Typographic, gramatic and/or clarification corrections.

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l 29, w 5, change lowered to lower

p iv, l 12, w 1-2, change A series ... to Series ...

p 2, l 10-11, w 11-end, change ...ultimately depends upon an ... to ... can be aided by an ...
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p 70, l 20, w 6-8, change ... is thought to ... to ... may ...

p 90, l 16, w 11-12, change ... the same ... to ... a similar ...

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p 126, l 1, w 13, change were to was

p 134, l 6, w 5, change utilized to used

p 139, l 1, w 11, change phtosynthetic to photosynthetic

p 142, l 22, w 1-2, change without without to without
l 29, w 4, change enhaced to enhanced

p 155, l 9, w 4, change Pflanzengewben to Pflanzengeweben

p 160, l 25, w 8, change Wechselverhaltnis to Wechselverhältnis
w 10, change Tierund to Tier_und

EFFECTS OF SHORT-TERM APHID INFESTATION ON RESPIRATION, PHOTOSYNTHESIS, GROWTH AND PRODUCTION OF THEIR LEGUMINOUS HOSTS

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A thesis submitted for the degree
of Doctor of Philosophy
at The Australian National University

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DECLARATION

This thesis contains no material which has been submitted for the award of any degree or diploma at any university. It contains no material previously published, except where due reference is made in the thesis.

C D Hawkins

Christopher D. B. Hawkins

01 May 1986

PREFACE

Some decisions had to be made in the presentation of this thesis.

Chapters 2 through 9, representing the experimental results of the investigation, have been prepared as manuscripts for submission to recognized journals. At the time of thesis submission, all had been sent to journals, one has been published, two have been accepted for publication, and five are in the hands of editors. It was decided to present these experimental chapters in the form in which they were submitted to the respective journals, except that all references cited appear in a single section at the end of the thesis and acknowledgements are contained in a general statement at the beginning. As journal policy differed on abbreviation lists, a comprehensive list is presented at the begining of the thesis as well as in the applicable chapters. Page numbers are sequential throughout the thesis to avoid confusion with journal pagination of individual chapters, but full reference to the journal status of the chapter is given as a footnote on the title page of each chapter.

Inevitably some reiteration of points will be evident in the introduction and discussion sections of experimental chapters because of the need for internal consistency in each chapter. The general introduction (Chapter 1), which sets the background to the investigation, and the final discussion (Chapter 10), which assesses the contributions of the investigation in that context, are designed to tie together the separate strands.

The experimental work was carried out entirely by the candidate (C.D.B. Hawkins) under the guidance of the supervisors of the Ph.D. program (M.I. Whitecross and M.J. Aston). The writing up as manuscripts was also done by the candidate, again with appropriate discussion between candidate and supervisors. The submitted papers thus have joint authorship with the candidate as senior author in each instance and supervisors as junior authors.

ABSTRACT

Three well fertilized, non-nodulated legume species [broad bean (*Vicia faba* L. cv. Aquadulce), cowpea [*Vigna unguiculata* (L.) Walp. cv. Caloona] and garden pea (*Pisum sativum* L. cv. Victory Freezer)] were infested with various initial densities of two aphid species [cowpea (*Aphis craccivora* Koch) and pea [*Acyrthosiphon pisum* (Harris)]] to determine the short-term (10 days) effect(s) of aphid feeding on plant growth, to test whether there was a critical initial aphid density, to gauge whether the response of the legumes to aphid feeding was general or plant-aphid species specific. Early experiments showed that short-term aphid infestation significantly reduced plant growth in all combinations used and that the reduction in plant biomass was greater | than could be accounted for by the increase in aphid biomass, thus indicating possible increased rates of respiration and/or | decreased rates of photosynthesis. Initial aphid density appeared to be unimportant in determining final plant dry weight, so that, in all future experiments an initial aphid density of 10 adult aphids per plant was chosen as the most economical number of aphids to ensure significant growth reductions. The overall plant response to aphid feeding was general rather than plant-aphid species specific.

A series of studies were conducted on nitrogen (N) and phosphorus (P) accumulation, root respiration, shoot respiration, translocation, and photosynthetic CO_2 gas exchange to determine where biomass was being lost in non-nodulated aphid-infested plants, and if any of these physiological processes were plant-aphid species specific. After 10 days of infestation, there was no significant difference (nsd) between control and infested plants for N and P accumulation on a percentage basis but on an absolute basis, control plants accumulated significantly more N and P. The pattern of N and P accumulation was specific for each plant species. Root respiration was significantly reduced in aphid-infested plants because of a reduced translocate flux to the roots. The longer the aphid infestation, the less was the activity of the alternative respiratory pathway in the roots of infested plants. Shoots of aphid-infested plants had greater rates of respiration, alternative pathway capacity, and photosynthesis, and lower | alternative pathway activity than their respective controls. The increases in photosynthesis and shoot respiration, suggesting

a delay of senescence, were probably associated with aphid-induced alterations in source-sink relationships and these were possibly due to changes in the concentrations of plant growth substances.

To determine whether the increased rates of photosynthesis in aphid-infested shoots could result in a long-term compensatory growth response, cowpea aphids were removed from cowpea seedlings after 20 days infestation and the plants were grown to maturity. There was nsd in biomass between control and formerly infested plants after four months, even though control plants were significantly larger at the time of aphid removal. It appears that the aphid-induced enhancement of growth resulted from changes in the levels of endogenous plant growth substances.

Series of plant tissue extractions and thin layer chromatograms were conducted to determine whether aphids were injecting foreign substances into the plant. No compound of unequivocally aphid origin was found, but there were differences in the amounts of various substances present in host plants. From early studies, it appeared that aphid feeding caused broad bean to lose its apical dominance. To determine if this was an aphid-induced cytokinin type response, BAP (6-Benzyl-aminopurine) was sprayed on shoots or poured into the root zone of broad bean seedlings. Aphid-treated and BAP-treated plants had significantly more lateral branches than controls and BAP-treated plants had lowered rates of root respiration, higher rates of shoot respiration and of alternative pathway activity in shoots than controls.

These results indicate that host plants respond to aphid infestation in a general way rather than in a plant-aphid species specific manner and growth responses mirror this trait. The changes in translocation patterns, increases in photosynthesis and increases and decreases in shoot and root respiration, respectively, appear to be due to aphid-induced alterations in endogenous plant growth substance concentrations.

The aphid-induced alterations to host plant physiology are capable of resulting in a compensatory growth response if the aphids are removed.

ACKNOWLEDGEMENTS

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I would also like to thank the following people for their time and helpful comments and criticisms of the various chapters: Drs. C. Critchley (Chapters 2 through 9), D.A. Day (Chapters 4 through 7) and H. Lambers (Chapter 2), Mr. S.C. Brown (Chapters 2 through 9), Ms. S.H. Laszlo (Chapters 1 through 10), and the editors and referees of the various journals.

Mr. R.B. Cunningham of the Statistics Department cheerfully gave statistical advice, Mr. B. Bennett of the Forestry Department provided instruction on and access to the Technicon Auto Analyzer$^{(R)}$, Dr. S.C. Wong of the Department of Environmental Biology loaned the oxygen electrode and related chambers, Mr. T.J. Murphy provided excellent technical assistance with the translocation project, Mrs. E.A. Gallagher drew the majority of the figures, Mr. M.C. Commons did all of the photographic work associated with this investigation, Messrs. A.S. Carter and G. Serbov maintained the plants and aphids during my absences, and Mr. K.P. Hubert and Mrs. N. Plovanic assisted with many phases of the research. To all of you, thank you for your invaluable contributions.

Finally, I wish to thank the Botany Department for providing facilities and funding and The Australian National University for awarding the postgraduate scholarship.

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Abbreviations

ANOVA,	Analysis of variance
BAP,	6-Benzyl-aminopurine
CCCP,	Carbonyl cyanide-m-chlorophenyl-hydrazone
CER,	Net CO_2 exchange rate
CP/BB,	Cowpea aphids on broad bean plants
CP/CP,	Cowpea aphids on cowpea plants
DW,	Dry weight
$\overline{E}$,	Mean unit leaf or mean net assimilation rate
$\overline{F}$,	Mean leaf area ratio
Hepes,	N-2-hydroxyethylpiperazine-N'-2 ethanesulfonic acid
LAW,	Leaf area to leaf weight ratio
LSD,	Least significant difference
Mes,	2-(N-morpholino)ethane-sulfonic acid
P/BB,	Pea aphids on broad bean plants
P/P,	Pea aphids on pea plants
%N,	Nitrogen content as a percentage of plant or tissue DW
%P,	Phosphorus content as a percentage of plant or tissue DW
%TPR,	Percentage of total plant recovered radioactivity
$\overline{R}$,	Mean relative growth rate
$\overline{R}_A$,	Mean relative aphid growth rate
R_D,	Dark respiration rate
RGR,	Mean relative growth rate
SE,	Standard error of the mean
SHAM,	Salicylhydroxamic acid
TCA-cycle,	Tricarboxylic acid cycle
V_{alt},	Alternative respiratory pathway activity
V_{cyt},	Cytochrome respiratory pathway activity
V_{res},	Residual component of respiration
V_T,	Total rate of respiration

CHAPTER 1

INTRODUCTION

Aphids are one of the most defenceless yet most destructive economic pests of agricultural crops around the world (Bornman and Botha 1973). The loss to the pastoral industry in Australia due to aphid feeding was estimated in 1982 at over $A 100 million per annum (Lehane 1982). Infestations of aphids may cause the plants to be stunted or killed prematurely; though often the damage is less obvious but nevertheless significant (Blackman 1974). Little is known of aphid-host plant interactions. Most plant biologists are familiar with the use of aphid stylets to tap sieve tube elements in translocation studies (Zimmermann 1960; Bornman and Botha 1973; Dixon 1975; Richardson 1975) and are aware that their roses can be severely damaged by aphids. Breeding of plant resistance to aphids, however, can be aided by an understanding of the underlying physiological responses to aphid probing and feeding (Southwood 1973; Dixon 1977; de Ponti 1982; Kowalski and Visser 1983).

1.1 Aphid characteristics

Aphids or Aphididae belong to the large order of hemimetabolous insects (insects with incomplete metamorphosis) known as the Hemiptera or bugs (Blackman 1974). They belong to the suborder Homoptera which also includes froghoppers, leafhoppers, scale insects and white flies (Dixon 1973).

Most aphids are polymorphic: that is, within a single species several distinctly different forms or morphs of individuals are produced (Dixon 1973). Adult aphids can either be winged (alate) or wingless (apterous) and the quality of their food is thought to be partially responsible for determining this change of condition (Blackman 1974).

1.1.1 Aphid morphology and reproduction

The morph of aphid familiar to most people is the summer form, both apterae and alatae, and they are all females (Dixon 1973). They are viviparous, that is, they give birth to live young, and the young are produced from unfertilized ova by parthenogenesis (Blackman 1974). It is this method of reproduction that enables aphids to multiply at such a tremendous rate in the summer to exploit short-term food supplies (Blackman 1979). The nymphs can start feeding as soon as they are born. Most aphids do have a sexual phase in their life cycle (Dixon 1973) but some, in countries with mild winters (Australia) or in the tropics, seem to have dispensed with it (Blackman 1974; Maelzer 1981). The aphids utilized in this study were maintained in the parthenogenetic phase.

Before birth the female aphid nymph already has her daughters developing inside her (Blackman 1974) and it is this telescoping of generations or paedogenesis which confers a reproductive advantage on the aphids (Dixon 1985). As Blackman (1974) points out; if you start with an ovum of an aphid and of a sexually reproducing insect, and assume they both produce 50 offspring per female in each generation, in two generations the aphid will have produced 127,550 individuals and in the same time, the other insect will have produced 50 individuals. Therefore, it is not surprising that aphid infestations are capable of inflicting considerable damage to plants.

Parthenogenetic individuals are a genetic clone, meaning that aphids should be very similar if not identical from one experiment to the next. However, parthenogenetic aphids have a remarkable ability to adapt to a wide range of environments and host plants (Dixon 1985). The adaptation arises through phenotypic expression and genetic mutation as there is no unequivocal evidence for endomeiosis in parthenogenetic females (Blackman 1979).

1.1.2 Aphid feeding

All the Hemiptera are piercing and sucking insects which feed on plant sap, or in some cases, blood (Blackman 1974). Their mouthparts are modified for this purpose (Pollard 1973). The mandibles and maxillae, which form the jaws of insects which bite food, in the Hemiptera, form two pairs of fine, long, bristle-like stylets which can be seen only under a microscope (Auclair 1963). The beak or rostrum, which is characteristic of Hemiptera and has four segments in an aphid, is more easily observed (Blackman 1974). There is a dorsal groove in the rostrum in which the stylets run (Pollard 1971, 1973). At its apex, the stylet groove becomes a tube which encloses the stylets tightly and guides their movements (Dixon 1973). The two pairs of stylets come together in the groove forming an interlocking bundle (Blackman 1974). The inner faces of the maxillary stylets form two canals; the central food-canal for the uptake of plant translocate, and a fine duct through which saliva is injected into the plant (Auclair 1963; Dixon 1973; Pollard 1971, 1973, 1977). The mandibular stylets protect and support the maxillary stylets, and aid in the piercing and penetration of plant tissue (Blackman 1974). There are sensory nerve dendrites running inside the stylets and this may indicate that the stylet tip also serves a chemosensory function (Parish 1967).

An aphid's stylets can probe through plant tissue and tap the phloem sieve tubes rich in plant translocate. In a healthy plant, the phloem is under considerable pressure (Zimmermann 1960) and once a sieve tube element is tapped, translocate will flow up the food-canal of the aphid into its pharynx, with no effort on the part of the aphid (Kennedy and Mittler 1953). There is no doubt that aphids can suck up food when a plant wilts and the phloem loses turgor because they have a muscular food pump at the entrance to the pharynx (Mittler and Dadd 1962; Auclair 1963).

Phloem feeding aphids probably use their food pump as a regulatory valve so as to have control over the rate of translocate uptake (Mittler and Dadd 1962). Not all aphids are phloem feeders. The violet aphid *Myzus ornatus* Laing, is obliged to suck up its food because it feeds from mesophyll cells near the edge of

the leaf lamina (Lowe 1967). In actively growing leaves, parenchymatous cells are richer in nutrients than the phloem and this may compensate for the energy expended in obtaining the food (Blackman 1974). Some aphids also feed from the xylem (Dixon 1973).

1.1.2.1 Stylet penetration and saliva composition

The stylets may follow an indirect route to reach the phloem sieve tube elements, usually passing between the plant cells rather than through them (Zimmermann 1960). The aphid ejects saliva down the salivary-canal as it penetrates the plant tissue (Auclair 1963). The saliva contains a polyphenoloxidase which appears to be an invariable component (Miles 1968a), a pectin polygalacturonase (Laurema and Nuorteva 1961; McAllan and Adams 1961) which probably dissolves the middle lamella, and a cellulase so that the stylet tips can pass through the plant tissues more easily (Adams and Drew 1963). Otherwise, only a few sugar hydrolyzing enzymes have been found in the saliva of bugs that feed on phloem or xylem sap (Nuorteva 1958). There is also good evidence that aphid saliva also contains natural phytohormones (Nuorteva 1955, 1956; Hussain *et al.* 1974).

The saliva sets into a gel as it flows out near the tip of the penetrating stylet and eventually forms a salivary sheath around the stylets (Auclair 1963). *Aphis craccivora* Koch has been reported to secrete two types of saliva, a watery liquid and a more viscous material which forms the stylet sheath (Miles 1959). Saliva is probably secreted only during feeding at times when ingestion is not possible (Miles 1968a). Salivation ceases when the stylets penetrate a sieve tube element, although it occurs during penetration and removal of the stylets from the plant tissue (Kinsey and McLean 1967). It is thought that the saliva may contain in its combination of components, substances which can be 'toxic' to the plant (Auclair 1963; Miles 1968a; Dixon 1973; Blackman 1974;).

1.1.3 Aphid host plant selection

Kennedy and Booth's (1951) proposed theory of host plant selection involves two kinds of stimuli from the host plant, 'token' and 'nutrient' stimuli. Token or flavour stimuli provide information about the kind or species of plant on which the aphid has arrived and nutrient stimuli inform the aphid about the physiological condition of the plant and its value as food. The stimuli probably enable the aphid to optimize its feeding location on the plant.

1.1.4 Phloem feeding and aphids

The N to C ratio of plant translocate is probably sub-optimal for the growth of phloem feeders (Raven 1983). They must pass a large amount of translocate through their body to extract the nitrogenous compounds which are essential for growth and reproduction (Mattson 1980). Therefore, the honeydew or sugary liquid which the aphid excretes through its anus, is almost equal in quantity to the translocate it imbibes at the other end (Mittler 1957). Under ideal conditions, an aphid apparently takes in more nitrogen than it can use because most of the amino acids and amides that are found in translocate are usually present, in smaller quatities, in the aphid's honeydew (Mittler 1958). Plant hormones are also present in the honeydew of aphids (Hussain *et al.* 1974). Changes in concentration of nitrogenous substances in the phloem translocate of the plant are the major reason why aphids change or alternate host plants (Blackman 1974). Phloem feeding aphids are usually found on the youngest leaves (Mittler 1957). These leaves, even without the aphids, are in fact an energy drain to the plant because of their import of nutrient rich translocate (Harris 1973).

1.2 Host plant-aphid interactions

There is a considerable volume of information available on the effect of the host plant upon the physiology of the aphid, ranging from host plant selection (Kennedy and Booth 1951; Kennedy and Stroyan 1959; van Emden *et. al.* 1969; Dixon and Wratten 1971; Dixon 1973; van Emden 1973; Blackman 1974) to effects of aphid diet on osmoregulation (Kennedy and Fosbrooke 1973). Much is known

about the effect of aphid vectored plant viruses on the physiology of the host plant (Kennedy *et al.* 1962; Merrett and Bayley 1969; Swenson 1973; Harris and Maramorosch 1977; Pollard 1977; Kurstak 1981). However, the information available on the effects of aphid feeding on the physiology of the host plant is often at best, scarce, and at worst, contradictory.

1.2.1 Reduced plant growth and the effect of translocate removal

One thing that most workers agree about is that severe to moderate aphid infestations will reduce yield and total plant biomass on a short- and a long-term basis, in both herbaceous and woody plant species (Harrington 1941; Allen 1947; Harvey and Hackerott 1958; Howe and Pesho 1960; McMurtry 1962; Dixon 1971a,b; Forrest *et al.* 1973; van Emden 1973; Galecka 1977; Barlow *et al.* 1977; Kain *et al.* 1977, 1979; Mallott and Davy 1978; Wu and Thrower 1981; Barlow and Mesmer 1982; Harper and Kaldy 1982; Petitt and Smilowitz 1982; Tedders *et al.* 1982; Havličkova and Němec 1983; Rohitha and Penman 1983; Singh *et al.* 1983; Bishop 1984; Choudhury 1984; Sirur and Barlow 1984; Summers and Coviello 1984; Burton *et al.* 1985; Koritsas and Garsed 1985). There have been about as many mechanisms attributed to these reductions in growth and reproductive output as there are physiological phenomena to be investigated.

The removal of translocate by aphids causes them to act as physiological 'sinks' for plant nutrients drawn from distant plant organs (Way and Cammell 1970). In fact, they can be a considerable energy drain on the plant. A single willow aphid can account for the photosynthetic products of 5 to 20 cm^2 of leaf (Mittler 1958). One average adult pea aphid represents about 8 percent of the daily net primary production of a 0.15 g dry weight pea plant (Randolph *et al.* 1975). On a lime tree, an average seasonal aphid infestation of 5 aphids per leaf consumes 19 percent of the tree's net annual production (Llewellyn 1975). Clearly, aphids can become a major drain or sink on infested plants and this in itself has the potential to cause severe damage to the plant.

Wu and Thrower (1981) reported that aphids could divert 20 percent of the translocate of a leaf from its normal destination. There also can be a decrease in

transport from roots to shoots with an aphid infestation of the shoots (Forrest *et al.* 1973; Hussain *et al.* 1973). Altered translocation patterns may be a significant contributor to the damage observed in aphid infested plants (Daly 1976). The imbibing of translocate by aphids can also reduce starch and carbohydrates in roots (Tedders *et al.* 1982) and shoots (Kloft 1960; Tedders *et al.* 1982). In lime and sycamore trees the energy drain of the aphids, that is, removal of translocate, accounts for only one-third of the observed reduction in growth (Dixon 1971a,b). For another homopteran, in a leafhopper infested grass, translocate removal accounted for about two-thirds of the observed reduction in plant growth (Andrzejewska 1967). Finally, there is some evidence that aphids feeding from the phloem do not cause phloem injury in branches of woody species (Evert *et al.* 1968) but do cause callose residues to clog the phloem in leaves (Tedders and Thompson 1981; Wood *et al.* 1985), the exact cause and result of this direct response to aphid feeding is still not known (Wood *et al.* 1985).

1.2.2 Plant response to aphid saliva

Aphids cannot be regarded as simply imbibers of phloem sap because they secrete substances into the sieve tube elements (Green 1971) which cause changes in growth and translocation (Edwards and Wratten 1980), either directly or indirectly (Southwood 1973). Many of the aphid-induced changes are to the aphid's advantage but not always (Dixon 1975). For example, some substances in hemipteran saliva may produce local proteolysis and increase free amino acids in the region where the insect is feeding (McNeill and Southwood 1978).

Some aphids induce the development of structural abnormalities called galls in their host plant. The plant tissue grows around and surrounds the aphid and its progeny, then later in the season the gall opens and the aphids leave to find another host (Dixon 1973). Early workers were able to stimulate gall formation by using sawfly or leaf-miner larval excrement (Cosens 1912; LaRue 1937) and also by applying heteroauxin (LaRue 1937). This lead to the use of extracts of non-galling aphids in the search for auxin.

Link *et al.* (1940) demonstrated curvature responses in *Avena* coleoptiles with

ether extracts of aphids but were unable to determine if the auxin's origin was plant or aphid. Allen (1947) concluded that aphids either inject or withdraw a substance which increases or decreases the activity of the plant's response to growth substances. Allen (1947) also considered there was a similarity in growth changes caused by plant hormones and insect feeding, and found that bean plants treated with hormones failed to show the expression of insect damage after insect feeding. It was then demonstrated that substances in the saliva of aphids could be translocated throughout the entire plant (Lawson *et al.* 1954). The similarity in disturbance to the plant caused by insect feeding and plant hormone treatments was noted again (Nuorteva 1955). Nuorteva (1956) then observed growth inhibiting substances in homopteran saliva and later concluded that they may be hormones and enzymes previously drawn out of the plant (Nuorteva 1958). Later it was shown that aphid feeding could reduce the auxin content of its host (Maxwell and Painter 1962a) and that aphids concentrate the auxins in their honeydew (Maxwell and Painter 1962c). The auxin present in pea aphids was reduced by a short period of food deprival prior to analysis for auxins (Maxwell and Painter 1962b). This demonstrated that the auxin was plant derived rather than aphid synthesized. Maxwell and Painter (1962b) concluded that the 'toxins' may be concentrated auxins or plant growth inhibitors which had their origin from substances extracted by the aphid during feeding.

Aphid infestation can result in increased levels of growth inhibitors in shoots and increased levels of cytokinins in roots, with decreased levels of growth promoters in the shoots (Hussain *et al.* 1973) but the imbalance of growth inhibiting hormones can not be accounted for by indiscriminate translocate removal by aphids (Hussain *et al.* 1974). Perhaps, there is an interaction between the non-hormonal plant growth regulators (which may or may not be present in aphid saliva) and the plant hormones, as suggested by Kefeli and Dashek (1984). It has also been hypothesized that seasonal correlation of insect growth and reproduction to the environment is influenced by the changing levels of plant hormones (Visscher Neumann 1982). There certainly is a hormonal involvement between the plant and the aphid.

However, even after 70 years, the mechanisms and modes of action of this involvement with respect to the basic metabolic processes of the plant such as nitrogen uptake, respiration, photosynthesis, and plant water relations are still uncertain.

1.2.3 Aphids and plant nitrogen accumulation

Nitrogen uptake by plant roots is an energy requiring process (Pate 1983). In aphid infested plants total N has been shown to be reduced (Macfoy and Dabrowski 1984; Sirur and Barlow 1984; Koritsas and Garsed 1985) or remain unchanged (Forrest *et al.* 1973). There is some confusion as to whether aphid feeding results in increased or decreased content of percentage nitrogen in infested tissue (Harper and Kaldy 1982; Summers and Coviello 1984). The uncertainty of the percentage nitrogen content results could be related to the variability in the respiratory and photosynthetic rates observed in aphid infested plants (see below). Possibly, the response of a plant to an aphid attack is species-species specific, even though the overall visible growth response is general for most plant-aphid combinations that have been examined.

1.2.4 Aphids and plant respiration

Respiration in infested and infected plants has been shown to increase (Allen 1954; Kloft and Ehrhardt 1959; Scott and Smillie 1966; Daly 1976; Uritani and Asahi 1980), remain unchanged until the plant tissue became moribund and then decrease rapidly (Wu and Thrower 1981), or to decrease by up to 25 percent (Wood *et al.* 1985). Leafhoppers (also Homoptera) have also been shown to increase the rate of respiration (Ladd and Rawlins 1965).

The purpose of respiration is two fold: to provide carbon skeletons for the biosynthesis of primary and secondary plant products and to provide energy in the form of ATP (Millerd and Scott 1962; Uritani and Asahi 1980). Increases in respiration should indicate that more energy is available for nutrient uptake and more carbon precursors are available for biosynthetic work, while decreases in respiration could indicate the opposite. Most respiration measurements have been carried out on homopteran infested shoots, ignoring the roots where most of the

nutrient uptake occurs. Little is known about the respiratory apparatus of diseased plants (Daly 1976) or about the regulation of the various respiratory pathways (Uritani and Asahi 1980), such as, the ATP producing cytochrome pathway and the non-ATP producing alternative respiratory pathway (Day *et al.* 1980). For these reasons, a considerable portion of this study will investigate respiration and its regulation in the roots and shoots of infested and control plants.

1.2.5 Aphids and plant photosynthesis

Photosynthetic rates in aphid infested plants have been observed to increase (Way and Cammell 1970), remain unchanged (van Emden 1973), or decrease (Kloft and Ehrhardt 1959; Daly 1976; Mallott and Davy 1978; Wood *et al.* 1985). Leafhoppers have only been shown to decrease photosynthesis (Ladd and Rawlins 1965; Womack 1984). Maggs (1964) and Sweet and Wareing (1966) suggested that the photosynthetic rates of most plants are below the maxima of which they are capable, so, the various photosynthetic rates observed above, may all be correct, under the experimental conditions in which they were obtained.

Some workers (Randolph *et al.* (1975) believe that some plants are able to compensate for aphid consumption by increasing production. This could be brought about by increases in photosynhtesis and/or decreases in respiration. To increase photosynthesis, increased levels of cytokinins, synthesized in the roots (Kende 1965), would be translocated to the shoots because cytokinins have been shown to stimulate photosynthesis in both expanding and expanded leaves (Li and Proctor 1984). The stimulation is thought to arise because the composition and activity of the photosynthetic apparatus is under phytohormonal control, primarily cytokinins, and then auxins, with the gibberellins having little or no effect (Buschmann and Lichtenthaler 1977). To reduce photosynthesis, it could be something as simple as decreased light transmission to the photochemical apparatus. Tedders and Smith (1976) demonstrated that sooty moulds growing on the honeydew of aphids could reduce light transmission by up to 25 percent. If a plant can increase production in response to aphid feeding, this probably is the result of a complex interaction between levels of plant hormones and translocate within various regions of the plant.

1.2.6 Aphids and plant water relations

The fact that aphids take large amounts of liquid food from the plant suggests the question of whether plant water relations are altered in response to aphid feeding. Galecka (1977) reported that severe aphid infestations on potato plants had no effect on plant water relations, and suggested that the wilted appearance of aphid infested plants may be due to other causes. Van Emden *et al.* (1969) observed that aphid infested plants had a markedly raised moisture content. They intimated that the wilting of aphid infested plants was ascribable to a reduction in root tissue rather than the removal of translocate by aphids. This does not imply that the water relations are not affected in plant-aphid systems.

1.3 Aim of research

In order to improve our understanding, the plant must be regarded as an important and variable part of the environment of an aphid, rather than just as a source of food (Dixon 1977). The understanding of the physiology of the host plant and the host plant-aphid relationship is critical to form a reservoir of knowledge to produce practical advice on crop management and to develop an enhanced understanding of plant resistance and breeding. The aim of this study is to determine the short-term effects of two wide ranging agricultural pest aphid species on the growth, nutrient mobilization, respiration, translocation and photosynthesis of three economically important legume species. The water relations of one of the plant-aphid combinations will also be investigated. Further, the effects of short-term aphid infestation on long-term plant growth will be examined.

1.3.1 Aphids used in the research

Of all the aphid species present in Australia, less than 10 percent, 11 species, are native or specific to native plants (Eastop 1966). The rest have been introduced from Asia, Europe and the Americas. The two phloem feeding aphid species to be utilized in this study, the cowpea aphid, *Aphis craccivora* Koch, and the pea aphid, *Acyrthosiphon pisum* (Harris), are both non-native cosmopolitan pest species (Eastop 1966; Blackman and Eastop 1984) but the latter was only

introduced to Australia in 1980 (Milner 1982). *A. craccivora* has been reported to feed on members of 28 different host plant families but it feeds primarily on the stems and leaves of the Leguminosae (Kennedy *et al.* 1962; Eastop 1966). *A. pisum* feeds on the stems and leaves of at least six genera in the Leguminosae but there have been reports of occasional hosts in other plant families (Eastop 1966). Both aphid species can be important virus vectors (Kennedy *et al.* 1962) but every attempt will be made to keep the stock colonies free of viruses.

1.3.2 Plants used in the research

After the Gramineae, the Leguminosae are the most important family of cultivated crop plants in both the tropical and temperate world (Langer and Hill 1982). In Australia and New Zealand where little nitrogen fertilizer is used, plants rely on the nitrogen fixed by legumes for their growth. Two temperate legumes, the garden pea, *Pisum sativum* L., and the broad, tick, horse, field or faba bean, *Vicia faba* L., and one tropical legume, the cowpea, *Vigna unguiculata* (L.) Walp., will be utilized in this study.

