psi$) measured midday were on average 0.78 MPa lower than $\Psi$ measured predawn (Fig. 16 and Table 13). Average $\Psi_o$-values at midday, however, were only 0.16 MPa lower than at predawn. Therefore, $\Psi_p$ at midday was on average 0.62 MPa lower than at predawn. Predawn $\Psi$ and $\Psi_o$ correlated well with midday $\Psi$ ($r=0.79$, P<0.001) and $\Psi_o$ ($r=0.93$, P<0.001), respectively, whereas $\Psi_p$ did not ($r=0.34$, P<0.01).
Fig. 16. Predawn and midday leaf water ($\Psi$), osmotic ($\Psi_o$) and turgor potentials ($\Psi_p$) of apple plants in relation to soil water potentials ($\Psi_{soil}$) in Exp. 3. Regression equations, $R^2$- and P-values are given.
Fig. 17. Predawn leaf water ($\Psi$), osmotic ($\Psi_o$) and turgor potentials ($\Psi_p$) of apple plants in relation to soil water potentials ($\Psi_{soil}$) in Exp. 4. Regression equations, $R^2$- and $P$-values are given.

Relating $\Psi_o$ and $\Psi_p$ of uninfested and with aphid infested plants measured at harvest in the three treatments with respective $\Psi$ also demonstrated the ability of plants to adjust osmotically. In Figs. 18 and 19, $\Psi_o$ and $\Psi_p$ were plotted against $\Psi$, and regression lines for each treatment fitted and $R^2$-values calculated. In Fig. 18, +A and -A plants were examined separately.

In both experiments, $\Psi_p$ of water stressed plants could be maintained at higher values at decreased $\Psi$ than $\Psi_p$ of well-watered plants. This was due to a steeper decrease in $\Psi_o$ with decreasing $\Psi$ (steeper slope) and/or to generally lower $\Psi_o$ (lower intercept) in water stressed than in well-watered plants. Measurements of the components of plant water potential in both experiments suggested that in uninfested plants grown under low levels of water stress (i.e. mildly stressed in Exp. 4 and moderately stressed in Exp. 3), $\Psi_p$ maintenance was not only due to generally decreased $\Psi_o$ (due to active osmotic adjustment) but also due to a steeper decrease of $\Psi_o$ with declining $\Psi$, indicating an increase in tissue elasticity.
Fig. 18. Predawn leaf osmotic ($\Psi_o$) and turgor ($\Psi_p$) potentials in relation to leaf water potentials ($\Psi$) of aphid infested (+A) and uninfested (-A) apple plants grown under well-watered (control), moderately and severely water stressed conditions in Exp. 3. For illustration purposes symbols have been removed. The regression equation for each treatment and corresponding $R^2$- and P-values are shown below.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Equation</th>
<th>$R^2$-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf osmotic potential ($y$) as related to leaf water potential ($x$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control +A</td>
<td>$y = -1.23 + 0.47 x$</td>
<td>0.17</td>
<td>ns</td>
</tr>
<tr>
<td>Control -A</td>
<td>$y = -1.27 + 0.06 x$</td>
<td>0.12</td>
<td>ns</td>
</tr>
<tr>
<td>Moderate +A</td>
<td>$y = -1.38 + 0.62 x$</td>
<td>0.14</td>
<td>ns</td>
</tr>
<tr>
<td>Moderate -A</td>
<td>$y = -1.40 + 0.82 x$</td>
<td>0.58</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Severe +A</td>
<td>$y = -1.43 + 0.61 x$</td>
<td>0.85</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Severe -A</td>
<td>$y = -1.99 + 0.25 x$</td>
<td>0.44</td>
<td>ns</td>
</tr>
<tr>
<td>Leaf turgor pressure ($y$) as related to leaf water potential ($x$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control +A</td>
<td>$y = 1.23 + 0.53 x$</td>
<td>0.21</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>Control -A</td>
<td>$y = 1.72 + 0.94 x$</td>
<td>0.73</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Moderate +A</td>
<td>$y = 1.38 + 0.38 x$</td>
<td>0.06</td>
<td>ns</td>
</tr>
<tr>
<td>Moderate -A</td>
<td>$y = 1.40 + 0.18 x$</td>
<td>0.06</td>
<td>ns</td>
</tr>
<tr>
<td>Severe +A</td>
<td>$y = 1.43 + 0.39 x$</td>
<td>0.71</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Severe -A</td>
<td>$y = 1.99 + 0.75 x$</td>
<td>0.87</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>
Fig. 19. Predawn leaf osmotic ($Y_o$) and turgor ($Y_p$) potentials in relation to leaf water potentials ($Y$) of uninfested apple plants grown under well-watered (control), mildly, moderately 1 and moderately 2 water stressed conditions in Exp. 4. The regression equation for each treatment and the corresponding $R^2$- and $P$-values are shown.

Since, at harvest, the components of plant water potential in Exp. 3 and Exp. 4 were measured at the peak of a drying cycle, mean $Y$, $Y_o$ and $Y_p$ at maximum water stress in the different water regimes could be calculated (Tables 13 and 14). In both experiments, $Y_{soil}$ values at harvest were significantly different between the different water regimes (treatments), although variability within each category was high.
Table 13. Predawn and midday leaf water ($\Psi$), osmotic ($\Psi_0$) and turgor potentials ($\Psi_p$) as well as their corresponding soil matric potentials ($\Psi_{s\text{os}}$) of aphid infested (+A) and uninfested (-A) apple plants grown under well-watered (control), moderately and severely water stressed conditions measured at the peak of drying cycles at harvest in Exp. 3*. Means ±SE (in parenthesis) are shownb.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$\Psi$ (MPa)</th>
<th>$\Psi_0$ (MPa)</th>
<th>$\Psi_p$ (MPa)</th>
<th>$\Psi_{s\text{os}}$ (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>predawn</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+A</td>
<td>-A</td>
<td>+A</td>
<td>-A</td>
</tr>
<tr>
<td>Control</td>
<td>-0.64 a (± 0.06)</td>
<td>-0.79 a (± 0.05)</td>
<td>-1.53 a (± 0.07)</td>
<td>-1.77 a (± 0.03)</td>
</tr>
<tr>
<td>Moderate</td>
<td>-0.71 a (± 0.03)</td>
<td>-0.88 a (± 0.06)</td>
<td>-1.82 b (± 0.05)</td>
<td>-2.09 b (± 0.05)</td>
</tr>
<tr>
<td>Severe</td>
<td>-1.51 b (± 0.10)</td>
<td>-1.60 b (± 0.10)</td>
<td>-2.35 c (± 0.06)</td>
<td>-2.39 c (± 0.04)</td>
</tr>
<tr>
<td></td>
<td>midday</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+A</td>
<td>-A</td>
<td>+A</td>
<td>-A</td>
</tr>
<tr>
<td>Control</td>
<td>-1.26 x (± 0.05)</td>
<td>-1.46 x (± 0.05)</td>
<td>-1.69 x (± 0.05)</td>
<td>-1.93 x (± 0.03)</td>
</tr>
<tr>
<td>Moderate</td>
<td>-1.73 y (± 0.07)</td>
<td>-1.77 y (± 0.07)</td>
<td>-2.04 y (± 0.06)</td>
<td>-2.25 y (± 0.06)</td>
</tr>
<tr>
<td>Severe</td>
<td>-2.24 z (± 0.08)</td>
<td>-2.40 z (± 0.05)</td>
<td>-2.53 z (± 0.07)</td>
<td>-2.52 z (± 0.06)</td>
</tr>
</tbody>
</table>

+a A plants, number of replicates: control=15, moderate=12, severe=12
- A plants, number of replicates: control=19, moderate=13, severe=6
c Means in a column followed by the same letter are not significantly different (P ≤ 0.05).

In Exp. 3, a significant reduction in predawn $\Psi$ was measured between well-watered/moderately stressed and severely stressed plants. Predawn $\Psi_o$ decreased significantly with each increase in intensity of water stress. This resulted in significantly higher $\Psi_p$ in moderately stressed plants compared with the other two treatments. Both $\Psi$ and $\Psi_o$, when measured at midday decreased significantly with increasing intensities of water stress and were significantly lower than at predawn (P ≤ 0.05). Leaf turgor pressure of water stressed plants measured at midday was not significantly different from that of well-watered plants (exception: severely water stressed, uninfested plants).
The components of plant water potential of aphid infested (+A) and uninfested (-A) plants in Exp. 3 were affected differently by soil water stress. Uninfested plants under well-watered and moderately stressed conditions had significantly lower $\Psi$ and $\Psi_o$ than +A plants (mean difference in $\Psi$=0.14 MPa and in $\Psi_o$=0.20 MPa, $P \leq 0.05$; see Table 13). Turgor pressures, particularly under moderately water stressed conditions, were higher in -A than +A plants (mean difference in $\Psi_p$=0.10 MPa). In severely stressed plants, significant differences in $\Psi$, $\Psi_o$ and $\Psi_p$ between +A and -A plants were not observed.

Table 14. Predawn leaf water ($\Psi$), osmotic ($\Psi_o$) and turgor potentials ($\Psi_p$) as well as their corresponding soil matric potentials ($\Psi_{soil}$) of uninfested (-A) apple plants grown under well-watered (control), mildly, moderately 1 and moderately 2 water stressed conditions measured at the peak of drying cycles at harvest in Exp. 4; n indicates number of replicates. Means ±SE (in parenthesis) are shown.*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>$\Psi$ (MPa)</th>
<th>$\Psi_o$ (MPa)</th>
<th>$\Psi_p$ (MPa)</th>
<th>$\Psi_{soil}$ (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>-0.64 a (± 0.07)</td>
<td>-2.03 a (± 0.08)</td>
<td>1.39 a (± 0.12)</td>
<td>0.00 a (± 0.00)</td>
</tr>
<tr>
<td>Mild</td>
<td>12</td>
<td>-1.02 b (± 0.07)</td>
<td>-2.13 ab (± 0.06)</td>
<td>1.11 b (± 0.07)</td>
<td>0.69 b (± 0.072)</td>
</tr>
<tr>
<td>Moderate 1</td>
<td>9</td>
<td>-1.15 b (± 0.11)</td>
<td>-2.12 ab (± 0.08)</td>
<td>0.97 b (± 0.08)</td>
<td>1.20 c (± 0.10)</td>
</tr>
<tr>
<td>Moderate 2</td>
<td>9</td>
<td>-1.17 b (± 0.11)</td>
<td>-2.31 b (± 0.07)</td>
<td>1.14 ab (± 0.09)</td>
<td>1.60 d (± 0.20)</td>
</tr>
</tbody>
</table>

* means in a column followed by the same letter are not significantly different ($P \leq 0.05$).

In Exp. 4, $\Psi$ of leaves of well-watered plants was significantly higher than in water stressed plants (Table 14). Significant reductions in $\Psi_o$ were observed between well-watered and Mod. 2 stressed plants only. Leaf turgor pressure was significantly reduced between well-watered and water stressed plants. Since components of plant water potentials were measured only in -A plants, the effect of aphid infestation on plant water relations could not be examined in this experiment.
3.3.3 Effects of Water Stress on Development and Growth of Aphid Populations

In Fig. 20, the size of aphid populations as well as their total fresh and dry weights, after 22 days of development, are plotted against the different water regimes in Exp. 3. Populations which had developed on the upper parts of the plants, i.e. above leaf A (10th leaf counted from the apex at the beginning of the experiment), are shown separately from those which had developed on the lower parts, i.e. below leaf A.

Aphid populations which developed on well-watered plants, whether on the upper or lower parts, were significantly larger than on water stressed plants. Further, a slight decrease in the size of aphid populations with an increase in the intensity of water stress was observed. The decline in an aphid population from well-watered to moderately stressed plants was much steeper than the decline from moderately to severely stressed plants, although the bigger drop in soil matric potential was between the moderate (\(\psi_{\text{soil}} = -0.7\) MPa) and the severe treatment (\(\psi_{\text{soil}} = -2.4\) MPa). This observation suggested that a critical moisture level for aphid development existed between the well-watered and the moderately stressed treatment, below which the development of aphid populations was severely impaired.

As in Exp. 1 and Exp. 2, aphid development on the upper and lower parts of the plant differed. The size of an aphid population was significantly larger on the upper parts, particularly of well-watered plants, compared with the lower parts. The difference in population size between treatments and/or between position in the plant, decreased with increasing intensities of water stress. Hence population size was affected both by the water supply level (treatment) and by the feeding position in a plant (leaf age), but the former effect tended to dominate at high levels of water stress.

Aphid densities of upper and lower parts of plants grown under different water regimes were calculated per unit shoot length (cm) or per leaf. On the upper parts of well-watered plants, aphid density amounted to 52.5 aphids/cm shoot length (or 104 aphids/leaf), of moderately stressed plants to 21.5 aphids/cm shoot length (or 42 aphids/leaf) and of severely water stressed plants to 13.0 aphids/cm shoot length (or 28 aphids/leaf). These differences in aphid densities on the upper parts of the plants were significant between well-watered and water stressed plants (\(P \leq 0.05\)). On the lower parts of the plants, aphid densities amounted to 30 aphids/cm shoot length (or to 47 aphids/leaf) on well-watered plants, to 14.5 aphids/cm shoot length (or to 22
Fig. 20. Size as well as fresh and dry weights of aphid populations feeding on the upper and lower parts of well-watered (control), moderately and severely water stressed apple plants collected after 22 days. Upper parts of the plant refers to plant parts above leaf A (10th leaf counted from the apex at the beginning of the experiment); lower parts refers to parts below leaf A. Means (6 replicates) labelled by the same letter are not significantly different (P ≤ 0.05).
aphids/leaf) on moderately stressed and to 5.0 aphids/cm shoot length (or to 8 aphids/leaf) on severely stressed plants. The differences in aphid density per leaf on the lower parts of the plant were significant between well-watered and water stressed plants ($P \leq 0.05$). Aphid density, whether on the upper or lower parts of the plant, tended to decrease with an increase in intensity of water stress.

Aphid density on the upper parts of the plant correlated positively with leaf number ($r=0.81$, $P<0.001$) as well as shoot length ($r=0.82$, $P<0.001$). Aphid density on the lower parts did not correlate with shoot length or leaf number. On the upper as well as on the lower parts of the plant in Exp. 3, individual body weights of aphids correlated positively (and highly) with aphid density ($r=0.75$, $P<0.001$): the higher the observed aphid density, the heavier the aphids, probably indicating that both effects were the result of the same nutritional cause.

By dividing fresh and dry weight of the different aphid stages of development by the corresponding number of individuals, mean body fresh and dry weight of aphid individuals under well-watered and water stressed conditions were calculated (Table 15). In Table 15, body weights of aphids collected from the upper parts of the plants, i.e. above leaf A (10th leaf counted from the apex at the beginning of the experiment), are shown separately from those collected from the lower parts, i.e. below leaf A.

Under water stress conditions, mean body weights of the apterous aphid stages were significantly lower, both on the upper and lower parts of the plant, compared with well-watered conditions. Under increasing water stress conditions, mean aphid body weight tended to decrease further.

Aphid body weights on the upper parts of well-watered plants were significantly higher than on the lower parts ($P \leq 0.05$). This difference in body weights decreased with an increase in intensities of water stress.