The pea crop is one of the four most important grain legume crops (Davies 1976) and constitutes an important source of protein (seed crude protein about 22 percent) for human consumption (Langer and Hill 1982). The origin of the pea is not certain but it would seem that it first entered cultivation in Ethiopia, the Mediterranean, and central Asia, with a secondary source of diversity in the Near East (Vavilov 1949). *V. faba* is the major grain legume of northern Europe and its seed crude protein is around 25 percent (Bond 1976; Langer and Hill 1982). Its supposed centre of origin was the Near East with the species then radiating out in four directions to create secondary centres of diversity (Cubero 1974). The cowpea is an ancient crop now grown as a pulse, a vegetable, or for fodder throughout the tropics and subtropics (Steele 1976). The bulk of the world's crop is grown in Africa with Nigeria producing 61 percent of it (Langer and Hill 1982). The seed crude protein of cowpea is about 25 percent (Langer and Hill 1982). The plant is of tropical African origin and evidently reached Egypt, Arabia and India at an early date as there is a written record of the cowpea in Sanskrit (Steele 1976; Langer and Hill 1982).

CHAPTER 2

APHID-INDUCED CHANGES IN GROWTH INDICES OF THREE LEGUMINOUS PLANTS: UNRESTRICTED INFESTATION[1]

2.1 Introduction

Aphids are important pests of forage crops around the world and severe infestations may cause the plants to be stunted or killed prematurely (Blackman 1974). In contrast to leaf-eating insects, aphids cause little obvious damage to the leaves of the host and consequently the magnitude of their effects on plants is not fully appreciated (Dixon 1971b). Plants can respond very rapidly to an aphid attack even in organs removed from the site of feeding (Dixon 1975). However, the mechanisms by which the aphid infestations initiate their deleterious effects on plants are poorly understood (Petitt and Smilowitz 1982).

It is generally accepted that aphid infestations reduce the achieved total plant dry weight (Galecka 1977; Barlow *et al.* 1977; Mallott and Davy 1978; Wu and Thrower 1981; Barlow and Mesmer 1982; Harper and Kaldy 1982; Petitt and Smilowitz 1982; Havličkova and Němec 1983; Rohitha and Penman 1983; Lloyd *et al.* 1983) and the ultimate leaf area (van Emden 1973; Mallott and Davy 1978; Wu and Thrower 1981; Barlow and Mesmer 1982; Rohitha and Penman 1983). Barlow *et al.* (1977) speculated that the reduction in plant biomass resulted from reduced total photosynthesis because of decreased leaf area or because components of aphid saliva brought about changes in plant hormones. However, Mallott and Davy (1978) attributed reduction in biomass solely to removal of translocate by the aphids. Kain *et al.* (1977) suggested that the reduction in plant biomass was a combination of the effects of saliva components and translocate removal.

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[1]THIS CHAPTER WAS PUBLISHED IN THE *CAN. J. BOT.* 63: 2454-2459 AND IS REFERRED TO IN THE THESIS AS HAWKINS *ET AL.* {1985}

Some workers (Dixon 1971b; Galecka 1977; Wu and Thrower 1981) felt that the plant response to aphid feeding was specific to a particular plant-aphid system. This idea is supported by reports that various aphid-infested plants have increased (Way and Cammell 1982), unchanged (Mallott and Davy 1978) or decreased (Wu and Thrower 1981)rates of photosynthesis.

The analysis of components of plant growth (Causton and Venus 1981; Hunt 1982) can be used to determine changes in the partitioning of assimilates and this may indicate particular systems that are stressed by aphid feeding.

The following study was conducted to determine the sites of any primary effects and the time required for physiological responses to be severely impaired and to observe whether plant responses to aphid attack are specific for the six combinations of plant-aphid species.

2.2 Materials and methods

Seeds of cowpea (*Vigna unguiculata* (L.) Walp. cv. Caloona) were obtained from Arthur Yates Seed Company, Rockhampton, Queensland; seeds of broad bean (*Vicia faba* L. cv. Aquadulce) and garden pea (*Pisum sativum* L. cv. Victory Freezer) were obtained from M. F. Hodge and Sons, Adelaide, South Australia. Seeds were potted in vermiculite at a density of 1 seed per 12.5 cm diameter pot. Plants were grown in a clear glasshouse for 2 weeks after planting, divided according to size into 5 blocks with 12 or 9 plants per block, depending on the number of aphid densities, and then transferred to a LB growth cabinet, described by Morse and Evans (1962), for the experimental period.

Plants growing in the glasshouse received from 65 to 80% of the outdoor incident photosynthetically active radiation depending on the time of day, measured with a Licor quantum probe (model LI-185A, Lambda Instrument Corp., Lincoln, NB, U.S.A.). Air conditioning and under-bench heating allowed the shaded bench-top air temperature to range from 11 to 35°C (Pernix Thermohygrograph, Wilh. Lambrecht, KG, Göttingen, Federal Republic of Germany). Glasshouse relative humidity was not regulated and ranged from 30 to 90%. The growth cabinet was maintained on a 16 h light: 8 h dark cycle with a day temperature of 23.0 $\pm$ 0.5°C and a photon flux density of 350 μmol.m^{-2}.s^{-1}, supplied by 28 140-W cool white fluorescent (95%) and four 100-W incandescent (5%) lamps, while the night temperature was 18.0 $\pm$ 0.5°C. The relative humidity in the growth cabinet could not be regulated and ranged between 50 and 75%. Water and nutrient (modified Hoaglands solution) schedules were identical in control and experimental plants.

Cowpea aphids (*Aphis craccivora* Koch (Homoptera: Aphididae)) and pea aphids (*Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae)) were obtained from Commonwealth Scientific and Industrial Research Organization, Division of Entomology, Black Mountain, Canberra, Australian Capital Territory, Australia (courtesy of R. Hughes, R. Milner and T. Woolcock) and each was maintained on the three plant species in a growth cabinet under the environmental conditions described above.

On experiment day 0, the 12 plants per block were randomly divided into 4 groups of 3, 1 control and 3 experimental treatments, while the 9 plants per block were randomly divided into 3 groups of 3. Various densities of 8-day-old (±6 h) adult aphids from synchronous colonies were transferred to the experimental plants using a fine, moist, camel-hair brush. This was repeated for the other blocks. Clear plastic collars, 18 cm tall, fitting closely to the top of the pot, confined the aphids to an individual plant. When plant branches extended beyond the edge of the plastic collar, aphids could leave their plants but had great difficulty in gaining access to others. Control plants were checked daily for aphids that may have gained access.

Experiments were continued for 10 days with one plant per treatment per block harvested on day 0 and one control and two or three aphid-infested plants harvested per block on days 5 and 10. Leaf area was measured on an automatic area meter (Hayashi Denko Co., Ltd., Tokyo, Japan). Other parameters measured were leaf number, shoot (stem, petiole and leaf) and root dry weight, and aphid fresh and dry weights. Each plant-aphid combination experiment was replicated at least once.

Calculations based on the equations described by Hunt (1982) were used to determine the following indices. The mean relative growth rate, $\overline{R}$, for a period of time is

$$\overline{R} = (\ln W_2 - \ln W_1)/(T_2 - T_1) \quad (1)$$

where W_1 and W_2 are the total plant dry weights at the beginning, T_1, and end, T_2, of the time period. This index is very sensitive to the whole environmental relationship of the plant (Hunt 1982). A mean relative aphid growth rate ($\overline{R}_A$) index was also calculated. The mean unit leaf rate or net assimilation rate, $\overline{E}$, for a given time period is

$$\overline{E} = ((W_2-W_1)/(T_2-T_1)) \times ((\ln A_2-\ln A_1)/(A_2-A_1)) \quad (2)$$

where A_1 and A_2 are the leaf areas at T_1 and T_2. The mean unit leaf rate is an approximate measure of the net photosynthetic rate if respiration is ignored and if mineral uptake is either neglected or allowed for (Causton and Venus 1981). For plants grown in a constant environment, $\overline{E}$ can be considered an index of the plants' productive efficiency (Williams 1946). The mean leaf area ratio, $\overline{F}$, for a period of time, T_1 to T_2 is

$$\overline{F} = ((A_1/W_1) + (A_2/W_2))/2 \quad (3)$$

This term is an index of plant leafiness and assumes that the leaves are the sole assimilatory organs. Based on this definition, the stems and roots are unproductive (Causton and Venus 1981). In a broad sense, $\overline{F}$ represents the ratio of photosynthetic to respiring tissue within the whole plant (Hunt 1982). The root to shoot ratio on a dry weight basis was also calculated.

Two-way analysis of variance (ANOVA) was carried out using the GENSTAT package (Statistics Department, Rothamsted Experimental Station, U.K.) and treatment means were compared using the protected LSD (least significant difference) at $\alpha = 0.05$, as described by Snedecor and Cochran (1980).

2.3 Results

All data presented are representative of the results of replicate experiments.

In all instances except one (three cowpea aphids on pea plants), the control plants had significantly higher dry weights than infested plants by day 10 (Fig. 1), including the dry weights of their aphids (Table 1).This effect could be seen for some plant-aphid combinations by day 5 (Fig. 1).

The mean relative growth rate, $\overline{R}$, was greater in control than in aphid-infested groups by day 5, and this relationship was usually significant by day 10 (Table 2). There was a decline in $\overline{R}$ between days 5 and 10 and the percentage decline was usually larger in experimental groups. Control groups generally had a greater mean unit leaf rate, $\overline{E}$, than the experimental groups by day 5 (Table 2). This relationship was significant by day 10 for all trials except pea aphids on cowpea plants (Table 2). There were no significant differences in the mean leaf area ratio, $\overline{F}$, for any of the plant-aphid combinations (Table 2).

The mean relative aphid growth rate, $\overline{R}_A$, was greater in the initial low aphid density plants than in the high density ones for all plant-aphid combinations on days 5 and 10 except for pea aphids on broad bean plants on day 5 (Fig. 2). Aphid dry weights initially increased rapidly and then gradually increased at a slower rate for all aphid densities in all six combinations (data not shown).

Figure 1. Average plant dry weight and LSD on the days shown, for cowpea, pea, or broad bean plants infested on day 0 with the number of cowpea or pea aphids indicated at the base of each bar.

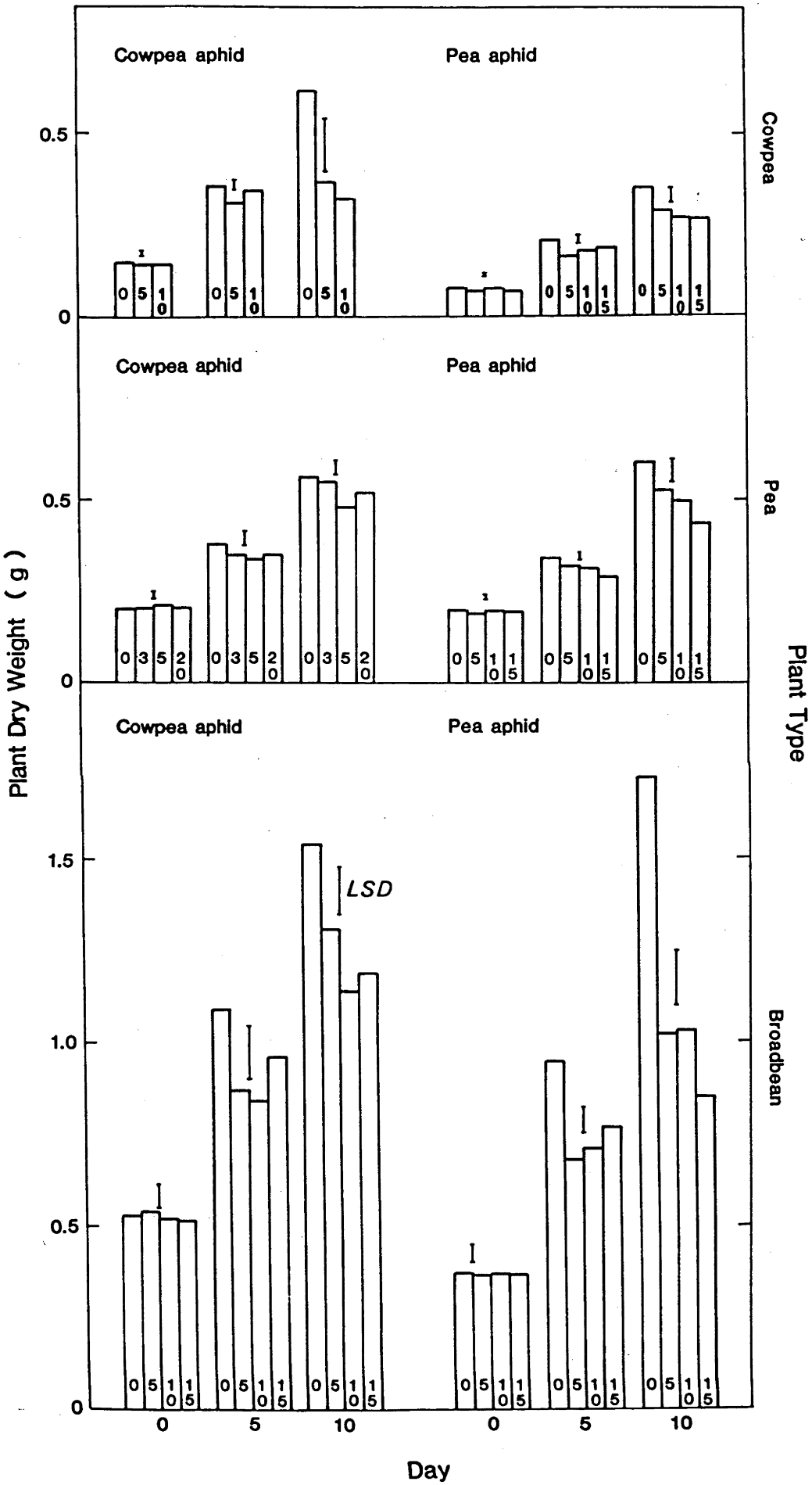

Cowpea aphid
Pea aphid
Cowpea
Pea
Broadbean
Plant Type
Plant Dry Weight (g)
LSD
Day
0
0.5
1.0
1.5
5
10

Table 1. Mean dry weights of plants (cowpea, pea or broad bean) plus aphids (cowpea aphid or pea aphid) after 10 days.

	Cowpea aphid		Pea aphid	
Plant	No.*	DW,$ g	No.*	DW,$ g
Cowpea	0	0.624a	0	0.349a
	5	0.377b	5	0.296b
	10	0.334b	10	0.271b
			15	0.278b
LSD		0.148		0.029
Pea	0	0.566a	0	0.606a
	3	0.551ab	5	0.538b
	5	0.486c	10	0.510b
	20	0.525bc	15	0.457c
LSD		0.040		0.037
Broad bean	0	1.541a	0	1.726a
	5	1.375b	5	1.130b
	10	1.203c	10	1.170b
	15	1.268bc	15	0.977c
LSD		0.125		0.152

* Initial number of aphids per plant on day 0

$ Mean plant plus aphid dry weight (DW). Means followed by the same letter not significantly different, P = 0.95.

Table 2. Average net relative growth rate ($\overline{R}$), mean unit leaf rate ($\overline{E}$), mean leaf ratio($\overline{F}$), and the LSD of 2-week-old cowpea, pea, or broad bean plants infested with different levels of cowpea or pea aphids for 5 and 10 days.

Plant*	Aphid$	Day	No.¢	$\overline{R}$, $mg.mg^{-1}.day^{-1}$	$\overline{E}$, $mg.m^{-2}.day^{-1}$	$\overline{F}$, $m^2.mg^{-1}$
CP	CP	5	0	0.177a	12 790a	0.000 014 3a
			5	0.156b	11 250b	0.000 014 1a
			10	0.175a	13 580a	0.000 013 2a
	LSD			0.013	870	**
		10	0	0.138a	9 690a	0.000 014 8a
			5	0.082b	6 010b	0.000 014 5a
			10	0.073b	5 370b	0.000 014 4a
	LSD			0.009	870	**
CP	P	5	0	0.199a	8 400a	0.000 025 4a
			5	0.164a	6 640a	0.000 025 8a
			10	0.181a	7 300a	0.000 025 8a
			15	0.183a	7 590a	0.000 025 3a
	LSD			**	**	**
		10	0	0.150a	6 320a	0.000 025 4a
			5	0.137ab	5 860a	0.000 025 4a
			10	0.128b	5 870a	0.000 024 0a
			15	0.130b	6 140a	0.000 023 5a
	LSD			0.014	**	**
P	CP	5	0	0.121a	10 780a	0.000 011 3a
			3	0.106a	8 890a	0.000 011 9a
			5	0.098a	8 570a	0.000 011 4a
			20	0.085a	8 880a	0.000 011 6a
	LSD			**	**	**
		10	0	0.102a	9 070a	0.000 011 3a
			3	0.099a	8 840a	0.000 011 2a
			5	0.084b	7 260b	0.000 011 6a
			20	0.093ab	8 370a	0.000 011 2a
	LSD			0.010	1 000	**
P	P	5	0	0.108a	8 900a	0.000 012 8a
			5	0.103a	8 530ab	0.000 012 1a
			10	0.095ab	7 400cb	0.000 012 8a
			15	0.082b	6 550c	0.000 012 4a
	LSD			0.019	1 480	**
		10	0	0.110a	8 470a	0.000 012 9a
			5	0.100b	7 760ab	0.000 012 8a
			10	0.093b	7 020b	0.000 013 1a
			15	0.081c	5 890c	0.000 013 5a
	LSD			0.008	870	**
BB	CP	5	0	0.148a	19 190a	0.000 007 2a
			5	0.093b	11 510b	0.000 008 2a
			10	0.097b	11 850b	0.000 008 2a
			15	0.124ab	16 510ab	0.000 007 5a
	LSD			0.040	5 530	**
		10	0	0.108a	13 350a	0.000 007 9a
			5	0.091ab	11 310b	0.000 007 9a
			10	0.077b	9 160c	0.000 008 3a
			15	0.083b	10 260cb	0.000 008 0a
	LSD			0.018	1 900	**
BB	P	5	0	0.197a	29 740a	0.000 006 7a
			5	0.119b	17 480b	0.000 006 8a
			10	0.130b	19 830b	0.000 006 7a
			15	0.148b	21 570b	0.000 006 9a
	LSD			0.038	7 190	**
		10	0	0.159a	22 720a	0.000 006 9a
			5	0.099b	13 320b	0.000 007 4a
			10	0.104b	15 730b	0.000 006 9a
			15	0.085b	11 360b	0.000 007 4a
	LSD			0.025	5 080	**

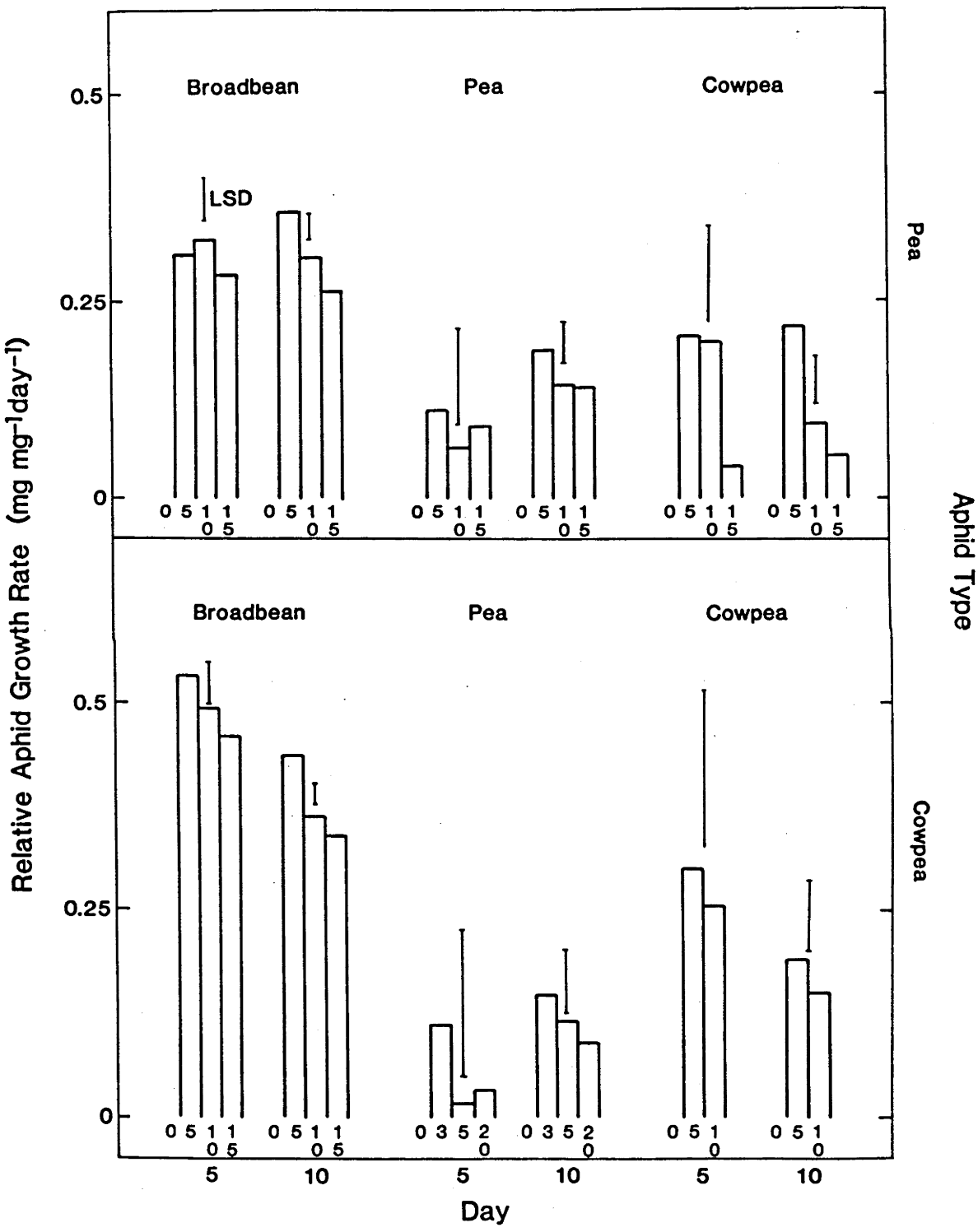
Note: Means followed by the same letter are not significantly different (P=0.95). **, ANOVA not significant, therefore no LSD.

*, Abbreviations: CP, cowpea; P, pea; BB, broadbean.

$, Abbreviations: CP, cowpea aphid; P, pea aphid.

¢, Initial number of aphids per plant.

Figure 2. Average net relative aphid growth rate ($\overline{R}_A$) and LSD for pea and cowpea aphids after 5 or 10 days of infestation on broad bean, pea, and cowpea plants. Initial aphid numbers are indicated at the base of each bar.


Broadbean
Pea
Cowpea
LSD
0.5
0.25
0
Pea
Broadbean
Pea
Cowpea
0.5
0.25
0
Cowpea
Relative Aphid Growth Rate (mg mg-1day-1)
Aphid Type
5
10
5
10
5
10
Day

2.4 Discussion

The general decrease in plant dry weight of aphid-infested plants by day 5 and for all plant-aphid combinations but one by day 10 (Fig. 1) indicates the severity with which small initial numbers of aphids can cause deleterious effects on plant growth. It also indicates that initial aphid density has little or no effect on final dry weight. van Emden (1973) reported that the degree of damage was similar for brussels sprouts (*Brassica oleracea*) regardless of whether the aphid infestation (*Brevicoryne brassicae*) was small or large. Reductions in aphid-infested plant dry weights have been reported for *A. pisum* on *P. sativum* (Barlow *et al.* 1977; Barlow and Mesmer 1982; Havličkova and Němec 1983) and for the green peach aphid (*Myzus persicae*) on potato (*Solanum tuberosum*) on both a long- and short-term basis (Galecka 1977; Pettit and Smilowitz 1982), for the bean, *Vigna sesquipedalis*, infested with *A. craccivora* (Wu and Thrower 1981), for alfalfa (*Medicago sativa*) infested with various aphid species (Harper and Kaldy 1982; Rohitha and Penman 1983; Lloyd *et al.* 1983), and for barley (*Hordeum vulgare* infested with the bird cherry-oat aphid (*Rhopalosiphum padi*)(Mallott and Davy 1978). Wu and Thrower (1981) observed that the maximum stress to the leaf on which the aphids were feeding occurred on the 6th or 7th day of infestation. This would account for the large change observed between days 5 and 10 (Fig. 1).

The dry weight of aphid-infested plants plus the associated aphid dry weight was significantly less than their respective controls for the higher aphid densities (Table 1), which supports Raven's (1983) observation that net production by the unparasitized host exceeds that of the host plus parasite. This also indicates that the aphids were doing more than merely removing translocate from the phloem stream as proposed by Mallott and Davy (1978) because the aphids' conversion efficiency of photosynthate to dry matter, 43 to 65% (Dixon 1975), was similar to that reported for herbaceous plants (Lambers 1985).

Significant decreases in mean relative growth rates and mean unit leaf rates for aphid-infested plants with respect to their controls (Table 2) have also been reported by Mallott and Davy (1978) and Barlow and Mesmer (1982). The 27%

reduction in $\overline{R}$ for 15 pea aphids on pea plants was much greater than the 8% reduction reported by Barlow and Mesmer (1982) for 25 aphids on 14-day-old plants, probably because their plants were larger when infested (day 10 control leaf area 0.1219 versus 0.0872 m^2 in the present study). The greater decrease in $\overline{R}$ for smaller or younger plants infested with aphids was reported by Barlow and Mesmer (1982) while Howe and Pesho (1960) observed that alfalfa resistance to aphid attack increased with increased plant age or size.

The percentage decline in $\overline{R}$ (Table 2) between days 5 and 10 was much greater in experimental groups than control, indicating that aphid infestation does alter the plant's growth processes. The increased rate of reduction of $\overline{R}$ between days 5 and 10 in the aphid-infested plants indicates that the result of the severe stress was first observed during this time.

The reductions in $\overline{E}$ (Table 2) were similar to the reductions in $\overline{R}$, but this was to be expected, as mathematically $\overline{E}$ depends on $\overline{R}$ and the leaf area ratio. Aphid infestations decreased $\overline{E}$ up to 50% in 10 days, illustrating the severe stress a small number of aphids were capable of causing. Therefore, aphid infestation was capable of reducing significantly the net rate of assimilation either by increasing respiration and (or) decreasing photosynthesis in all cases but one within 10 days.

The absence of a significant difference in leaf area ratio between control and infested groups (Table 2) was also reported by Barlow and Mesmer (1982). However, they reported a trend for increased $\overline{F}$ with increased levels of aphid feeding, while Mallott and Davy (1978) found it was slightly, but consistently, higher in infested plants after 3 weeks. Even though total leaf area was reduced, aphid feeding had not reduced the proportion of assimilates that were available for leaf expansion.

The greater mean relative aphid growth rates found for the initial low aphid density plants compared with the initial high density ones (Fig. 2) probably occurred because the greater the aphid density, the fewer the number of young that are produced per adult (Barlow and Mesmer 1982). The rapid initial increase in aphid dry weight and subsequent slowing has been reported by Mallott and Davy

(1978) and may be attributed to a decrease in the host plant's susceptibility (Dixon and Wratten 1971). The apparent decrease in host susceptibility was probably linked with the decreased $\overline{R}_A$ found for the high aphid density plants after 10 days. A greater $\overline{R}_A$ could be an indicator of decreased resistance by that host plant to the aphid species with the greatest $\overline{R}_A$.

The instantaneous growth rate equals the product of the instantaneous unit leaf rate and the instantaneous leaf area ratio, or

(4) $$R = E \times F$$

but, except in certain circumstances,

(5) $$\overline{R} \neq \overline{E} \times \overline{F}$$

(Hunt 1982). A plant can have a large $\overline{R}$ because of an increased $\overline{E}$ and (or) $\overline{F}$. Therefore, $\overline{R}$ is proportional to $\overline{E} \times \overline{F}$ (Causton and Venus 1981). The usual significant decrease in $\overline{R}$ for aphid-infested plants (Table 2) was not due to the reduction in photosynthetic surface area or plant leafiness, $\overline{F}$, but was due to the significant reduction in $\overline{E}$ (Table 2). This raises the question of whether the aphid-infested plants became less efficient at converting light to chemical energy or whether the aphids caused new assimilate sinks (in addition to themselves) increasing respiration, thus resulting in the significant reduction in the mean unit leaf rate. Further studies will be required to elucidate whether or not the light or dark reactions of photosynthesis or the plant respiratory processes are the primary targets of aphid feeding or whether some combination of impairment of all of these processes causes lesser production of new tissue.

This study has demonstrated significant reductions in plant dry weight, leaf area, $\overline{R}$, and $\overline{E}$. These effects were not due solely to the removal of photosynthate from the phloem. The effects of aphid feeding can be observed within 10 days of infestation, and there is little indication that initial aphid density is important in determining final plant dry weight. The physiological mechanisms that underlie

these aphid-induced changes are likely initiated within the plant shortly after the aphid infestation begins. The overall growth responses, as measured at day 10, of all legume-aphid systems examined here were similar rather than plant-aphid system specific, even though the underlying physiology could have differed. It will therefore be beneficial to look at short-term aphid-induced physiological changes.

CHAPTER 3

INTERACTIONS BETWEEN APHID INFESTATION, PLANT GROWTH AND UPTAKE OF NITROGEN AND PHOSPHORUS BY THREE LEGUMINOUS HOST PLANTS[1]

3.1 Introduction

It has been suggested that changes in the plant's respiratory and photosynthetic processes are the primary means by which growth is reduced in aphid-infested plants (Hawkins *et al.* 1985). Aphids remove sugar from the phloem via their stylets and this lost translocate could reduce the energy supply available for nutrient uptake by the roots (Bowling and Dunlop 1978) and root growth and respiration. Both nitrogen and phosphorus uptake in the roots are energy requiring processes (Bidwell 1974). Cowpea plants (*Vigna*) reduce the bulk of absorbed nitrate in their shoots (Atkins *et al.* 1980), while pea (*Pisum*) and broad bean (*Vicia*) plants possess highly active nitrate reductases in their roots (Pate 1973). It is thought that internal nitrogen levels can also affect respiratory processes (Marek 1984).