Carroll and Hoyt (1986) reported medium birth weights of 20-28 µg for A. pomi progeny of apterae reared at 20°C. The authors also reported an apterae weight of 0.712 mg on growing leaves and of 0.580 mg on mature leaves. In Exp. 3 of this study, the weights of apterae feeding on well-watered plants (upper and lower parts) were lower than reported by Carroll and Hoyt (1986). This reduction is probably explained by the higher ambient air temperatures of 23-24°C reached in the present study (at midday) compared with 20°C utilized by Carroll and Hoyt. Fresh weights of A. pomi reported by
Table 15. Body fresh and dry weights of aphid individuals in the different stages of development feeding on the upper and lower parts of well-watered (control), moderately and severely water stressed apple plants in Exp. 3. Upper parts of the plant refers to plant parts above leaf A (10th leaf counted from the apex at the beginning of the experiment); lower parts refers to parts below leaf A. Means ±SE (in parenthesis) are shown a,b.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Fresh weight (mg)</th>
<th>Dry weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control upper parts</td>
<td>moderate severe</td>
</tr>
<tr>
<td>alatae</td>
<td>0.439 a (±0.045)</td>
<td>0.292 a (±0.000)</td>
</tr>
<tr>
<td>L3+L4 winged</td>
<td>0.333 a (±0.015)</td>
<td>0.284 a (±0.032)</td>
</tr>
<tr>
<td>adults apterous</td>
<td>0.642 a (±0.034)</td>
<td>0.357 b (±0.039)</td>
</tr>
<tr>
<td>L3+L4 apterous</td>
<td>0.397 a (±0.011)</td>
<td>0.154 b (±0.020)</td>
</tr>
<tr>
<td>L1+L2</td>
<td>0.076 a (±0.005)</td>
<td>0.050 b (±0.004)</td>
</tr>
</tbody>
</table>

mean of 6 replicates for apterous stages only
means in a row followed by the same letter are not significantly different (P ≤ 0.05).

Rutz (1993) at 20°C/16°C (sinuous day/night temperature curve, similar environmental conditions as in the study presented here) ranged between 0.50 - 0.58 mg and 28 - 35 μg for apterous adults and L1, respectively.
Aphids had significantly higher water contents (%) when feeding on the upper parts of plants compared with the lower parts, particularly on well-watered plants (P ≤ 0.05; data not shown). This difference diminished with increasing intensities of water stress and became insignificant on severely stressed plants. The body size of aphids seemed to be affected by water stress and by aphid feeding position in the plant. Therefore, aphids appeared to react to water stress by reproducing less and attaining lower body weight. A range of body weights was observed on the upper parts of the plant, particularly on well-watered plants. It was noticed, however, that the more severe the water stress, the lower the aphids were positioned on a plant and the smaller the size and more uniform the weight of an aphid became. This was most clearly observable on adult aphids.

As demonstrated in Fig. 21, analyzing aphid populations into their various stages of development (L1+L2, apterous L3+L4, apterous adults, winged L3+L4 and alatae), showed the age distribution of an aphid population after 22 days of development on the upper parts of the plants, i.e. above leaf A (10th leaf counted from the apex at the beginning of the experiment), and on the lower parts, i.e. below leaf A.

The most obvious difference between populations on the upper and lower parts of the plant was in the number of winged forms. On the upper parts, the number of alatae and winged L3+L4 was higher and decreased with an increase in intensities of water stress. On the lower parts alatae were only just starting to develop on the well-watered plants.

On the upper parts, the trend within an aphid population was to increase the proportion of apterous L3+L4 and adults and to decrease the proportion of L1+L2 (and winged L3+L4) with water stress (data not shown). On the lower parts, a significant increase in percentage apterous L3+L4 and a significant decrease in percentage L1+L2 with an increase in intensity of water stress was observed. Aphids feeding on stressed plants had most probably a longer generation time and/or were less fecund.

A similar approach as in Exp. 3 was used in Exp. 4. In Fig. 22, the size as well as total fresh and dry weights of aphid populations were plotted against the different soil water regimes. In contrast to Exp. 3, where aphid populations remained for 22 days on the host, aphid populations in Exp. 4 had different lengths of developmental period. Aphids were collected when the host plant stopped growing due to a high population density.
Age distribution in aphid populations feeding for 22 days on the upper and lower parts of well-watered (control), moderately and severely water stressed apple plants in Exp. 3. Upper parts of the plant refers to plant parts above leaf A (10th leaf counted from the apex at the beginning of the experiment); lower parts refers to parts below leaf A. Means (6 replicates) labelled by the same letter are not significantly different (P ≤ 0.05).
Size, fresh and dry weight as well as development time of aphid populations feeding on well-watered (control), mildly, moderately 1 and moderately 2 water stressed apple plants (until host had stopped growing). Means (3 replicates) labelled by the same letter are not significantly different (P ≤ 0.05).
The period of population development ranged between 20 to 39 days depending on treatment (well-watered: 35, 37, 39 days; mild stress: 31, 21, 23 days; Mod.1 stress: 21, 19, 20; Mod.2 stress: 27, 22, 27 days). The mean developmental period on well-watered plants was significantly higher than on water stressed plants (P ≤ 0.05). Most notable was that the plants in treatment Mod. 1 stopped growing first (20 days).

The size of aphid populations feeding on water stressed plants were significantly smaller on well-watered compared with stressed plants. The smallest populations, although not significantly, were collected from Mod. 1 stressed plants which also had stopped growing first. Mean fresh and dry weights of aphid populations decreased significantly between the well-watered and Mod. 1/Mod. 2 water stressed treatments. From both plant and aphid growth parameters, the Mod. 1 plants appeared to be under more severe growth restraints than the Mod. 2 plants.

Aphid density (per unit of shoot length or per leaf) tended to decrease with an increase in water stress. A significant decrease in density was observed between the well-watered and the Mod. 1 stressed treatment (P ≤ 0.05). Aphid densities measured in the four treatments were: Control: 134 aphids/cm (235.5 aphids/leaf); Mild: 97.5 aphids/cm (168 aphids/leaf); Mod. 1: 69 aphids/cm (126 cm/leaf); Mod. 2: 81 aphids/cm (141 aphids/leaf).

Aphid density correlated positively with number of leaves (r=0.76, P<0.01) and shoot length (r=0.62, P<0.05). However, these correlations were lower compared with those in Exp. 3 indicating, that another factor beside plant size was affecting aphid population development under the conditions of Exp. 4. Body weights of aphid individuales correlated highly with population densities (r= -0.84, P<0.01; exception: L2). In contrast to Exp. 3, this correlation was negative. The higher the density of aphids in a population, the smaller the body weight of individuals became, probably indicating that weight here was being adversely affected by population size.

As in Exp. 3, mean body fresh and dry weight of aphid individuals under well-watered and water stressed conditions were calculated (Table 16). In contrast to Exp. 3, fresh and dry weight of the different stages of aphid development did not decrease with increasing water stress. The body weight of aphids tended to increase with an increase in stress, were lowest on well-watered plants and highest on Mod. 1 stressed plants (differences were significant, except for L2, see Table 16). These differences among treatments became more distinct when dry instead of fresh weights were compared.
Table 16. Body fresh and dry weights of aphid individuals in the different stages of development feeding on well-watered (control), mildly, moderately 1 and moderately 2 water stressed apple plants in Exp. 4. Means ±SE (in parenthesis) are shown a,b.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Fresh weight (mg)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Mild</td>
<td>Moderate 1</td>
<td>Moderate 2</td>
</tr>
<tr>
<td>alatae</td>
<td>0.198 b (±0.006)</td>
<td>0.225 ab (±0.020)</td>
<td>0.252 a (±0.014)</td>
<td>0.224 ab (±0.019)</td>
</tr>
<tr>
<td>L3+L4 winged</td>
<td>0.220 b (±0.017)</td>
<td>0.250 ab (±0.027)</td>
<td>0.304 a (±0.012)</td>
<td>0.263 ab (±0.005)</td>
</tr>
<tr>
<td>adult aperous</td>
<td>0.257 b (±0.017)</td>
<td>0.384 ab (±0.056)</td>
<td>0.441 a (±0.036)</td>
<td>0.387 ab (±0.044)</td>
</tr>
<tr>
<td>L4 aperous</td>
<td>0.165 b (±0.011)</td>
<td>0.221 ab (±0.016)</td>
<td>0.257 a (±0.008)</td>
<td>0.270 a (±0.029)</td>
</tr>
<tr>
<td>L3 aperous</td>
<td>0.113 c (±0.003)</td>
<td>0.120 bc (±0.010)</td>
<td>0.153 a (±0.007)</td>
<td>0.149 ab (±0.015)</td>
</tr>
<tr>
<td>L2</td>
<td>0.055 a (±0.004)</td>
<td>0.068 a (±0.007)</td>
<td>0.078 a (±0.003)</td>
<td>0.080 a (±0.016)</td>
</tr>
<tr>
<td>L1</td>
<td>0.021 b (±0.002)</td>
<td>0.028 ab (±0.004)</td>
<td>0.033 a (±0.004)</td>
<td>0.029 ab (±0.003)</td>
</tr>
</tbody>
</table>

| Dry weight (mg) | | | | |
|-----------------|---|---|---|
| alatae | 0.073 c (±0.004) | 0.079 bc (±0.003) | 0.096 a (±0.008) | 0.091 ab (±0.005) |
| L3+L4 winged | 0.073 b (±0.004) | 0.076 b (±0.004) | 0.093 a (±0.002) | 0.086 a (±0.002) |
| adult aperous | 0.091 b (±0.004) | 0.119 ab (±0.013) | 0.138 a (±0.014) | 0.125 ab (±0.014) |
| L4 aperous | 0.055 b (±0.003) | 0.068 b (±0.003) | 0.082 a (±0.003) | 0.086 a (±0.007) |
| L3 aperous | 0.041 b (±0.001) | 0.040 b (±0.002) | 0.050 a (±0.003) | 0.050 a (±0.003) |
| L2 | 0.020 a (±0.001) | 0.023 a (±0.001) | 0.025 a (±0.002) | 0.028 a (±0.004) |
| L1 | 0.008 b (±0.00) | 0.009 ab (±0.001) | 0.011 a (±0.002) | 0.010 ab (±0.001) |

a mean of 3 replications
b means in a row followed by the same letter are not significantly different (P ≤ 0.05).
The body water content (%) of aphids did not differ significantly among treatments or among the different aphid stages of development (data not shown). The larval instars, however, tended to have lower water contents on well-watered than on stressed plants. In Exp. 3, aphid populations only just began to reach their maximum size, particularly those on water stressed plants, whereas in Exp. 4 the maximum size of a population had already been reached and populations showed signs of collapsing. Population density seemed to be the main factor in determining body weight of aphid individuals (under favorable nutrition).

Analyzing aphid populations into their various stages of development as presented in Fig. 23, showed the age distribution of an aphid population after plants had stopped growing probably as a result of high aphid densities. In contrast to Exp. 3, in which populations started off with two to three L4 instars, populations in Exp. 4 started off with a predefined age structure.

![Aphid Stage of Development](image)

**Fig. 23.** Age distribution in aphid populations feeding on well-watered (control), mildly, moderately 1 and moderately 2 water stressed apple plants (until plants stopped growing) in Exp. 4. Means (of 3 replications) labelled by the same letter are not significantly different (P ≤ 0.05).
Larval instars significantly decreased in number from well-watered to stressed plants. The number of apterous adults significantly decreased only between the well-watered and the Mod. 1 stressed treatments. Alatae and winged L3+L4 tended to decrease in number with an increase in water stress. Except for L1 and apterous L3, the differences in the proportion of aphid stages among treatments were not significant (data not shown). The proportion of L3 in populations feeding on well-watered plants was significantly higher than on Mod. 1 stressed plants (16.1% vs 11.5%; P ≤ 0.05). The proportion of L1 feeding on well-watered plants was significantly lower than on Mod. 1 stressed plants (27.3% vs 35.9%, P ≤ 0.05).

The trend in age distribution under water stress described for Exp. 4 was opposite to that observed in Exp. 3. Compared with the upper parts of the plants in Exp. 4, populations in Exp. 4 had a lower proportion of L1 and L2 and a higher proportion of alatae, winged L3+L4, apterous L3 and L4 (data not shown). An explanation is almost certainly that aphid populations in Exp. 3 had not reached their maximum size, particularly those on well-watered plants, whereas in Exp. 4 the maximum size had been reached and populations showed signs of collapsing. Population density seemed to be a main factor in determining population age structures.

Age distribution in a population among replicates of a treatment were highly variable. One reason for this variability is the observed variation in density among replicates. Wennergren and Landin (1993) mention that variations in the local environment of insect herbivores, such as crowding, can also change the structure of populations. Moreover, towards the end of Exp. 4, a very large dispersion of the large size apterous adults into the pot was observed (particularly in the well-watered treatment). This dispersion changed the structure of aphid populations on the plants: the proportion of adults and newly born progeny (L1) decreased within a population. Comparably, Vehrs et al. (1992) observed that most of the decrease in the *Myzus persicae* (Sulzer) population was due to the high numbers of dispersing apterous aphids found in the water bath below the plants. The authors explained this dispersal behavior by crowding effects.
3.4 Discussion

3.4.1 Effects of Water Stress on Plant Development

In both experiments, growth (stem elongation and production of new leaves), accumulation of fresh and dry weight as well as total leaf area of uninfested plants decreased with water stress (see Tables 10, 11 and 12). In Exp. 3, a critical moisture stress level for the growth of uninfested plants was observed between the well-watered (mean \( \Psi_{soil} \) at harvest = 0.00 MPa, range: -0.00 to -0.07 MPa) and the moderately stressed treatment (mean \( \Psi_{soil} \) at harvest = -0.69 MPa, range: -0.46 to -1.43 MPa; see Table 13). In Exp. 4, the critical moisture stress level for the growth of plants was observed between the mildly (mean \( \Psi_{soil} = -0.69 \) MPa, range: -0.18 to -1.60 MPa) and the Mod. 1 stressed treatment (mean \( \Psi_{soil} = -1.20 \) MPa, range: -0.28 to -2.48 MPa; see Table 14). Hence, the critical moisture stress level, below which the growth of apple plants in pots under artificial environmental conditions was significantly affected, developed below \( \Psi = -0.65 \) MPa. Under conditions of intermittent drying, i.e. those imposed in this series of experiments, however, it was difficult to define an exact critical moisture stress level. For example, plant growth among other plant parameters decreased with an increase in water stress within a treatment and increased again when plants were rewatered to start a new drying cycle (see Fig. 15).

In the study presented here, new leaf production was not affected at moderate stress levels but was impaired with a further increase in stress intensity. In both experiments 3 and 4, internodal length decreased to a certain moisture level indicating that stem elongation was restricted to a greater extent than leaf production. Beyond this moisture level, leaf production was also strongly affected which was expressed by longer internodes (\( \Psi_{soil} \) for Exp. 3 < -0.69 MPa and \( \Psi_{soil} \) for Exp. 4 < -1.20 MPa). A similar reaction of internodal lengths to water stress was observed in Exp. 2.

One of the traditional responses reported to be associated with water stress in plants is a significant reduction of the above ground biomass. Water stress greatly alters the biomass distribution of trees, decreasing shoot growth and increasing the root to shoot ratio (Dorschner et al., 1986; Schnider, 1989; Sritharan and Lenz, 1989; Steinberg et al., 1989; Honek, 1991; Buwalda and Lenz, 1992). Under conditions of water stress, the production of new leaf area is reduced or completely halted, thereby reducing the transpiring surface,
whereas root production is maintained (Sritharan and Lenz 1989; Steinberg et al., 1989; Buwalda and Lenz, 1992), favoring the search for water. In addition, direct effects of water stress on leaf area expansion may be the cause for restricted photosynthesis and hence growth (Buwalda and Lenz, 1992).

Different water regimes clearly affected the reaction of the components of plant water potential to soil drying. At a certain stress intensity (e.g. \( \Psi = -1.00 \) MPa), \( \Psi^0 \) in uninfested plants were lower in water stressed than in well-watered plants (difference \( \Delta \) in \( \Psi^0 \) ca 0.45 MPa; see Figs. 18 and 19). Leaf turgor pressure of water stressed plants was therefore maintained at higher values at decreased \( \Psi \) than in well-watered plants. This was due to a steeper decrease in \( \Psi^0 \) with decreasing \( \Psi \) and/or to a generally lower \( \Psi^0 \) in water stressed than in well-watered plants. A steeper decrease in \( \Psi^0 \) with decreasing \( \Psi \) may indicate an increase in tissue (cell wall) elasticity, whereas generally lower \( \Psi^0 \) with declining \( \Psi \) are consistent with an active accumulation of solutes, i.e. to osmotic adjustment to conditions of water stress. Predawn measurement of the components of water potential in both experiments indicated that, namely in treatments with low stress intensities, maintenance of turgor pressure was due not only to generally lower \( \Psi^0 \) at a certain \( \Psi \), but also to increases in tissue (cell wall) elasticity. Under severe water stress regimes, a distinct (active) osmotic adjustment seemed to take place. These results support and clarify the conclusions of Davies and Lakso (1979a) who suggested a combination of active osmotic adjustment and increases in tissue elasticity to account for the high drought tolerance of apple trees.

In Exp. 3, predawn \( \Psi_p \) in moderately stressed plants was higher than in well-watered plants (see Fig. 18 and Table 13), although average \( \Psi_{soil} \) reached significantly lower levels (see Table 13). This was probably due to the fact that at night, plant roots which had grown into still wet areas of the soil could provide a temporary relief of the water stress for the plant, which was reflected in only insignificant lower \( \Psi \)-values in moderately stressed compared with well-watered plants (see Table 13). Since the moderately stressed plants had adjusted osmotically, turgor pressures were significantly higher in these than in well-watered plants. Similar effects of temporary stress relief were also observed in Exp. 4, where predawn plant \( \Psi \) and \( \Psi_p \) of the three stressed treatments were very similar (see Fig. 19), although average \( \Psi_{soil} \) of the different treatments were significantly different (see Table 14). Even though predawn \( \Psi_p \) was maintained, the growth of plants in both moderately water
stressed treatments was reduced. This might be due to significantly lower midday $\Psi$ of moderately stressed plants compared with well-watered plants. In addition, changes in cell wall extensibility and the minimum turgor threshold for growth may further decrease growth rates of water stressed plants (e.g. Mathews et al., 1984 cf. Steinberg et al., 1989).

In Exp. 3, components of plant water potential measured at predawn gave higher values than at midday (mean difference for $\Psi$= 0.78, $\Psi_o$= 0.16, $\Psi_p$= 0.62 MPa). Steinberg et al. (1989) reported that, at midday, $\Psi_o$ of peach leaves under well-watered conditions had decreased by 0.5 to 0.8 MPa from predawn values. Because this decrease was accompanied by a relatively high $\Psi_p$, the authors suggested that both active solute build-up as well as water loss from the leaf tissue had taken place. Davies and Lakso (1979a) even reported a decrease in $\Psi_o$ in leaves of apple by as much as 1.65 MPa during the day which they attributed to a combination of (active) osmotic adjustment and tissue dehydration. In the present study, the decreases in $\Psi_o$ determined at midday, compared with predawn, were not very distinct and probably mostly due to a concentration of solutes in plant sap by dehydration, i.e. passive osmotic adjustment, rather than to an active accumulation of more solutes, since leaf water contents were generally lower at midday than at predawn (data not shown). The decrease in osmotic potential at midday could not keep up with the loss of water, however, and this was reflected in the reduced leaf turgor pressures that were observed.

3.4.2 Effects of Aphid Populations on Plant Development

Growth (as measured by the increases in shoot length and number of leaves as well as fresh and dry weights, leaf area, and the length of internodes) of aphid infested (+A) and uninfested plants (-A) did not differ significantly. Growth parameters were always smaller in infested than in uninfested plants, however, and significantly so in the mildly water stressed treatment in Exp. 4. Adding the production of aphid biomass to the production of plant biomass did not alter these relationships, since aphid biomass accounted for less than 6.5% of the total biomass production in the system (even in heavily infested control plants).

These results show that under conditions of mild water stress ($\Psi_{soil} > -0.65$ MPa) an infestation with aphids exerted an additive stress on the plants besides water stress. Whereas well-watered plants probably could
compensate to a great extent the "damage" (loss in assimilates by aphid feeding) due to even high densities of aphids (stimulation by a positive feedback of an additional sink), plants under even mild water stress suffered significantly reduced growth even when attacked by much smaller densities of aphids. Aphids drained from the plant assimilates which were needed for osmotic adjustment to maintain growth at low stress intensities. Significantly lower $\Psi_0$ in leaves of uninfested than infested plants confirmed these observations. This drain of assimilates needed by the plants can also explain the higher critical moisture stress level, below which plant growth is significantly reduced, in plants which were infested with aphids ($\Psi_{\text{soil}} > -0.69$ MPa) compared with uninfested plants ($-0.69 > \Psi_{\text{soil}} > -1.2$ MPa) in Exp. 4. At high intensities of water stress, the differences in plant growth between aphid infested and uninfested plants decreased and became insignificant. This can be due to two "reasons" or a combination of both: (1) With an increase in stress intensity, the dominant limiting factor became water stress and/or (2) with an increase in intensities of water stress, aphid population density remained below the critical level that would have affected plant development. In addition, the loss in productive leaf area (photosynthetically active leaf surfaces) due to leaf curling and honeydew production of $+A$ plants could well have affected plant growth. Since water stressed plants had lower aphid densities, leaf curling and honeydew would have affected leaf area less.

The aphids, depending on density and length of the infestation period, seemed to be more damaging on young tissue. *Aphis pomi* definitely prefers growing tissues as a feeding site and depends on food from actively growing tissues to increase populations to great numbers (Baker and Turner, 1916; Lathrop, 1923; Cutright, 1930; Oatman and Legner, 1961; Specht, 1972). In young trees, the ratio between growing and mature tissues is much higher than in older trees; it follows that young trees should therefore suffer more than older trees.

Results of previous work concerning effects of *A. pomi* on the growth of apple trees are scarce and contradictory. Oatman and Legner (1961) found that the green apple aphid reduced shoot growth of 7-year-old "Red Delicious" and "Cortland" apple trees. Hamilton et al. (1986) found that *A. pomi* did not affect growth of terminal shoots of mature "Red Delicious" trees, but had a limited effect on mature "Golden Delicious" trees. Hugentobler (1990) reported that *A. pomi* decreased the growth of 1-year old "Golden Delicious" plants only slightly (due to reductions in leaf area) after 10 days of infestation. Kaakeh et al. (1992, 1993) reported that the accumulation of fresh and dry weight in
leaves, lateral shoots, rootstock and roots of one year old "Redchief Delicious" apple trees grown in pots during the first growing season (3-months) were negatively affected by *A. pomi* and *Aphis spiraeola* (Patch.) In contrast, Varm and Pfeiffer (1989) reported that dry matter accumulation was not affected by *A. spiraeola* (infestation period = 40 days). Differences in materials and methods (cultivars, plant age, experimental conditions etc.) among these studies make generalized conclusions difficult.

In Exp. 3, the components of water potential measured at predawn and at midday (as well as tissue water content) were lower in -A plants compared with +A plants (see Table 13). This difference became insignificant, however, under severely water stressed conditions. Leaves of +A plants under well-watered and moderately stressed conditions maintained larger aphid populations and densities, the leaves were therefore also covered with more honeydew of lesser viscosity than severely stressed plants. This may well indicate that large aphid populations with high honeydew production may actually affect gas exchange between leaves and environment causing less transpiration (higher $\Psi$) and decreased photosynthesis. Many authors have reported detrimental effects of honeydew on plant functioning (e.g. Camell, 1981; Wood et al., 1988; Rossing and Van de Wiel, 1990; all cf. Hurej and Van der Werf, 1993), whereas experiments of Hurej and Van der Werf (1993) showed no effect on growth and yield when artificial honeydew was sprayed on sugar beet. Decreased transpiration might also explain higher percentage water contents of leaves in +A compared with -A plants in Exp. 3.

In Exp. 3, leaf turgor pressures ($\Psi_p$) were higher in -A than in +A plants in the well-watered and moderately stressed treatments (see Table 13). This was due to generally lower $\Psi_o$ at a given $\Psi$ in -A than in +A plants (see Fig. 18), which indicated that an infestation with aphids may reduce the plants' ability to adjust osmotically to water stress. In addition, a steeper decrease of $\Psi_o$ with declining $\Psi$ in water stressed compared with well watered plants was only observed in leaves of -A trees. This probably indicates that tissue elasticity in -A plants increased with increasing stress intensity, whereas in +A plants, no changes in tissue elasticity occurred. The differences in $\Psi_o$ and $\Psi_p$ between +A and -A plants decreased within as well as among treatments with increasing intensity of water stress.

Dorschner et al. (1986) reported that in wheat plants subjected to *Schizaphis graminum* (Rondani) and drought stress, the aphids caused increased (less negative) $\Psi_o$ compared with plants subjected to drought stress alone, i.e. an
infestation with aphids reduced osmotic adjustment to drought stress. Assimilate drain by aphids can be quite significant (Hurej and Van der Werf, 1993), and Riedell (1989) demonstrated that +A plants accumulate less proline and glycinebetaine during a drought stress period than -A plants and therefore an infestation with aphids limits the capacity of plants to adjust successfully to drought stress (will be discussed in section 4). The results of the present study show that aphids might impair turgor maintenance in water stressed plants: (a) by decreasing the plants’ ability to adjust osmotically, and/or (b) by possibly impairing increases in tissue elasticity under water stress.

In Exp. 4, aphid populations were allowed to develop until host plants stopped growing. This density threshold for plant growth decreased with water stress, and water stressed plants, therefore, had stopped growth earlier (after 20 - 25 days) than in well-watered plants (after 37 days), although aphid populations developed more slowly under conditions of water stress. According to Kieckhefer and Gellner (1992), aphid density thresholds for significant yield loss differ with environmental conditions, plant and aphid genotype.

3.4.3 Effects of Water Stress on the Population Development of Aphids

In both experiments, plant growth (see Table 10) as well as the size (Figs. 20 and 22) and densities of aphid populations decreased with increasing intensities of water stress. Obviously, the development of aphid populations was affected by plant growth.

In Exp. 4 and on the upper parts of plants in Exp. 3, the size of aphid populations correlated well with the increase in shoot length and in leaf number: e.g. in Exp. 3, r=0.95 between number of aphids and shoot length; P<0.001 and in Exp. 4, r=0.88 between number of aphids and leaf number; P<0.001). On the lower parts of plants in Exp. 3, such a correlation was not found. This could be explained if the growth rate, i.e. the production of young tissue, is more important to the growth of aphid populations than the total size of the plant. The fact that the proportion of growing to mature tissue is higher in small compared with large plants may be the reason that young plants are more susceptible to an infestation with aphids than old plants.
Water stress generally affects plant growth by decreasing plant water potentials, i.e. by reducing turgor pressure. It could be speculated that it is not plant growth per se, but changes in turgor pressure that decreased the size and density of aphid populations under water stress. Nevertheless, analysis of the data revealed that no specific component of plant water potential could explain the changes in size or density of aphid populations.

Since on the lower parts of the plants in Exp. 3, neither plant growth nor turgor pressure correlated with the decrease in the size and density of aphid populations, probably the nutritional quality of leaves for aphids was negatively affected by water stress. Even on the upper parts of plants some leaf quality characteristics changed with water stress, since even here aphid densities decreased with an increase in stress. In contrast, Dorschner et al. (1986) showed that, although the total number of aphids declined with an increase in water stress, the density of the infestation could increase on water stressed wheat. Most probably the response of aphids to water stress differs with the type and intensity of water stress and with aphid and plant genotype.

Food quality (assimilate production and transport) beside food quantity (plant size) plays an important role in the development of aphid populations (Atkinson, 1979; Honek, 1991) and becomes especially obvious when alatae production and aphid size are considered. Leather (1989) reported that suboptimal conditions manifested themselves by constraints in size and the reproductive potential of apterae and by the production of alatae.

In Exp. 3, the body size of aphids (fresh and dry weight) decreased with water stress (see Table 15) whereas in Exp. 4, aphid size increased with water stress (see Table 16). The difference between the two experiments was that aphid densities in Exp. 4 were much higher than in Exp. 3 and most probably had reached their maximum size (past the carrying capacity of the plant). Variation in adult size is seen as a measure of coping with a variable environment (Cockburn, 1991 cf. Kindlmann and Dixon, 1992) or as a consequence of maximizing population growth (Kindlmann and Dixon, 1992) under a particular combination of temperature and food quality (Atkinson, 1979; Dixon, 1985; Kindlmann and Dixon, 1992; Thomas, 1993). This is true for Exp. 3: water stress had probably changed leaf nutritional quality and leaf surface temperatures and these changes affected aphid body size negatively. Further, food quality and availability were strongly influenced by aphid feeding and could be related to the level of competition among the members of the population on the host (density, crowding)(Baugh and Phillips Jr., 1991;
Honek, 1991; Kaakeh et al., 1992; Kindlmann and Dixon, 1992; Thomas, 1993). This was the case in Exp. 4. One way in which aphids respond to lower food quality and quantity is to maintain lower body weights (see Table 16) and decreased fecundity (see Fig. 23). Thus fecundity is positively correlated with adult size and/or weight (Calow, 1978 cf. Dixon, 1985; Kouamé and Mackauer, 1992). It follows that in the present study, the first constraint on well-watered plants which affected aphid body size was crowding, whereas under water stress conditions it was water stress via the change in food quality. On mature leaves the main constraint for aphid size was the quality of their food. The ability of aphids to respond to changes in the quantity of phloem sap and thereby to anticipate the onset of adverse nutritional conditions is of great adaptive significance (Dixon, 1985).

Another way to adapt to lower food quantity and quality for an aphid population is to produce winged morphs. Alatae are produced by many aphids in response to deteriorating conditions in their local environment such as crowding or reduction in food quality (Lees, 1966 cf. Baugh and Phillips Jr., 1991; Watt and Dixon, 1981 cf. Wiktelius, 1992; Dixon, 1985; Leather, 1989; De Barro, 1992). In Exp. 3, formation of alatae could be primarily related to aphid density (r=0.72 between density and number of winged larvae; P < 0.001); and not to an assumed reduction in the quality of plants consequent upon an increase in water stress (to be discussed in Section 4). In Exp. 4, alatae production was also related to aphid density (r=0.59 between density and number of alatae; P < 0.05) as well as to the reduction in food quality arising from both crowding and water deficiency. A decrease in the carrying capacity of the plant under water stressed conditions (indicated by a decrease in maximum aphid density in a population) decreased the density threshold below which alatae production is initiated. Baugh and Phillips Jr. (1991) found a positive correlation between alatae production and leaf water potential of wheat with maximum alatae production between Ψ = -0.76 and Ψ = -1.03 MPa. The authors conclude that the host itself is involved in alatae production and not population density alone.