An extreme variablity is displayed among higher plant species with respect to total nitrogen content (Pate 1983). There is also the general view that severe aphid infestations reduce total nitrogen content (Macfoy and Dabrowski 1984; Sirur and Barlow 1984; Koritsas and Garsed 1985) though little is known of their effect on total phosphorus. However, there is some doubt as to whether aphid feeding results in increased or decreased nitrogen and phosphorus as a percentage of plant dry weight (Harper and Kaldy 1982; Summers and Coviello 1984).

The following study was conducted to determine the short-term (10 day) effect

[1]THIS CHAPTER WAS SUBMITTED TO *CAN. J. BOT* ON 31 OCT 85 AND IN REVISED FORM ON 10 APR 86 AND IS REFERRED TO IN THE THESIS AS HAWKINS *ET AL.* (SUBMITTED 1985)

of aphid feeding on the uptake of nitrogen and phosphorus by 3 well fertilized, non-nodulated legume species and to observe if their response to aphid infestation was general or plant-aphid species specific.

3.2 Materials and methods

Seedlings of cowpea (*Vigna unguiculata* (L.) Walp. cv. Caloona), broad bean (*Vicia faba* L. cv. Aquadulce), and garden pea (*Pisum sativum* L. cv. Victory Freezer) were raised, and cowpea and pea aphids, *Aphis craccivora* Koch and *Acyrthosiphon pisum* (Harris), respectively, both Homoptera:. Aphididae, were cultured as previously described (Hawkins *et al.* 1985). Both aphid species had been maintained on the plant species utilized for a minimum of one year prior to the initiation of these experiments.

On experiment day 0, two-week-old plants were divided according to size into 5 blocks of 6. Each block was randomly divided into 3 pairs of plants, one of each pair being a control, and one an experimental plant with 10, 8 day-old adult aphids placed on it. One pair of plants in each block was harvested on days 0, 5, and 10. The experiment was carried out in a growth cabinet as previously described (Hawkins *et al.* 1985). Plant-aphid combinations utilized were cowpea aphids on cowpea and broad bean seedlings and pea aphids on pea, broad bean and cowpea seedlings, and each combination was replicated at least once.

The following parameters were assessed on each harvest day: aphid dry weight; leaf number; leaf area (Automatic Area Meter, Hayashi Denko Co., Ltd., Tokyo, Japan); and leaf, stem and petiole, and root dry weights. Total nitrogen, organic and ammonium, and phosphorus were estimated from the oven-dried samples of leaf, stem and petiole (these 3 parts comprise the shoot), and roots by a semi-micro Kjeldahl digestion and a molybdenum blue technique (Allen *et al.* 1974), respectively, determined in a Technicon Auto Analyzer $^{(R)}$ (Technicon Industrial Systems, Tarrytown, NY, USA), and expressed on a dry weight basis. The mean relative growth rate, $\overline{R}$, the mean unit leaf or mean net assimilation rate, $\overline{E}$, and the mean leaf area ratio, $\overline{F}$, were calculated (Hunt 1982), as was the mean relative growth rate of the aphids, $\overline{R}_A$, (Hawkins *et al.* 1985).

Two-way analysis of variance (ANOVA) was carried out using the GENSTAT package (Statistics Department, Rothamsted Experimental Station, UK) at $\alpha = 0.05$. The protected LSD (Least Significant Difference) was calculated for each pair of means, at $\alpha = 0.05$ (Snedecor and Cochran 1980).

3.3 Results

The data presented are representative of the results of replicate experiments.

The dry weights of the component plant parts and the entire plant, leaf areas, and root-to-shoot ratios were not significantly different between control and infested plants after 5 days infestation (data not shown) but were at 10 days for all leaf areas and dry weights, except for leaf, root, and total plant dry weight of pea plants, and for cowpea leaves infested with cowpea aphids (Table 1). However, the differences in root-to-shoot ratios remained insignificant (Table 1). Aphid feeding significantly reduced $\overline{R}$, and $\overline{E}$ in broad bean and cowpea but not in pea seedlings after 10 days (Table 2). The mean leaf area ratio, $\overline{F}$, was not affected by aphid feeding (Table 2). The increase in aphid biomass from day 0 to day 10 was significant for all plant-aphid combinations and $\overline{R}_A$ was similar for both aphid species on broad bean, pea aphids on pea, and cowpea aphids on cowpea plants, but was much lower for pea aphids feeding on cowpea (Table 3).

The percent nitrogen (%N) content (on a dry weight basis) was significantly greater in infested than in control broad bean plants but was the same or significantly less in infested than in control cowpea and pea seedlings after 10 days (Table 4). Percent phosphorus (%P) content (on a dry weight basis) was similar to that of %N but most comparisons were not significant (Table 5). Component plant part %N was similar to the whole plant relationship with infested broad bean having a higher value than controls, except for cowpea aphids on broad bean stems, and infested cowpea and pea having the same or lower values than controls after 10 days (Table 4). The variability was much greater for %P of component plant parts and no clear pattern emerged (Table 5). After 10 days of infestation, %N and %P for control and infested broad bean had decreased in comparison to day 5, cowpea had increased, and pea changed little (Fig. 1). The absolute content of N and P increased rapidly in all control plants from days 0 to 10, and in all infested plants from days 0 to 5, and then increased at a slower or a negative rate from days 5 to 10 in infested plants (Fig. 2).

Table 1. Mean leaf, root, stem and total plant dry weights, leaf area, root to shoot ratio, and the LSD for control plants and cowpea or pea aphids on broad bean and cowpea seedlings, and pea aphids on pea seedlings after 10 days infestation.

Plant[#]	Aphid[$]	No.[¢]	Dry weight mg				Leaf area m^2	Root:shoot Ratio
			Leaf	Stem	Root	Plant		
BB	P	0	494	369	640	1503	0.0164	0.750
		10	304	191	389	884	0.0087	0.800
LSD			113	112	109	230	0.0040	ns
BB	CP	0	395	260	555	1211	0.0124	0.875
		10	246	130	279	654	0.0073	0.765
LSD			72	105	95	230	0.0022	ns
CP	P	0	175	77	93	345	0.0055	0.372
		10	128	53	66	247	0.0042	0.350
LSD			15	6	23	30	0.0006	ns
CP	CP	0	143	62	91	295	0.0043	0.455
		10	95	33	70	198	0.0030	0.551
LSD			ns	12	17	78	0.0011	ns
P	P	0	133	72	200	405	0.0060	0.994
		10	96	48	174	318	0.0051	1.174
LSD			ns	19	ns	ns	0.0006	ns

Note: If the means are not significantly different (ns) by ANOVA ($\alpha = 0.05$) the LSD is not presented ($\alpha = 0.05$).

#, Abbreviations: BB, broad bean; CP, cowpea; P, pea.

$, Abbreviations: P, pea; CP, cowpea.

¢, Initial number of aphids per plant.

Table 2. Average net relative growth rate ($\overline{R}$), mean unit leaf rate ($\overline{E}$), mean leaf area ratio ($\overline{F}$), and the LSD of control plants and broad bean, cowpea, and pea seedlings after 10 days infestation with cowpea or pea aphids.??

Plant#	Aphid$	No.¢	$\overline{R}$ $mg.mg^{-1}.day^{-1}$	$\overline{E}$ $mg.m^{-2}.day^{-1}$	$\overline{F}$ $m^2.mg^{-1}$
BB	P	0	0.118	5 766	0.000 013 6
		10	0.075	2 882	0.000 014 5
LSD			0.287	1 801	ns
CP	CP	0	0.056	4 643	0.000 012 1
		10	0.016	1 314	0.000 012 5
LSD			0.032	2 539	ns
P	P	0	0.144	10 290	0.000 013 7
		10	0.115	7 790	0.000 014 7
LSD			ns	ns	ns

Note: If the means are not significantly different (ns) by ANOVA ($\alpha = 0.05$) the LSD is not presented ($\alpha = 0.05$).

??, Similar results to those presented were obtained for the broad bean and cowpea seedling-aphid combinations not presented.

#, Abbreviations: BB, broad bean; CP, cowpea; P, pea.

$, Abbreviations: P, pea aphids; CP, cowpea aphids.

¢, Initial number of aphids per plant.

Table 3. Mean total dry weight of the 10 aphids placed on the experimental plants on day 0 and the average net relative aphid growth rate of cowpea and pea aphids after 10 days growth on cowpea, broad bean, or pea seedlings.

Plant	Aphid	$DW^{\$}$ mg	$\overline{R}_A{}^{\$}$ $mg.mg^{-1}.day^{-1}$
Cowpea	Pea	2.42	0.104 *
Cowpea	Cowpea	1.50	0.212 *
Broad bean	Pea	7.41	0.231 *
Broad bean	Cowpea	2.60	0.256 *
Pea	Pea	2.15	0.224 *

Note: *, change in total aphid biomass was significant from days 0 to 10 (ANOVA at $\alpha = 0.05$).

$, Abbreviations: DW, dry weight; $\overline{R}_A$, average net relative aphid growth rate.

Table 4. Mean nitrogen content (%N) for total plant, and leaf, stem, and root component parts, and the LSD for control plants and pea or cowpea aphids on broad bean and cowpea seedlings, and pea aphids on pea seedlings after 10 days infestation.

Plant[#]	Aphid[$]	No.[¢]	%N Plant	Leaf	Stem	Root
BB	P	0	4.63	6.86	3.13	3.79
		10	5.80	7.98	4.64	4.68
LSD			0.24	0.43	1.02	0.43
BB	CP	0	4.06	5.56	4.38	2.73
		10	5.54	6.83	3.60	5.48
LSD			1.01	ns	ns	ns
CP	P	0	3.23	4.71	2.07	1.44
		10	2.80	3.45	2.29	2.03
LSD			0.40	0.57	ns	ns
CP	CP	0	3.37	3.61	1.91	2.61
		10	3.37	3.64	2.18	2.58
LSD			ns	ns	ns	ns
P	P	0	3.74	4.46	2.21	3.82
		10	2.78	3.09	2.26	2.78
LSD			0.78	0.95	ns	ns

Note: If the means are not significantly different (ns) by ANOVA ($\alpha = 0.05$) the LSD is not presented ($\alpha = 0.05$).

#, Abbreviations: BB, broad bean; CP, cowpea; P, pea.

$, Abbreviations: P, pea aphids; CP, cowpea aphids.

¢, Initial number of aphids per plant.

Table 5. Mean phosphorus content (%P) for total plant, and leaf, stem, and root component parts, and the LSD for control plants and pea or cowpea aphids on broad bean and cowpea seedlings, and pea aphids on pea seedlings after 10 days infestation.

Plant[#]	Aphid[$]	No.[¢]	%P			
			Plant	Leaf	Stem	Root
BB	P	0	0.491	0.630	0.432	0.420
		10	0.566	0.547	0.456	0.525
LSD			ns	ns	ns	ns
BB	CP	0	0.501	0.494	0.755	0.375
		10	0.580	0.483	0.401	0.688
LSD			ns	ns	0.348	ns
CP	P	0	0.392	0.630	0.177	0.112
		10	0.326	0.455	0.197	0.185
LSD			ns	0.105	ns	ns
CP	CP	0	0.345	0.412	0.194	0.217
		10	0.286	0.327	0.199	0.178
LSD			0.039	0.053	ns	0.038
P	P	0	0.330	0.348	0.201	0.364
		10	0.219	0.191	0.173	0.249
LSD			0.055	0.102	ns	0.099

Note: If the means are not significantly different (ns) by ANOVA (α = 0.05) the LSD is not presented (α = 0.05).

#, Abbreviations: BB, broad bean; CP, cowpea; P, pea.

$, Abbreviations: P, pea aphids; CP, cowpea aphids.

¢, Initial number of aphids per plant.

Figure 1. The %N and %P for controls and pea aphids on broad bean and pea plants and cowpea aphids on cowpea seedlings at 0, 5, and 10 days of aphid infestation. Similar relationships were observed for the two plant-aphid combinations not shown. Control plants, ● ○; infested plants, ■ □; %N, ● ■; %P, ○ □; and the LSD at $\alpha = 0.05$ is indicated by the vertical bar, significantly different for %N, ▲ and %P △.

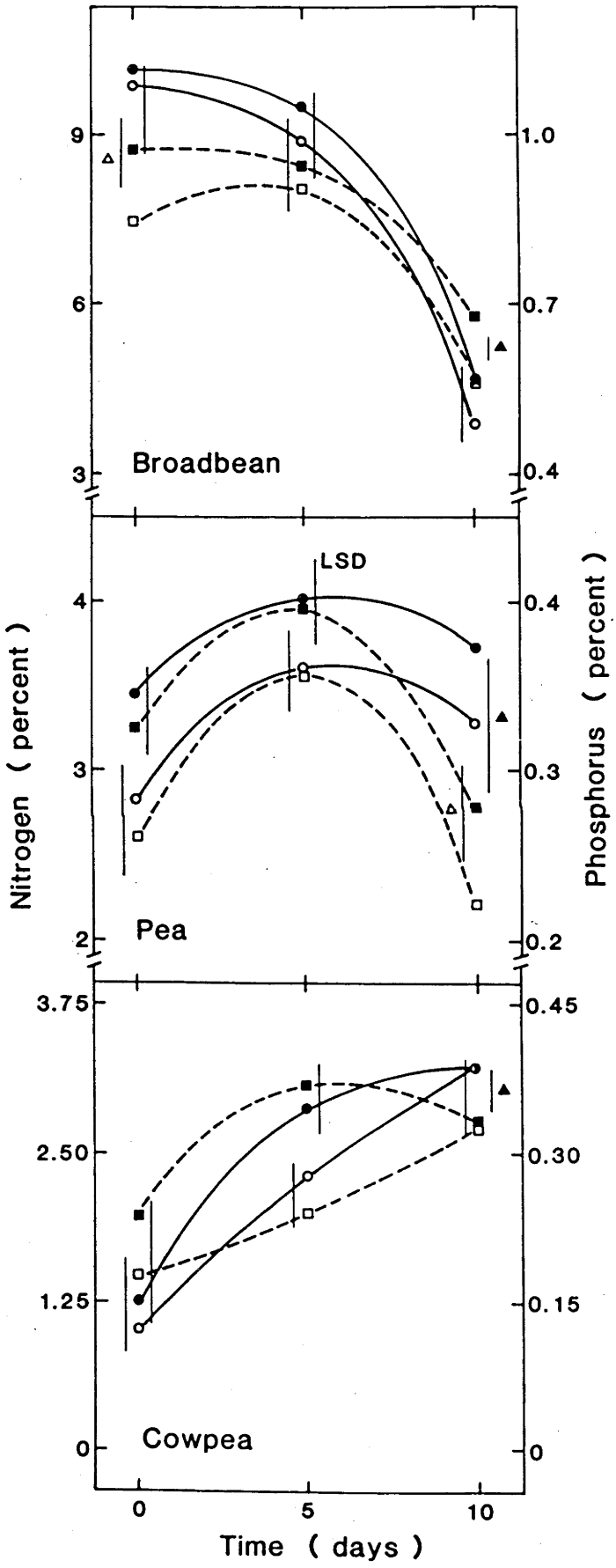
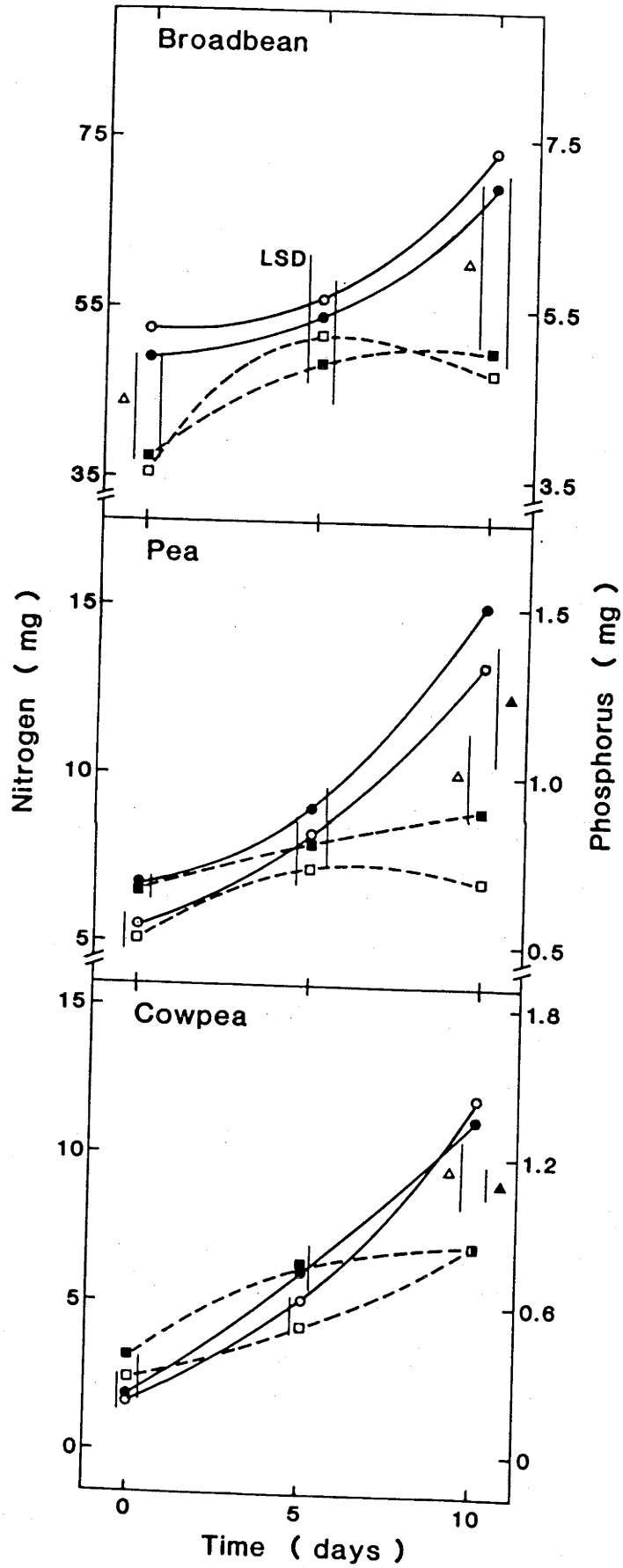

Broadbean
LSD
Pea
Cowpea
Nitrogen (percent)
Phosphorus (percent)
Time (days)
9
6
3
1.0
0.7
0.4
4
3
2
0.4
0.3
0.2
3.75
2.50
1.25
0
0.45
0.30
0.15
0
0
5
10

Figure 2. The absolute content of N and P (mg) for controls and pea aphids on broad bean and pea seedlings and cowpea aphids on cowpea plants at 0, 5, and 10 days of aphid infestation. Similar relationships were observed for the two plant-aphid combinations not shown. Control plants, ● ○; infested plants, ■ □; N, ● ■; P, ○ □; and the LSD at $\alpha = 0.05$ is indicated by the vertical bar, significantly different for N, ▲ and P, △ .


Broadbean
Pea
Cowpea
LSD
75
55
35
15
10
5
15
10
5
0
7.5
5.5
3.5
1.5
1.0
0.5
1.8
1.2
0.6
0
0
5
10
Time (days)
Nitrogen (mg)
Phosphorus (mg)

3.4 Discussion

The significant reductions in plant dry weight and leaf area in the infested plants (Table 1) are typical of those which have been reported previously for many plant-aphid combinations including those utilized in the present experiment (Galecka 1977; Mallott and Davy 1978; Wu and Thrower 1981; Barlow and Mesmer 1982; Rohitha and Penman 1983; Hawkins *et al.* 1985). The difference in size between species' control groups (Table 1) was a function of the glasshouse environmental regime prior to initiating the experiment. The reductions, significant and otherwise, for aphid-infested leaf and stem dry weights, in the five plant-aphid combinations examined (Table 1), was probably the result of the aphids imbibing translocate that would normally have been used for growth by these tissues. Translocate removal by the aphids and/or a decreased supply of photosynthate because of reduced leaf area would result in a decreased flux of photoassimilate to the roots and this could cause the reduction in root biomass (Table 1). The lack of root-to-shoot ratio changes (Table 1) has been observed for other herbaceous plant-aphid systems (Wu and Thrower 1981; Rohitha and Penman 1983). This indicates that the proportion of assimilates available for shoot and root growth was the same in control and infested plants.

Cowpea and broad bean seedlings both had significantly reduced $\overline{R}$ (mean relative growth rate) and $\overline{E}$ (mean unit leaf or net assimilation rate) in infested plants (Table 2) and this has been observed for several combinations of plants and aphids (Mallott and Davy 1978; Barlow and Mesmer 1982; Hawkins *et al.* 1985). Neither $\overline{R}$ nor $\overline{E}$ was significantly reduced for pea seedlings (Table 2) because the aphid density was apparently not sufficient to reduce plant growth (Table 1). The similarity in $\overline{F}$ (mean leaf area ratio) between treatments for all plant-aphid combinations (Table 2) is also consistent with the findings of Mallott and Davy (1978), Barlow and Mesmer (1982) and Hawkins *et al.* (1985). The coupling of this result with the lack of significance for the root-to-shoot ratios (Table 1), indicates that the infested plants did not reallocate their assimilate resources in response to aphid feeding, as proposed by Raven (1983).

Aphid biomass increased significantly for all plant-aphid combinations from days 0 to 10 (Table 3) indicating that the plants were adequate hosts. The $\overline{R}_A$ for pea aphids on cowpea seedlings was significantly less than that observed for the other 4 combinations suggesting that this cultivar of cowpea was not the most suitable host for the pea aphid. All 5 $\overline{R}_A$ values were similar to those reported by Hawkins *et al.* (1985) for the same plant-aphid combinations.

Nitrogen and P are expressed as a percentage of the dry weight of the plant or component plant part because aphid feeding had reduced the dry weights, usually significantly (Table 1).

Plant %N and %P were greater in infested broad bean and the same or less in infested cowpea and pea seedlings (Tables 4 & 5), suggesting that the individual plant species responded differently with respect to N and P accumulation. Harper and Kaldy (1982) found no difference between control and infested alfalfa plants for %N, but did find a greater %P in infested ones. Summers and Coviello (1984) also reported no difference for %N between control and infested alfalfa plants. The present data (Tables 4 & 5) indicate that infested broad bean expended more energy on a weight basis to acquire N and P than the controls while infested cowpea or pea expended the same or less energy.

The lack of a significant difference, in most of the plant-aphid combinations, for leaf and stem %N and %P (Table 4 & 5) indicates that generally aphid feeding does not result in a lowered incorporation of N and P into growing tissue. Furthermore, it is unlikely that aphids preferentially remove N and P from the contents of the phloem. However, when the reductions in stem and leaf dry weights are taken into account (Table 1) there is an obvious reduction of absolute N and P in leaf and stem tissue. This supports the observations of Macfoy and Dabrowski (1984), Sirur and Barlow (1984) and Koritsas and Garsed (1985) for N, though little is known about P. The values for %N and %P of non-nodulated roots were significantly different in only one case after 10 days (Tables 4 & 5), suggesting that usually sufficient translocate reached the roots to maintain %N and %P on a root tissue weight basis. Perhaps, resources were directed towards maintenance of %N

and %P at the expense of other root physiological processes. The greater variation observed for P than N (Tables 5 & 4) was also reported by DeJong (1982). The data for the component plant parts (Tables 4 & 5) indicate that even though there were for the most part significant changes for whole plant %N and %P, they could not be attributed to a single organ but were the cumulative result of aphid feeding.

A different mechanism in the accumulation of N and P, as a percentage of plant dry weight, appears to be in operation in each plant species over the 10 days of the study (Fig. 1). The early differences, days 0 to 5, could be related to cotyledon size or different means of nitrate reduction. Broad bean has very large cotyledons, pea intermediate size ones, and cowpea tiny ones. Broad bean and pea are amide producing legumes which have high activities of nitrate reductase in their roots (Pate 1973) compared to cowpea which forms ureide and reduces the bulk of nitrate in its shoots (Atkins *et al.* 1980). The much greater R observed for broad bean compared to cowpea (Table 2) could account for the decrease in %N and %P in broad bean and the increase in cowpea (Fig. 1). However, pea had an even greater R than broad bean, suggesting that accumulation of plant %N and %P may be related to the speed and efficiency with which active uptake of N and P is initiated, or it may be a combination of the above factors. The %N and %P values at day 10 are within the normal range of values for legumes (Allen *et al.* 1974). This suggests that the species differences (Fig. 1) were due to developmental rather than aphid-induced causes.

The change in absolute N and P up to day 5 was similar for control and infested plants (Fig. 2) and may also indicate that early changes in %N and %P were developmental rather than aphid related. The change in the rate of accumulation of N and P in the infested plants from days 5 to 10 (Fig. 2) indicates the stress that aphid feeding was putting on the plants. A significant reduction in the total root respiration of infested plants has also been observed during this time period (Hawkins *et al.* 1986) and they attributed it to a reduced flux of translocate to the roots. Perhaps, the significant reduction of absolute N and P in aphid-infested plants was due to the significant decrease of their root respiration.

DeJong (1982) has shown that CO_2 assimilation was proportional to peach leaf N and P content. The variability of %N and %P, indicating increased or decreased amounts of N and P on a tissue weight basis, could account for the photosynthetic anomalies attributed to aphids, such as increased (Way and Cammell 1970), unchanged (Mallott and Davy 1978), or decreased (Wu and Thrower 1981) rates of photosynthesis. Marek (1984) observed that the rate of dark respiration in the light was significantly greater in low N treatment plants. It would be interesting to determine if infested species could have greater rates of photosynthesis and lower rates of dark respiration in the light than their controls.

This study has shown that plant dry weight, leaf area, total N, and total P were reduced for all plant-aphid combinations by 10 days of aphid feeding; that the feeding itself did not result in an altered incorporation of N and P into plant tissues, as a percentage of dry weight; and that %N and %P accumulation into the plant were specific to the plant species being investigated. It appears that the effect of aphids upon root respiration and on shoot respiration and photosynthesis are important areas to be investigated before the primary and causal effects of aphid feeding on host plant physiology can clearly be understood.

CHAPTER 4

SHORT-TERM EFFECTS OF TWO APHID SPECIES ON PLANT GROWTH AND ROOT RESPIRATION OF THREE LEGUMINOUS SPECIES[1]

4.1 Introduction

Hawkins *et al.* (1985) suggested that aphid-induced changes in respiratory and photosynthetic processes of infested plants were the primary means by which plant growth was reduced. Most studies on the effects of aphids on respiration have been concerned with shoot respiration (Kloft and Ehrhardt 1959, Daly 1976, Wu and Thrower 1981, Wood *et al.* 1985). The removal of phloem translocate by the aphids could reduce the energy supply available to root metabolism or alter the efficiency of root metabolism.

In roots of higher plants, there are two respiratory pathways which may or may not function simultaneously (Day and Lambers 1983). The operation of both the alternative respiratory pathway and the phosphorylating cytochrome respiratory pathway *in vivo* implies that the cytochrome pathway is either saturated or restricted by adenylates (Day *et al.* 1980, Laties 1982). The alternative pathway *per se* is not coupled to ATP synthesis (Day *et al.* 1980) but is so widely distributed that it must play a role of physiological significance in plants (Lance 1981). Lambers (1980, 1982, 1985) has suggested that the physiological significance of the alternative pathway in the roots of higher plants is its function as an "energy overflow" when carbohydrate supply to the roots is in excess of energy requirements in the roots for structural growth, energy production, storage and osmoregulation.

The present study was conducted to determine the short-term (10 days) effect

[1]THIS CHAPTER WAS ACCEPTED FOR PUBLICATION IN *PHYSIOLOGIA PLANTARUM* ON 05 MARCH 1986 AND IS REFERRED TO IN THE THESIS AS HAWKINS *ET AL.* {1986}

of aphid feeding on root respiration in three well fertilized, non-nodulated legume species, and to observe whether the response of root respiration to aphid infestation was general or species specific. In particular, the effect of aphid infestation on the capacity and engagement of the alternative respiratory pathway was monitored.

Abbreviations-

CP/BB, cowpea aphids on broad bean plants; CP/CP, cowpea aphids on cowpea plants; P/BB, pea aphids on broad bean plants; P/P, pea aphids on pea plants; RGR, mean relative growth rate; SHAM, salicylhydroxamic acid; V_{alt}, alternative path activity; V_{cyt}, cytochrome path activity; V_{res}, residual component of respiration; V_T, total rate of respiration.

4.2 Materials and methods

Seeds of cowpea [*Vigna unguiculata* (L.) Walp. cv. Caloona], broad bean (*Vicia faba* L. cv. Aquadulce), and garden pea (*Pisum sativum* L. cv. Victory Freezer), and cowpea and pea aphids, *Aphis craccivora* Koch and *Acyrthosiphon pisum* (Harris), respectively, both Homoptera: Aphididae, were obtained and raised as described by Hawkins *et al.* (1985).

On the day prior to commencement of the experiment, the plants were placed in a growth cabinet (16 h light: 8 h dark cycle with a day temperature of 23 $\pm$ 0.5°C and a photon flux density of 350 μmol.m^{-2}.s^{-1}, while the night temperature was 18 $\pm$ 0.5°C) and maintained as described by Hawkins *et al.* (1985). The two-week-old plants were divided according to size into 5 blocks of 5 plants on experimental day 0. The respiration rate of the roots of one plant randomly selected from each block was determined on day 0. The remaining 4 plants were randomly divided into 2 pairs; each comprising a control, and an experimental plant with 10, 8-day-old adult aphids placed on it. Root respiration was determined for one pair of plants on experimental days 5 and 10. This procedure was repeated for the other 4 blocks. Plant-aphid combinations utilized were CP/BB, CP/CP, P/BB and P/P. The other two combinations were not used because of the poor growth displayed by the aphids.

Plants were removed from the growth cabinet for respiration determinations after a 7 to 9 h period of photosynthesis. The vermiculite was washed off the roots with water (24°C) prior to measurement of respiratory rates. The whole root system was cut from the shoot [excision of the shoot does not affect root respiration during the experimental periods used here (de Visser and Lambers 1983)] and placed into a dark, temperature controlled (24 $\pm$ 1°C), 110 ml capacity cuvette fitted with a Clark type oxygen electrode and a protected stir bar. A fully aerated nutrient solution [modified Hoagland (Hoagland and Arnon 1938, solution 2)] without the iron component, which chelates with SHAM (Schonbaum *et al.* 1971), provided the bathing medium for the roots and the oxygen concentration changes were determined polarographically.

After V_T was determined (5 to 10 min) the effects of KCN and/or SHAM were observed. This was achieved by slowly injecting KCN (0.22 M in water) or SHAM (1.37 M in 2-methoxyethanol) into the original solution to give final concentrations of 0.2 mM KCN and 15 mM SHAM. The high concentration of SHAM used has been shown to be sufficient to block the CN^--insensitive alternative oxidase without affecting the cytochrome pathway (de Visser and Blacquière 1984). After SHAM addition, respiration was monitored for a maximum of 15 to 20 min (Lambers 1985). Respiration rates were expressed per g dry weight of roots dried for 2 days at 70 °C, after which further weight change was insignificant.

The capacity of the alternative pathway (including V_{res}) was assumed to be fully expressed in the presence of CN^- (Lambers 1982, Laties 1982). SHAM inhibition of respiration measures the actual V_{alt} (Lambers 1982, Lambers *et al.* 1983). Other responses to SHAM have also been observed in root tissue and are aptly discussed by de Visser and Blacquière (1984) and Møller and Bérczi (1985). Lambers *et al.* (1983) defined the degree of engagement of the alternative pathway as the reduction in respiration due to SHAM divided by the rate of respiration in the presence of CN^- (including V_{res}).

Shoot (leaf, stem and petiole) dry weights were determined as for roots. RGR was calculated for roots and for whole plants (see Hawkins *et al.* 1985) as this index is very sensitive to the whole environmental relationship of the plant (Hunt 1982).

Two-way analyses of variance (ANOVA), at $\alpha = 0.05$, were carried out on the various data and linear regression was done on respiration rates using the GENSTAT package (Statistics Department, Rothamsted Experimental Station, U.K.). The protected least significant difference [LSD, as described by Snedecor and Cochran (1980)] was calculated for each pair of means, at $\alpha = 0.05$.

4.3 Results

The results presented are representative of at least one replicate experiment.

The RGR of the entire plant was significantly reduced in all cases for aphid-infested plants between days 0 and 10 but this was not necessarily the case between days 0 and 5, or days 5 and 10 (Tab. 1). The root RGR was also significantly reduced in the aphid-infested plants between days 0 and 10, with similar relationships to those observed for the whole plant between days 0 and 5, or 5 and 10 (Tab. 1).

The rates of total root respiration were less in the aphid-infested plants after 5 days and were significantly so in all cases after 10 days (Fig. 1). For all plant-aphid combinations, but the control plants of P/BB, V_T declined with time (Fig. 1). For the replicate experiments of P/BB, both control and infested plant V_T and V_{cyt} decreased with time (data not shown). When the SHAM-inhibited portion of respiration (alternative pathway) was deducted from V_T, control plants had greater V_{cyt} (including V_{res}) than aphid-infested ones, in all cases but one (P/P), after 5 days (Fig. 2). This relationship was significant for all plant-aphid combinations after 10 days (Fig. 2). The respiration rate of control P/BB did not change with time when only the cytochrome pathway portion (V_{cyt} + V_{res}) of respiration was considered (Fig. 2).

The alternative pathway capacity (including V_{res}) was quite variable, but by day 10 all the roots of aphid infested plants, except for P/P, had a significantly lesser capacity than their respective controls (Tab. 2). The percentage engagement of the alternative pathway was greater in control roots on day 10 and this was usually also the case on day 5 (Tab. 3). It generally took 10 days for the dry weight of the infested plant roots to become significantly reduced (Tab. 3). The residual rate of respiration (SHAM + KCN or KCN + SHAM) also decreased with time and was generally greater in aphid-infested roots than in those of the controls (data not shown). After 10 days, V_{res} for control and infested plants, respectively, were 15 and 22 % for CP/CP, 14 and 21 % for CP/BB, 12 and 25 % for P/BB, and 9 and 12 % for P/P.

Table 1. Mean relative plant and root growth rates (RGR) with the LSD at the 95% level, for control (C) and aphid-infested (A) plants, for cowpea aphids on cowpea (CP/CP) and broad bean (CP/BB) plants, and pea aphids on broad bean (P/BB) and pea (P/P) plants on days 0-5, 5-10 and 0-10.

	RGR, $mg.mg^{-1}.day^{-1}$											
	Root						Plant					
Parameter	Days 0-5		Days 5-10		Days 0-10		Days 0-5		Days 5-10		Days 0-10	
	C	A	C	A	C	A	C	A	C	A	C	A
CP/CP	0.009	-0.012	0.243	-0.016	0.126	-0.014	0.167	0.140	0.187	0.085	0.177	0.112
LSD	0.040		0.040		0.017		0.020		0.027		0.015	
CP/BB	0.137	0.089	0.153	0.088	0.145	0.089	0.166	0.124	0.147	0.125	0.156	0.124
LSD	0.068		0.061		0.024		0.046		0.045		0.010	
P/BB	0.127	0.073	0.152	0.021	0.140	0.047	0.149	0.074	0.158	0.048	0.154	0.061
LSD	0.109		0.173		0.049		0.069		0.066		0.030	
P/P	0.264	0.214	0.086	0.083	0.175	0.148	0.226	0.181	0.118	0.097	0.172	0.139
LSD	0.014		0.019		0.010		0.016		0.032		0.009	

Figure 1. Change in total root respiration (total respiration (V_T) = cytochrome path (V_{cyt}) + alternative path (V_{alt}) + residual respiration (V_{res})) for the four plant-aphid combinations investigated. The linear regression correlation coefficient is r. Vertical lines to the right of each pair of means are the LSD (P = 0.95) and the vertical line to the left of each mean is the standard error of the mean (SE) for five determinations from one experiment. Cowpea aphids on cowpea (CP/CP) and broad bean (CP/BB), pea aphids on broad bean (P/BB) and pea plants (P/P), control (◯) and aphid-infested (□) plants.

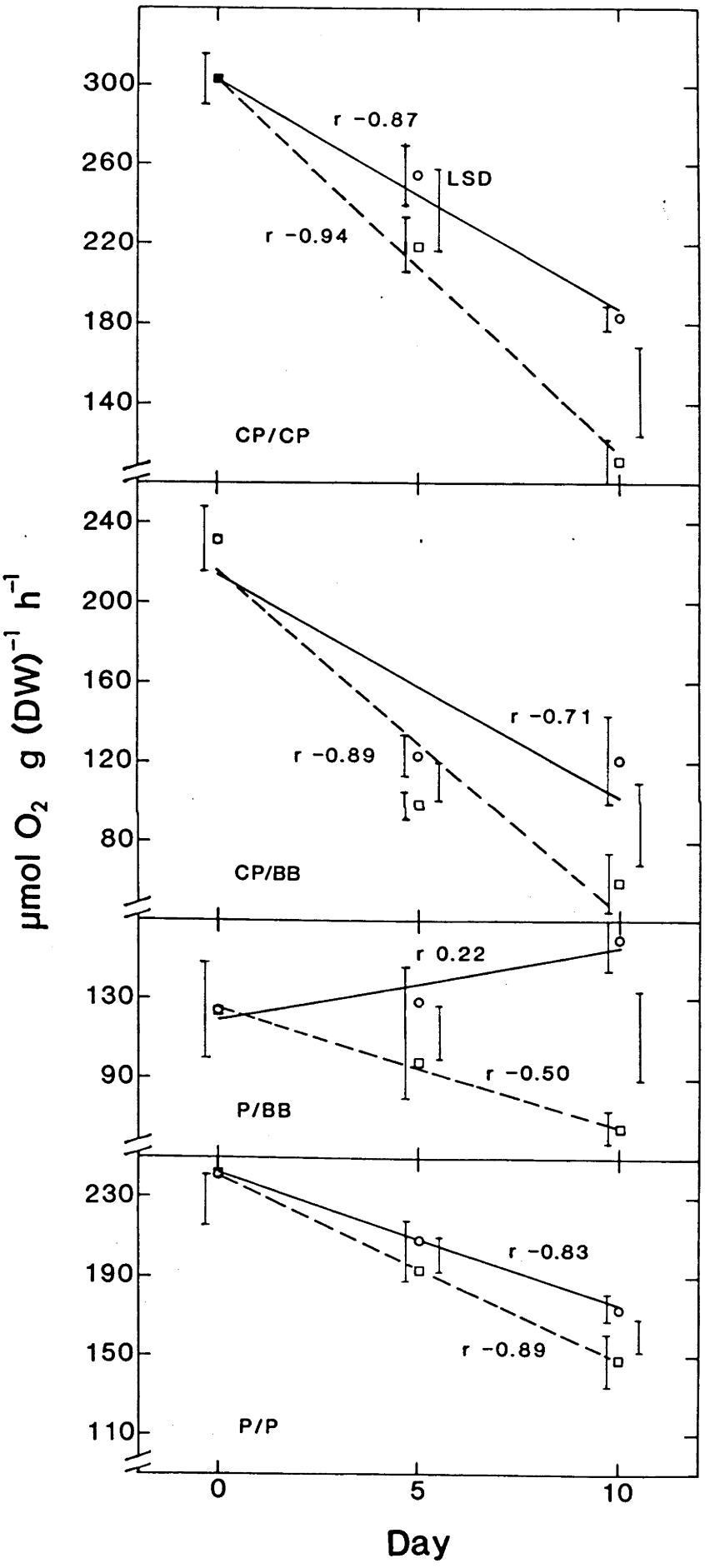

300
260
220
180
140
r -0.87
LSD
r -0.94
CP/CP
240
200
160
120
80
r -0.71
r -0.89
CP/BB
130
90
r 0.22
r -0.50
P/BB
230
190
150
110
r -0.83
r -0.89
P/P
0
5
10
Day
µmol O2 g (DW)-1 h-1

Figure 2. Change in root cytochrome pathway respiration ($V_{cyt} + V_{res}$) measured in the presence of 15 mM SHAM for the four plant-aphid combinations investigated. The linear regression correlation coefficient is r. Vertical line to the right of each pair of means is the LSD (P = 0.95) and the vertical line to the left of each mean is the SE. Abbreviations as in Fig. 1; control (◯) and aphid-infested (□) plants.

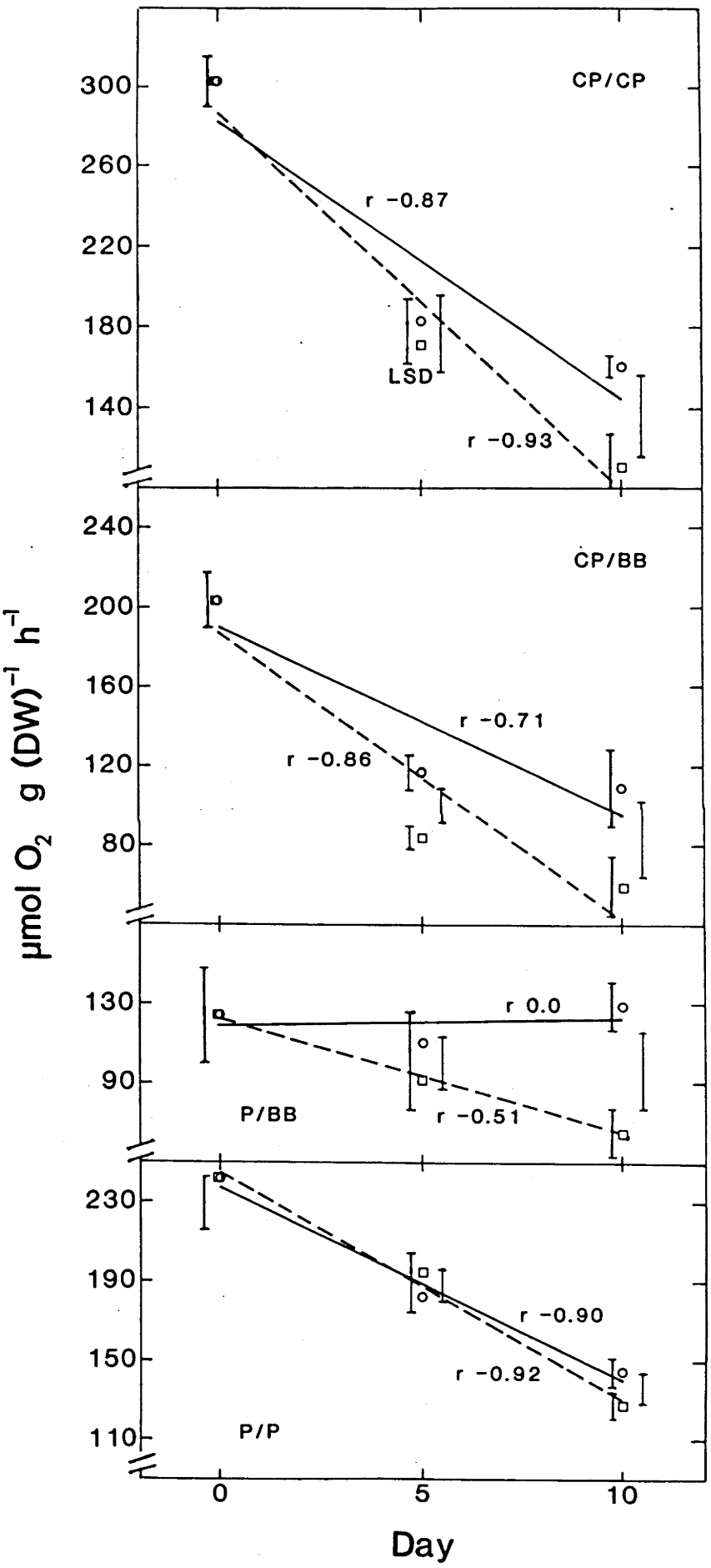

CP/CP
r -0.87
LSD
r -0.93
CP/BB
r -0.71
r -0.86
r 0.0
P/BB
r -0.51
r -0.90
r -0.92
P/P
µmol O2 g (DW)-1 h-1
Day
0
5
10

Table 2. Capacity of the alternative pathway in the presence of 0.2 mM KCN (alternative + residual respiration) in roots of control (C) and aphid-infested (A) plants for cowpea aphids on cowpea (CP/CP) and broad bean (CP/BB), and pea aphids on broad bean (P/BB) and pea (P/P) plants on experimental days 0, 5 and 10. An *, indicates the mean capacity of the alternative path was significantly different between C and A plants, LSD at the 95% level, while ns denotes no significant difference.

Trial	Capacity μmol $O_2 \cdot g(DW)^{-1} \cdot h^{-1}$					
	Day 0		Day 5		Day 10	
	C	A	C	A	C	A
CP/CP	232	232	196	169	140	111
	ns		ns		*	
CP/BB	185	185	134	109	142	80
	ns		*		*	
P/BB	128	128	140	126	128	57
	ns		ns		*	
P/P	142	142	122	94	99	110
	ns		*		ns	

Table 3. Percentage engagement of the alternative pathway ((total respiration - reduction with 15 mM SHAM) / (reduction with 0.2 mM KCN)) in roots of control (C) and aphid-infested (A) plants for cowpea aphids on cowpea (CP/CP) and broad bean (CP/BB), and pea aphids on broad bean (P/BB) and pea (P/P) plants on experimental days 0, 5 and 10. An *, indicates that the mean root dry weight was significantly greater in C than A plants, LSD at the 95% level, while ns denotes no significant difference. The difference in alternative path engagement was only significant in those cases where root weights were significantly greater.

	Engagement, %					
Trial	Day 0		Day 5		Day 10	
	C	A	C	A	C	A
CP/CP	0	0	39	29	17	0
	ns		ns		*	
CP/BB	15	15	4	13	8	0
	ns		ns		*	
P/BB	0	0	14	6	25	0
	ns		ns		*	
P/P	0	0	23	0	30	20
	ns		*		*	

4.4 Discussion

Similar changes in plant RGR with time to those presented here (Tab. 1) have been reported by Hawkins *et al.* (1985) for the plant-aphid combinations used. They observed that it took between 5 and 10 days for the aphids to cause significant alterations to plant physiological processes, and attributed the alterations to translocate removal by the aphids and decreases in the efficiency of photosynthesis and/or respiration in the infested plants. The decrease in root RGR of aphid-infested plants by day 10 was relatively larger than the reduction in plant RGR (Tab. 1). This may have occurred by translocate imbibed by the aphids being lost to the root system. Translocation rates to the roots in these plant-aphid combinations have been observed to decrease within 5 days and to be significantly decreased after 10 days of infestation (C.D.B. Hawkins, unpublished results).

The significant decrease in V_T of aphid-infested plants after 10 days infestation compared with controls (Fig.1), could be the result of a reduced carbohydrate supply to the roots of the infested plants. This is suggested by the decreased RGR for both infested plants and their roots (Tab. 1). Lambers *et al.* (1980) suggested that higher rates of root respiration could result partly from a better photosynthetic performance in the shoots and partly from a higher capacity of the roots to attract carbohydrates. The shoots of the control plants have been shown to possess better photosynthetic abilities than those infested with aphids for all the plant-aphid combinations used here (Hawkins *et al.* 1985). Although it has been reported that aphid infestation increases plant respiration rates (Kloft and Ehrhardt 1959; Daly 1976), such infestations have also been shown to have no short-term effect on shoot respiration (Wu and Thrower 1981) or even to decrease shoot respiration by up to 25 % (Wood *et al.* 1985). Preliminary measurements of shoot respiration rates for these plant-aphid combinations indicates that infested shoots have greater rates of O_2 uptake in the dark than the controls (C.D.B. Hawkins, M.I. Whitecross & M.J. Aston unpublished results).

The decrease in root respiration with age (Fig. 1) has been reported previously for other root systems (Blacquière and Lambers 1981, Lambers *et al.* 1981) and was

associated with decreased alternative pathway activity. This appears to be the case in this instance also (Tabs. 2 and 3).

Cytochrome pathway respiration (V_{cyt} + V_{res}) in control plants was significantly faster than in aphid-infested plants (Fig. 2). This difference was probably due to carbohydrate supply and to regulation of the cytochrome pathway.

The absence of a significant difference between control and infested root V_T after 5 days of infestation (Figs. 1 and 2) could help to explain why C.D.B. Hawkins, M.I. Whitecross and M.J. Aston (unpublished results) found no difference in total N and P accumulation for these plants. However, they did find significant reductions in total N and P in infested plants after 10 days of infestation just as significant reductions in respiration were found here (Figs. 1 and 2).

The unchanged respiration observed for control plants of P/BB (Fig. 2) may indicate that physiologically these roots were more mature than usual due to the glasshouse regime prior to their transfer to the growth cabinet. The day 0 V_T and V_{cyt} (Figs. 1 and 2) were in the range observed for replicate experiments on days 5 and 10 (data not shown).

Even though the capacity of the alternative pathway was considerable (Tab. 2), in only one case was it engaged in 14-day-old seedlings, but 19-day-old seedlings had V_{alt} in all cases except for infested P/P (Tab. 3). Infested plants had no V_{alt} after 10 days of infestation except for P/P. The difference in P/P from the other plant-aphid combinations was probably due to the significant reduction in root dry weight (Tab. 3) and root RGR (Tab. 1) after 5 days for P/P, while these differences did not become significant until day 10 for the other combinations. The low percentage engagement of the alternative pathway (Tab. 3) is typical for legume species (de Visser and Blacquière 1984). The greater engagement of the alternative pathway in roots of control plants indicates that these roots are less efficient than the roots of infested plants in terms of energy conversion (Lambers 1982, de Visser and Lambers 1983).

The decline in residual respiration with time and for control versus aphid-infested plants is not easily explained. It should be noted that the inhibitors are

not 100 % efficient at the concentrations used (Lance *et al.* 1985) and that treatments involving applications of inhibitors to intact tissue is questionable on the grounds of imprecision (Møller and Bĕrczi 1985).

The increased respiratory sink postulated by Hawkins *et al.* (1985) for aphid-infested plants was not present in the roots of these infested plants. In fact, the roots of the infested plants represent a decreased respiratory sink because aphids drain carbohydrates and less are available to the roots. This investigation has also shown that aphids cause a reduction in root respiration and an increase in the efficiency of energy conversion in the roots. The response of root respiration to aphid feeding was a general rather than a plant-aphid species specific. However, further and longer time scale studies are required with both aphid-infested and control plants to examine more closely the regulatory mechanisms of root metabolism *in vivo*.

CHAPTER 5

SHORT-TERM EFFECTS OF TWO APHID SPECIES ON PLANT GROWTH AND SHOOT RESPIRATION OF THREE LEGUMES[1]

5.1 Introduction

Hawkins *et al.* (1985) suggested that observed short-term, aphid-induced reductions in plant growth were the result of increased energy utilization and/or decreased energy production, in addition to the removal of translocate by the aphids. In a previous report, Hawkins *et al.* (1986) showed that aphid infestation resulted in decreased rates of root respiration, probably via decreased levels of substrate transport to the roots. The roots, therefore, were not a site of increased energy utilization.

Most studies concerning the effects of aphids on shoot respiration have employed infrared gas analyzer techniques (Kloft and Ehrhardt 1959; Daly 1976; Wu and Thrower 1981; Wood *et al.* 1985) which do not allow the regulation and contribution of different respiratory pathways to be determined. Further, respiration of a single leaf was often deemed representative of the entire shoot and this ignores different respiration rates associated with different leaf ages (Azcón-Bieto *et al.* 1983a).

Azcón-Bieto *et al.* (1983b) demonstrated that the energy producing cytochrome pathway and the non-phosphorylating alternative respiratory pathway (Day *et al.* 1980) can function simultaneously in leaves of several plant species. The simultaneous operation of both pathways implies that the cytochrome pathway is either saturated or restricted by adenylates (Day *et al.* 1980; Laties 1982).

Although the physiological significance of the alternative respiratory pathway

[1]THIS CHAPTER WAS SUBMITTED TO *PHYSIOLOGIA PLANTARUM* ON 10 MAR 86 AND IS REFERRED TO IN THE THESIS AS HAWKINS *ET AL.* [SUBMITTED 1986A]

remains to be elucidated (Lambers 1980, 1982, 1985; Lance 1981), the contribution of the two respiratory pathways to respiration can be determined polarographically using respiratory inhibitors and uncouplers (Azcón-Bieto *et al.* 1983b). The capacity of the alternative pathway (including residual respiration) is assumed to be fully expressed in the presence of CN^- (Lambers 1982; Laties 1982). The actual activity of the alternative pathway is measured by the SHAM inhibition of the total rate of respiration (Lambers 1982; Lambers *et al.* 1983). The engagement of the alternative pathway is calculated by dividing its activity by its capacity (Lambers *et al.* 1983). Phosphorylation is uncoupled from electron transport in the presence of CCCP which acts to reduce the electrochemical proton gradient ($\Delta\mu_{H^+}$) across the inner mitochondrial membrane to zero (Moore 1978). This allows maximum electron transport by the respiratory pathways (Moore 1978).

This study was conducted to determine the short-term (10 day) effect of aphid feeding on plant growth and on the respiration of the entire shoot (leaf, stem and petiole) in three well fertilized, non-nodulated legume species, and to observe whether the response of shoot respiration to aphid feeding was general or plant-aphid species specific (see Hawkins *et al.* 1985 for discussion). In particular, the regulation, activity and capacity of the two respiratory pathways in response to aphid infestation was monitored.

Abbreviations:

CCCP, carbonyl cyanide-m-chlorophenyl-hydrazone; CP/BB, cowpea aphids on broad bean plants; CP/CP, cowpea aphids on cowpea plants; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; Mes, 2-(N-morpholino)ethane-sulfonic acid; P/BB, pea aphids on broad bean plants; P/P, pea aphids on pea plants; SHAM, salicylhydroxamic acid; TCA-cycle, tricarboxylic acid cycle.

5.2 Materials and methods

Seeds of cowpea (*Vigna unguiculata* (L.) Walp. cv. Caloona), broad bean (*Vicia faba* L. cv. Aquadulce), and garden pea (*Pisum sativum* L. cv. Victory Freezer), and cowpea and pea aphids, *Aphis craccivora* Koch and *Acyrthosiphon pisum* (Harris), respectively, both Homoptera: Aphididae, were obtained as previously described (Hawkins *et al.* 1985). Plants and aphids were raised as described by Hawkins *et al.* (1985) except that the potting medium was changed from vermiculite to washed river sand. The change in medium had no effect on the relationship between plant growth, aphid growth and their interactions as shown by two-way analyses of variance.

The plants were placed in a growth cabinet one day prior to the commencement of the experiment and maintained as described by Hawkins *et al.* (1985). On experiment day 0, the two-week-old plants were divided according to size into five blocks of five plants. The shoot respiration rate of one plant randomly selected from each block was determined on day 0. The remaining plants in each block were randomly divided into two pairs; each pair comprised a control plant and an experimental plant with 10, eight-day-old adult aphids placed on it. Shoot respiration was measured for one pair of plants on experimental days 5 and 10. This procedure was repeated for the other four blocks. The aphid-plant combinations utilized were P/P, CP/CP, P/BB and CP/BB.

Plants were removed from the growth cabinet for respiration determinations after a 7 to 9 h period of photosynthesis. The whole shoot system was cut from the roots, intact leaves separated from the stem, and both placed into a dark, temperature controlled (24 $\pm$ 1°C), 110 ml capacity cuvette fitted with a Clark type oxygen electrode and a protected stir bar. A reaction medium of 10 mM Hepes, 10 mM Mes buffer (pH 6.6) and 0.2 mM $CaCl_2$ in equilibrium with air (Azcón-Bieto *et al.* 1983b) provided the bathing solution for the entire shoot and changes in oxygen concentrations were determined polarographically.

After measuring the total rate of respiration, aliquots of the uncoupler, CCCP (0.33mM in ethanol), the cytochrome pathway inhibitor, KCN (0.22M in water)

and/or the alternative pathway inhibitor, SHAM (1.37M in 2-methoxyethanol) were slowly injected into the reaction medium to give final concentrations of 2 μM CCCP, 0.2 mM KCN and 15 mM SHAM. Higher concentrations of CCCP or KCN did not give any additional effect; nor did up to 25 mM SHAM.

Respiration rates were expressed per g dry weight of shoot dried for two days at 70 °C. Root dry weights were similarily determined.

The various data were analyzed using two-way analysis of variance (ANOVA) at $\alpha = 0.05$, and the linear regression of respiration rates were done using the GENSTAT package (Statistics Department, Rothamsted Experimental Station, U.K.). For each pair of means, the protected least significant difference [LSD, as described by Snedecor and Cochran (1980)] was calculated at $\alpha = 0.05$.

5.3 Results

The results presented are representatives of replicate experiments.

Control plants had significantly higher dry weights than aphid-infested plants by day 10 (Fig. 1). This effect could be seen for CP/BB alone at day 5 (Fig. 1). Shoot dry weights were also significantly greater in control plants than in aphid-infested plants after 10 days but again, this relationship only held for CP/BB at day 5 (Fig. 1). The root dry weights of aphid-infested plants were significantly less than in controls after 10 days (not shown; but see Fig. 1).

The rates of total shoot respiration were greater in the aphid-infested plants after five days and were significantly so in all cases by day 10 (Fig. 2). After 10 days, using data from all experiments, the increase in respiration of the infested plants ranged from 133 to 199 percent of their respective controls (not shown). The rate of total shoot respiration declined with time (Fig. 2) for all plant-aphid combinations. This relationship was observed for both cytochrome pathway and alternative pathway activity (Fig. 2). Simple linear regression of total and cytochrome pathway respiration (as presented in Fig. 2) in control plants showed a high coefficient of correlation with all values being greater than -0.8. However, the same regressions on the aphid-infested plants displayed much more variability than on control plants and the correlation to linearity was always less for the aphid treated plants.

The capacity of the alternative respiratory pathway (including residual respiration) in all plants decreased with time but was higher in aphid-infested shoots, compared to controls, by day 10 (Tab. 1), even though it was no longer engaged (Fig. 2). However, when the capacity of the alternative pathway was expressed as a percentage of the total shoot respiration it was much more variable (not shown). The uncoupled rate of the cytochrome pathway (using CCCP) also decreased with age and by day 10, aphid-infested shoots had greater rates than controls (Tab. 2).

Little change in the percentage of residual respiration (O_2 uptake in the presence of both SHAM and KCN) was observed with time for either treatment

Figure 1. Increase in total plant (entire histogram), root (upper part of histogram), and shoot (lower part of histogram) dry weights with time for control (histogram on left) and and pea (P) or cowpea (CP) aphid-infested (histogram on right) pea (P), cowpea (CP) and broad bean (BB) plants. The interval (I) to the right of a pair of histograms is the LSD for plant dry weight, while the interval to the left (I) is the LSD for shoot dry weight, both at α = 0.05. The same sample was used for control and aphid-infested plants on day 0. Each histogram and segment thereof represents the mean of five independent samples. Control shoot,  and root,  dry weights; and aphid-infested shoot,  and root,  dry weights.