A further point of interest was to consider when an aphid population starts to suffer from water stress. Sumner et al. (1983) mentioned that a critical moisture level (Ψ = -0.75 MPa) existed, below which the fecundity and longevity of S. graminum were sharply decreased. In the present study, the size and density of aphid populations decreased significantly between well-watered and moderately stressed plants in Exp. 3 (see Fig. 20) and between well-watered and mildly stressed plants in Exp. 4 (Ψ_{soil} > -0.69 MPa; Fig. 22).
If the assumption is true that water stress changes plant quality then a relationship between specific aphid load (aphid abundance, density) and plant quality (carrying capacity of the plant) should exist. The carrying capacity of a plant decreased significantly with water stress. The critical moisture stress level for the development of aphid populations and the threshold of population density for a significant reduction in biomass of hosts did not coincide, however, as shown by aphid size, particularly on well-watered plants (Tables 15 and 16). Thus, in Exp. 4, well-watered plants which were infested with aphids stopped growing at an aphid density at which the aphids themselves had long since begun to suffer decreased body weights (of all morphs) as well as lower fecundity, the dispersion of apterae and the production of alatae. According to references cited in Price et al. (1989), the carrying capacity of a plant will be well below the level where damage to the plant approaches a lethal threshold or even becomes very conspicuous.

In conclusion, Exps. 3 and 4 clearly showed that the rate of aphid population development was negatively affected by water stress. In contrast to the slightly higher \( r_m \) found under water stress as compared with well-watered conditions in Exps. 1 and 2, the size and density of aphid populations decreased significantly with water stress. Reduced plant growth and, thus, reduced production of new plant tissue, changes in leaf surface temperature as well as supposed changes in phloem characteristics, particularly in sap quality, affected population development by decreasing the carrying capacity of the plant. The next section (section 4) will examine in detail the quality of the aphids' source of nutrition (phloem sap) under different water regimes.
Leer - Vide - Empty
4

**Effects of Water Stress on Population Dynamics of the Green Apple Aphid: Changes in Phloem Sap Quality**

4.1 Introduction

As also implied from the results presented in the previous section, a major factor controlling the population dynamics of aphids, and thus their attack and damage of crops, is the quantity and quality of the food offered by the potential host plant. Aphids feed primarily on the phloem sap of plants and presumably get all their essential starting materials for the metabolites they need for growth and reproduction from the phloem sap of their hosts (carbohydrates, amino acids, minerals, vitamins, water, etc.) (Dadd, 1985 cf. Slansky Jr. and Scriber, 1985; Srivastava, 1987). Although phloem sap is considered to contain relatively high amounts of nitrogen (1-2.3 %) compared with other plant tissues (e.g. xylem) (Pollard, 1973), N is nevertheless limiting in the nutrition of insects (Auclair, 1965 cf. Prosser and Douglas, 1992; SenGupta and Miles, 1975; Mattson, 1980 cf. Klinglaufer, 1987, Hugentobler, 1990). The phloem sap of plants is often described as a very dilute, nutrient-poor, nutritionally incomplete and unbalanced food source, the most important limiting factor being nitrogen present in the form of amino acids (Raven, 1983 cf. Prosser and Douglas, 1993; Slansky Jr. and Scriber, 1985; Klinglaufer, 1987). Although the phloem sap is rich in carbohydrate and poor in amino
acids and minerals essential for insect nutrition (Byrne and Miller, 1990; Prosser and Douglas, 1992), one advantage of feeding on this source is that all nutrients are in soluble and renewable form (Slansky Jr. and Scriber, 1985; Risebrow and Dixon, 1987). In order to obtain and accumulate sufficient amounts of the scarce components, however, aphids must process large amounts of liquid (Llewellyn, 1977 cf. Dorschner 1993; Mullin, 1986; Risebrow and Dixon, 1987; Slansky Jr. and Scriber, 1985; Byrne and Miller, 1990). The excess (e.g. carbohydrates) is excreted by the aphids in the form of honeydew.

The quantity and quality of food available to aphids fluctuates and may depend on factors such as water supply. Typical plant responses to water stress are probably highly relevant for the feeding success of aphids. Nitrogen metabolism is among the processes most sensitive to water stress (Hsiao et al., 1976). In general, water stress disturbs nitrogen metabolism so that the amount of protein decreases while amino acids increase in concentration (increased protein hydrolysis and reduced plant growth) (Kramer, 1983 cf. Mattson and Haack, 1987; cf. Louda, 1986; Klinglauf, 1987; Srivastava, 1987). Further, water stress disturbs carbohydrate metabolism so that starch levels generally decrease while sugar levels increase (cf. Mattson and Haack, 1987). Other plant responses to water stress which may be relevant for the feeding success of aphids are changes in the distribution of assimilates as water stress increases, reduction of carbohydrate translocation and changes in secondary metabolite concentrations (cf. Louda, 1986).

Not all plants or plant parts are equally nutritionally suitable for phloem feeders (Slansky Jr. and Scriber, 1985; Risebrow and Dixon, 1987; Srivastava, 1987; Douglas, 1993). Many aphids showed preferences for young or early senescent leaves (Dixon, 1985; Klinglauf, 1987; cf. Srivastava, 1987). It is widely accepted that for most deciduous trees the amino acid content of phloem sap is relatively high when the foliage is growing or senescing (nutrients are then being actively translocated into or out of the leaves) but low in mature leaves (e.g. Douglas, 1993). Further, aphids are capable of altering the nitrogen balance of their host plants to a certain extent in ways which can be beneficial to themselves ("additional sink effect") (Klinglauf, 1987; Risebrow and Dixon, 1987; Miles, 1989a; Dorschner, 1990). Few studies exist on phloem sap composition of apple trees (Bieleski, 1969 cf. Wermelinger, 1985; Kluge, 1967 cf. Kollar and Seemüller, 1990; Hugentobler, 1990). The most thorough and extensive study was conducted
by Kollar and Seemüller (1990). Next to carbohydrates and amino acids, the authors found organic acids (citric and malic acid), inorganic constituents (K⁺, Mg²⁺, Na⁺, SO₄⁻, etc.), proteins, lipids, and RNA nucleotide. It is not entirely certain, however, that all these components (e.g. proteins) are freely available in the bulk flow of phloem sap available to the aphids (P.W. Miles, University of Adelaide, South Australia, pers. comm.). The effect of water stress on the concentration and composition of nutrients in the phloem sap of apple and its relevance to the performance of *Aphis pomi* De Geer has not been studied yet.

In this study, changes in the concentration and composition of carbohydrates and amino acids in phloem sap of plants grown under different water regimes were examined, in order to speculate on how they relate to the development of aphid populations and their growth. Further, since aphids developed significantly differently on growing leaves compared with mature leaves (sections 2 and 3), it was of interest to examine differences in nutritional quality within the plant. Last but not least changes in phloem sap composition due to aphid infestation were investigated.

### 4.2 Materials and Methods

**4.2.1 Collection of Phloem Sap**

Precise quantitative and qualitative data on pure phloem sap composition can be obtained by severing the stylets of feeding aphids by means of a high frequency microcautery or a laser technique (e.g. Fisher and Frame, 1984; Weibull et al., 1986; Kuo-Sell, 1989; Girousse et al. 1991). However, as a preliminary experiment to this study had shown, this approach (using radiofrequency microcautery) was unsuitable for the relatively small *A. pomi* feeding on water stressed plants. Under conditions of water stress, the phloem sap seemed more viscous and probably the turgor pressure in the sieve tubes was also less positive, rendering the flow through the severed stylet more tedious (also found by U. Hugentobler, Institute of Plant Sciences, ETH Zürich). Stylectomy has been successful mainly if at all on herbaceous plants (Gruppe, 1989; P.W. Miles, University of Adelaide, South Australia, pers. comm.). Therefore, the technically more simple and convenient EDTA-exudation method was used in this study (King and Zeevart, 1974). Diethylene diamine tetraacetic acid (EDTA) is known to inhibit callose sealing
of the sieve tubes by complexing the calcium ions which are essential for this process (Costello et al., 1982; Bolsinger and Flückiger, 1986), allowing therefore to collect the exuding phloem sap. The drawbacks of this method are sap dilution and possible contamination from surrounding cells (Girousse et al., 1990; Weibull et al., 1990). In a preliminary experiment, the EDTA-exudation technique was optimized for the apple plants grown under the given conditions based on method descriptions by Groussol et al. (1986), Girousse et al. (1990), Hugentobler (1990), Miles (1990, technical report) and Weibull et al. (1990).

4.2.2 Plant Material

Phloem sap was collected from the upper and lower parts of plants grown under well-watered and water stressed conditions in experiment 3. The samples were taken from uninfested plants as well as from plants, that were previously infested with aphids. For a detailed description of the experimental conditions and layout see pages 65 and 66. The leaf samples for the phloem collection of phloem sap were taken at midday (around 1:00 pm) on the day of plant harvest and at the peak of the current drying cycle. Aphid populations had already been collected on the day of harvest. Samples from the upper parts of the plant consisted of the decapitated apex with 5 apical leaves (referred to as apex), and that from the lower parts consisted of the first 3 consecutive leaves from leaf A (referred to as basal leaves; ranged between leaf 10 for stressed and leaf 30 for well-watered plants, counted from the apex; see page 65).

4.2.3 Exudation Technique

Immediately after excision of the apices and the basal leaves at the base of their petioles, the samples were dipped into vials containing 0.750 ml of an EDTA-buffer solution. About 0.5-1.0 cm of the basal part of the petiole was immersed in the solution. The EDTA-buffer solution consisted of 3.5 mM disodium EDTA (Titriplex) and 5 mM di- and monosodium phosphate with a pH of 6.7. During the time of exudation, the samples were kept in darkness, at 23 °C and in high relative humidity in a growth cabinet. The stems were sealed into the top of the vials below the level of the leaf blades with Parafilm. Exudation time was 4 hrs. After exudation, the collected solutions were heated in a water bath to 90 °C for two minutes (to stop enzyme
activity) and then frozen at -30 °C until the assay of sugars and amino acids. Leaf area, fresh and dry weight of the tissue samples were measured.

4.2.4 Analysis of Carbohydrates

An aliquot of 200 µl from the exudate solution was microfiltered and directly analyzed by HPLC (Series 3B, Perkin Elmer Corp., USA) with a RI detector (ERC 7510, Erma Optical Works Ltd., Japan). The separation of the sugars sucrose, glucose and fructose and the sugar alcohols arabitol and sorbitol (all referred to below as sugars or carbohydrates) was achieved with an Aminex HPX-87C column (300 x 7.8 mm i.d., Bio Rad, CA, USA) maintained at 85 °C in a column heater. A guard column (Micro Guard DeAsh Cat. 1 and An. 2, Bio Rad, Ca, USA) at room temperature was used between the injector and analytical column. The mobile phase was water and sample flow rate amounted to 0.6 ml/min. Sugars were identified by comparing retention times of samples and standards. Arabitol at a concentration of 2.5 mM served as an internal standard, since arabitol is unlikely to be found in phloem sap (Tarczynski et al., 1992); F. Mächler, Institute of Plant Sciences, ETH Zürich, pers. comm.). The internal standard was added to the EDTA-buffer solution before exudation had started. This allowed correction for changes in the volume of the EDTA-solution during the time of exudation, since arabitol as well as components which were exuded into the solution remain in the solution and do not enter through xylem tissue as a result of transpiration (Byrne and Miller, 1990).

4.2.4 Analysis of Nitrogenous Substances

Amino acids and amides (referred to below as amino acids) were analyzed by automated gradient HPLC (Waters 600 E, MA, USA) using the Pico Tag amino acid analysis system (Waters, Division of Millipore Corpor., MA, USA). Prior to amino acid quantification, samples were dried and derivatized. An aliquot of 100 µl from the exudate solution was dried under vacuum in a Pico Tag work station. Redrying was accomplished by adding 15 µl of 2:2:1 methanol:1M Na-acetate:triethylamine (TEA) and drying under vacuum. Twenty µl of 7:1:1:1 methanol:H₂O:TEA:phenylisothiocyanate (PITC) were added to each redried sample and allowed to react for 20 min at room temperature. Samples were then dried and reconstituted in 100 µl sample solution (phosphate buffer: 5 mM sodium phosphate with 6% acetonitrile).
Amino acid standards (Fluka, Buchs SG Switzerland) were derivatized in a similar manner. The internal standard used was methionine sulfone at a concentration of 30 µM and was added to the sample just prior to drying and derivatisation. Standards and samples were microfiltered before they were analyzed. The separation of the amino acids was achieved with a Pico Tag column for free amino acid analysis (reversed phase, 3.9×30 cm, Waters, Millipore Corpor., MA) maintained at 46 °C column temperature. Peak detection occurred at the wavelength of 254 nm in a UV detector maintained at 64 °C (Waters 486, MA, USA). The mobile phases were Eluent A (sodium acetate buffer and TEA) and Eluent B (acetonitrile; Waters; MA, USA) run at a gradient and the sample flow rate was 1.0 ml/min.

The amino acids measured were aspartic acid (ASP), glutamic acid (GLU), serine (SER), asparagine (ASN), glycine (GLY), histidine (HIS), threonine (THR), alanine (ALA), arginine (ARG), proline (PRO), tyrosine (TYR), valine (VAL), methionine (MET), cysteine (CYS), isoleucine (ILE), leucine (LEU), phenylalanine (PHE), tryptophan (TRP), ornithine (ORN) and lysine (LYS). Glutamine (GLN), although an amino acid commonly found in the phloem sap of several plants and also initially detected in the exudate solutions in this study, was not quantified due to the fact that it was missing in the standard mix.

4.2.5 Statistical Analysis

Differences among the treatments were evaluated using analysis of variance (ANOVA), and the means were tested by a least significant difference range test (LSD) at a 5 % level of significance if not otherwise mentioned. The software used was SAS (SAS release 6.04, Institute Inc. Cary, NC, USA).
4.3 Results

The phloem exudation technique using EDTA allowed the assessment of the concentration of total sugars and amino acids in the exudate and the measurement of the relative content of the different components. The exudation technique does not allow the determination of the absolute amount of collected phloem sap nor the flow rate.

4.3.1 Sugars

Figure 24 presents the concentration of total sugars (mM) measured in the exudate solution of the phloem of uninfested (-A) plants and of plants previously infested with aphids (+A) that were grown under different water regimes. Concentrations for the apices and basal leaves are shown separately, since the size of the leaf samples differed.

![Graph showing total sugar concentration in apices and basal leaves of apple plants under different water stress conditions. The graph includes bars for control, moderate, and severe water stress, as well as symbols indicating treatments with letters (a, b, etc.) representing significant differences. The data includes +A and -A plants.](image)

**Fig. 24.** Total sugar concentration in the exudates of phloem sap collected from apices and basal leaves of apple plants previously infested with aphids (+A) and without aphids (-A) grown under well-watered (control), moderately and severely water stressed conditions. Means (5 replicates) with the same letter are not significantly different (P ≤ 0.05); letters on top of bars indicate differences among treatments (+A and -A separately) and letters within bars between +A and -A plants within a treatment.
Mean area of apex samples was smaller (ca 35 cm$^2$) than that of basal leaves (ca 74 cm$^2$). This may partly explain why the concentration of total sugars found in the phloem exudate collected from apices was significantly lower than in the exudate collected from basal leaves. Even so, by any manipulation of the data, the higher sugar content of the exudates from the basal leaves of aphid infested plants appeared to be disproportionately large as discussed below. The size of the samples from apices and basal leaves was highly variable even within a water stress treatment.