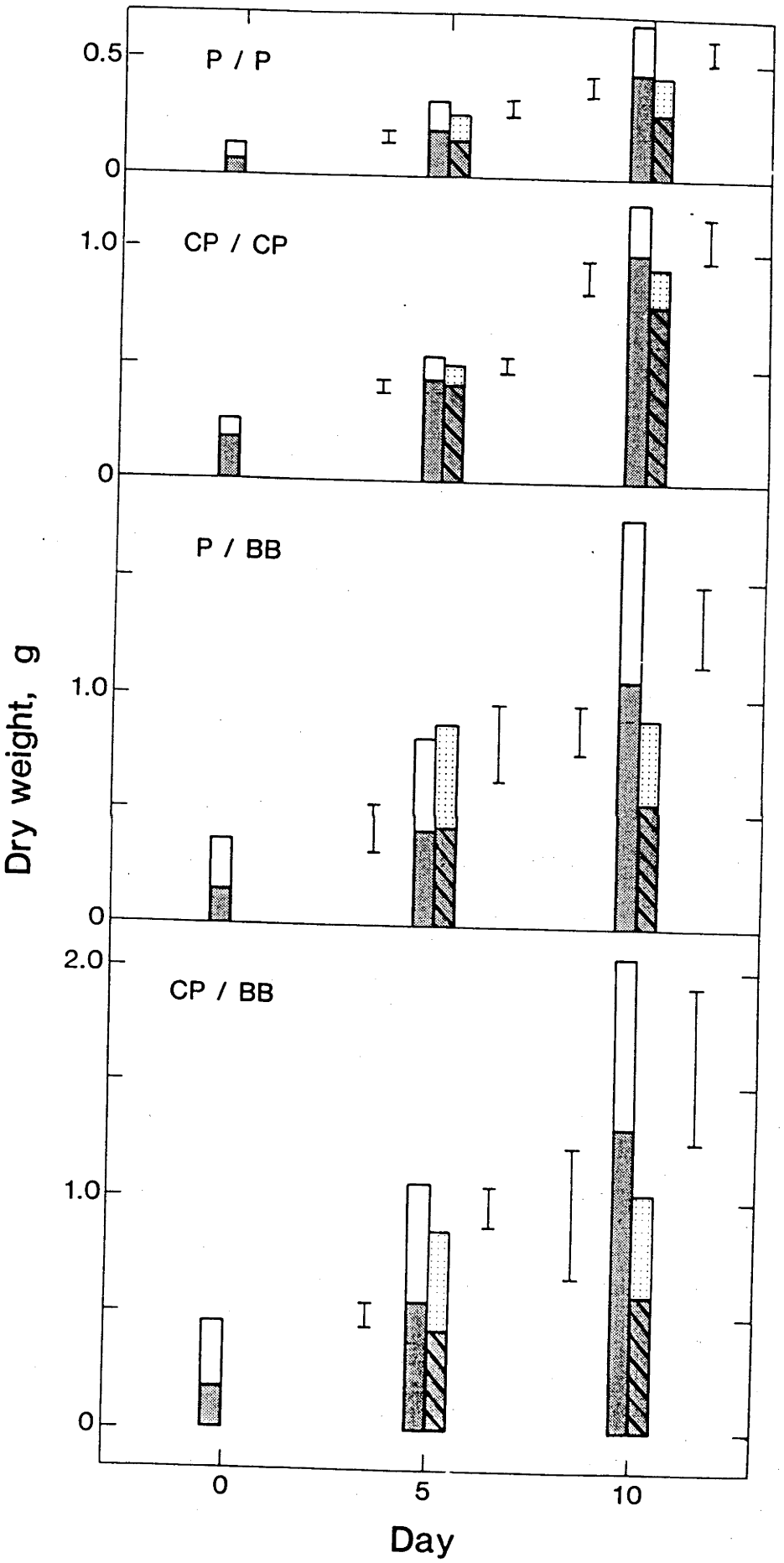

P / P
CP / CP
P / BB
CP / BB
0.5
1.0
2.0
0
Dry weight, g
0
5
10
Day

Figure 2. Change in total shoot respiration (entire histogram) and the activities of the alternative (upper part of histogram) and the cytochrome (lower part of histogram) respiratory pathways with time for control (histogram on left) and pea (P) or cowpea (CP) aphid-infested (histogram on right) pea (P), cowpea (CP) and broad bean (BB) plants. The interval to the left of a histogram pair (I) is the LSD for total shoot respiration at $\alpha = 0.05$. Each histogram represents the mean of five independent determinations. Control plant cytochrome,  and alternative,  pathway activity; and aphid-infested cytochrome,  and alternative pathway,  activity.

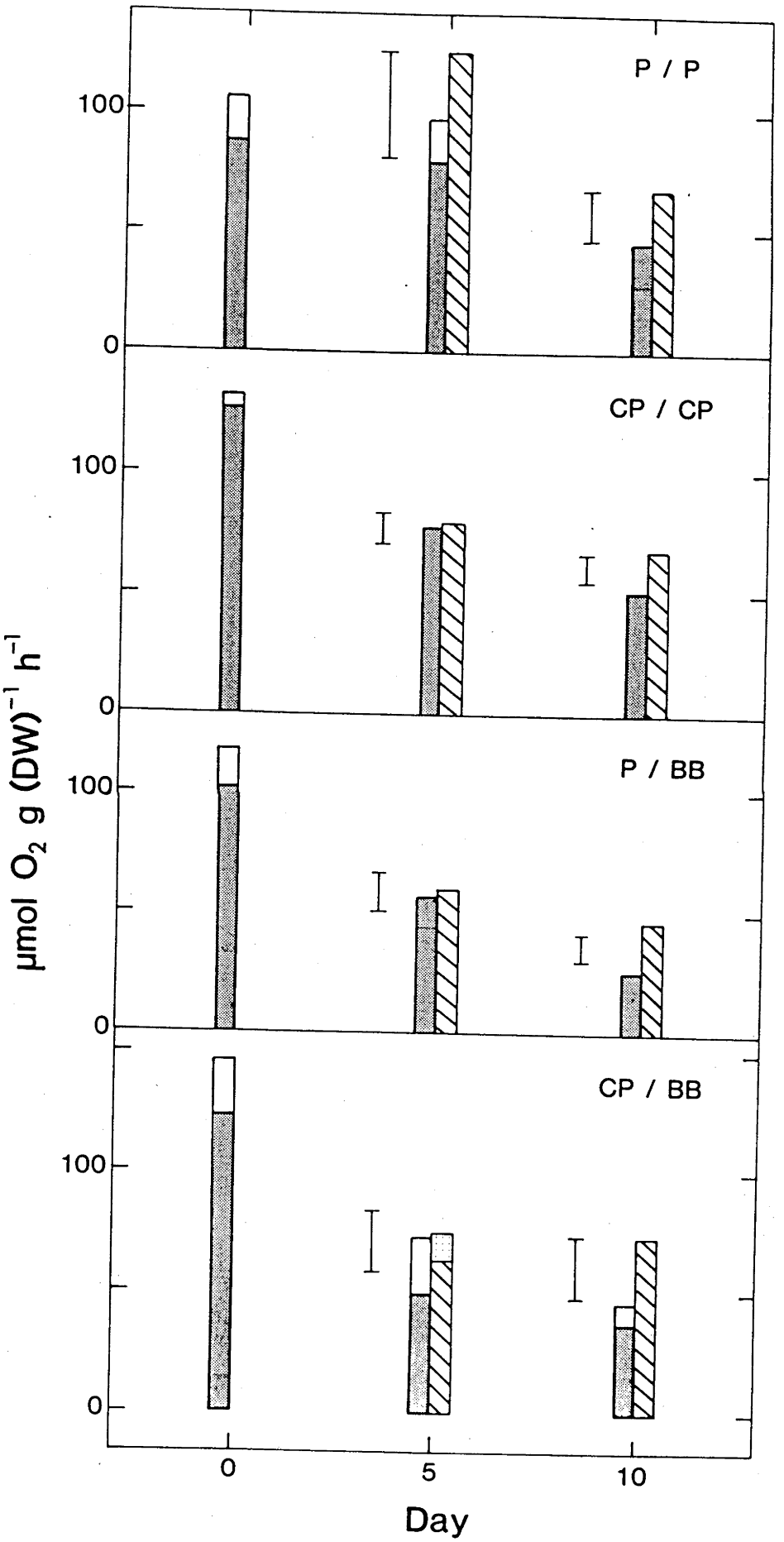

P / P
CP / CP
P / BB
CP / BB
100
0
µmol O_2 g (DW)$^{-1}$ h^{-1}
0
5
10
Day

Table 1. Change with time in the capacity (μmol O_2.g(DW)$^{-2}$.h^{-1}) of the alternative respiratory path (including residual respiration) in shoots of control (C) and aphid-infested (A) pea (P), cowpea (CP), and broad bean (BB) plants infested with pea (P) and/or cowpea (CP) aphids.

	Day of trial				
	0	5		10	
treatment	C/A	C	A	C	A
P/P	94.5	88.4	91.9	63.7	97.8
CP/CP	92.2	61.3	65.1	42.6	47.7
P/BB	108.3	56.6	53.9	25.4	39.1
CP/BB	109.1	49.4	44.2	27.8	55.3

Table 2. Change with time in the uncoupled rate (μmol O_2.g $(DW)^{-1}.h^{-1}$) of shoot respiration in control (C) and aphid-infested (A) pea (P), cowpea (CP), and broad bean (BB) plants infested with pea (P) and/or cowpea (CP) aphids.

	Day of trial				
	0	5		10	
Treatment	C/A	C	A	C	A
P/P	166.7	141.8	130.9	70.9	97.0
CP/CP	133.8	97.6	101.9	61.2	73.0
P/BB	142.4	58.2	64.4	33.4	58.1
CP/BB	157.5	77.7	75.6	43.6	70.9

(not shown). The mean residual rates of shoot respiration for control and infested plants respectively, were 25 and 22 percent for P/P, 17 and 16 percent for CP/CP, 25 and 23 percent for P/BB and 20 and 18 percent for CP/BB after 10 days.

5.4 Discussion

The significant decrease in plant, shoot and root dry weights by day 10 (Fig. 1) has been reported previously for these plant-aphid combinations (Hawkins *et al.* 1985, 1986). Hawkins *et al.* (1985) proposed that the decrease in plant growth was due to an increase in energy utilization and/or a decrease in energy production, in addition to the removal of translocate by the aphids. The major change observed in plant growth between infested and control plants from days 5 to 10 for these (Hawkins *et al.* 1985) and other plant-aphid systems (Wu and Thrower 1981) has previously been reported. The observed aphid-induced changes in plant growth are obviously typical for these systems and this assumption is also made regarding the effects of aphid feeding on shoot respiration.

A significant increase in aphid-infested shoot respiration (Fig. 2) has been reported previously (Kloft and Ehrhardt 1959; Daly 1976) and also for shoots infested with other organisms (Allen 1954; Ladd and Rawlins 1965; Uritani and Asahi 1980). Other changes to shoot respiration in response to aphid feeding have also been reported. Wood *et al.* (1985) observed decreases in respiration of up to 25 percent while Wu and Thrower (1981) noted unchanged respiration rates until the tissue became moribund, at which time respiration decreased rapidly. The differences reported for the effects of aphids on shoot respiration suggest that this may be a species specific response, rather than a general one.

The aphid-induced enhancement of shoot respiration by 133 to 199 percent indicates that this is a major energy drain for the plant. The underlying mechanism is probably a combination of many factors.

Increased rates of photosynthesis are usually accompanied by proportionally increased rates of respiration (McCree 1970) and these can be associated with increased alternative pathway activity (Azcón-Bieto *et al.* 1983b). The increased rates of respiration observed for the infested shoots (Fig. 2) could therefore be in response to aphid-induced increases in the rate of photosynthesis. The phenomenon of increased photosynthetic rates for aphid-infested leaves was reported by Way and Cammell (1970) but the reported 'norm' is for aphids to decrease photosynthetic

rates (Kloft and Ehrhardt 1959; Daly 1976; Wu and Thrower 1981; Wood *et al.* 1985). The increased rates of respiration could also be a response to metabolically active substances secreted by the aphids into the phloem, which eventually interacts with the shoot respiratory system. Aphid secretion into the phloem is well documented (Edwards and Wratten 1980).

The decrease in shoot respiration with age (Fig. 2) has been reported previously for other shoot systems (Azcón-Bieto *et al.* 1983a,b) and was ascribed to decreased cytochrome pathway activity. This was also the case in this instance (Fig. 2). Simple linear decreases in short-term root respiration was reported for these plant-aphid systems (Hawkins *et al.* 1986) but for root respiration the correlation to linearity was best for the rates observed for the roots of aphid-infested plants, rather than for control shoots. This lends support to Lambers' (1985) observation that respiration studies on shoots and roots should be conducted separately. The decrease in total shoot respiration was likely a function of tissue ageing because at an older age, the respiration rates become constant until the tissue becomes senescent (Azcón-Bieto *et al.* 1983a).

The capacity of the alternative respiratory pathway was greater in aphid-infested plant shoots than in control plant shoots by day 10 (Tab. 1), even though this pathway was no longer engaged in aphid-infested shoots (Fig. 2). Therefore, the increased respiration in aphid-infested shoots was not due to higher alternative pathway activity resulting from increased substrate supply. Azcón-Bieto *et al.* (1983b) hypothesized that this was one of the major causes of increased shoot respiration in their system. This in turn could indicate that the increased respiration found in the aphid-infested plants was not a result of increased rates of photosynthesis.

The effect of the uncoupler, CCCP, on shoot respiration (Tab. 2) indicated there was a general decrease in respiration with age and that after 10 days the infested plants had greater uncoupled respiratory rates. This is probably a direct result of their higher cytochrome pathway activity (Fig. 2).

There are three responses that may be observed when shoot respiration is uncoupled:

A) CCCP will not stimulate respiration if glycolysis and the TCA-cycle are substrate limited and not controlled by ADP levels (Blacquière and de Visser 1984).

B) CCCP will stimulate respiration via a Pasteur effect when glycolysis is restricted by adenylates (Wiskich 1980).

C) CCCP will stimulate respiration directly when the mitochondrial cytochrome chain is limited by adenylates (Azcón-Bieto *et al.* 1983c).

With this in mind, it becomes clear that the effect of aphid feeding on cytochrome pathway respiration was quite variable (Tab. 2, Fig. 2).

On day 5, respiration was stimulated by the addition of CCCP (indicating adenylate control) for control P/P and both treatments of CP/CP. Therefore, in three of the four aphid treatments, the cytochrome pathway was substrate limited by substrate supply to the mitochondria. By day 10, the regulation had changed with the cytochrome pathway, for both treatments of P/P and P/BB and the control of CP/CP, being limited by the supply of ADP. Now, mitochondria in only two aphid treatments were substrate limited. This suggests, that with time, as growth slows, cytochrome pathway activity becomes less regulated by substrate availability and more by adenylate control. The above also indicates that *in vivo* regulation of cytochrome pathway respiration is a complex phenomenon (Blacquière and de Visser 1984) and that aphid infestation was not affecting respiratory regulation *per se*.

The increased respiration, activity and capacity observed in aphid-infested plants (Fig. 2, Tab. 1, Tab. 2) could also be the result of a delay in senescence of the infested shoots. If a substance injected by the aphids into the phloem (Edwards and Wratten 1980) caused an alteration in the plants hormonal balance, senescence could be delayed and respiration rates would be maintained for a longer time. The higher rates of respiration, without alternative pathway engagement, would tend to increase ATP production. This, presumably because it is not used for increased

growth (Fig. 1), could be used for cellular maintenance associated with the aphids' feeding on the shoot. The increased energy put into maintenance could confer some advantage on the plant in the future, such as upon aphid removal or their natural decline in numbers.

The constancy of the residual respiration rate in shoots is in marked contrast to its decline observed for roots of the same species (Hawkins *et al.* 1986). This may indicate that penetration of the inhibitors and uncoupler at the concentrations used is easier or more efficient in shoot than in root tissue.

In summary, this study has shown that short-term aphid infestation does cause an increase in shoot respiration, a decrease in the carbon economy of the shoot, and that the increase in respiration was not associated with the alternative respiratory pathway. How the increase in shoot respiration was initiated is unclear, but it may be via increased rates of photosynthesis, responses to toxins injected by the aphids and/or delayed senescence. Further studies investigating some of these proposals are worthwhile and have been initiated to examine in more detail the *in vivo* regulation of shoot respiration.

CHAPTER 6

THE EFFECT OF SHORT-TERM APHID FEEDING ON THE PARTITIONING OF $^{14}CO_2$-PHOTOASSIMILATE IN THREE LEGUME SPECIES[1]

6.1 Introduction

Hawkins *et al.* (1986) proposed that the observed decreases in root growth and root respiration, due to short-term aphid infestations, were the result of decreased carbohydrate (translocate) flux to the roots. This conclusion was based on growth analyses of the infested plants (Hawkins *et al.* 1986) rather than on more sophisticated techniques such as sugar analyses and patterns of translocate distribution.

Translocation studies using $^{14}CO_2$ have been performed on aphid-infested plants (Thrower and Thrower 1966; Way and Cammell 1970; Wu and Thrower 1973; Veen 1985) but these workers have primarily been concerned with the effects of aphid feeding on the partitioning of ^{14}C-translocate in the shoot and not transport to or from the root. The same authors all observed that, to varying degrees, short-term aphid infestation did cause alterations in shoot translocation patterns. Dixon (1975) proposed that some of the aphid-induced changes in assimilate partitioning could be to the advantage of the aphid.

Change in the translocation patterns of non-aphid-infested shoot tissue is a complex phenomenon (Wardlaw 1985). It may involve hormonal interactions (Starck 1983) for phloem loading (Marre *et al.* 1974; Herold 1980; Patrick 1982), and source-sink, end-product feedback regulation (Neales and Incoll 1968; Thrower 1974; Geiger 1975; Geiger and Giaquinta 1982; Baker 1985; Wardlaw 1985).

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[1]THIS CHAPTER WAS SUBMITTED TO *CAN. J. BOT.* 15 APR 86 AND IS REFERRED TO IN THE THESIS AS HAWKINS *ET AL.* (SUBMITTED 1986B)

This investigation was conducted to determine the effect(s) of short-term aphid infestation on whole plant translocate partitioning, with special emphasis on the roots, in three well fertilized, non-nodulated legume species. A secondary aim was to observe whether the response of whole plant translocation to aphid feeding was general or plant-aphid species specific (*cf.* Hawkins *et al.* 1985).

6.2 Materials and methods

Cowpea and pea aphids, *Aphis craccivora* Koch and *Acyrthosiphon pisum* (Harris), respectively, both Homoptera: Aphididae, were obtained and maintained as previously described (Hawkins *et al.* 1985). Seeds of cowpea (*Vigna unguiculata* (L.) Walp. cv. Caloona), broad bean (*Vicia faba* L. cv. Aquadulce), and garden pea (*Pisum sativum* L. cv Victory Freezer) were obtained and raised as described by Hawkins *et al.* (1985, 1986).

The glasshouse grown plants were placed in a growth cabinet under a 16:8 h light: dark cycle (Hawkins *et al.* 1985) one day prior to the commencement of each experiment. The 14-day-old plants were divided according to size into five blocks of four plants on experiment day 0. The four plants of each block were randomly divided into two pairs; each comprising a control plant, and an experimental plant which was infested with 10, eight-day-old adult aphids. Translocation patterns were determined and the aphid distribution was recorded for one pair of plants on experimental days 5 and 10. This procedure was repeated for the other four blocks. No translocation patterns were determined on experimental day 0 because the majority of the 14-day-old plants or their leaves were too small to accommodate the translocation apparatus. The aphid-plant combinations used were P/P (pea aphids on pea), P/BB (pea aphids on broad bean), CP/CP (cowpea aphids on cowpea) and CP/BB (cowpea aphids on broad bean).

Growth analyses were not determined because it has been demonstrated repeatedly for these aphid plant combinations that 10 adult aphids placed on a 14-day-old plant significantly reduce plant growth in 10 days (Hawkins *et al.* 1985, 1986).

At noon on the day preceeding the experiment, the 10 plants to be sampled in one translocation determination were moved to an experimental growth cabinet which operated under identical environmental conditions to the holding growth cabinet (Hawkins *et al.* 1985). This ensured at least 6 h of photosynthesis prior to labelling. On the morning of the experiment, 200μl of 7.54×10^4 Bq (2μCi) $NaH^{14}CO_2$ [specific activity 6.36×10^7 Bq.mmol^{-1} (59.3 mCi.mmol^{-1}) Radiochemical

Centre, Amersham, U.K.] was put into each of 10, 24 ml capacity vials (Fig. 1) and a seal was effected between the leaf and vial. The leaf surface area exposed over the vial was 2.40 cm^2. The leaves fed were one of the primary leaves of cowpea and one of the leaves from the third or fourth leaf pair, of broad bean and pea seedlings, respectively.

The $^{14}CO_2$ was generated in the sealed vial by injecting 1.5 ml of 50% lactic acid into the vial through the rubber diaphragm. It took about three min to treat the 10 plants. The $^{14}CO_2$ was fed to the leaves for 20 min at which time it was absorbed from the atmosphere of the vial by injecting 2.0 ml of 1M KOH into the vial through the diaphragm. The vials were removed from the fed leaves and the plants were then left in the growth cabinet, in the light, for 4 h of translocation. Both feeding and translocation were done at a light intensity of 350 $\mu mol.m^{-2}.s^{-1}$.

The 10 plants were removed from the growth cabinet after 4 h and divided into component parts: leaf tissue from above and below the fed site, tissue from the fed leaf, root tissue, and a representative sample of aphids (some aphids from all areas of the plant) were collected where applicable. No aphids were taken from the fed leaf or the opposite member of the pair. Leaf tissue was sampled with a punch that removed 0.30 cm^2 discs; stems, petioles and roots were sampled using a razor blade; and aphids were removed with a fine, camel hair brush. Two leaf discs were taken from each fed leaf and one leaf disc was taken from the mid-lateral portion of all other leaves and pooled for samples from above and below the fed site. In cowpea, the below sample was from the cotyledon scar region of the stem. Roots were washed in water, blotted dry and spread out and divided into upper, mid, and lower root zones. 0.5 cm samples of root tip and of root base were randomly selected from roots originating in each of the three zones, giving a total root sample of three tips and three bases. All plants were sampled systematically: all aphid samples first, then all above fed site samples, etc. Upon removal, the sampled tissue was immediately placed in tared, closed weighing bottles for fresh weight determinations. The maximum tissue sample was usually about 50 mg.

After weighing, each tissue (except for aphids) was sliced into 1 mm strips or

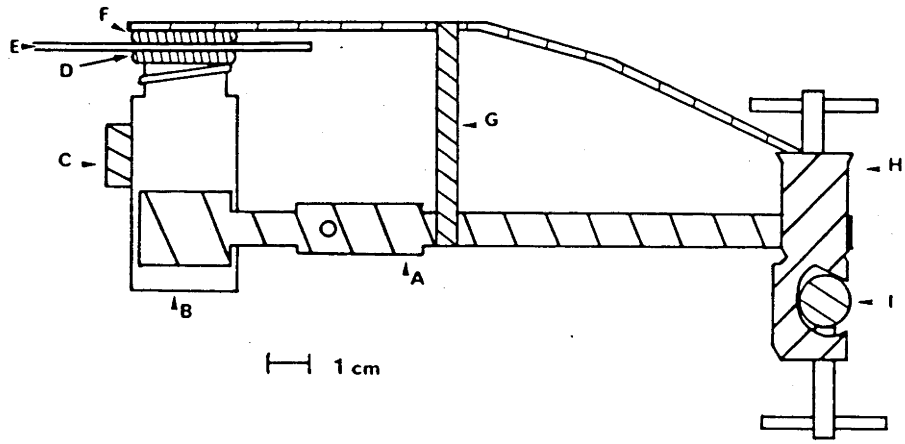

Figure 1. $^{14}CO_2$ feeding apparatus for translocation determinations. A 24 ml vial (B) held in a retort stand clamp (A) was manoeuvred under the leaf to be fed (E). The leaf was placed on top of the neoprene 'O' ring (D) and a second 'O' ring (F; that was fastened to a wire loop) was placed on the leaf directly over the 'O' ring on the vial. The seal between the leaf and the vial was effected by the tension (which did not damage the leaf tissue) provided by the elastic band (G). Chemicals were injected into the vial through the rubber diaphragm (C). Five such apparatus were attached by a clamp (H) to a supporting bar (I), one of which was on each side of the growth cabinet.

segments and placed separately into a scintillation vial. 1.5 ml of NCS^{TM} tissue solubilizer (Amersham/Searle, Arlington Heights, IL, U.S.A.) was then added to each sample. The vials containing tissue and solubilizer were placed in a water bath at 50 $\pm$ 1°C for 48 h at which time they were removed and 450 μl of benzoyl peroxide decolorizer (1.0 g benzoyl peroxide in 5 ml of toluene) was added. The samples were digested for another 1 h, removed from the water bath and cooled to room temperature, after which, 10.0 ml of the scintillation cocktail [44 ml Permafluor$^{(R)}$ 1 (Packard Instrument Co., Downers Grove, IL, U.S.A.; 125 g.l^{-1} PPO (2,5-diphenyloxazole), 2.5 g.l^{-1} POPOP (1,4 bis[5-phenyl-2-oxazolyl]-benzene)), 319 ml Triton-X100, and 637 ml toluene] was added. Samples were allowed to settle overnight, in the dark, at room temperature before counting was begun.

Sample radioactivity was determined with a Beckman$^{(R)}$ LS-7500 liquid scintillation spectrometer (Beckman Instruments, Irvine, CA, U.S.A.) with automatic quench compensation activated. The ^{14}C was measured in the 397-655 energy window at efficiencies of 75-85 percent.

The radioactivity of each sample was corrected for background and normalized to one g fresh weight of tissue. Radioactivity for each sample was summed to give total plant (including aphids where applicable) recovered radioactivity. Each sample was then expressed as percentage of total plant recovered radioactivity.

Calculation of means and two-way analysis of variance (ANOVA) at $\alpha = 0.05$, on %TPR were done using the GENSTAT package (Statistics Department, Rothamsted Experimental Statiom, U.K.). Analysis of residual versus fitted values for the ANOVA model indicated that data transformation was not required (Netter and Wasserman 1974). The protected LSD (least significant difference) was calculated (Snedecor and Cochran 1980), at $\alpha = 0.05$, for each pair of means.

6.3 Results

All data presented are representative of the results of replicate experiments (2 treatments X 5 blocks).

The percentage of total plant recovered radioactivity was not significantly different between fed leaves of control and aphid-infested plants (data not shown). However, fed leaf radioactivity accounted for such a large amount of the total recovered plant radioactivity that it was not included (*cf.* Cralle and Heichel 1985) in the %TPR (total plant recovered radioactivity) data that follow.

Control plants had significantly greater %TPR in the roots than infested plants of P/BB and CP/CP by day 5 and this relationship was significant for all plant-aphid combinations by day 10 (Fig. 2). By day 5, control CP/CP had significantly higher %TPR for tissue from below the fed leaf, whereas the relationship was variable and not significant for the other combinations (Fig. 2). All control plants had higher %TPR in tissue from below the fed site than aphid-infested ones by day 10, but this was only significant for P/P and CP/BB (Fig. 2). The %TPR for tissue from above the fed leaf was significantly greater in control plants for P/BB and CP/BB and in aphid-infested plants for CP/CP by day 5 (Fig. 2). By day 10, all the control plants had significantly higher %TPR for tissue from above the fed leaf than the aphid-infested plants (Fig. 2). A large portion of the %TPR was found in the aphids on day 5 and this increased significantly (data not shown) for all plant-aphid combinations between days 5 and 10 (Fig. 2).

A brief description of where the aphids were distributed on the plants on days 5 and 10 and any differences observed in growth form between aphid-infested and control plants are presented in Table 1.

Aphid-infested plant %TPR were recalculated, ignoring the radioactivity ingested by the aphids, to determine if translocation partitioning was changed when the basis of comparison was the same for control and aphid-infested plants. The %TPR going to the roots was the same or greater in controls on day 5 and was greater in all cases on day 10, but on day 10 it was only significant for P/P and

Figure 2. %TPR on experimental days 5 and 10 for aphid-infested and control plants' root tissue, tissue from below the fed leaf, tissue from above the fed leaf, and for aphids of all four plant-aphid combinations. The LSD ($\alpha = 0.05$) for the experimental tissue and the control tissue to its left is the vertical line over the experimental tissue. P/P, P/BB, pea aphids on pea and broad bean plants; CP/CP, CP/BB, cowpea aphids on cowpea and broad bean plants. Control tissue,  ; experimental tissue,  ; aphid tissue,  .

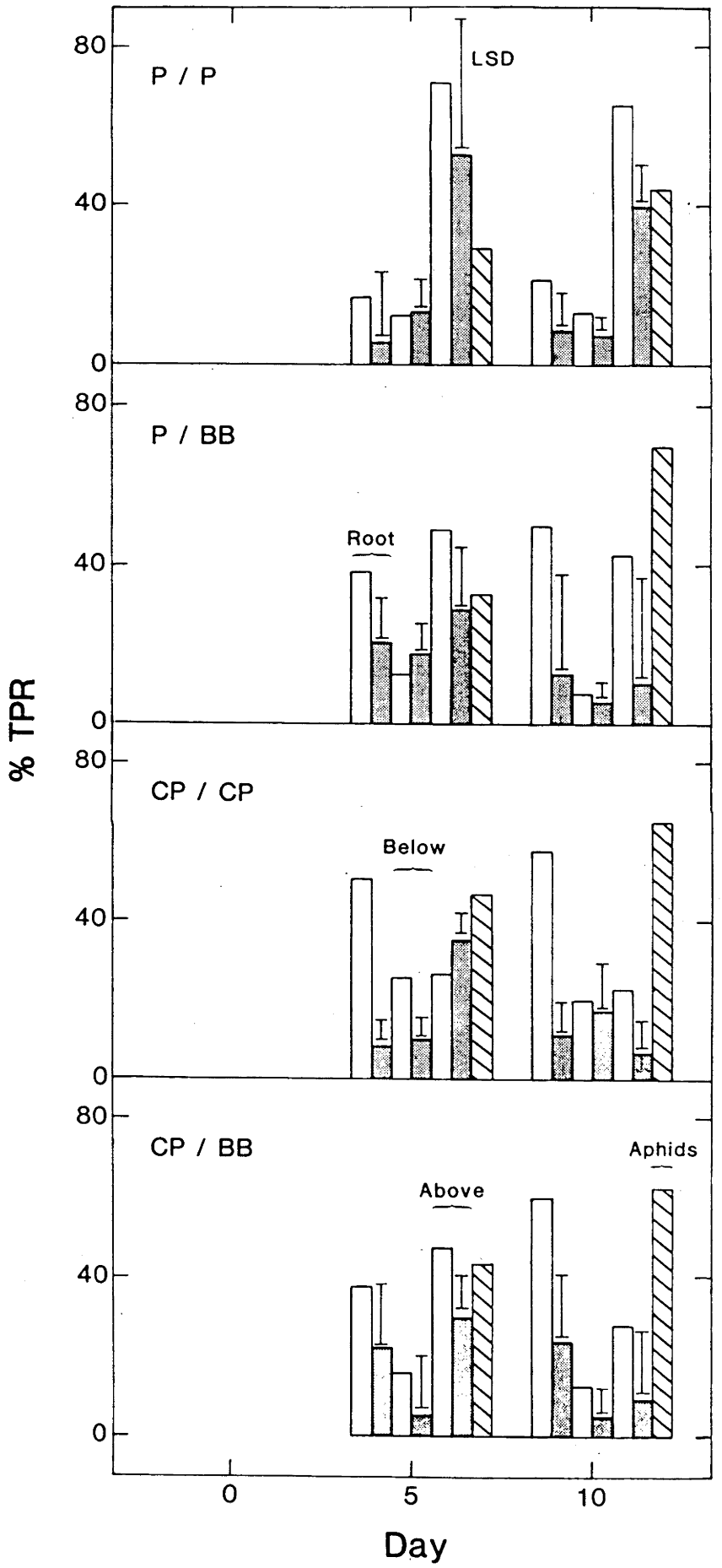
P / P
LSD
P / BB
Root
CP / CP
Below
CP / BB
Above
Aphids
80
40
0
% TPR
0
5
10
Day

Table 1. Descriptions of the plants and the location of the aphids for the four plant-aphid combinations on experimental days 5 and 10.