In apical phloem exudates, the concentration of total sugars increased with water stress, which was significant only in uninfested plants (Fig. 24). In the phloem exudate collected from basal leaves of plants not infested with aphids, the concentration of total sugars did not differ significantly among water stress treatments probably because of the variability in the size of samples mentioned above. The high concentration of sugars measured in the exudate solutions of the basal leaves of well-watered, with aphid infested plants was probably due to contamination with honeydew which also covered the petioles of leaves (honeydew was not washed off the petioles before immersion into the exudation solution). Despite the effort to exclude any surface contamination from exudates, this conclusion seemed inevitable from quantitative analyses (see below). A higher aphid density (see page 85) and a less viscous honeydew on well-watered plants led to a large amount of honeydew production and its uniform distribution onto the lower leaves. This contamination with honeydew could be inferred from relevant HPLC chromatogram: before the sucrose peak an extra and large peak was detected. According to the retention time it was identified as due to melezitose, an oligosaccharide typical of and exclusive in honeydew. Honeydew of A. pomi feeding on similar apple plants grown under different levels of nitrogen fertilization was reported to contain sorbitol, melezitose, fructose, sucrose and glucose in decreasing order (Hugentobler, 1990). Therefore, the high sugar concentrations measured in the exudates of these basal leaves can be attributed to the contamination of the exudate solution by honeydew.

The phloem exudate collected from plants which were previously infested with aphids contained higher concentrations of total sugars than uninfested plants, although this difference reached significance only in the well-watered treatment.
An attempt was made to allow for the size of apex and basal leaf samples to show in the expression of the concentration of total sugars found in the phloem exudate. For this purpose, the concentration of total sugars (mM) was divided by the respective leaf area (cm²) of the sample (data not shown). In plants uninfested with aphids, apices still gave lower concentrations of total sugar than basal leaves, particularly under well-watered conditions. Further, the concentration of total sugars increased significantly with water stress. Moderately water stressed plants tended to give the highest concentrations per cm², indicating either a higher concentration of the pure phloem sap or a faster flow rate. Even when allowance was made for sample size, plants previously infested with aphids generally gave higher concentrations of total sugar than plants not infested with aphids, particularly in the well-watered treatment.

The main carbohydrate component found in the phloem exudates of all samples was the sugar alcohol sorbitol (ranged between 47 to 82 % of total sugar). The other components were sucrose, glucose and fructose. Since phloem sap is considered to contain only non-reducing sugars and sugar alcohols, the amount of glucose and fructose recovered indicates contamination of the exudate solution with either invertase, or with the reducing sugars themselves, from extra-phloem tissue sap, as discussed in detail on page 122. The dominance among these three sugars in the exudate solution depended on the water stress treatment, the presence of aphids and the position within the plant. Table 17 presents the relative content (%) of each sugar component in the exudate of phloem sap. For illustration purposes, the percentage sugar composition of the phloem exudates is also presented graphically (Fig. 25).

The relative content of sucrose, glucose and fructose in the phloem sap varied between apices and basal leaves (whether infested or not); the differences, however, were significant in the well-watered treatment only (Table 17). The relative content of sucrose and fructose was significantly lower and glucose significantly higher in the apex. The relative content of sorbitol was not significantly affected by the position of the sample within a plant.

In plants which were previously infested with aphids, the relative content of sucrose and fructose in the exudate solution was significantly higher than in the exudate of aphid uninfested plants. Further, previously infested plants showed significantly lower proportions of sorbitol in their phloem exudate collected both from the apex and basal leaves compared with that of
Table 17. Relative content (%) of sugar components in the phloem exudate of apices and basal leaves of apple plants previously infested with aphids (+A) and without aphids (-A) under well-watered (control), moderately and severely water stressed conditions; means ±SE are shown *.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>-A Control</th>
<th>Moderate</th>
<th>Severe</th>
<th>+A Control</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Apex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorbitol</td>
<td>65.90 ±1.75</td>
<td>74.94 ±4.03</td>
<td>77.88 ±2.11</td>
<td>49.79 ±3.09</td>
<td>55.69 ±8.69</td>
<td>69.81 ±2.74</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4.59 ±0.90</td>
<td>6.26 ±2.60</td>
<td>4.54 ±0.68</td>
<td>10.74 ±1.24</td>
<td>11.38 ±2.46</td>
<td>10.11 ±1.65</td>
</tr>
<tr>
<td>Glucose</td>
<td>26.30 ±2.27</td>
<td>13.25 ±2.27</td>
<td>12.35 ±0.67</td>
<td>24.51 ±2.33</td>
<td>18.06 ±3.73</td>
<td>11.00 ±0.49</td>
</tr>
<tr>
<td>Fructose</td>
<td>3.20 ±1.02</td>
<td>5.55 ±1.38</td>
<td>5.23 ±0.37</td>
<td>14.96 ±1.10</td>
<td>14.86 ±3.42</td>
<td>9.08 ±0.92</td>
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<td><strong>Basal Leaves</strong></td>
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<tr>
<td>Sorbitol</td>
<td>71.70 ±2.26</td>
<td>73.98 ±1.67</td>
<td>81.76 ±2.50</td>
<td>49.90 ±2.60</td>
<td>47.03 ±5.74</td>
<td>54.37 ±6.66</td>
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<tr>
<td>Sucrose</td>
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<tr>
<td>Glucose</td>
<td>11.40 ±0.84</td>
<td>12.19 ±1.32</td>
<td>8.93 ±1.66</td>
<td>14.69 ±2.06</td>
<td>15.83 ±2.92</td>
<td>13.53 ±1.88</td>
</tr>
<tr>
<td>Fructose</td>
<td>9.43 ±1.22</td>
<td>8.03 ±0.82</td>
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<td>20.30 ±0.60</td>
<td>15.04 ±1.80</td>
<td>12.89 ±1.60</td>
</tr>
</tbody>
</table>

* means (5 replicates) in a row followed by the same letter are not significantly different (P ≤ 0.05).

uninfested plants, irrespective of the water stress treatment (Fig. 25). Although the interpretation of these data is complicated by the evidence of contamination of the phloem sap as discussed above, one conclusion that can be drawn is that aphid infestation had considerably increased the "leakiness" of the tissue (surrounding cells).
Fig. 25. Relative content (%) of sugar components in the phloem exudate of apices and basal leaves of apple plants previously infested with aphids (+A) and without aphids (-A) under well-watered (C), moderately (M) and severely (S) water stressed conditions.

To study the effect of water stress on the percentage of each sugar component in the phloem exudate alone, only the plants not infested with aphids were considered (Table 17). A significant increase in the relative content of sorbitol in the exudate collected from apices and basal leaves with water stress was observed. Fructose content of basal leaf exudates and glucose contents from apical exudates decreased significantly with water stress. Therefore the most important effect of water stress on the carbohydrate composition of phloem exudates was the increase in the proportion of sorbitol.

In plants which were previously infested with aphids, the relative content of sorbitol significantly increased only in the exudates collected from the apex. Similar to the results from uninfested plants, the relative content of fructose in exudates collected from basal leaves and of glucose from apices decreased significantly with water stress, possibly because lower water contents impeded the leakiness of tissues suggested above.

Another difference with respect to the carbohydrate composition in the exudates collected from aphid infested and uninfested plants was the
presence or absence of the oligosaccharides stachyose and raffinose. In the HPLC chromatograms, particularly of phloem exudates collected from plants which were previously infested with aphids, two extra peaks were detected and identified as stachyose and raffinose. The raffinose peak was in general larger than the stachyose peak.

4.3.2 Amino Acids

Figure 26 demonstrates the total concentration of amino acids measured in the phloem exudate of plants which were previously infested with aphids (+A) and of uninfested plants (-A) grown under different water regimes. Data of the samples from apices and basal leaves are again shown separately due to differences in their size.

The concentration of total amino acids in the exudates of phloem sap from apices and basal leaves of apple plants previously infested with aphids (+A) and without aphids (-A) grown under well-watered (control), moderately and severely water stressed conditions. Means with the same letter are not significantly different (P ≤ 0.05); letters on top of bars indicate differences among treatments (+A and -A separately) and letters within bars between +A and -A plants within a treatment.
In contrast to total sugars, the concentration of amino acids in exudates from apices and basal leaves of water stressed plants did not differ significantly. In phloem exudates collected from the apex, the concentration of total amino acids decreased significantly with an increase in water stress, whether previously infested with aphids or not. In the exudate of basal leaves, total amino acids concentrations did not change significantly with water stress. The high concentration measured in the exudate solution of the basal leaves of well-watered, aphid infested plants was probably again due to the contamination with honeydew. Hugentobler (1990) stated that total amino acid concentration in honeydew was about one third compared with the phloem. An attempt was made to include the size of the sample used for exudation in the expression of total amino acid concentration found in the corresponding exudate solutions. For this purpose, total amino acid concentrations (μM) were divided by the respective leaf area (cm², data not shown). The exudate solutions collected from apex samples still gave higher concentrations of amino acids/cm² than from basal leaves. The samples taken from plants which were previously infested with aphids also contained higher concentrations of total amino acids than from uninfested plants. The concentration of total amino acids in the exudates decreased with increasing intensity of water stress, except in exudates collected from the basal leaves of uninfested plants. In the latter, the concentration of total amino acids /cm² increased with water stress, the highest difference being between well-watered and moderately stressed plants. Whether these changes in the concentrations of amino acids in the phloem exudate were due to variable concentrations in the phloem itself or due to changing flow rates could not be determined.

The major components of amino acids found in the phloem exudates of all samples were ASN (12 - 80% of the total content), THR (2 - 30%), CYS (1.4 - 28%), GLU (4% - 19.4%) and ASP (1.4 - 8.1%). These made up 70 to 90 % of the concentration of total amino acids. The high proportions of ASN might be overestimated since GLN, which often appears together with ASN although at lower proportions (Hugentobler, 1990), was not measured. Of the 20 amino acids measured, only arginine was not detected in the phloem exudate and will be excluded from further discussions. Table 18 presents the relative content (%) of each amino acid detected and measured in the exudates of phloem sap of apple plants. Since phloem exudates from the apex and the basal leaves differed significantly in their amino acid composition, these results will be presented separately.
In the exudate collected from the apex, the relative content of ASN was significantly higher, whereas that of THR, ALA, ILE, LEU and PHE were not significantly different in relative amounts from those found in exudate collected from the basal leaves. All the remaining amino acids were significantly lower in the apical exudate.

In the phloem exudate collected from the apex of plants previously infested with aphids, the relative content of ASN tended to be higher, whereas that of ASP, CYS and PHE was significantly lower (VAL, not significantly) than in uninfested plants. In the exudate collected from the basal leaves of plants previously infested with aphids, the relative content of ASN was significantly higher, whereas that of ASP, GLU, SER, HIS, LEU, PHE, LYS was significantly lower than in uninfested plants.

To study the effect of water stress on the amino acid composition of the phloem exudate, only samples of plants not infested with aphids were considered (Table 18). For illustration purposes, the proportion of the major amino acids found in phloem exudates is also presented graphically (Fig. 27).

![Graph showing amino acid composition in phloem exudate](image)

**Fig. 27.** Relative content (%) of the major amino acids in the phloem exudate of apices and basal leaves of apple plants previously infested with aphids (+A) and without aphids (-A) under well-watered (C), moderately (M) and severely (S) water stressed conditions.
Table 18. Relative content (%) of amino acid components in the phloem exudate of apices and basal leaves of apple plants previously infested with aphids (+A) and without aphids (-A) under well-watered (control), moderately and severely water stressed conditions; means and ±SE are shown.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>-A Apex</th>
<th>Control</th>
<th>Moderate</th>
<th>Severe</th>
<th>+A Apex</th>
<th>Control</th>
<th>Moderate</th>
<th>Severe</th>
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<tr>
<td>ASN</td>
<td>121.18±1.67</td>
<td>55.27±15.68</td>
<td>27.94±12.72</td>
<td>77.16±2.92</td>
<td>64.86±9.78</td>
<td>49.26±11.18</td>
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</tr>
<tr>
<td>THR</td>
<td>8.95±1.88</td>
<td>12.18±6.16</td>
<td>27.91±6.60</td>
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<td>9.82±4.71</td>
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<tr>
<td>CYS</td>
<td>5.06±1.11</td>
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<td>16.75±4.96</td>
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<tr>
<td>GLU</td>
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<td>8.85±1.48</td>
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</tr>
<tr>
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<tr>
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<td>1.36±1.10</td>
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<tr>
<td>SER</td>
<td>0.68±0.66</td>
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<td>0.43±0.42</td>
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<td>0.77±0.74</td>
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<tr>
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<td>PRO</td>
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<tr>
<th>Amino acid</th>
<th>-A Basal Leaves</th>
<th>Control</th>
<th>Moderate</th>
<th>Severe</th>
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<td>PHE</td>
<td>1.10±0.55</td>
<td>0.79±0.11</td>
<td>0.62±0.07</td>
<td>0.29±0.03</td>
<td>0.42±0.08</td>
<td>0.37±0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIS</td>
<td>0.89±0.48</td>
<td>1.06±0.06</td>
<td>1.03±0.24</td>
<td>0.22±0.04</td>
<td>0.60±0.14</td>
<td>0.53±0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYR</td>
<td>0.89±0.25</td>
<td>1.00±0.36</td>
<td>0.49±0.06</td>
<td>0.23±0.05</td>
<td>1.06±0.31</td>
<td>0.37±0.10</td>
<td></td>
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</tr>
<tr>
<td>LEU</td>
<td>0.89±0.15</td>
<td>0.73±0.09</td>
<td>0.64±0.14</td>
<td>0.15±0.05</td>
<td>0.51±0.16</td>
<td>0.71±0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORN</td>
<td>0.31±0.20</td>
<td>0.36±0.13</td>
<td>0.34±0.10</td>
<td>0.13±0.01</td>
<td>0.34±0.22</td>
<td>0.25±0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRP</td>
<td>0.28±0.14</td>
<td>0.27±0.08</td>
<td>0.23±0.05</td>
<td>0.06±0.01</td>
<td>0.21±0.05</td>
<td>0.22±0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MET</td>
<td>0.20±0.09</td>
<td>0.13±0.04</td>
<td>0.22±0.13</td>
<td>0.19±0.05</td>
<td>0.15±0.06</td>
<td>0.09±0.06</td>
<td></td>
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</tr>
</tbody>
</table>
In the apical exudate, the proportion of ASN was observed to decrease significantly with increasing intensity of water stress, whereas that of THR, CYS, ASP, HIS (VAL, LEU, ORN) increased significantly. In the phloem exudate collected from the basal leaves, GLU and ASP decreased with water stress, whereas THR tended to increase. In these samples, therefore, the exudates collected from the apex and from the basal leaves, besides having a completely different amino acid composition, also appeared to indicate totally different reactions to water stress.

With respect to plants which were previously infested with aphids, phloem exudates from the apex contained significantly decreasing relative contents of ASN and significantly increasing contents of GLU and ASP (THR and LEU) with increasing intensity of water stress. In phloem exudates collected from the basal leaves, ASN decreased significantly, whereas THR, CYS, LEU, HIS and ASP (GLU) increased significantly between the well-watered and water stressed treatments. These results must be treated with some caution, in new of the evidence of contamination of samples from aphid infested plants with honeydew as well as with the general contamination of the samples with the leaked contents of extra-phloem tissue sap, as noted above in relation to sugar concentrations. Nevertheless, in conclusion, water stress seemed to decrease the relative content of ASN in the exudate solution, whereas HIS and THR (to a lesser extent LEU and VAL) increased. For the other major amino acids the change with water stress depended on sample position within the plant and on whether the plant was previously infested with aphids or not.