Trial[#]	Day 5	Day 10
P/P	Most aphids below FS	Most aphids below FS
P/BB	Aphids all over plant but more below FS Some secondary mainstem branching	As for day 5, but greater proportion below FS More secondary mainstem branching
CP/CP	Most aphids above FS	Most aphids above FS but some between primary leaves and cotyledon scar
CP/BB	Aphids approximately equally distributed over plant Some secondary mainstem branching	Aphids distributed as on day 5 More secondary mainstem branching

#, Abbreviations: P/P and P/BB, pea aphids on pea and broad bean plants, respectively; CP/CP and CP/BB, cowpea aphids on cowpea and broad bean plants, respectively; FS, feeding site where the $^{14}CO_2$ was fed to the plant.

CP/CP (Fig. 3). The %TPR from below the fed leaf was variable, with only control CP/CP being significantly higher on day 5 (Fig. 2). By day 10, all the aphid-infested plants had higher %TPR for tissue from below the fed site than controls and this was significant for P/BB and CP/CP (Fig. 3). The partitioning of %TPR above the fed leaf was variable (Fig. 3). Control CP/CP %TPR was significantly greater on day 5 while on day 10, %TPR from above the fed site of control P/BB and CP/CP and aphid-infested P/P and CP/BB were greater than their respective counterparts (Fig. 3).

The distribution of %TPR in the major organs of cowpea, both control and aphid-infested plants, on day 10 is presented in Table 2. Again, the aphids accounted for the bulk of %TPR and control plant roots had significantly higher %TPR, either accounting for or ignoring uptake by the aphids (Table 2). The difference observed in partitioning when the aphids were removed from the calculation between this experiment (Table 2, AR) and the other CP/CP trials (Fig. 3) was that the aphids were concentrated above the fed leaf in this experiment (data not shown).

Figure 3. %TPR recalculated for tissue from aphid-infested plants (omitting the label taken up by the aphids) and for control plants' (same as Fig. 2) root tissue, tissue from below the fed leaf, and tissue from above the fed leaf on experimental days 5 and 10 for all four plant-aphid combinations. The LSD ($\alpha = 0.05$) for the experimental tissue and the control tissue to its left is the vertical line over the experimental tissue. Abbreviations as in Fig. 2. Control tissue,  ; experimental tissue,  .

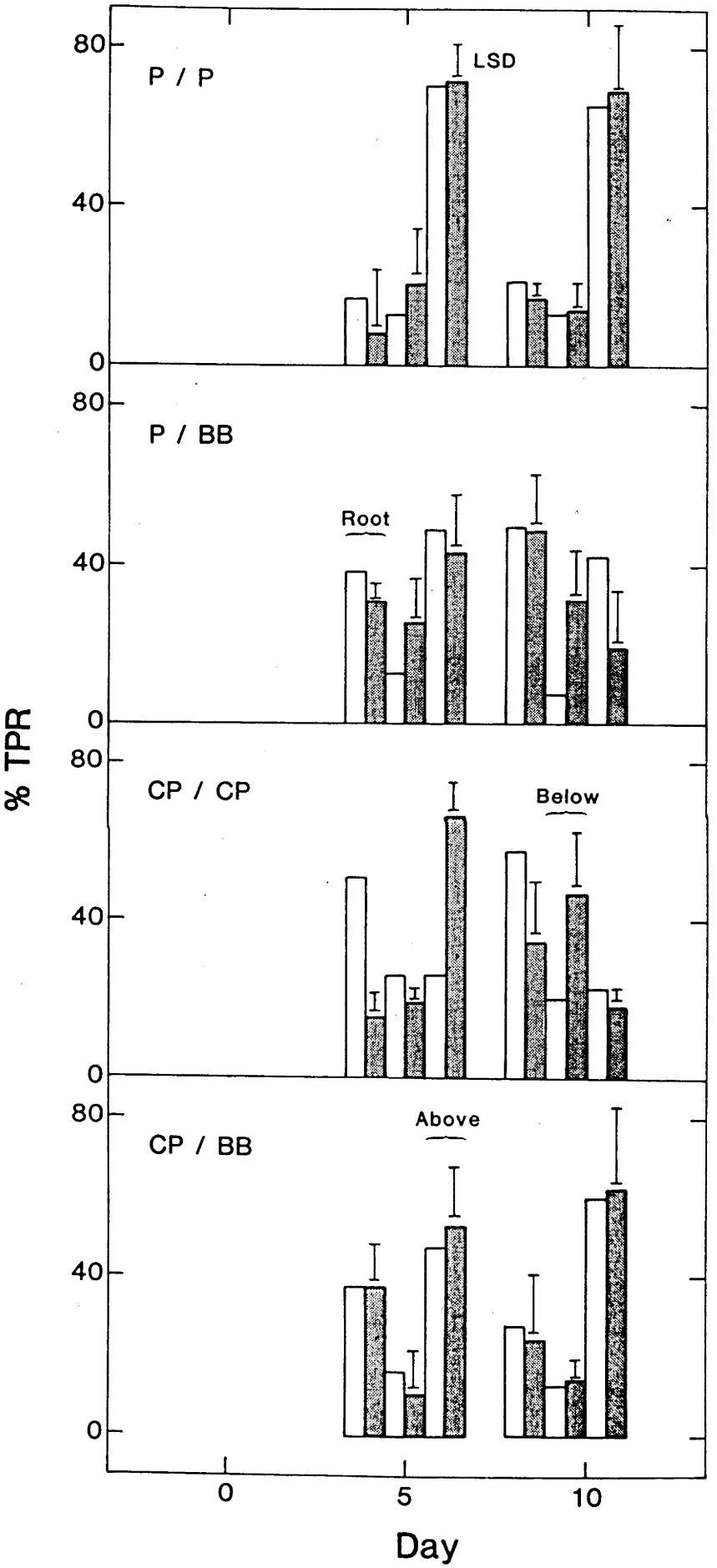
80
40
0
P / P
LSD
P / BB
Root
CP / CP
Below
CP / BB
Above
% TPR
0
5
10
Day

Table 2. Partitioning of photoassimilate into each tissue region of the plant and the LSD for aphid-infested and control cowpea plants where $^{14}CO_2$ was fed to one of the primary leaves after 10 days infestation.

Tissue region	%TPR# A	%TPR# C	%TPR# AR
3rd trifoliate		25.06	
" "		17.09	
2nd trifoliate	4.16	3.49	18.50
LSD	15.85	4.36	
" " petiole	6.55	3.76	26.54
LSD	13.52	23.36	
" " " aphids	6.83		
1st trifoliate	2.38	1.25	9.30
LSD	9.69	12.88	
" " aphids	1.36		
" " petiole	2.47	4.15	14.38
LSD	13.06	19.04	
" " " aphids	43.82		
opposite primary	0.51	2.16	3.16
LSD	5.52	5.46	
" " aphids	1.57		
cotyledon scar	3.17	12.51	12.86
LSD	17.65	17.09	
" " aphids	22.78		
upper root zone	2.59	11.57	9.80
LSD	8.88	3.31	
lower root zone	0.78	18.95	5.47
LSD	5.97	12.91	
	Summary$		
above fed site	15.56	54.80	68.72
below fed site	3.68	14.67	16.02
root zone	3.37	30.52	15.27
aphids	77.36	0.0	

Abbreviations: %TPR, percentage plant recovered radioactivity; A, aphid-infested plant; C, control plant; AR, aphid radioactivity removed from %TPR calculation to allow a direct comparison between C and AR plant tissue.

$ A summary of the data from above is presented for the four main tissue regions

6.4 Discussion

The significant reduction in %TPR (percentage of total plant recovered radioactivity) after 10 days for the aphid-infested plants (Fig. 2) in comparison to controls indicates that aphid feeding was reducing the flow of assimilate to the roots. Aphids could reduce translocate flux to the roots either by imbibing carbohydrates normally destined for the roots, by redirecting the flow of translocate within the plant away from the roots, or both. The decline in assimilate flux to the roots (Fig. 2) supports the proposal of Hawkins *et al.* (1986) that the observed decline in infested plant root respiration was a result of a decreased supply of carbohydrates to the roots. The marked decrease in translocation to the roots between days 5 and 10 (Fig. 2) parallels and possibly explains the significant reductions in root growth and root respiration that occurred between those days in previous experiments (Hawkins *et al.* 1985, 1986). Wu and Thrower (1981) also observed that similar major perturbations to their plant-aphid system were manifested during this time period.

The higher %TPR found in control plants compared with aphid-infested ones for tissue taken from below the fed leaf after 10 days (Fig. 2) was a result of a greater translocate flux to the roots of control plants since aphid feeding reduced that in the experimental plants. Under normal conditions, mature leaves below the fed site are not sinks but sources (Thrower 1974; Ismail and Sagar 1981). This probably explains non-significant differences of %TPR from below the fed site in plants other than cowpea. In cowpea, the sample site was not a leaf but the cotyledon scar region of the stem and hence the higher %TPR of samples from this region in control plants was probably a reflection of the amount of translocate going to the roots.

Initially, the change in %TPR from the region above the fed leaf was variable (Fig. 2), but again, by day 10 the control plants all had significantly higher %TPR than infested ones. This indicates that aphid feeding prevented translocate moving from the fed source leaf to its natural sinks, the expanding leaves above (Geiger and Giaquinta 1982). The simplest and most obvious explanation is that the

aphids removed this translocate from the phloem stream and thereby prevented it from reaching the expanding leaves. This is supported by the significant proportion of the total label found in the aphids on days 5 and 10 (Fig. 2).

There was a significant increase in %TPR found in the aphids between days 5 and 10 (Fig. 2). There are at least two possible explanations for the increase:

i) On day 5, the aphid population consisted of few adults and more but about equal numbers of first through fourth instar nymphs while on day 10, there were a large number of adults and first and second instar nymphs with smaller numbers in the other instars. If the larger adults ate proportionally more than the nymphs, possibly due to a greater development of their cibarial pump, the aphid %TPR for the day 10 sample should be greater than for the day five one.

ii) As the aphid sample was representative of the aphid population structure on the plant and did not include aphids from the fed leaf, it is possible that the stylet length of the nymphs prevented them from reaching the larger phloem elements. It is known that stylet length is very important in determining the feeding site in aphids (Gibson 1972; Dixon and Logan 1973; Dixon 1975) and for a gall forming aphid, *Neothoracaphis yanonis* (Matsumura), the adults feed on the phloem but the young can not because their stylets are too short (Sorin 1966). This too could account for the higher %TPR in the day 10 aphid sample.

The advantage of removing the aphids' contribution to total recovered plant radioactivity from the calculation is that control and infested plants are then being compared on a more equitable basis. Control plants still had greater fluxes of carbohydrates to the roots than aphid-infested ones on days 5 and 10 (Fig. 3), even though the difference was only significant for P/P and CP/CP on day 10. A possible reason for the control broad bean plants not having significantly higher root %TPR than roots of infested plants is that roots of non-infested broad bean are capable of re-exporting labelled assimilate to the high demand sinks of the shoot within 1 to 3 h of labelling the fed leaf (Ismail and Sagar 1981). The significant reductions observed for aphid-infested broad bean root growth and root respiration

(Hawkins *et al.* 1985, 1986) makes it unlikely that these plants would be re-exporting carbohydrates to the shoot. Nevertheless, the reduced flux of photoassimilate to the roots of all aphid-infested plants, with time, should account for the reductions in root growth and root respiration of these plant-aphid combinations (Hawkins *et al.* 1985, 1986).

By day 10 in the aphid-infested plants, all of the tissue from below the fed site had higher %TPR than their respective controls (Fig. 3) when the aphid contribution to %TPR was removed but the difference was only significant for P/BB and CP/CP. This was surprising because the result for this tissue region was the opposite when aphid ^{14}C-label was taken into account (Fig. 2). The change in this relationship between control and infested tissue indicates that the aphids were inducing a change in the partitioning of photoassimilate. The absence of a significant difference for P/P (Fig. 3) could be related to the lower aphid %TPR found for these plants (Fig. 2). The almost even aphid distribution over the shoots of CP/BB (Table 1) could be responsible for the similar values obtained in this system (Fig. 3).

In both cases where the difference for %TPR of tissue from below the fed leaf were significant and for P/P (Fig. 3), there was a noticeable number of aphids feeding in the region (Table 1). This could indicate that at least in two, if not all cases, the aphids were inducing leaves that normally would be sources, to become translocate sinks. The region of the plant below the fed site is not normally a sink (Thrower 1974; Ismail and Sagar 1981). Imbibition of radioactivity by aphids feeding on sink leaves has been reported previously for other aphid-plant systems (Thrower and Thrower 1966; Canny and Askham 1967; Way and Cammell 1970; Wu and Thrower 1973). Tissue from below the fed leaf accounts for the smallest %TPR in control plants (Fig. 3) while in the aphid-infested ones, P/P and CP/BB responded as controls did but for P/BB and CP/CP the shoots accounted for the smallest %TPR. This indicates that aphids can induce a redirection in the normal partitioning of assimilate. Starck (1983) proposed that a redistribution of translocate was a primary response to a change in the supply-demand balance of the plant.

The distribution of %TPR in tissue from above the fed leaf was quite variable on day 10 (Fig. 3) and appears to be related to where the aphids fed on the plant (Table 1). The amount of translocate moving up the plant was significantly reduced when aphids fed primarily below the $^{14}CO_2$-fed site except for P/P (Fig. 3). These data indicate that aphid feeding can result in a redirection of the normal source-sink translocation relationships. A closer look at the CP/CP system may illuminate some possible mechanisms for the redirection of translocate.

The flow of translocate to the roots was significantly reduced (Table 2), whether aphids were included in the calculation or not, especially to the lower, actively growing root zone. The large amount of label accounted for in the %TPR of the aphids is another prominent feature of the system (Table 2). The topmost trifoliate leaf of both control and experimental plants accounted for more than 40 percent of the %TPR but in the aphid-infested tissue, the petiole had a higher %TPR than the trifoliate (Table 2). This occurred because in cowpea, as opposed to the other three aphid-plant systems, aphids feed preferentially on petioles, stems and then leaves (data not shown). This again indicates that aphids can induce a change in the partitioning of plant photoassimilate. The similarity in %TPR for tissue from below the fed leaf (Table 2, AR) arose because very few aphids were feeding in this region. These data (Table 2) illustrate that feeding aphids are capable of preventing significant translocate from reaching the roots, of redirecting translocate from normal sinks in the shoot region, and of inducing the conversion of sinks to sources. The question of what possible physiological mechanisms could account for these observations remains.

Roots are a considerable distance from their sources and the downward flux of translocate could be reduced by the aphids imbibing assimilate in passage and destined for the roots. This would require no direct biochemical interaction between aphids and roots and probably would be sufficient to account for the reduction in root %TPR (Figs. 1 and 2, Table 2) and root respiration (Hawkins *et al.* 1986).

A redirection of photoassimilate in the shoot and/or conversion of former sources to sinks requires some kind of direct biochemical communication between the

aphid and the host plant because source-sink interactions are more than direct regulation by end-product inhibition (Wardlaw 1985). For example: it is known that the allocation of photosynthate is under hormonal control (Starck 1983); that phloem loading [the definition of a source (Baker 1985)] and the transport of photosynthate can be enhanced by cytokinins and auxins (Marre *et al.* 1974; Herold 1980; Patrick 1982); that the rate of phloem transport is dependent on the activity of the sink (Thrower 1974; Geiger and Giaquinta 1982); that aphids can inject plant hormones into phloem elements *via* their saliva (Miles 1968a,b); and that aphid infestation can result in increased levels of cytokinins in the roots and growth inhibitors in the shoots, and decreased concentrations of growth promoters in the shoots (Hussain *et al.* 1973). The increased lateral branching observed in aphid-infested broad bean (Table 1) also indicates aphid-induced changes in the cytokinin to auxin ratios of these plants (Matthysse and Scott 1984).

If phloem loading is regulated by cytokinins and auxins, for aphids to 'turn off' a source and convert it into a sink, they would have to initiate a decrease in local cytokinin and auxin concentrations. This does seem to be a distinct possibility (Hussain *et al.* 1973). Aphids, in themselves, are considered to be 'physiological sinks' on the plant (Way and Cammell 1970). This coupled with the ability to turn sources off (aphids receive not only the photosynthate of the source but that which it can import as well) would alter the normal partitioning of translocate.

The idea of inducing sources to become sinks seems counter-productive to the well-being of the aphid (*cf.* Dixon 1975). However, it is generally agreed that an increase in the sink to source ratio (the case here) will lead to increased export from the sources (Geiger and Giaquinta 1982) which in turn can lead to increased photosynthesis (Sweet and Wareing 1966; Neales and Incoll 1968; Peet and Kramer 1980; Wardlaw 1985). An increase in photosynthesis is compatible with increased phloem loading and transport because it has been demonstrated that cytokinins and auxins can promote photosynthetic unit synthesis (Buschmann and Lichtenthaler 1977) and increased net rates of photosynthesis (Li and Proctor 1984). This implies that if the auxin and cytokinin concentration is lowered in the leaves that are

converted to sinks it must be increased in those leaves that remain sources. Wareing *et al.* (1968) concluded that an increase in the sink-source ratio could promote the export of cytokinins from the roots.

Hawkins *et al.* (submitted 1986a) have observed significant increases for whole shoot respiration in aphid-infested plants of all these plant-aphid combinations and attributed it to either increased rates of photosynthesis and/or maintenance respiration. Unfortunately, the design of the present study precludes any direct comparison being made between shoot translocation and whole shoot respiration. The phloem loading step involves a protonated carrier (Baker 1985) and the proton gradient is maintained by a proton extrusion pump (Geiger and Giaquinta 1982; Giaquinta 1983). Therefore, it is attractive to speculate that the increase in infested shoot respiration (Hawkins *et al.* submitted 1986a) is in response to increased phloem loading in the remaining source leaves.

This investigation has shown that short-term aphid feeding results in a decreased flux of translocate to the roots of affected plants, a change in the partitioning of assimilate in affected shoots, and the induction of sources to become sinks. Some of these effects appear to be directly related to the imbibing of translocate by aphids, while others appear to be a complex combination of interactions involving aphid saliva, plant hormones and source-sink levels of photoassimilate. The effect of aphid feeding on translocation appears to be a plant-aphid species specific phenomenon except for the decreased rate of translocation to the roots. The proposal that aphid feeding results in increased rates of photosynthesis in source leaves is being pursued.

CHAPTER 7

SHORT-TERM EFFECTS OF APHID FEEDING ON PHOTOSYNTHETIC CO_2 GAS EXCHANGE AND DARK RESPIRATION IN LEGUME LEAVES[1]

7.1 Introduction

Hawkins *et al.* (submitted 1986a) proposed that the observed increase in legume shoot dark respiration in response to short-term aphid infestation was paralleled by an increase in net photosynthesis. This was based on McCree's (1970) observation that increased rates of photosynthesis are accompanied by proportionate increases of dark respiration. However, the view generally held is that aphid feeding reduces the rate of photosynthesis and increases the dark respiration rate (Kloft and Ehrhardt 1959; Daly 1976; Uritani and Asahi 1980). Wood *et al.* (1985) did report decreases in rates of both net photosynthesis and dark respiration for aphid-infested pecan leaves, while Way and Cammell (1970) reported that aphid infestation could increase photosynthesis.

If aphid feeding does increase the sink-source ratio of infested plants, this will lead to increased export from the source leaves (Geiger and Giaquinta 1982; Giaquinta 1983). This can, in turn, lead to increased rates of photosynthesis (Wardlaw 1985) because normally leaves are not operating at peak capacities (Maggs 1964). The notion of photosynthetic rate being regulated by leaf carbohydrate levels was first proposed over a hundred years ago (Boussingault 1868), recently refined (Neales and Incoll 1968), and is now accepted with the proviso that it also involves phytohormone regulation (Starck 1983).

This study was conducted to determine the effects of short-term (6 to 9 days)

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aphid infestation on the rates of net photosynthesis and dark respiration in single attached leaves (which had undertaken most of their development under conditions of aphid infestation) of three well fertilized, non-nodulated legume species. Another aim was to observe whether these responses were plant-aphid species specific or general.

Abbreviations:

CER, net CO_2 exchange rate; CP/BB, cowpea aphids on broad bean; CP/CP, cowpea aphids on cowpea; IRGA, infra-red gas analyzer; LAW, leaf area to leaf weight ratio; P/BB, pea aphids on broad bean; P/P, pea aphids on pea; and R_D, dark respiration rate.

7.2 Materials and methods

Seeds of cowpea [*Vigna unguiculata* (L.) Walp. cv. Caloona], broad bean (*Vicia faba* L. cv. Aquadulce), and garden pea (*Pisum sativum* L. cv. Victory Freezer) and pea and cowpea aphids, *Acyrthosiphon pisum* (Harris) and *Aphis craccivora* Koch, respectively, both Homoptera: Aphididae, were obtained and raised as described and modified by Hawkins *et al.* (1985, 1986).

The plants were moved from the glasshouse to a growth cabinet on the day prior to the commencement of the experiment and were then grown under the environmental conditions described by Hawkins *et al.* (1985). For each aphid-plant combination the 14-day-old plants were divided according to size into five blocks of two plants on experimental day 0. Each pair of plants was randomly divided to include a control, and an experimental plant with 10, 8-day-old adult aphids placed on it. Plant aphid combinations utilized were CP/BB, CP/CP, P/BB and P/P.

On the day prior to photosynthetic and dark respiration rate determinations, the pairs of plants to be measured the following day were moved from the holding growth cabinet to the experimental growth cabinet which was under a similar environmental regime as the holding cabinet. This insured that all plants had a minimum period of 6 h of photosynthesis in the experimental growth cabinet prior to determinations of photosynthesis and dark respiration rates (*cf.* Hawkins *et al.* 1986). Rate measurements were carried out after 6, 7, 8 or 9 days of aphid feeding because this is when major physiological changes start to manifest themselves (Wu and Thrower 1981; Hawkins *et al.* 1985). Three pairs of plants were randomly selected for rate determinations of each plant-aphid combination and no more than two pairs of plants from any one plant-aphid combination were tested on any one day. This procedure was repeated in its entirety at a later date.

Measurements of steady-state net CO_2 exchange rates (CER) and dark respiration rates (R_D) of the centre leaflet of the first trifoliate, and one leaf from the third or fourth leaf pair, for cowpea, broad bean and pea plants, respectively, were made using an open gas exchange system. This ensured that leaves from infested plants had undertaken most, if not all, of their development while the plant

was aphid-infested. The appropriate attached leaf (with aphids removed if present) was sealed into a 23 ml Plexiglass(R) leaf chamber. Temperature was regulated to 23 $\pm$ 1 °C by water circulated from a water bath. Prior to connecting the leaf chamber to the IRGA (Infra-red gas analyzer, Model 225 MKII, Analytical Development Co., Ltd., Hoddeson, U.K.) system, the enclosed leaf was allowed to equilibrate for at least 20 min at 23 °C in the light (photon flux density of 315 μmol.m^{-2}.s^{-1}), with air from within the growth cabinet being pumped past the leaf at a rate of 2.0 l.min^{-1}. After equilibration, the leaf chamber was connected to the IRGA system whose source of CO_2 variable air was drawn from inside the growth cabinet.

The above design allowed steady-state CER (determined twice) and R_D to be measured under the CO_2, light (315, instead of 350 μmol.m^{-2}.s^{-1}), temperature (23.0 $\pm$ 0.5 °C) and relative humidity (not regulated in these cabinets) regimes that the plants had been growing under since the aphids had been placed on the experimental plants.

After rate determinations, the leaf(let) was removed from the plant and its area and dry weight were determined (Hawkins *et al.* 1985). CER (mean of the two steady-state rates) and R_D were calculated on both an area and a dry weight basis. The mean rates for each treatment of each plant-aphid combination were calculated using both experiments (3 + 3 plants). Analyses of variance (ANOVA) were used to compare means, $\alpha = 0.05$, and protected least significant differences (LSD) were calculated (Snedecor and Cochran 1980), $\alpha = 0.05$.

7.3 Results

Net CO_2 exchange rates (CER) were significantly greater in leaves from aphid-infested plants when expressed on a weight or an area basis, except for P/P when the area calculation was used (Table 1). The percentage increases in infested leaf CER over that of controls on a weight and an area basis were respectively 66 and 76 % for CP/CP, 14 and 25 % for CP/BB, 24 and 30 % FOR P/BB, and 23 and 9 % for P/P.

The leaf area to weight ratio (LAW), the inverse of specific leaf weight, was not significantly different between control and experimental leaves (Table 2).

CP/CP and P/P aphid-infested plants had significantly greater dark respiration rates (R_D) than controls, whether expressed on a weight or an area basis (Table 3). The percentage increases in infested leaf R_D over control leaf R_D on a weight and an area basis were 116 and 129 % for CP/CP, 53 and 61 % for CP/BB, 106 and 113 % for P/BB, and 110 and 176 % for P/P. R_D as a percentage of CER was greater in aphid-infested than in control plants (Table 4).

When the mean net daily carbon gain of the leaf $[(CER.h^{-1} \times 16h.day^{-1}) - (R_D.h^{-1} \times 8h.day^{-1})]$ was calculated for each plant-aphid combination (Table 5), leaves from aphid-infested plants had greater net daily gains than control leaves. Again, except for P/P, the percentage increase was greater when expressed on a leaf area basis.

Table 1. Mean net CO_2 exchange rates (CER) of control (C) and aphid-infested (A) plants. Each value is the mean of 6 independent observations. Results are expressed on both a leaf weight and a leaf area basis.

Trial	CER			
	$\mu mol\ CO_2.h^{-1}.g(DW)^{-1}$		$\mu mol\ CO_2.h^{-1}.dm^{-2}$	
	C	A	C	A
CP/CP¢	489	809	22.4	39.4
LSD$	85		5.4	
CP/BB	516	588	25.5	31.8
LSD	40		1.5	
P/BB	505	623	26.2	34.0
LSD	84		5.7	
P/P	568	700	28.9	31.6
	38		(4.0)*	

¢, Abbreviations: CP/CP and CP/BB, cowpea aphids on cowpea and broad bean; P/BB and P/P, pea aphids on broad bean and pea.

$, LSD at $\alpha = 0.05$.

*, If LSD is enclosed, ( ), it is not significant.

Table 2. Mean leaf area to leaf weight ratio (LAW) of control (C) and aphid-infested (A) plants. Each value is the mean of 6 independent observations.

Trial	LAW $dm^2 \cdot g(DW)^{-1}$		LSD
	C	A	
CP/CP¢	21.95	21.56	(3.03)$*
CP/BB	20.48	18.51	(1.69)
P/BB	19.27	18.67	(1.89)
P/P	19.71	22.64	(4.43)

¢, Abbreviations: CP/CP and CP/BB, cowpea aphids on cowpea and broad bean; P/BB and P/P, pea aphids on braod bean and pea.

$, LSD at $\alpha = 0.05$.

*, If LSD is enclosed, ( ), it is not significant.

Table 3. Mean dark respiration (R_D) of control (C) and aphid-infested (A) plants. Each value is the mean of 6 independent observations. Results are expressed on both a leaf weight and a leaf area basis.

Trial	R_D			
	μmol $CO_2.h^{-1}.g(DW)^{-1}$		μmol $CO_2.h^{-1}.dm^{-2}$	
	C	A	C	A
CP/CP¢	114.5	247.1	5.21	11.93
LSD$	48.6		2.88	
CP/BB	63.9	98.0	3.37	5.43
LSD	(38.3)*		(2.57)	
P/BB	59.3	122.7	3.13	6.66
LSD	(66.7)		(3.78)	
P/P	72.6	153.0	3.84	6.76
LSD	49.4		2.23	

¢, Abbreviations: CP/CP and CP/BB, cowpea aphids on cowpea and broad bean; P/BB and P/P, pea aphids on broad bean and pea.

$, LSD at $\alpha = 0.05$.

*, If LSD is enclosed, ( ), it is not significant.

Table 4. Mean dark respiration rate as a percentage of mean net CO_2 exchange rate calculated on both a leaf weight and a leaf area basis for control (C) and aphid-infested (A) plants.

Trial	Percentage			
	Weight		Area	
	C	A	C	A
CP/CP¢	23	31	23	30
CP/BB	12	17	13	17
P/BB	12	20	12	20
P/P	13	22	13	21

¢, Abbreviations: CP/CP and CP/BB, cowpea aphids on cowpea and broadbean; P/BB and P/P, pea aphids on broadbean and pea.

Table 5. Mean net daily carbon gain ((CER x 16h) - (R_D x 8h)) and percentage increase in this value for aphid-infested (A) plants over controls (C). Results are expressed on both a leaf weight and a leaf area basis.

Trial	Mean net daily carbon gain			
	µmol $CO_2.h^{-1}.g(DW)^{-1}$		µmol $CO_2.h^{-1}.dm^{-2}$	
	C	A	C	A
CP/CP¢	6908	10967	316	535
%?	59		69	
CP/BB	7745	8624	381	466
%	11		22	
P/BB	7606	8986	394	491
%	18		25	
P/P	8507	9976	431	452
%	17		5	

¢, Abbreviations: CP/CP and CP/BB, cowpea aphids on cowpea and broad bean; P/BB and P/P, pea aphids on broad bean and pea.

?, Percentage increase in A over their respective C

7.4 Discussion

Increased CER (net CO_2 exchange rates) for leaves of aphid-infested plants (Table 1) has been reported previously (Way and Cammell 1970) but the general view is that aphid feeding reduces CER (Kloft and Ehrhardt 1959; Daly 1976; Mallott and Davy 1978; Wu and Thrower 1981; Veen 1985; Wood *et al.* 1985). This view is also held for another sucking bug, the potato leafhopper (*Empoasca fabae*), and its effect on CER (Ladd and Rawlins 1965; Womack 1984; Walgenbach and Wyman 1985). It has also been reported that aphid infestation had no effect on CER (van Emden 1973). The discrepancy in the literature for the response of CER to aphid feeding could indicate that CER is a response specific of the plant-aphid system being investigated.

The significant increase observed for CER of leaves from aphid-infested plants (Table 1; except for P/P on an area basis) could result from an increase in the assimilate sink to source ratio (Geiger and Giaquinta 1982; Giaquinta 1983) because aphids are considered to be 'physiological sinks' on the plant (Way and Cammell 1970). Experimental manipulations of sink to source ratios have shown that increases or decreases in the ratio result in respective increases or decreases of CER (Herold 1980; Peet and Kramer 1980; Wardlaw 1985). The regulation or fine tuning of this relationship is thought to be under hormonal control (Starck 1983), particularly, cytokinins and auxins (Marre *et al* . 1974; Herold 1980; Patrick 1982).

It did not make any difference whether CER or the dark respiration rate (R_D) were expressed on a leaf weight or a leaf area basis because the leaf area to leaf weight ratio (LAW) was not significantly different between control and infested plant leaves (Table 2). This indicates that after 6 to 9 days, aphid feeding had not resulted in a re-organization of leaf structural matter. The lack of significance for CER of P/P when expressed on an area basis (Table 1) was due to an increased, rather than a decreased LAW for the infested P/P leaf (Table 2). The expression of CER on both a leaf area and a leaf weight basis allows comparisons with most values in the literature, however, it is most biologically meaningful to express rates on an area basis because leaf area can be associated with light interception (Wilhelm and Nelson 1985).

Greater R_D values for leaves of aphid-infested plants (Table 3) have also been reported in whole shoot respiration studies of the same plant-aphid combinations (Hawkins *et al.* submitted 1986a). An increased dark respiration rate is considered to be the general response of aphid-infested (Kloft and Ehrhardt 1959; Daly 1976; Uritani and Asahi 1980) and leafhopper-infested (Ladd and Rawlins 1965) plant tissue. However, there are reports of decreased (Wood *et al.* 1985) and unchanged [until the tissue became moribund, when R_D decreased (Wu and Thrower 1981)] R_D.

The increased R_D of aphid-infested leaves was not due to increased alternative respiratory pathway activity (Hawkins *et al* submitted 1986a). However, if increased sink demand stimulates CER as proposed above, this would be accompanied by increases in phloem loading and phloem transport. The phloem loading step requires that a proton gradient be maintained by a proton extrusion pump (Geiger and Giaquinta 1982; Giaquinta 1983). This increased use of energy could account for the increase in R_D. Another possibility is that the maintenance energy requirements of the leaf have been increased due to increased rates of photosynthesis and cellular damage caused by aphid feeding. The increased maintenance requirement of the aphid-infested leaves may be indicated when R_D was expressed as a percentage of CER because the value was greater for all infested tissue (Table 4). If the increase in R_D was due only to an increase in phloem loading, and CER increased proportionately, the percentage of R_D to CER should be the same for control and infested leaves. Therefore, the increased R_D observed for leaves from aphid-infested plants was likely caused by increased phloem loading and maintenance requirements.

When the mean net daily carbon gain for leaves from each plant-aphid combination was calculated, leaves from aphid-infested plants had a greater acquisition than those of control plants (Table 5). However, this excess of production, which can be considerable, was not allocated to plant growth but rather to aphid ingestion. There were in fact significant reductions in short-term growth for the plant-aphid combinations used (Hawkins *et al.* 1985, 1986). Whether the observed compensatory responses can be extrapolated from the leaf to the plant is

unknown but the response of R_D of a single leaf was the same as for the whole shoot (Table 3; Hawkins *et al.* submitted 1986a). However, in this case the magnitude of the difference between control and infested leaves was much greater than for whole shoot measurements.

This study has shown that there is an increase in CER, R_D and net daily carbon gain in the leaves of aphid-infested plants. It was proposed that CER increased due to increased assimilate sink demand and that R_D increased to meet the increased energy requirements of phloem loading and cellular maintenance associated with aphid feeding. The compensatory carbon acquired by infested leaves was apparently consumed by the aphids. The response of CER and R_D to aphid feeding appeared to be general rather than plant-aphid species specific. The possibility that compensatory CER in infested plants can result in long-term recovery after aphid removal is being investigated for the CP/CP system.

CHAPTER 8

LONG-TERM EFFECTS ON COWPEA PLANT GROWTH OF A SHORT-TERM COWPEA APHID INFESTATION[1]

8.1 Introduction

Aphids are important agricultural pests that feed on plants by inserting their stylets into the phloem (Raven 1983). It is generally accepted that short-term (less than 3 or 4 weeks) aphid feeding results in reduced plant biomass (Galecka 1977; Wu and Thrower 1981) and reduced relative growth rates (Barlow and Mesmer 1982; Hawkins *et al.* 1985). Long-term (more than 3 or 4 weeks) aphid feeding results in decreased relative growth rates (Mallott and Davy 1978) and severely decreased plant height and biomass or even death (Kennedy and Stroyan 1959; Mallott and Davy 1978; Harper and Kaldy 1982; Rohitha and Penman 1983; Singh *et al.* 1983; Macfoy and Dabrowski 1984; Choudhury 1984). Short-term aphid infestation followed by aphid removal and subsequent long-term aphid-free growth results in reduced total vegetative and reproductive production (Kain *et al.* 1977, 1979; Petitt and Smilowitz 1982; Rohitha and Penman 1983; Bishop 1984; Summers and Coviello 1984).

Losses of translocate from the phloem to the aphid may affect the plant in 2 ways; directly, by removing the amount actually ingested, and indirectly, by lost production that would have resulted from the consumed translocate (Barlow *et al.* 1977). The mechanisms underlying the deleterious damage caused by the aphids acting as adventitious sinks are only poorly understood (Kennedy and Stroyan 1959; Petitt and Smilowitz 1982).

Compensatory growth, that can occur in plants fed upon by grazers, has not

[1]THIS CHAPER WAS ACCEPTED FOR PUBLICATION IN *CAN. J. BOT.* ON 25 MAR 86 AND IS REFERRED TO IN THE THESIS AS HAWKINS *ET AL.* (IN PRESS 1986)

been observed for aphid-infested plants, but this may be because the aphid densities used have been too great (Raven 1983). In addition the net biomass production by the unparasitized host generally exceeds that of the host plus its parasite (Raven 1983). There is some evidence that aphid infestations can promote growth (Miles and Lloyd 1967; Miles 1968b; Dyer 1980) and leaf photosynthesis (Way and Cammell 1970), suggesting the possibility of a compensatory response.

Aphids might possibly be seen as analogous to grazing herbivores. McNaughton (1983) delineated 3 broadly contrasting effects of herbivores on the fitness of affected plants. If this classification is applied to aphid feeding, 3 possible outcomes can be postulated: (i), aphid feeding is always detrimental to the plant; (ii), plants can compensate for low levels of aphid feeding; and (iii), moderate levels of aphid feeding may result in overcompensation by the plant. Which response is displayed by a plant is likely to be a function of aphid population density and the susceptibility of the plant species.

The study reported here was conducted to determine if there were any short-term or long-term compensatory responses to a short-term aphid infestation and to observe if any mechanisms for plant adaptation to aphid feeding could be discerned.

8.2 Materials and methods

Seeds of cowpea [*Vigna unguiculata* (L.) Walp. cv. Caloona], obtained from Arthur Yates Seed Company, Rockhampton, Queensland, were potted in vermiculite at a density of 1 seed per 12.5 cm diameter pot. Plants were grown in a clear glasshouse for two weeks after planting and then divided according to size into 5 blocks with 15 (planted in December) or 19 (planted in January) plants per block. Plants grown in a clear glasshouse, from December to early June, received 65 to 80 % of the outdoor photosynthetically active radiation (Hawkins *et al.* 1985). Air coolers and under-bench heating allowed a relative humidity range of 30 to 90 % with a shaded bench-top air temperature of 16 to 37 C (Pernix Thermohygrograph, Wilh. Lambrecht, KG, F.R.G.).

Cowpea aphids [*Aphis craccivora* Koch (Homoptera: Aphididae)] obtained from CSIRO, Division of Entomology, Black Mountain, Canberra, Australia (courtesy of R. D. Hughes and L. T. Woolcock) were maintained in a growth cabinet under the environmental conditions described by Hawkins *et al.* (1985). Ten, 8 day-old $\pm$ 6 h adult aphids were transferred to the experimental plants using a fine, moist camel hair brush on experiment day 0. Aphids were confined to an individual plant by the use of clear plastic collars described by Hawkins *et al.* (1985). Control plants were checked daily for adults that may have gained access. Water and nutrient (modified Hoaglands) regimes were identical between control and experimental plants.

On experiment day 0, the 15 two-week-old plants per block were randomly divided and treated as shown in Figure 1. To determine if the insecticide had any effect on plant growth and to replicate the initial trial, an experiment was started in which 19 plants per block were randomly divided as per Figure 1, except that: on day 0, one control (c) was not sprayed with Rogor$^{(R)}$ and one experimental (e) plant was; a c and an e plant were harvested and a c and an e were sprayed on days 5, 10, 15, and 20; the sprayed plants were harvested at the end of the experiment. This was repeated for the other 4 blocks. The clear plastic collars were removed from the 30 or 50 plants remaining on day 20 and these plants were allowed to grow for about 3 more months until they were harvested.

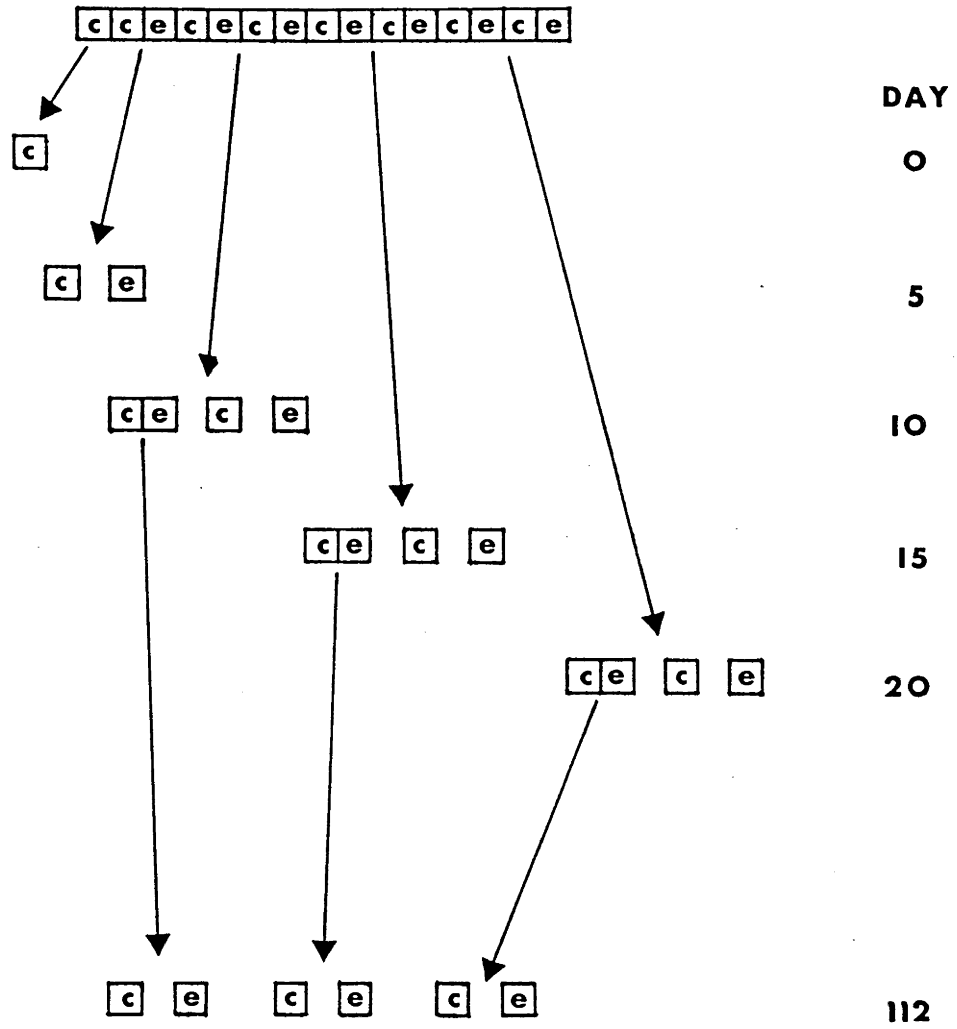

Figure 1. Flow chart of experimental design. On experimental day 0, 15, 2-week-old cowpea plants were randomly divided into 8 control (c) and 7 experimental (e) plants. One plant was harvested on day 0, a c and an e plant were harvested on days 5, 10, 15, and 20. On days 10, 15, and 20, a c and an e plant were each sprayed with the systemic insecticide Rogor(R) (active constituent dimethoate, 10 ml per 10 l water, Chemspray Pty. Ltd., Marayong, N.S.W., Australia) to remove the aphid infestation, and all remaining plants were harvested on day 112. This was repeated for the other 4 blocks.

On harvest days the following parameters were assessed: leaf number; number of nodes; internodal distances at the end of the trial; leaf area (Automatic Area Meter, Hayashi Denko Co., Ltd., Tokyo, Japan); leaf, stem and petiole, and root dry weights; and number of mature and immature seed pods and seeds produced. The equations described by Hunt (1982) and discussed recently (Hawkins *et al.* 1985) were used to calculate the mean relative growth rate, $\overline{R}$, the mean unit leaf rate(mean net assimilation rate) $\overline{E}$, and the mean leaf area ratio, $\overline{F}$.

Two-way analyses of variance (ANOVA) were carried out using the GENSTAT package (Statistics Department, Rothamsted Experimental Station, U.K.) and treatment means were compared using the protected LSD [least significant difference (if ANOVA F ratio is not significant, then neither are the means)] at $\alpha = 0.05$, as described by Snedecor and Cochran (1980).

8.3 Results

The data presented are primarily from the 5 x 15 plant trial. However, identical relationships were observed in a 5 x 19 plant replicate.

At the end of the trial, there was no difference in $\overline{R}$ (mean relative growth rate) and the biomass of experimental plants was 100 % that of the controls (Table 1). Control plant dry weights had been significantly greater than those of the infested plants by day 10 and remained so until day 20 when infested plant biomass was 67 % that of controls (Table 2). Control plant $\overline{R}$ and $\overline{E}$ (mean unit leaf rate) were significantly greater than the experimental ones after 10 days. There was only one occasion when experimental plants had a significantly greater $\overline{F}$ (mean leaf area ratio) than controls (Table 2). Leaf number was significantly greater in controls by day 15 and remained so until about day 80, and there was no difference at the end of the trial (Table 3). The ratio of root-to-shoot dry weights was not significantly different between control and experimental plants for any of the harvests (Table 3). Because of leaf drop between aphid removal and the end of the study, $\overline{E}$ and $\overline{F}$ were not calculated for the entire period. The aphid-infested plants had a significantly greater $\overline{R}$ from the time of aphid removal until the end of the study (Table 1). $\overline{R}$ values for component plant parts were all significantly greater in experimental plants from the time of aphid removal until the end of the experiment (Table 4). There was no significant difference between treatments for reproductive, vegetative, or total biomass produced (Table 5) at the end of the experiment.

By the end of the experimental period, there was no difference in total number of main axis nodes produced for either treatment but between days 15 and 80 the controls produced significantly more nodes and did not produce any more nodes after day 80 (Table 3). Internode lengths prior to day 30 were significantly longer in controls, then there was no further difference until about day 65 when formerly infested internodal lengths were significantly longer, and finally, there was no difference between treatments for the last 4 internodal lengths (data not shown).

Flowering was first observed in controls on day 65 and in experimental plants on day 72. Control plants produced significantly more seed pods by day 80 but

Table 1. Average net relative growth rate ($\overline{R}$) of cowpea plants for the three trials and the LSD for control and aphid-infested plants from day 0 to day 112 and from removal of the cowpea aphids until day 112.

Day[#]	Condition[$]	$\overline{R}_{0-112}$, $mg.mg^{-1}.day^{-1}$	$\overline{R}_{r-112}$, $mg.mg^{-1}.day^{-1}$
10	C	0.0499a	0.0495ac
	A	0.0500a	0.0533b
15	C	0.0499a	0.0489a
	A	0.0498a	0.0536b
20	C	0.0500a	0.0481a
	A	0.0500a	0.0524bc
LSD		ns	0.0031

Note: Means followed by the same letter are not significantly different ($\alpha = 0.05$). ns, ANOVA not significantly different, therefore no LSD.

#, Day aphids were removed with insecticide

$, Abbreviations: C, control plants; A, aphid-infested plants; r, day of aphid removal, either day 10, 15 or 20.

Table 2. Average net relative growth rate ($\overline{R}$), mean unit leaf rate ($\overline{E}$), mean leaf area ratio ($\overline{F}$), plant dry weights, and the LSD for control and aphid-infested cowpea plants on experimental days 5, 10, 15 and 20.

Day[#]	Condition[$]	$\overline{R}$, $mg.mg^{-1}.day^{-1}$	$\overline{E}$, $mg.m^{-2}.day^{-1}$	$\overline{F}$, $m^2.mg^{-1}$	DW[$] mg
0-5	C	0.0093	948	0.000 009 94	172.6
	A	0.0111	1 120	0.000 009 92	175.0
LSD		ns	ns	ns	ns
0-10	C	0.0536	4 692	0.000 011 47	276.3
	A	0.0172	1 387	0.000 012 43	193.7
LSD		0.0099	872	ns	14.4
0-15	C	0.0564	5 545	0.000 010 27	389.8
	A	0.0250	2 084	0.000 012 04	238.7
LSD		0.0153	2 661	0.000 000 92	97.2
0-20	C	0.0587	5 741	0.000 010 17	525.6
	A	0.0389	3 541	0.000 010 53	356.9
LSD		0.0197	2 133	ns	167.8

Note: If the means are not significantly different (ns) by ANOVA ($\alpha = 0.05$) the LSD is not presented.

#, Days between which the various parameters were calculated.

$, Abbreviations: DW, dry weight; C, control plants; A, aphid-infested plants.

Table 3. Mean number of leaves, ratio of root-to-shoot dry weights, number of main axis nodes produced, and the LSD for control and aphid-infested cowpea plants at various times during the trial.

Day[#]	Condition[$]	Leaf number	Root to shoot ratio	Node number
0	C	5.0	0.430	3.0
	A	5.0	0.436	3.0
LSD		ns	ns	ns
15	C	10.6	0.379	5.0
	A	8.4	0.317	4.4
LSD		0.5	ns	0.3
80	C	60.4	??	18.5
	A	52.0	??	17.5
LSD		4.3		0.7
112	C	38.5	0.195	18.5
	A	40.5	0.165	19.4
LSD		ns	ns	ns

Note: If the means are not significantly different (ns) by ANOVA ($\alpha = 0.05$) the LSD is not presented.

#, Day of experiment counted from when the aphids were initially placed on the plants.

$, Abbreviations: C, control plants; A, aphid-infested plants.

??, No data as harvest not made.

Table 4. Leaf, stem and root average net relative growth rate ($\overline{R}$) and the LSD for control and aphid-infested plants from the day of aphid removal until the end of the experiment, day 112.

Day[#]	Condition[$]	$\overline{R}$, $mg.mg^{-1}.day^{-1}$ Leaf	Stem	Root
10	C	0.0387ac	0.0517ac	0.0360ad
	A	0.0430b	0.0571b	0.0408bc
15	C	0.0382ac	0.0504a	0.0365acd
	A	0.0426b	0.0577b	0.0434b
20	C	0.0367a	0.0484a	0.0350a
	A	0.0411bc	0.0544bc	0.0399bcd
LSD		0.0034	0.0039	0.0046

Note: Means followed by the same letter are not significantly different ($\alpha = 0.05$).

#, Day aphids removed with insecticide.

$, Abbreviations: C, control plants; A, aphid-infested plants.

Table 5. Final vegetative, reproductive, and total dry weights, and the LSD for control and aphid-infested plants which had the aphids removed on days 0, 5, 10, 15 and 20.

Day[#]	Condition[$]	DW[$]g Vegetative	Reproductive	Total
0	C	20.76a	23.45a	44.21a
	S	20.62a	22.21a	42.83a
5	C	22.76a	19.25a	42.01a
	A	19.89a	18.85a	38.74a
10	C	19.18a	22.73a	41.94a
	A	21.42a	21.29a	42.71a
15	C	21.32a	21.44a	42.76a
	A	21.64a	21.45a	43.09a
20	C	20.39a	21.32a	41.71a
	A	20.98a	20.71a	41.69a
LSD		4.08	3.46	4.58

Note: Means followed by the same letter are not significantly different ($\alpha = 0.05$).

#, Day aphids removed with insecticide.

$, Abbreviations: DW, dry weight; C, control plants; S, sprayed with insecticide, no aphids; A, aphid-infested plants.

there was no difference in total seed or seed pod production by the end of the trial (Table 6). Controls had significantly more ripe seed pods and seeds and significantly less unripe seed pods and seeds while experimental plants had significantly more ripe and unripe seeds per seed pod (Table 6). The water content of the mature seeds was 5 % for both treatments and the mean seed dry weight of mature seeds was 63.0 mg and 65.9 mg for control and infested plants, respectively (not significantly different).

Table 6. Mean number of seed pods produced, total, ripe and unripe; mean number of ripe and unripe seeds per pod; mean number of seeds produced per control and aphid-infested cowpea plant; and the LSD for plants with aphids removed on days 10, 15 and 20.

Trial[#$]		Mean number Pods day 80	Pods day 112	Ripe pods day 112	Ripe seeds per pod	Unripe pods day 112	Unripe seeds per pod	Ripe seeds per plant
10	C	18.2a	38.0a	32.8a	8.22a	5.2a	2.88a	267a
	A	1.2b	41.8a	12.6b	10.68bc	29.2b	6.18b	129b
15	C	15.6a	39.2a	32.4a	8.69a	6.8a	3.59ac	279a
	A	0.2b	39.8a	7.2b	11.96b	32.6b	6.14bd	84b
20	C	10.8ac	38.4a	29.8a	8.71a	8.6a	4.27cd	258a
	A	4.8bc	40.0a	15.0b	10.12	25.0b	6.30b	147b
	LSD	8.3	ns	10.2	1.31	12.3	1.90	87

Note: Means followed by the same letter are not significantly different ($\alpha = 0.05$). ns, ANOVA not significantly different, therefore no LSD.

#, Day of experiment that the aphids were removed with insecticide.

$, Abbreviations: C, control plants; A, aphid-infested plants.

8.4 Discussion

That the final plant biomass and $\overline{R}$ (mean relative growth rate) were the same for control and formerly infested plants (Table 1) was unexpected, since it is generally accepted that long-term aphid infestation does significantly reduce plant dry weight (Barlow *et al.* 1977; Mallott and Davy 1978; Harper and Kaldy 1982; Singh *et al.* 1983). Lucerne (alfalfa) infested with aphids and then sprayed in the fall to remove them, in New Zealand, Australia or California, still had not recovered by the following spring (Kain *et al.* 1977, 1979; Rohitha and Penman 1983; Bishop 1984; Summers and Coviello 1984). Petitt and Smilowitz (1982) observed that potato plants were unable to resume normal foliar growth after early damage and subsequent aphid removal. Summers and Coviello (1984) reported that even though prior aphid infestation significantly reduced the first cut, there was no effect on second and subsequent cuts of alfalfa. This possibly indicates that there is no residual long-term effect for alfalfa in response to aphid feeding. McNaughton (1983) noted that removal of a portion of the vegetative tissue is rarely translated into a commensurate proportional reduction in yield, provided there is an intervening period of growth. Perhaps, this type of phenomenon was observed here.

Significant short-term reductions in aphid infested cowpea dry weights, $\overline{R}$, and $\overline{E}$ (mean unit leaf rate), with $\overline{F}$ (mean leaf area ratio) being similar between treatments (Table 2), has been previously reported by Hawkins *et al.* (1985), and for pea plants (Barlow and Mesmer 1982; Hawkins *et al.* 1985). Significant short-term reductions in plant dry weights have been reported for potatoes (Galecka 1977), beans (Wu and Thrower 1981), and alfalfa (Rohitha and Penman 1983) infested with aphids. The reduction in $\overline{R}$ was due to a decrease in $\overline{E}$ with no change in $\overline{F}$ (Table 2), indicating changes in activity of the plants' anabolic and/or catabolic pathways (see Hawkins *et al.*, 1985, for more detail). The significant difference in $\overline{F}$ on day 15 (Table 2) does indicate a change in the partitioning of photosynthate but the difference was insignificant again on day 20.

The short-term reduction in leaf number and unchanging ratio of root-to-shoot dry weights (Table 3) for various plant-aphid combinations has also been noted by

Wu and Thrower (1981) and Rohitha and Penman (1983). The decline in leaf number has been attributed to decreased resources being available for leaf initiation in the infested plants (Wu and Thrower 1981). The unchanging root-to-shoot ratio, and $\overline{F}$, indicates that the infested plants were not re-allocating their resources to alleviate the stress of aphid attack.

The relative growth rate of infested plants was significantly greater than that of controls (Table 1), as was $\overline{R}$ of the component plant parts (Table 4), from the time of aphid removal until the end of the experiment. This indicates that the infested plants had managed to overcome the deleterious effects associated with aphid infestation. This result, coupled with insignificant differences for root-to-shoot ratios (Table 3), reproductive and vegetative growth, and total biomass production (Table 5), suggests that, again, as was the case for short-term infestation, the primary causal factor was to be found in $\overline{E}$. The unit leaf rate may change either by increases in photosynthetic and/or decreases in respiratory processes after aphid removal. This effect is the opposite of that observed for the short-term infestation. The enhancement in plant growth may be specific to the cowpea aphid-cowpea plant system. Perhaps, in another plant-aphid system there would have been no enhanced plant growth after aphid removal. These data also indicate that the duration of infestation, up to 20 days, had no effect on the final development of plant biomass.

The increase in plant growth from aphid removal until the end of the experimental growth period indicates a compensatory response on the part of the experimental plants. There is some evidence that 'hormones' present in animal saliva and transferred to the plants during feeding may promote growth (Miles and Lloyd 1967; Miles 1968b; Dyer 1980), while Way and Cammell (1970) have shown that leaves are capable of increasing the rate of photosynthesis in response to aphids feeding on adjacent leaves. Rates of photosynthetic oxygen evolution, implying CO_2 uptake, have been observed in control cowpea plants to be as little as one-half those found in infested plants (C.D.B. Hawkins unpublished data). This would indicate compensation by the plant in response to aphid feeding.

The total plant dry weights were the same for both day 0 treatments (Table

5) indicating that the insecticide had no long-term effect on plant growth. McNaughton (1970) also found that Rogor(R) did not affect growth of sycamore seedlings.

The gradual increase in node production between aphid removal and the end of the study for experimental plants (Table 3), and the changes observed for internodal lengths, also suggest an enhancement in plant growth after aphid removal. Continued node production in the experimental plants after day 80 (Table 3) could indicate delay of senescence or altered development in response to the reduction in plant biomass from the prior aphid infestation (Table 2), or just compensatory growth. Most aphid infestations are believed to promote senescence (Kennedy and Stroyan 1959). Therefore, it is most likely that either a delay in development or else compensatory growth was being observed.

The one-week delay in flowering for the formerly infested plants may be the result of a developmental delay in response to aphid feeding. It also could be due to 'hormonelike' substances (Miles 1968a) that may be injected into the phloem by the aphid.

It was surprising that the total number of seed pods and seeds produced was the same for both treatments (Table 6) because reduced yield is generally associated with aphid infestations (Mallott and Davy 1978; Choudhury 1984; Burton *et al.* 1985). However, the partitioning of seed pod and seed production was significantly different (Table 6). This suggests a developmental delay in experimental plants as a result of reduced biomass at the time of aphid removal or an aphid induced hormonal alteration. The greater mean number of seeds per pod on the experimental plants (Table 6) indicates either more fecund ovaries were produced or fewer ovules were aborted during development; both explanations imply hormonal involvement. Similar water content and mean mature seed dry weight for both treatments indicates that there was no final developmental difference for the mature seeds.

Macfoy and Dabrowski (1984) reported higher concentrations of total phenols in infested than in noninfested cowpea stems and suggested that this was probably

an active part of the cowpea defence mechanism against *A. craccivora* infestation. This is supported by Dreyer and Jones (1981) who have shown that phenolics can be a deterrent to aphid feeding. The enzyme polyphenoloxidase is an invariable component of the saliva of all phytophagous bugs (Miles 1968a) and could result in increased phenolics in the plant.

Phenolics are non-hormonal factors which serve as coordinators of phytohormone regulation and they behave non-specifically, modifying the actions of auxins, gibberellins, and cytokinins upon growth (Kefeli and Dashek 1984). The composition and activity of the photosynthetic apparatus (Buschmann and Lichtenthaler 1977) and the partitioning of photosynthate (Starck 1983) are both under phytohormone control. The 'catch up' in growth observed after aphid removal could be the result of compensatory growth which resulted from non-hormonal regulator / plant hormone interactions.