Proline, often mentioned in context with water stress and the ability of plants to adjust osmotically, tended to increase in proportion with water stress, but to different degrees depending on the specific comparisons. In exudates from apices, the relative content of proline increased between the moderately and severely stressed treatments, whereas in the exudates from basal leaves, the relative content of proline already increased significantly between the well-watered and the moderately stressed treatment. The proline data overall had a particular high variance, however, which was probably due to the highly variable water stress development even within a treatment. The relative content of proline was in general higher in the exudates from basal leaves compared with apical exudates (exception: well-watered, -A plants) as well as in plants which were previously infested with aphids compared with uninfested plants.
4.4 Discussion

4.4.1 Sugars

The sugar alcohol sorbitol was clearly the major carbohydrate component found in the phloem of the apple plants in the study presented here. Most other plant species, however, have predominantly sucrose in their phloem sap (5-25% of the sap) (Pollard, 1973; Klinglauf 1987); sucrose is therefore commonly regarded as the main transport sugar in plants (cf. Kuo-Sell, 1989; cf. Byrne and Miller, 1990). For species such as corn (Ohshima et al., 1990), wheat (Hayashi and Chino, 1986 cf. Girousse et al. 1991), rice (Kawabe et al. 1978 cf. Girousse et al., 1991), oats (Kuo-Sell, 1989), cotton (Tarczynski, et al., 1992), alfalfa (Girousse et al., 1991), peas (Rochat and Boutin, 1991), and most leguminous species (Girousse et al. 1991) sucrose was reported to constitute the predominant or exclusive carbohydrate. On the other hand, Wang and Stutte (1992) reported that sorbitol constitutes 60 to 80 % of the translocated carbohydrate in most woody Rosaceae species. Hugentobler (1990) found almost exclusively sorbitol (70% of total sugar) and sucrose in the pure phloem sap of apple plants collected by stylectomy. Also Bieleski (1969 cf. Wermelinger, 1985) reported that sorbitol comprised 80 % of total sugars found in the phloem of apple. Besides sucrose and sorbitol, oligosaccharides of the raffinose type (raffinose, stachyose, ribose) have been identified in the stylet sap of different plant species (Ziegler, 1975; Klinglauf, 1987; Tarczynski et al., 1992). For example, evergreen ash (Costello et al., 1982) and pumpkin (Byrne and Miller, 1990) were reported to contain predominantly raffinose and stachyose in addition to some sucrose.

As mentioned in the introduction, the most comprehensive and complete study on the phloem sap of apple was done by Kollar and Seemüller (1989). The authors reported that beside the main sugars sorbitol and sucrose, the phloem sap contained low concentrations of stachyose, raffinose and fructose, whereas glucose was completely missing. Also in the study presented here, fructose was measured and stachyose and raffinose were identified in the phloem extract, particularly of samples taken from aphid infested plants. But also glucose was found in phloem extracts of apple plants, although it has been suggested that, generally, glucose was unlikely to be found in the phloem sap of any plant species (e.g. Tracynzki et al., 1992).

The relatively high amounts of glucose (ranging between 9 and 26 % of total
sugar) and, to a lesser extent, of fructose (3-20 %) in relation to sucrose (4.5-22 %) found in the exudate solutions indicated that the solution contained more than just pure phloem sap. Girousse et al. (1991) reported that recovered hexoses in the EDTA-exudate are to be considered as artefacts resulting from the cutting of the petioles: Leakage of compounds from the cells surrounding the phloem may contaminate the phloem extract. Groussol et al. (1986), showed that leakage from surrounding cells under the employed experimental conditions was negligible in comparison with the amount of sugars collected from the phloem sap. Another important source of contamination is the enzymatic inversion of sucrose to glucose and fructose by invertase during the time of exudation. Since the main carbohydrate component found in the phloem of apple was sorbitol, however, and since it is not susceptible to hydrolysis, a great proportion of sucrose was probably hydrolyzed by invertase in the sampling procedure employed in the present study. It has to be noted, though, that the glucose fraction in phloem exudates was on an average two-fold higher than the fructose fraction (data from Hugentobler, 1990 show similar results). Sucrose hydrolysis should produce equimolar amounts of glucose and fructose; if leakage from surrounding cells can indeed be considered negligible, the question arises, from where originates this surplus in glucose? The results of the present experiment do not, unfortunately, suggest an answer.

In the study presented here, the concentration of total sugar in the phloem sap exudate tended to increase with water stress, particularly when the phloem was collected from the apex of water stressed plants (Fig. 24). This is in agreement with most of the literature reporting changes in the content of carbohydrates induced by water stress (e.g. cf. Mattson and Haack, 1987; Irigoyen et al., 1992). Because sorbitol constitutes a major carbohydrate component in apple trees (cell sap and phloem) Wang and Stutte (1992), hypothesized that it has an important role in the osmotic adjustment during water stress. They demonstrated that in mature apple trees, sorbitol accounted for over 50% of total osmotic adjustment. In potted young cherry trees, Ranney et al. (1991) found that the change in sorbitol concentration alone accounted for the total solute accumulation. Wang and Stutte (1992) give several possible explanations for the effect of water stress on sorbitol accumulation. Water stress may (1) increase the partitioning of newly fixed carbon into sorbitol (2) induce the enzymatic pathways that break down starch and sucrose which increases sorbitol synthesis (3) decrease the rate of transport of sorbitol, relative to sucrose from the leaf.
The present study revealed that the relative content of sorbitol increased in the phloem exudate when apple plants were subjected to water stress. Normally, however, osmotic adjustment refers to solute accumulation on a cellular basis; solutes and assimilates needed for this accumulation have to be transported to the respective cells. If changes in leaf/cell carbohydrate composition occur due to water stress, similar changes in the phloem sap composition are to be expected, since osmotic adjustment, namely of growing tissues, depends to a great extent on the import of recent photosynthates (e.g. Boyer, 1988 cf. Studer, 1993). An increase in the sorbitol fraction in the phloem sap of water stressed apple plants, therefore confirms the important role of sorbitol in osmotic adjustment to water stress.

Leaves of different stage of development are considered to exhibit differential sensitivity (ability to adjust osmotically) to plant water stress (e.g. O'Neill, 1983 cf. Premachandra and Joly, 1992, Lakso et al., 1984; Steinberg et al., 1989; Wang and Stutte, 1992). In section 2 of the study presented here, it was suggested that growing tissues (the five apical leaves) may show a reduced ability to adjust osmotically to water stress (lower turgor pressure) as compared with mature leaves of the same plant.

Depending on plant species and plant organ under consideration, carbohydrate components other than sorbitol such as sucrose and glucose can be considerably involved in the process of osmotic adjustment. Therefore, the proportions of the compounds involved in osmotic adjustment may be of importance. In this study, no significant differences in phloem exudate composition between samples from the apex and from basal leaves of water stressed plants could be observed. Also, the relative content of sorbitol, probably the most important carbohydrate for osmotic adjustment in apple, was not different in exudates from the apex and basal leaves in any treatment. These results indicate that the carbohydrate composition of the phloem sap can not explain the different ability of apex and basal leaves, respectively, to adjust osmotically to water stress. Additionally, it has to be noted that carbohydrate composition within other than sieve cells might have been different between apex and basal leaves: the nature of sugars transported in the phloem may be different from the carbohydrate composition in sink and particularly source tissues (Ziegler, 1975).
The total concentration of carbohydrates was significantly higher in exudates from basal leaves than from the apex. Mature basal leaves produce great amounts of assimilates, a great part of which is exported to other plant parts, whereas growing tissues (apex) depend to a great extent on the import of assimilates. In this study, it was not possible to determine whether the higher amounts of carbohydrates in the phloem exudate solutions from basal leaves were due to a higher concentration in phloem sap or to a higher flow rate of the sap.

Coleman (1986) and Milburn (1974 cf. Girousse et al., 1991) mention that there are concentration gradients in the various components of the phloem sap along stems. These gradients depend on the proximity of a source or sink organ. In castor bean, sucrose was found to be highest in the phloem sap of the shoot apex and to decrease towards the base of the stem (Vreugdenhil and Kool-Grousveld, 1989). Klinglauf (1987) reports that as leaves age, their carbohydrate concentration decreases. Hugentobler (1990) on the other hand, found no difference in total sugar concentration but a higher relative content of sorbitol in exudates collected from source leaves as compared to sink leaves. Similarly, Kuo-Sell (1989) reported no difference in sucrose concentration in the phloem sap in the different parts of oats. Whether the concentration of carbohydrates in the phloem sap of apex and basal leaves are similar (according to Kuo-Sell, 1989 and Hugentobler, 1990) or higher in the sieve cells of the apex (according to Vreugdenhil and Kool-Grousveld, 1989, and Klinglauf, 1987), it would seem that higher amounts of carbohydrates in exudates of basal leaves compared with the apex are due to a higher flow rate of the phloem sap from basal leaves.

As mentioned in the introduction, aphids may change the food quantity and quality of their host. The concentration of total sugars was in general significantly higher in exudates collected from previously aphid infested than uninfested plants (see Fig. 24). Although it was not possible to determine whether this was due to a higher concentration in the phloem sap or a higher flow rate, this higher sugar concentration in exudates was probably due to the "sink" effect the aphids exerted on their host. As cited in Dorschner (1990), aphids act as powerful sinks which can direct assimilates and nutrients from distant parts of the plant towards the aphid colony. Dorschner (1990) proposed that salivary enzymes may increase the flow of nutrients and assimilates to the aphid colony because salivary secretions can be translocated within the host and thus affect long distance transport of nutrients within a plant. In addition, the larger the colony, the more powerful
the sink produced until the carrying capacity of the plant is reached (Miles, 1989a; Dorschner 1990). This was confirmed in the present study: the difference in total sugar concentration in the exudate collected from previously aphid infested and uninfested plants was significant only in well-watered plants, where the greatest aphid population size and density had developed (see Fig. 20). In moderately and severely stressed treatments, with smaller aphid populations, the differences in total sugar concentrations were smaller and not significant.

Not only the quantity of sugars, but also the carbohydrate composition of the phloem exudate was affected by aphid infestation. The nonphagostimulant sorbitol (Pollard, 1973) was significantly lower in the exudate of previously infested plants in favor of the phagostimulant sucrose (Srivastava, 1987) and/or in favor of raffinose and stachyose. These oligosaccharides were detected almost exclusively in exudates of aphid infested plants. As discussed on pages 100 and 101, a previous aphid infestation reduced the ability of apple plants to adjust osmotically under conditions of water stress. The significantly lower relative content of sorbitol in leaves which were previously infested with aphids compared with uninfested leaves might thus confirm the important role of sorbitol in osmotic adjustment. The mechanism by which aphids can change the composition of the phloem sap, their source of nutrition, is still not well understood. Dixon (1985) stated that aphids secrete substances into plants that rapidly affect the metabolism of their host plant leading to an improvement of the quality of the phloem sap. Miles (1968 cf. Dixon, 1975) even suggested that "aphids produce their own specific chemical organizer of plant growth" as well as unspecific plant hormones, which are then introduced into their host. Later Miles retracted the suggestion that aphids significantly add to the amounts of auxin present in plant tissues, but suggested (Miles, 1990) that general redox reactions induced by their salivary oxidases would tend to produce reactions simulating those in senescent tissues, presumably including the increase in mobilization of nutrients referred to above (P.W. Miles, University of Adelaide, South Australia, pers. comm.).

4.4.2 Amino Acids

In the study presented here, all common amino acids with the exception of arginine were detected in the phloem exudate of apple plants. The major amino acids found in the exudate solution were ASN, THR, CYS, GLU and
ASP which accounted for 70 - 90 % of the total amino acids found in the phloem exudates (see Table 18). The dominance among these components depended on the sample position within the plant, on the water stress treatment and whether the plant was previously infested with aphids or not. Comparable amino acid compositions were reported for apple and several other crops (Weibull et al. 1986, Weibull et al., 1990; Ohshima et al., 1990; Girousse et al., 1991). Kollar and Seemüller (1990) found that ASP and GLU together with their amides (ASN and GLN) were predominant in the phloem of different apple cultivars, whereas arginine and cysteine were not detected. The major amino acids found by Hugentobler (1990) in the phloem sap of apple plants (originating from the same clone as plants in this study) were ASN, GLN, HIS, ASP, GLU and SER. In contrast to this study, arginine was also detected in considerable amounts.

The ten essential amino acids for animal and insect nutrition are ARG, HIS, ILE, LEU, LYS, MET, PHE, THR, TYR and VAL (Fried and Dadd, 1982 cf. Hugentobler, 1990; Prosser and Douglas, 1992). However, as often mentioned in the literature, non-essential amino acids dominate in the phloem sap. Of the essential amino acids only THR was detected at appreciable amounts, whereas arginine was completely missing. The ability of aphids to grow well on a rather limited amino acid mixture in their diet is attributed to the presence of a large numbers of symbionts in their gut which upgrade the dietary non-essential amino acids (e.g. ASP, GLU and their amides) to essential ones and thus supply the missing amino acids (Houk, 1987; Sasaki et al., 1991 cf. Dorschner, 1993; Prosser and Douglas, 1992).

As one example: methionine, an essential amino acid often found in appreciable amounts in the phloem sap (e.g. Hugentobler, 1990) is an important source of the sulfur required by aphids. Nevertheless, methionine may occur in very low relative amounts in the phloem (present study; Kollar and Seemüller, 1990). In such situations, there are alternative ways for aphids to get the essential methionine and/or sulfur: It may be provided by cysteine, a non-essential sulfur amino acid, as was probably the case in this study, and/or in the form of inorganic sulfur which is also found in phloem, as was probably the case in the study of Kollar and Seemüller (1990). Another possibility is that methionine can be synthesized by the symbionts from inorganic sulfur and non-sulfur amino acids as was shown for Myzus persicae (Sulzer)(Douglas 1990 cf. Prosser and Douglas, 1992). Prosser and Douglas (1992) suggested that the symbionts of a specific aphid species may be specifically adapted to complement the amino acids in the phloem sap of
aphid's host plant. Therefore, whether the presence of an amino acid in the phloem should be considered as essential or not depends on plant species, the aphid, its symbionts and other circumstances, so that generalization is not possible.

Since amino acids rather than sugars determine the nutritional quality of phloem sap for aphids, the amino acid composition of the phloem is of great importance for aphid population development. As can be seen from Fig. 27, the different treatments affected phloem composition significantly in this study. Asparagine constituted with 68-77 % relative content the most important amino acid fraction in phloem exudate collected from apices of well-watered plants. As the brief literature overview of Girousse et al. (1991) shows and other studies report (Kuo-Sell, 1989; Douglas, 1993), asparagine is the major component in the phloem sap of several crops and is considered the chief nitrogen transport compound. This is probably linked to ASN's specific properties and to its position in nitrogen metabolism (high N/C ratio as mentioned by Miltin and Lea, 1977 cf. Girousse et al., 1991). The relative content of ASN in phloem exudates from basal leaves of uninfested, well-watered plants, however, amounted to only 20 %, i.e. the composition of the phloem exudates from the basal leaves was totally different from apical exudates. In exudates from the basal leaves, the relative contents of practically all other amino acids were higher, particularly of CYS and GLU as well as ASP and SER.

Water stress induced a striking reduction in the relative content of ASN in phloem exudate from the apex, whereas the fractions of the other major amino acids TYR, CYS, GLU, ASP as well as of HIS, ORN, VAL increased. In exudates from basal leaves, water stress exerted less significant changes in amino acid composition, namely on the relative content of ASN. A previous infestation with aphids also affected the amino acid composition of the phloem exudates. The relative content of ASN was significantly higher in plants that were previously infested with aphids than in uninfested plants, whereas the relative content of ASP and PHE were lower.

The results presented here may be of particular importance in explaining the development of aphid populations under different levels of water supply as well as for the understanding of the preference for young (growing) tissues by A. poni. Asparagine has been reported to support faster development of M. persicae, Brevicoryne brassicae (L.) and Sitobion avenae (F.) (van Emden and Bashford, 1977 cf. Kuo-Sell, 1989; Weibull et al., 1986). In addition, ASN has
the highest N/C ratio (2/4) of all the protein amino acids (cf. Girousse et al., 1991). This again is of advantage to the aphids, because N is arguably the most limiting chemical element in their nutrition. Since, additionally, symbionts in the aphids' gut may upgrade ASN to essential amino acids, ASN is probably an "optimal" compound in the aphids' diet. The relative content of ASN in the phloem might therefore be a determining factor influencing aphid performance and population development. The results of this study support this hypothesis in a number of ways: (1) *A. pomi* generally prefers young growing tissues as feeding sites. The relative content of ASN was significantly higher in phloem exudates from the apex than from basal leaves. Not only the relative content, but also ASN concentration (per unit sample tissue) was significantly higher in exudates from the apex than from basal leaves. Hugentobler (1990) similarly reported higher concentrations of all amino acids (except TYR) in the exudate collected from sink leaves as compared to source leaves. (2) Water stress of the host clearly reduces aphid population size and density. The relative content of ASN in the phloem exudates decreased significantly with increasing water stress. (3) An aphid infestation leads to higher relative content of ASN in the phloem exudates compared with uninfested plants. The greater the population size and density, the bigger were the differences in the ASN fraction. The ASN export from basal leaves seemed to be significantly increased by aphid infestation. The mechanisms by which aphids can influence their host's phloem composition are not clear (whether by their "sink" effect or actively by injecting salivary secretions into their host).

The relative content of other, even essential amino acids such as THR, HIS, LEU, VAL and CYS, GLU, ASP or ORN seemed not to affect aphid population size and density. Regarding the effects of water stress as well as of sample position, these amino acids actually increase in proportion while aphid population size decreased. On the other hand, it would be of interest to study the development of glutamine in this context. Glutamine, which could not be measured in this study, can occur in similar proportions as ASN in the phloem sap (Kollar and Seemüller, 1990) and was reported to support faster development of *M. persicae* (van Emden and Bashford, 1977 cf. Kuo-Sell, 1989).

Many plants respond to water stress by a marked increase in proline in their tissue as well in the phloem (Hsiao, 1973; Miles et al., 1982; cf. Mattson and Haack, 1987; cf. Holtzer et al., 1988). Miles et al. (1982) even stated that in absolute quantity proline overshadowed all other amino acids in some stressed plants. In the present study, the relative content of proline increased
with water stress. This increase, however, was not significant, probably due to several reasons: Proline seems to respond later than organic acids and sugars to water stress (Irigoyen et al., 1992) and the nature of water stress is known to affect amino acid composition. For example, under progressive water stress, proline (and glycinebetaine) were the dominant accumulated compounds measured in wheat extracts, whereas rapid stress resulted in the accumulation of a number of other amino acids, particularly amides (Naidu et al., 1990). Towards the end of the experiments of this study, plants became relatively big and their soil volume rather limited resulting in a fast water stress build up and shorter watering intervals, which could therefore have affected proline accumulation. Further, most amino acids including proline are completely metabolized upon rewatering (Naidu et al., 1990). Although the present study demonstrated no significant changes in the relative content of proline in the phloem exudates, the absolute amounts of proline might actually have increased (see Fig. 28 below).

It should be noted that according to some authors, proline correlates negatively with the growth of M. persicae (Van Emden and Bashford, 1977 cf. Kuo-Sell, 1989). Therefore, negative effects of proline on aphids can not be excluded. In addition, whether an amino acid acts as a phagostimulant or not probably depends on the aphid species, morph, physiological state of the host plant and other circumstances.

In general, levels of soluble nitrogen are reported to increase in organs and tissues under water stress (Mattson and Haack, 1987; Naidu et al., 1990). Miles et al. (1982) reported that water stress caused an increase in the content of all amino acids in rape leaf extracts as well as in pure phloem sap. In the study presented here, the amino acid concentration in the phloem exudate (as well as the concentrations calculated per unit of plant tissue) of apple plants decreased with increasing intensity of water stress (Fig. 26). The reason for the divergence in results of this study from those quoted above will be discussed below.

Using the EDTA-exudation technique allowed the assessment of the concentration of total sugar and total amino acids or any of their components only in the exudate solution. To assume that the concentration found in the exudates reflect absolute concentrations present in the phloem is probably wrong, particularly under conditions of water stress. Under conditions of water stress it can be expected that the flow rate out of the phloem into the solution decreases (higher phloem viscosity and lower turgor pressures). High phloem
viscosity was probably the reason why in a preliminary experiment it was not possible to collect phloem sap from severed stylets of aphids feeding on water stressed plants. Furthermore, the significant decrease in plant water potential with water stress (see page 83) must have an impact on the pressure relations in the phloem sap.

Figure 28 presents a hypothetical scheme relating the effect of varying phloem sap concentration and flow rate on the concentrations recovered in the phloem exudates (considering sample size). The assumed patterns of flow rate and concentration development are based on theoretical considerations and reports in the literature. Flow rate and concentration will mainly determine how much will flow out of the phloem sap into the solution and thus determine the solute concentrations measured in the exudates. In the hypothetical scheme presented in Fig. 28, the concentration of total amino acids in the phloem exudate decreases with increasing water stress. Total sugar, however, increases initially until moderately stressed and then decreases again as stress becomes more severe.

![Figure 28](image-url)

**Fig. 28.** Hypothetical scheme on the concentration of phloem sap, flow rate and the resulting concentration in the exudate solutions collected from the phloem of plants grown under different water regimes.
The concentration patterns observed in the phloem exudates (considering sample size) in this study conform to those presented in the model. It is therefore not unreasonable to assume that the flow rates and concentration patterns actually developed were much as presented in Fig. 28.

It can be concluded that changes in the composition of amino acids and in the overall composition of the aphids’ diet may be of greater importance for aphid population growth (size and density) than general changes in total amino acid (and/or carbohydrate) contents. Besides the fraction of specific sugars and particularly amino acids, also the ratio of total sugars to total amino acids in the phloem sap may be of great importance: this ratio increased up to 10-fold due to water stress in the phloem exudates, and was up to 10-fold higher in exudates from basal leaves than from the apex. These relativities are particularly important, since even if the phloem analysis were subject to contamination, the relative increase in amino acid content seems beyond doubt. Moreover, the much greater increase from the basal leaves would be of particular significance to the insects, since mature leaves are of inferior nutritional quality to the insects before infestation. The aphids’ response to changes in the phloem sap quality of the plant was probably conditioned by both physiological (nutritional) and behavioral (phagostimulant factors). Since the effect of water stress on aphid population size and density was less severe than the effect of leaf age (apex vs basal leaves), however, it can be assumed that other important leaf characteristics (e.g. content of phenols or other defensive compounds as well as physical leaf characteristics) not assessed in this study, must additionally have affected aphid population development.
Leer - Vide - Empty
Conclusions

An analysis of biophysical and biochemical changes in apple plants grown under well-watered and water stressed conditions may be expected to lead to a better understanding of the interrelationship between host (Malus domestica Borkh.) and aphid (Aphis pomi De Geer) under different levels of water supply. Nevertheless, the changes induced in the plant by ongoing water stress are not uniform within the plant and present a continuum of effects in time, some stages of which may be advantageous to the insects, whereas others are not.

5.1 Effects of Water Stress on the Host Plant

Water stress reduced plant growth and biomass production (FW, DW, LA) in all the experiments of this study. The extent of these effects was not the same in all four experiments. Factors which may have influenced the degree of reduction included among others the pattern of water stress (the rate, intensity and duration), experimental conditions (e.g. pot size and shape, plant size), and environmental conditions (e.g. soil fertility, light intensity). In experiments 1 and 2, plant growth decreased gradually with increasing intensity of water stress, whereas in experiments 3 and 4, a critical moisture stress level ($\Psi_{\text{soil}}$ ca -0.69 MPa) developed, below which plant growth was significantly reduced.

The osmotic potential of leaves decreased with a decrease in leaf water potential maintaining turgor pressure or at least delaying a decrease in turgor pressure in leaves, a process known as osmotic adjustment. In all four
experiments, plants were able to adjust osmotically to water stress, with the exception of plants in experiment 2 which showed very low osmotic adjustment. A greater part of the osmotic adjustment could be attributed to active accumulation of solutes. Osmotic adjustment was accompanied by an increase in the concentration and relative contents of stress substances in the phloem such as sorbitol, the major carbohydrate detected in the phloem of apple plants. Proline, although a minor contributor to the amino acids of the phloem sap also increased with water stress.

The total concentration of carbohydrates and amino acids in the phloem sap was shown to increase with increasing intensity of water stress. The total concentration of amino acids detected in phloem exudate collected in an EDTA-buffer solution, however, decreased and that of carbohydrates increased with water stress. Concentrations measured in the phloem exudate do not necessarily reflect the concentrations in the phloem sap, since the flow rate out of the phloem probably decreases with water stress (depending on size or diameter of the sieve tube, sap viscosity and pressure). The composition of phloem sap was shown to change under conditions of water stress compared with well-watered conditions. The concentration of carbohydrates and amino acids as well as their composition also varied with leaf position within plants, i.e. with leaf age or stage of maturity. Generally, the concentration of amino acids was higher in the phloem sap of the apex compared with mature, basal leaves, whereas the concentration of carbohydrates was lower. The relative content of ASN was higher and that of CYS and GLU lower in the phloem sap of the apex than in that of the basal leaves. Further, the changes in the amino acid composition of the phloem due to water stress were not the same in the apex and the mature, basal leaves (e.g. GLU, ASP, CYS).

Leaf surface temperature tended to increase with increasing intensity of water stress. The differences due to water stress did not exceed 3°C, and after rewatering, as well as at night, the differences disappeared. Leaves situated in the lower parts of the plant had lower leaf surface temperatures than leaves in the upper parts. Beside color, size, orientation of leaves, the distance to the source of artificial light in the growth chamber to a large extent determined leaf surface temperatures. This was a drawback in this study because changes in surface temperatures due to water stress in leaves situated in the upper parts of the plant were overshadowed by this artefact.
As in most experiments conducted in pots and under controlled environmental conditions, direct extrapolation of results to the field is problematic. Plants growing in pots are in general subject to more rapid and intense water stress than plants in the field which may reduce their ability to adjust osmotically and also affects phloem quality differentially than under a more gradual and uniform water stress development as generally encountered in the field. This certainly may affect not only overall plant growth, but particularly the development of aphids on these plants. Relating measurements of soil matric potentials at the peak of drying cycles to plant growth and characteristics can, therefore, be misleading, since the actual nature of the water stress is not described sufficiently by these measurements alone. Many plant characteristics such as plant growth, leaf surface temperatures, the components of plant water potential and probably also phloem sap characteristics (flow rate, pressure, quality) oscillate with the pattern of water stress. A more sensitive method than point measurements in time and space would be needed to characterize water stress, if stress intensity, duration and rate of development are to be successfully integrated into a comprehensive characterization of water stress as it affects both plant and insects. This would be particularly important for the extrapolation of results from studies in pots under controlled environmental conditions to field conditions.

As for the present study, it can be concluded that water stress resulted in changes in biophysical and biochemical characteristics of the plant which affected aphid population development and growth, although the kind and extent of such effects were not always easy to measure or interpret.

5.2 Effects of Water Stressed Plants on the Population Dynamics of Aphids

The effect of plant water stress on the population development and growth rate of *A. pomi* was studied using two approaches: the life table approach and the density-dependent population approach.

**Life table approach.** The *intrinsic rate of natural increase* $r_m$, a descriptor of population growth which combines information on the development time, survival rates and the age-specific fecundity, was generally not significantly affected by water stress in this study. Nevertheless, the development and pre-
reproductive time of larvae and aphids as well as the generation and doubling time of aphid populations tended to be shorter, whereas aphid fecundity was higher in water stressed plants compared with well-watered plants. Increases in leaf surface temperature which were mediated by water stress probably induced the slight changes in the population parameters of aphids. Possible reasons for only insignificant changes in $r_m$ under the severe water stress levels applied in this study were: Differences in leaf surface temperatures due to water stress were not high enough to affect aphid development significantly and/or the variability in temperature because of factors other than water stress, such as the distance to the source of light and ventilation within the growth chamber, overshadowed temperature differences due to water stress leading to an underestimation of the potential effect of changes in leaf surface temperature due to water stress. Another important reason is that aphids had a wide choice of microclimates and feeding sites within their host plant, which they could easily reach simply by moving short distances on the host plant.

A significantly lower $r_m$ was measured for aphid cohorts feeding on mature leaves situated in the lower parts of the plants compared with those feeding on growing leaves situated in the upper parts. Differences in leaf surface temperature alone, however, could not sufficiently explain the difference in $r_m$.

Life table construction and analysis are useful for comparing the potential rate of increase of individuals or cohorts of a species under defined conditions. However, extrapolation of the intrinsic rate of natural increase of individuals or cohorts to populations with high densities has to be done with caution, because population density may affect factors which in turn may influence $r_m$:

The nutritional quality of a host plant is affected by population density, and the mobility of individuals (choice of optimal feeding and birth sites) is restricted at high densities.

**Population approach.** The rate of aphid population development was negatively affected by water stress induced by intermittent drying. In contrast to the slightly higher $r_m$ under water stress compared with well-watered conditions found in experiments 1 and 2, the size and density of aphid populations decreased significantly with water stress in experiments 3 and 4. The critical moisture stress level for aphid development, below which the size of aphid populations was significantly reduced, seemed at $\Psi_{soil} < -0.69 \text{ MPa}$ (measured at the peak of a drying cycle). The effect of fluctuating experimental conditions on aphid population size represents a longer term
effect and therefore summarizes the corresponding changes in plant characteristics which change under intermittent drying. Plant growth and physiological factors which had changed under water stress and which possibly had an impact on aphid population development were: the rate of plant growth and production of new tissue, leaf surface temperatures, plant water relations and phloem feeding quality.

The reduction in aphid population size was closely related with a decrease in the rate of plant growth, i.e. the production of young tissue, which are greatly preferred sites for the nutrition of most aphid species. A decrease in aphid density per leaf or per unit of shoot length with water stress indicated that the magnitude of this response changed under water stress and that other factors beside shoot length affected the development of aphid populations.

Significantly smaller populations of aphids and lower densities on mature leaves of the lower parts of the plant further confirmed the importance of newly produced young tissue for aphid development. The preference of the green apple aphid for young tissue results in the higher susceptibility of apple trees to aphid infestation in spring compared with summer when growth has ceased and most leaves are mature. Further, it explains why young trees rather than older, bigger trees are more susceptible to aphid infestation.

The differences found in the population characteristics of aphids (size and density) under well-watered and water stressed conditions and/or within the plant could not be sufficiently explained by differences in leaf surface temperature due to water stress. Several phloem sap characteristics such as phloem sap viscosity, pressure, carbohydrate and amino acid concentration as well as composition are expected to vary under water stress conditions and intermittent drying and exert a more significant effect on aphid development than leaf surface temperatures.

Changes in phloem sap viscosity and pressure were expected to become limiting only under very severe water stress. The increase in the concentration of carbohydrates as was observed under conditions of water stress, increases the viscosity of phloem sap (Ziegler, 1975) and may thereby decrease sap flow. In addition the significant decreases in the components of plant water potentials with water stress measured in this study, may indicate a decrease in phloem sap pressure. Although turgor pressure of the sieve-tubes and capillarity may have a considerable impact on aphid feeding,
aphids are able to suck actively with the ciberial pharyngeal pump when necessary.

Although amino acid concentrations in the phloem sap increased with an increase in water stress, the carbohydrate to amino acid ratio increased up to 10-fold proving that carbohydrate concentrations increased to a greater extent than amino acid concentrations. A high carbohydrate to amino acid ratio renders the quality of phloem sap less favorable for aphids which then have to invest more energy in disposing of the excessive sugars consumed.

The most apparent changes in phloem sap composition with water stress, which were closely related to the size and density of aphid populations, were the increase in the relative content of sorbitol and the decrease in the fraction of ASN. The relative content of other amino acids was also affected by water stress. The fraction of amino acids such as CYS, GLU, ASP or ORN and even of essential amino acids such as THR, HIS, LEU or VAL increased with water stress. This, however, seemed to have no effect on aphid population development, probably because these amino acids were already found in sufficient quantities or because they may be supplemented by symbionts inhabiting the aphids' gut.

In this study, the relative content of ASN in the phloem was found to be a determining factor influencing the development of A. pomi populations. Asparagine (ASN) is present in high amounts in the phloem, is efficient in the transport of N because of its high N/C ratio and is a good precursor for amino acid upgrading by symbionts.

The size and density of aphid populations as well as body weights of aphid individuals were significantly higher on growing leaves compared with mature leaves situated in the lower parts of the plant, particularly in well-watered plants. The carbohydrate to amino acid ratio was up to 10-fold higher in mature leaves, and the relative content of ASN was significantly lower. This confirms that the apple plant is not a homogeneous source of nutrition for insects.

The content of phenols and other defensive compounds (allelochemicals) as well as physical leaf characteristics as leaf hairiness, toughness and thickness were not determined in this study. These factors may also be important limiting factors for aphid population growth and may be subject to changes under conditions of water stress and/or due to position within a
plant. Newly expanding leaves are reported to contain relatively low amounts of allelochemicals, whereas the mature leaves have relatively high levels of defensive allelochemicals. Water stress may exert differential effects on the production of allelochemicals, which may depend on the plant species, the compound under consideration, and environmental/experimental conditions.

It can be concluded that among the factors assessed in this study, the production of new and young plant tissue under well-watered conditions and changes in phloem sap quality under water stressed conditions were the main factors influencing aphid population development.

5.3 Effects of an Infestation with the Green Apple Aphid on the Host Plant

The effect of aphids on plant growth and development, the interaction of water stress and aphid infestation on the plant, as well as the possible feedback effects of aphid infestation and water stress may be of importance to understand the potential of aphid population growth and development.

At "moderate" aphid densities (as in experiment 3), overall plant growth was not significantly affected by an aphid infestation. At "high" aphid densities (as in experiment 4), however, mildly water stressed plants ($\Psi_{soil} \geq -0.69 \text{ MPa}$) suffered the most from an aphid infestation. In the well-watered and the two moderately stressed treatments in Exp. 4, plant growth was only reduced by aphids a few days before plant growth had completely stopped due to high aphid densities, whereas the growth of mildly stressed plants was clearly decreased by aphid infestation during the experiment and even at low aphid densities. The critical moisture level for growth of aphid infested plants ($\Psi_{soil} > -0.69 \text{ MPa}$) developed below that of aphid uninfested plants ($\Psi_{soil} < -0.69 \text{ MPa}$; Fig. 29). Thus, a critical moisture range was identified within which assimilate drain by feeding aphids reduced the plants' ability to adjust osmotically to water stress to an extent large enough to significantly decrease plant growth: although an infestation with aphids generally exerted a negative effect on osmotic adjustment of water stressed plants, this was translated into reduced plant growth only under conditions of mild water stress.
Under field conditions, plants are most likely growing in the range of critical moisture defined above most of the time. Under "unlimited" water supply, plants were able to compensate for the losses in assimilates withdrawn by aphids possibly due to positive feed back on photosynthesis caused by an additional sink and/or due to degradation and mobilisation of metabolites. In the range of critical water supply, however, even relatively low aphid densities significantly restricted plant growth by the consumption of assimilates and solutes needed for active osmotic adjustment to maintain more or less normal growth (as in uninfested plants). Under more severe water stress, plant growth was dominated by the effects of water stress, so that the extent of damage that an aphid infestation could exert became insignificant.

It follows that an apparent reduction of overall growth of the plant results when the drain of assimilates exceeds the capacities of the plant for compensatory growth, i.e. when the carrying capacity of a plant is surpassed. Thus, the aphid density threshold for significant loss in biomass production decreased with water stress due to a reduction in the carrying
capacity of a plant under water stress. Although aphids developed much slower on water stressed plants (see experiment 3), plant growth of water stressed plants stopped after a much shorter development time (a difference of 12 days in experiment 4) compared with well-watered plants.

The results of the study presented here confirm that an aphid infestation may affect both the concentration as well as the composition of phloem sap. Higher concentrations and significantly greater proportions of ASN, the major transport amino acid identified in this study, in phloem exudates of previously infested compared with uninfested apices confirmed that the aphids exerted a sink effect, hereby increasing transport of solutes to feeding sites. Additionally, the larger the colony, the more powerful the sink effect. Aphid infestation also significantly altered the composition of the phloem sap. The fraction of the nonphagostimulant sorbitol was significantly lower, whereas the fraction of ASN, apparently an important amino acid for A. poni nutrition, was significantly higher in phloem exudates collected from plant previously infested with aphids compared with uninfested plants. The mechanisms by which aphids can alter their nutritional basis in this way are not well understood. Possibly the injection of salivary secretions into the plant may induce changes in plant metabolism and/or degenerative changes within the plant due to an additional sink effect, i.e. in a direction that favors nutrition for the aphids.

An aphid infestation that is increasing presumably stimulates allelochemical defenses of the plant making it less attractive and nutritious. Eventually, however, the aphid population may reach a size where allelochemical defenses are overwhelmed cooperatively by the insects. Under conditions of water stress, either the concentration of allelochemicals increases in the plant making it more difficult to overwhelm the plant defenses or water stress decreases allelochemical concentrations but also reduces plant feeding quality to such a level that a population of aphids cannot build up to the point were allelochemicals can be overwhelmed (P.W. Miles, University of Adelaide, South Australia, pers. comm.).

Numerous biotic and abiotic factors which influence the development of aphid populations under increasing levels of water stress could be identified in this study. Due to the high complexity of the system, however, the complete elucidation of certain cause and effect relations would require additional work (e.g. metabolic pool model) to allow a complete understanding of the whole system.
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References


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# Appendix

Table 1. Propagation and rooting media modified according to Hugentobler (1990) used for the micropropagation of apple (cv. Golden Delicious) according to Wermelinger (1985).

<table>
<thead>
<tr>
<th></th>
<th>Propagation medium</th>
<th>Rooting medium</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macronutrients</strong></td>
<td>mM</td>
<td>mM</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>3.0</td>
<td>1.5</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>1.5</td>
<td>0.75</td>
</tr>
<tr>
<td>KNO₃</td>
<td>18.8</td>
<td>9.4</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>20.6</td>
<td>10.3</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.3</td>
<td>0.63</td>
</tr>
<tr>
<td>FeSO₄·7H₂O</td>
<td>0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Na₂-EDTA</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Micronutrients</strong></td>
<td>µM</td>
<td>µM</td>
</tr>
<tr>
<td>MnSO₄·H₂O</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>KI</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>Na₂MoO₄·2H₂O</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>0.1</td>
<td>0.05</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.1</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
<td>µM</td>
<td>µM</td>
</tr>
<tr>
<td>myo-inositol</td>
<td>560.0</td>
<td></td>
</tr>
<tr>
<td>thiamin-HCl</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td>mg/L</td>
<td>mg/L</td>
</tr>
<tr>
<td>IBA</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>6BA</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Sucrose</strong></td>
<td>g/L</td>
<td>g/L</td>
</tr>
<tr>
<td>Bacto-Agar</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>pH without agar</td>
<td>5.5</td>
<td>5.7</td>
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</tbody>
</table>
Table 2. Composition of the Hoagland solution (1N) modified according to Hugentobler (1990) and used in the present study.

<table>
<thead>
<tr>
<th>Macronutrients</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(NO₃)₂·4H₂O</td>
<td>2</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>1</td>
</tr>
<tr>
<td>KCl</td>
<td>0.025</td>
</tr>
<tr>
<td>KNO₃</td>
<td>3</td>
</tr>
<tr>
<td>NH₄H₂PO₄</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Micronutrients</th>
<th>µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe-EDTA</td>
<td>50</td>
</tr>
<tr>
<td>MnSO₄·4H₂O</td>
<td>5</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>25</td>
</tr>
<tr>
<td>Na₂Mo₄·2H₂O</td>
<td>0.25</td>
</tr>
<tr>
<td>CoSO₄·7H₂O</td>
<td>0.1</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Concentration of nutrients (in ppm):
Ca 80, N 105, Mg 24, S 32, K 118.5, Cl 0.9, P 15.5, Fe 2.8, Mn 0.275, Zn 0.033, B 0.27, Mo 0.024, Co 0.06, Cu 0.032.
### Abbreviations and Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
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<tbody>
<tr>
<td>$\Psi_g$</td>
<td>Gravitational potential</td>
<td>MPa</td>
<td>ALA</td>
<td>Alanine</td>
<td></td>
</tr>
<tr>
<td>$\Psi_m$</td>
<td>Matric potential</td>
<td>MPa</td>
<td>ASP</td>
<td>Aspartic acid</td>
<td></td>
</tr>
<tr>
<td>$\Psi$</td>
<td>Leaf water potential</td>
<td>MPa</td>
<td>ASN</td>
<td>Asparagine</td>
<td></td>
</tr>
<tr>
<td>$\Psi_o$</td>
<td>Leaf osmotic potential</td>
<td>MPa</td>
<td>ARG</td>
<td>Arginine</td>
<td></td>
</tr>
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<td>$\Psi_p$</td>
<td>Leaf turgor potential</td>
<td>MPa</td>
<td>CYS</td>
<td>Cysteine</td>
<td></td>
</tr>
<tr>
<td>$\Psi_{soil}$</td>
<td>Soil matric potential</td>
<td>MPa</td>
<td>GLN</td>
<td>Glutamine</td>
<td></td>
</tr>
<tr>
<td>$\Psi$</td>
<td>Leaf water potential</td>
<td>MPa</td>
<td>GLU</td>
<td>Glutamic acid</td>
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<tr>
<td>$\Psi$</td>
<td>Leaf water potential</td>
<td>MPa</td>
<td>GLY</td>
<td>Glycine</td>
<td></td>
</tr>
<tr>
<td>$\Psi$</td>
<td>Leaf water potential</td>
<td>MPa</td>
<td>HIS</td>
<td>Histidine</td>
<td></td>
</tr>
<tr>
<td>$x$</td>
<td>Development time</td>
<td>days</td>
<td>ILE</td>
<td>Isoleucine</td>
<td></td>
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<tr>
<td>$l_x$</td>
<td>Survival rate</td>
<td>%</td>
<td>LEU</td>
<td>Leucine</td>
<td></td>
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<tr>
<td>$m_x$</td>
<td>Age-specific fecundity</td>
<td>larvae/female</td>
<td>LYS</td>
<td>Lysine</td>
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<tr>
<td>$r_m$</td>
<td>Intrinsic rate of natural increase</td>
<td></td>
<td>MET</td>
<td>Methionine</td>
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<td>$R_0$</td>
<td>Net reproduction rate</td>
<td></td>
<td>ORN</td>
<td>Ornithine</td>
<td></td>
</tr>
<tr>
<td>$G$</td>
<td>Generation time</td>
<td>days</td>
<td>PHE</td>
<td>Phenylalanine</td>
<td></td>
</tr>
<tr>
<td>$D_T$</td>
<td>Population doubling time</td>
<td>days</td>
<td>PRO</td>
<td>Proline</td>
<td></td>
</tr>
<tr>
<td>L1-L4</td>
<td>First to fourth larval instar</td>
<td></td>
<td>SER</td>
<td>Serine</td>
<td></td>
</tr>
<tr>
<td>+A</td>
<td>Plants which were infested by aphids</td>
<td></td>
<td>THR</td>
<td>Threonine</td>
<td></td>
</tr>
<tr>
<td>-A</td>
<td>Plants which were not infested</td>
<td></td>
<td>TRP</td>
<td>Tryptophan</td>
<td></td>
</tr>
<tr>
<td>DW</td>
<td>Dry weight</td>
<td>g</td>
<td>TYR</td>
<td>Tyrosine</td>
<td></td>
</tr>
<tr>
<td>Exp.</td>
<td>Experiment</td>
<td></td>
<td>VAL</td>
<td>Valine</td>
<td></td>
</tr>
<tr>
<td>FW</td>
<td>Fresh weight</td>
<td>g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>Leaf area</td>
<td>cm$^2$</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mod.</td>
<td>Moderately water stressed</td>
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<td>ABA</td>
<td>Abscisic acid</td>
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<tr>
<td>PPFD</td>
<td>Photosynthetic photon flux density</td>
<td>μmol/m$^2$/sec</td>
<td>6BA</td>
<td>Benzyladenine</td>
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<tr>
<td>ETH</td>
<td>Eidgenössische Technische Hochschule, Zürich.</td>
<td></td>
<td>IBA</td>
<td>Indole butyric acid</td>
<td></td>
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<tr>
<td>WSL/FNP</td>
<td>Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft, Birmensdorf.</td>
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</tbody>
</table>
Curriculum Vitae

Name: Rima Mekdaschi Studer
Birthday: July 18, 1961
Place of Birth: Beirut, Lebanon
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Status: married to C. Studer

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I wish to express my profound gratitude to Prof. S. Dorn for her guidance, support and understanding during the writing phase of the thesis.

I am very thankful to Prof. J. Nösberger, Dr. J. Baumgärtner, and Dr. U. Schmidhalter for their critique and advice as co-examiners.

I thank Prof. J.J. Oertli for the opportunity given to work on a Ph.D. thesis in the plant nutrition group and in cooperation with the Entomology group.

My heartfelt thanks go to Prof. P.W. Miles (University of Adelaide, South Australia) and to Dr. C. Studer (Federal Institute of Technology Zurich, Switzerland) for many valuable comments and suggestions on both the experiments and the manuscript.

Most sincerely I thank my colleagues and friends at Eschikon for their help, technical support, advice, discussions, encouragement and the many nice moments we had. I like to mention a few names and apologize to those whom I may have overlooked: Sera, Michel, Peter, Fritz, Christoph, Urs H., Urs Sch., Charlotte, Yuncai, Felix, Hans D., Richi, Michael, Jeannette, Theres, Emil, etc.

Last but not least, I wish to thank my family and husband Christoph for their love, moral support and patience in all situations.