This study has shown that the short-term plant response to aphid feeding included reduction of $\overline{R}$ and $\overline{E}$, with the underlying causes being apparent decreases in photosynthesis and/or increases in respiration. The long-term response revealed no differences in vegetative growth, indicating compensatory growth in the formerly infested plants, and only changes in the timing of reproductive growth, suggesting hormonal changes. Whatever the underlying mechanisms are for the observed results, it is clear that some basic plant growth regulatory mechanisms are involved and require further investigation.

CHAPTER 9

SIMILARITIES BETWEEN THE EFFECTS OF APHID INFESTATION AND CYTOKININ APPLICATION ON DARK RESPIRATION AND PLANT GROWTH OF LEGUMES[1]

9.1 Introduction

Hawkins *et al.* (in press 1986) suggested that the observed long-term compensatory growth response of formerly aphid-infested cowpea plants might result from changes in concentration of various plant growth substances as a consequence of aphid feeding. These authors also observed that shoot respiration of aphid-infested plants decreased at a slower rate than in control plants and attributed this to a delay of senescence in the infested shoots (Hawkins *et al.* submitted 1986a). Delayed shoot senescence is a response promoted by cytokinins (Matthysse and Scott 1984, Fig. 6.3). Aphid infestation of broad bean has been shown to increase lateral branching (Hawkins *et al.* submitted 1986b) and this too is a cytokinin response. Hawkins *et al.* (submitted 1986b) also suggested that changes in translocation patterns of aphid-infested plants might be caused by changes in the endogenous concentrations of auxin and cytokinin.

The concept that aphids inject, into their host plants, substances which can interact with plant growth substances is not new (see e.g., Allen 1947). These substances may be of plant origin and may be concentrated by the aphid before injection back into the plant (Nuorteva 1955, 1958; Maxwell and Painter 1962b), or they may be of aphid origin (Allen 1947). Further, it has been suggested that the morphological characteristics of plant diseases and insect infestations could be best simulated with the applications of cytokinins (Thimann and Sachs 1966). There are

[1]THIS CHAPTER WAS SUBMITTED TO *CAN. J. BOT.* ON 24 APR 86 AND IS REFERRED TO IN THE THESIS AS HAWKINS *ET AL.* {SUBMITTED 1986D}

also reports that cytokinins have direct effects on dark respiration (Miller 1979, 1980; Dizengremel *et al.* 1982; Musgrave and Siedow 1985), that cytokinins can promote photosynthetic unit formation twice as effectively as auxins (Buschmann and Lichtenthaler 1977), and that cytokinins promote photosynthesis in both expanding and fully expanded leaves (Li and Proctor 1984). Many of the cytokinin-induced physiological changes are similar to those induced by aphid infestation.

Zimmerman and Hitchcock (1942) formulated the idea that the epinastic response of young tomato plants could be used as a bioassay to detect plant growth substances. This procedure was quantified (Synerholm and Zimmerman 1945, 1947), particularly for the auxins and their analogues, and is an effective, inexpensive method of checking for the presence or absence of plant growth substances in biological tissues.

The present investigation was conducted to examine the possibility that aphids inject some foreign substance(s) into their host plant, to study whether aphids contain any active plant growth substances whatever their origin, and to compare responses to foliar and root applications of cytokinins to see whether they simulate some observed aphid-induced plant physiological responses.

9.2 Materials and methods

9.2.1 Plant and aphid material

Cowpea and pea aphids, *Aphis craccivora* Koch and *Acyrthosiphon pisum* (Harris), respectively, both Homoptera: Aphididae, and broad bean (*Vicia faba* L. cv. Aquadulce), cowpea (*Vigna unguiculata* (L.) Walp. cv. Caloona), and garden pea (*Pisum sativum* L. cv. Victory Freezer) seedlings, were obtained, cultured and grown as described by Hawkins *et al.* (1985, 1986). Plants were placed in the standard experimental design (Hawkins *et al.* 1986) of 5 blocks (according to size) by 2 treatments (control and aphid-infested).

9.2.2 Examination of aphid-infested and control plants for foreign substances

After 10 days of aphid infestation, a randomly selected control and an experimental plant shoot (stem, petioles and leaves) were each homogenized for 60 s at high speed (Omni-mixer, Sorvall Inc., Newton, CT, U.S.A.) in 60% aqueous methanol. The extract was passed through a cotton wool column, collected, and the methanol evaporated. The column was washed twice with dichloromethane. This wash was combined with the water remaining from the evaporation and this mixture was extracted three times with dichloromethane. The dichloromethane was evaporated and the residue was dissolved in 2.5-5.0 ml of dichloromethane. 25 μl aliquots of control and aphid-infested shoot extracts were spotted on silica gel thin layer chromatography (TLC) plates (60 F-254, E. Merck, Darmstadt, F.R.G.). Each plate was first developed in 3% ethyl acetate in hexane (v:v); it was then developed in the same direction, three times, in 100% chloroform; and finally it was sprayed with concentrated sulphuric acid. This procedure was repeated for all eight plant-aphid combinations.

9.2.3 Assay for aphid-contained plant growth substances

Tomato seeds (*Lycopersicon esculentum* Mill. cv. Roma Teardrop) were obtained from the Henderson Seed Company (Lower Templestowe, Vic., Australia) and potted in a loam topsoil, Perlite mix (6:1) at a density of 2 seedlings per 15 cm pot. Plants were grown in a clear glasshouse, under conditions previously

described (Hawkins *et al.* 1985), for the duration of the experiment. When the seedlings had developed 2 or 3 internodes, extracts (in 60% methanol, 2 drops Tween 20, distilled water) of cowpea aphids, pea aphids, and cowpea plant tissue (all extracted after 10 days infestation on the same plant) were painted on the lateral aspect of the second internode or the petiole emerging from the third node. Extracts were made as concentrated as possible to maximize potential growth substance concentrations. This procedure was repeated six weeks later, but the internode utilized was then between the second and third nodes basal to the growing apex and the petiole used emerged from the second node. Additionally, the internode was encircled with cowpea aphid extract. A 10 mm ink line was placed on each side of all internodes immediately prior to their treatment to determine if there was any change in cellular elongation.

9.2.4 Simulation of aphid infestation by cytokinin application

In addition to the standard experimental design (5 blocks X 2 treatments), three additional treatments were instituted for some of the pea aphid on pea and cowpea aphid on broad bean plant trials. The three treatments were control, and foliar and root applied 44.4 μM 6-Benzyl-aminopurine (BAP; 10 mg dissolved in a few drops of 1N NaOH, 2 drops of Triton-X100, made up to 1 l with distilled water, see Henny and Fooshee (1985)). The BAP was either sprayed on the shoot until run-off on experimental days 0, 5, and 10 or a volume equal to that sprayed was poured into the root zone on days 0, 5, and 10. Control plants were sprayed with the carrier solution (less BAP) on days 0, 5, and 10. Root and shoot respiration measurements and plant weights, including component parts, were carried out as described by Hawkins *et al.* (1986, submitted 1986a) after 15 days of treatment.

Later the BAP trial was modified to include two levels of BAP, cowpea aphids and control treated broad bean plants to determine if the concentration of BAP had a significant effect on plant growth. The two concentrations of BAP utilized were 44.4 μM (10 $mg.l^{-1}$) and 222 μ M (50 $mg.l^{-1}$) and both were either applied to foliage or to the root zone on experimental days 0 and 5. The aphid treatment

was the placing of 10 adult cowpea aphids on the broad bean seedling shoots on day 0, and control plants were sprayed with the carrier solution on days 0 and 5. After 10 days, the plants were examined for changes in biomass and growth form.

9.2.5 Statistical analyses

Two-way analyses of variance (ANOVA), $\alpha = 0.05$, were done using the GENSTAT package (Statistics Department, Rothamsted Experimental Station, U.K.). The protected least significant difference (LSD, as described by Snedecor and Cochran (1980)) was calculated, $\alpha = 0.05$, between means.

9.3 Results

On one occasion chromatographic results showed that cowpea aphid-infested cowpea seedling shoots contained a substance (after the second development in 100% chloroform) which was not present in equivalent control shoots (Fig. 1), though this result could not be repeated on either of two subsequent occasions. Generally, no difference was found for the number of substances in the extracts between control and aphid-infested shoots for any of the plant-aphid combinations, either before or after spraying the chromatograms with sulphuric acid, except for that noted above (Fig. 1). There were differences in the amount of those substances present when comparing extracts from control and aphid-infested shoots (data not shown).

None of the cowpea tissue extracts painted on the petioles and/or internodes of tomato seedlings caused any change in growth form (data not shown). Both aphid extracts induced bending in treated petioles (changing angle between petiole and stem); increased growth on the treated side of the internode, causing a bending away from the treated side; and increased growth of the entire internode, without inducing a bending in it, when the internode was encircled with cowpea aphid extract (Table 1). The results were similar whether seedlings (second or third internode) or mature plants (between the second and third nodes back from growing tip) were used.

6-Benzyl-aminopurine (BAP), whether applied to the foliage or to the roots caused root respiration and root growth to decrease significantly compared with controls in broad bean (Fig. 2A). In plants subjected to the three treatments, alternative respiratory pathway activity was evident and it was not significantly different between treatments, but cytochrome pathway activity was significantly greater in the roots of control plants (Fig. 2A). Shoot respiration was significantly increased for both BAP treatments and they also demonstrated alternative pathway activity, which was not evident in the control broad bean shoots (Fig. 2B). Plant growth was reduced in BAP treated plants, root growth was reduced significantly, and shoot growth was enhanced with foliar application of BAP (Figs. 2A and 2B). Pea seedlings treated with BAP responded in a similar manner to broad bean

Figure 1. Photograph of the thin layer chromatography plate after a second development in 100% chloroform. The spots enclosed in circles on the right of the plate are from aphid-infested plant extracts. There are no corresponding enclosures for the control plant extracts on the left of the plate.

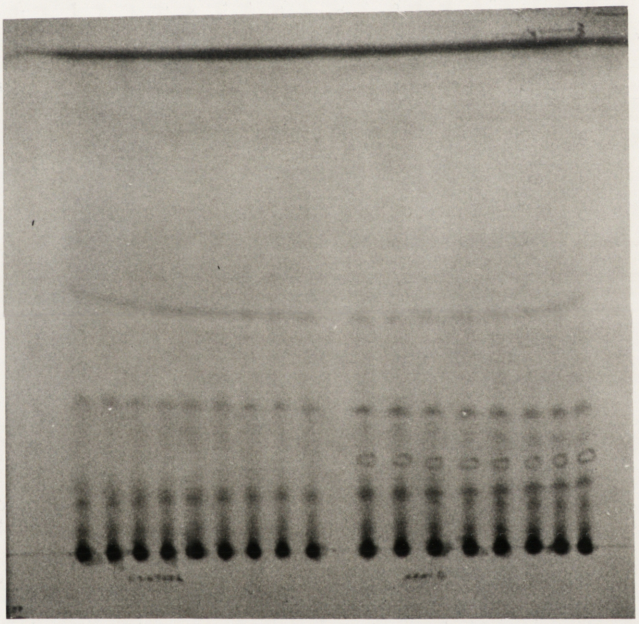

Table 1. Mean changes in the angle between stem and the petiole and in treated and untreated internodal lengths of tomato plants 24 h after application of various aphid and plant tissue extracts.

Treatment[$]	Application site	Change in angle	Internodal Lengths[¢]	
		Degrees	mm	
			UT	T
S	Mid-superior petiole	0.0		
C	None	0.0	10.7	10.7
P/BB	Mid-lateral internode		10.8	11.7
P/BB	Mid-superior petiole	+11.0		
DB	Mid-superior petiole	0.0		
CP/CP	Mid-lateral internode		10.2	11.8
CP/CP	Encircle internode		11.8	11.8
CP/CP	Mid-superior petiole	+5.4		
CP/CP	Mid-inferior petiole	-4.0		

$, Description: S, solvent (60% methanol, 0.05% Tween 20, water); C, control; P/BB, pea aphids (extracted in solvent) raised on broad bean; DB, dry paint brush; CP/CP, cowpea aphids (extracted in solvent) raised on cowpea. All applications on 2nd petiole basal to the growing tip and between nodes 2 and 3

¢, UT, untreated side of internode; T, treated side of internode; + and -, increase or decrease in angle between stem and petiole. All means based on six observations.

seedlings for root growth and root respiration but none of the differences was significant (data not shown). Both BAP treatments caused significant increases in shoot respiration of pea seedlings and there was no significant difference in shoot growth (Table 2). The activity of the alternative pathway (as a percentage of total respiration) was 0% in control pea shoots, 8% for pea shoots which had foliar applied BAP and 11% for those which had root applied BAP. Cytochrome pathway activity was greater in shoots from BAP treated plants (data not shown).

A five fold increase in BAP concentrations from 44.4 to 222 μM did not alter the earlier observed growth differences (Table 3 vs. Fig. 2 and Table 2). The number of lateral branches produced was significantly greater in aphid-infested and BAP treated plants compared to control plants (Table 3). The lower concentration of BAP, particularly root applied, was the more effective in inducing branching, while the effectiveness of the aphids was intermediate between the two BAP concentrations (Table 3).

Figure 2 A. Total root respiration (Resp) of control (C), foliar applied BAP (F), and root applied BAP (R) treatments for broad bean plants. Cytochrome pathway respiration is the lower portion of the histogram and alternative pathway respiration is the upper portion. Vertical bar to the right of the histograms is the LSD for total respiration and the one to the left is the LSD for cytochrome pathway respiration, both at $\alpha = 0.05$. Plant dry weight (Growth) is shown in the histograms on the right for C, F, and R treatments. Root dry weight is the lower portion of the histogram and shoot dry weight is the upper portion. Vertical bar to the right of the histograms is the LSD for plant dry weight and the one to the left is the LSD for root dry weight, both at $\alpha = 0.05$. Resp: cytochrome respiratory path; C,  F,  R  ; alternative respiratory pathway; C,  F,  R  . Growth: root dry weight; C,  F,  R  ; shoot dry weight; C,  F,  R,  .

B. Total shoot respiration (Resp) of C, F, and R treated broad bean plants. Cytochrome pathway respiration is the lower portion of the histogram and alternative pathway respiration is the upper portion. Vertical bars for the LSD as in Fig. 2A. Plant dry weight (Growth) is shown in the histograms on the right for C, F, and R treatments. Shoot dry weight is the lower portion of the histogram and root dry weight is the upper portion. Vertical bar to the right of the histograms is the LSD for plant dry weight and the one to the left is the LSD for shoot dry weight, both at $\alpha = 0.05$. Histogram code as in Fig. 2A.

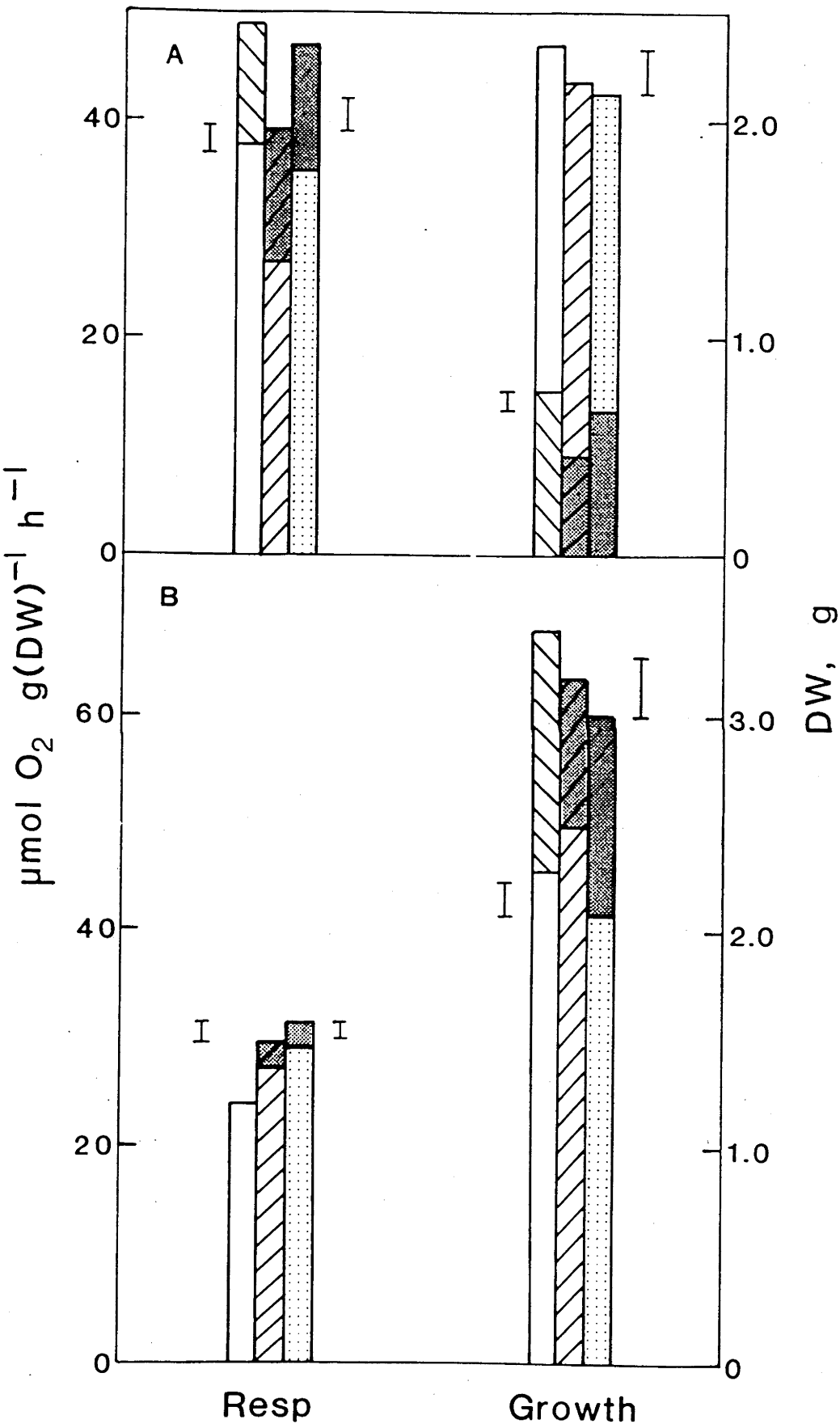
A
B
40
20
0
60
40
20
0
2.0
1.0
0
3.0
2.0
1.0
0
µmol O_2 g(DW)$^{-1}$ h^{-1}
DW, g
Resp
Growth

Table 2. Mean shoot respiration and mean shoot and plant dry weight for control or BAP treated pea plants 15 days after treatment, and the LSD for each parameter.

Treatment[$]	Shoot respiration $\mu mol\ O_2 . g(DW)^{-1} . h^{-1}$	Shoot DW[¢] g	Plant DW g
C	63.0a	0.980a	1.403a
F	72.5b	0.899a	1.215b
R	75.3b	0.862a	1.273ab
LSD	7.8	0.111	0.150

Note: Means followed by the same letter are not significantly different ($\alpha = 0.05$).

$, Abbreviations: C, control; F, foliar applied BAP (6-Benzyl-aminopurine); R, root applied BAP.

¢, Abbreviations: DW, dry weight.

Table 3. Mean plant and shoot dry weights and mean number of branches from the stem in control, aphid-infested and BAP treated broad bean 10 days after treatment was initiated and the LSD for each parameter.

Treatment[$]	Plant DW[¢] g	Shoot DW g	Branches No.
C	1.919a	1.367ab	0.4a
A	1.267b	0.998c	2.2bc
BAP50F	1.892a	1.465a	2.0bc
BAP50R	1.655a	1.166bc	1.8b
BAP10F	1.834a	1.385a	2.4bc
BAP10R	1.779a	1.300ab	2.6c
LSD	0.324	0.217	0.6

Note: Means followed by the same letter are not significantly different ($\alpha = 0.05$).

$, Abbreviations: C, control; A, 10 adult aphids per plant on day 0; BAP50F and BAP10F, foliar applied BAP(6-Benzyl-amino-purine) 50 mg and 10 mg per litre, respectively; BAP50R and BAP10R, root applied BAP 50 mg and 10 mg per litre, respectively.

¢, Abbreviations: DW, dry weight; No., number.

9.4 Discussion

The transient presence of a foreign substance in the extract from cowpea aphid-infested tissue (Fig. 1) could be an experimental artifact or its absence in replicate experiments may indicate that the compound was light sensitive or present in very minute amounts. The first assumption is the more likely because no foreign compounds were detected in extracts from the seven other aphid-plant combinations. The difference observed in the quantities of compounds present between control and aphid-infested tissue may indicate that any toxins associated with aphid infestation are not produced by the aphids but are rather of plant origin (Nuorteva 1955, 1958; Maxwell and Painter 1962b). The lack of difference between control and aphid-infested plant extracts indicates only that with this particular combination of solvents and developers no differences could be detected. Aphid-injected substances may still be present in subtle quantities which require more precise detection methods.

The petiole bending and internode elongation induced by the various aphid extracts (Table 1) indicates that there were plant growth substances present in the extracts (Synerholm and Zimmerman 1945, 1947), probably auxins because of the observed increased cell elongation (Thimann 1937). The absence of any induction of bending by the cowpea plant tissue extracts (Table 1) indicates that the plant growth substances in the aphids were present in higher than plant physiological concentrations. The increase in elongation of the entire internode without bending when encircled with aphid extract (Table 1) again suggests an auxin involvement. However, it has been demonstrated that cytokinins may induce localized increases in auxin concentration by the enhancement of the movement of auxin to the treated area (Lagerstedt and Langston 1967; Hemberg 1972). Whether the aphid extract contained increased concentrations of auxins and/or cytokinins, the cell elongation response is clearly auxin-induced (Thimann 1937).

The effects of foliar and root applied cytokinin on plant respiration, growth, and form were examined. A simulation using auxins and cytokinins would have been more appropriate but logistical constraints prevented this. The overall effect

of BAP (6-Benzyl-aminopurine) to decrease root respiration (Fig. 2A) and to increase shoot respiration (Fig. 2B) in broad bean was analogous to the respiratory response observed in the roots (Hawkins *et al.* 1986) and shoots (Hawkins *et al.* submitted 1986a) of aphid-infested plants. Root or foliar application of BAP did not affect the alternative pathway activity in roots but it did significantly reduce root cytochrome pathway activity (Fig. 2A), whereas, aphid infestation resulted in decreased activities of both respiratory pathways (Hawkins *et al.* 1986). The increased shoot respiration in BAP treated plants (Fig. 2B) resulted from increased activities of both respiratory pathways while in the aphid-infested shoots it was entirely due to increased cytochrome pathway activity (Hawkins *et al.* submitted 1986a). Hawkins *et al.* (submitted 1986a) attributed the aphid-induced increase in shoot respiration to increased rates of photosynthesis and the possibility that aphids can delay shoot senescence. It is known that cytokinin application increases the rate of photosynthesis (Li and Proctor 1984) and also delays shoot senescence (Matthysse and Scott 1984, Fig. 6.3) and these could account for the BAP-induced increase in shoot respiration.

In contrast to BAP application having no effect on alternative pathway respiration in roots (Fig. 2A), BAP application in shoots promotes alternative pathway activity (Fig. 2B). Miller (1979, 1980), Dizengremel *et al.* (1982) and Musgrave and Siedow (1985) observed that cytokinins inhibited rather than promoted alternative pathway activity but they used considerably greater concentrations of BAP. The promotion of alternative pathway activity in shoots may be associated with increased rates of photosynthesis and the proposed role of the alternative pathway functioning as an energy overflow (Lambers 1985).

The responses of pea seedling respiration to BAP application were similar (Table 2) to those of broad bean (Fig. 2) indicating that the effect of cytokinin on legume respiration is probably a general rather than a species specific response.

The significant reduction in root growth of foliar or root BAP treated broad bean (Fig. 2) was also characteristic of the response of roots of aphid-infested plants (Hawkins *et al.* 1985, 1986). BAP application apparently reduces the flux of

translocate to the roots just as aphid infestation does (Hawkins *et al* submitted 1986b) but the mechanisms must differ because aphid feeding removes translocate from the plant, and roots of aphid-infested plants had no alternative pathway activity (Hawkins *et al.* 1986), while BAP application did not affect root alternative pathway activity (Fig. 2A).

Shoot growth was enhanced with foliar application of BAP (Fig. 2) even though both BAP treatments (Fig. 2) and aphid infestation (Hawkins *et al.* 1985, 1986) resulted in significant reductions in plant growth. The BAP promotion of shoot growth was presumedly due to cytokinin enhanced photosynthesis (Li and Proctor 1984). Increased rates of shoot respiration with increased alternative pathway activity (Fig. 2) may account for the reduction in BAP treated plant growth. In aphid-infested plants, the reduction in plant growth was likely due to removal of translocate by the aphids and increased rates of shoot respiration.

Increasing the BAP concentration five fold did not alter the previously observed growth differences (Table 3 vs. Fig. 2 and Table 2) probably indicating that the higher concentration of BAP was still not an inhibitory level. The significant increase in lateral branching induced by both aphid infestation and BAP application (Table 3) suggests that aphids are capable of increasing cytokinin and/or decreasing auxin concentrations sufficiently (increase ratio of cytokinin to auxin) to overcome apical dominance. The apparent aphid-induced change in cytokinin to auxin ratio fell between that induced by the two known BAP concentrations because the effectiveness of the aphids in inducing lateral branching was intermediate to the two BAP concentrations (Table 3).

This series of experiments has shown that there are similarities between aphid-induced changes in root and shoot respiration, in plant growth, and in plant form and cytokinin-induced changes in the same parameters. However, even though cytokinins may be very important in effecting the changes induced by the aphids, it is more likely that the final physiological expression is the result of several plant growth substances interacting in conjunction with other factors, both external and internal. It would be worthwhile to attempt to simulate the effects of aphid

infestation using a 'cocktail' of plant growth substances in order to delineate the interaction(s) between aphid feeding, plant growth substances, and plant physiological responses.

CHAPTER 10

DISCUSSION

10.1 Experimental design, benefits and liabilities

All but one of the experimental studies (Hawkins *et al.* in press 1986) reported in the preceding chapters were conducted under identical strictly controlled growth cabinet conditions. The aphids used in all experiments were raised under the same growth cabinet conditions as above, since the colonies were first established in September and October of 1983 for cowpea and pea aphids, respectively. This design had the advantage of always providing aphids of known strains at the same adult stage of development when experiments were initiated. Furthermore, the stress effect of the aphid infestation upon the plants was very similar between experiments, provided the plants were of similar developmental stage [the same lot of each seed type was used for all the experiments reported here (see Hawkins *et al.* 1985)] on the day of aphid infestation. However, the greatest advantage of this design was that data from experiments distant in time could be directly compared with a high level of confidence because aphids, plants, and environmental conditions were as close as possible to being identical between experiments.

In Chapter 1, it was noted that aphids are important virus vectors (Kennedy *et al.* 1962) but that every attempt would be made to keep the aphid stock colonies free of viruses. No symptoms of viral infection were observed for any plants in the 10 or 15 day experiments. For the long-term growth experiments, no viral diseases were observed for any cowpea plants, but both treatments of broad bean suffered from periodic fungal disease after removal of the aphids. Therefore, the results from these experiments are likely the consequence of aphid feeding rather than secondary plant infections.

The primary disadvantage of the experimental methods utilized was that the

investigations were conducted in growth cabinets under conditions that were optimal for both plants and aphids. Under field or even glasshouse conditions, it is unlikely that much of the growing season would have conditions that are optimal for both host and parasite. Rather, such environments frequently alternate between conditions favouring host plant or aphid, or it may favour neither of them. The effect of the growth cabinet is to apply a constant stress to the experimental system, while under field conditions the experimental system would be exposed to a series of oscillating stresses. However, when determinations of photosynthesis and root respiration were conducted on glasshouse grown, aphid-infested cowpea and broad bean seedlings the response of these physiological measures to aphid infestation were almost identical (C.D.B. Hawkins, unpublished data) to those observed for the growth cabinet experiments.

It is therefore probably reasonable to assume that aphids induce similar changes in the physiology of their host plants whether the latter are grown in a growth cabinet or in the field. However, the amplitude of the response is possibly enhanced or diminished depending upon the field environmental conditions.

10.1.1 Plant water relations

One very important physiological process not dealt with in the body of the thesis was the effect of aphid feeding on plant water relations, even though it was noted in the introduction (Chapter 1) that aphids are capable of removing considerable volumes of fluid from their hosts. Experiments were carried out to check this phenomenon. It was found that cowpea aphid feeding had no effect on plant water potentials and stomatal conductances of cowpea plants (Appendix A, Figs. 1 and 2) grown in the glasshouse under light intensities and temperatures higher (on a cloudy day) than those used in growth cabinet experiments (350 μmol.m^{-2}.s^{-1}, 23 °C). In contrast, under full sunlight and much higher temperatures in the glasshouse, cowpea aphid feeding resulted in significant decreases in plant water potential and increases in stomatal conductance (Appendix A, Figs. 1 and 2). For the environmental conditions utilized for the growth cabinet experiments, altered plant water relations by aphid feeding does not appear to contribute

appreciably to changes in the physiology of host plants. This conclusion can only be drawn with confidence for cowpea plants because cowpeas are considered to be an extremely drought resistant species (pers. comm. Dr. M.M. Ludlow, CSIRO, St. Lucia, Queensland, Australia). That aphid feeding may alter plant water relations along with the other measured physiological responses, for pea and broad bean in the growth cabinet studies, should always be considered a possibility, when not directly measured. Under field or glasshouse conditions plant water relations would be expected to be an important parameter of the aphid-induced changes in physiological responses for all three plant species studies. In the field, altered water relations due to aphid infestation could result in an increased cost of plant maintenance: if the maintenance cost became too energetically expensive, the plants could die prematurely as Blackman (1974) reported.

10.2 Summary and integration of the major findings

Aphid-infested plant growth was reduced after 5 days but only significantly so after 10 days of infestation (Fig. 2-1). It was postulated that the underlying cause of the reduced growth was decreased energy production, or else increased energy consumption by non-growth processes (Hawkins *et al.* 1985). Such an alteration in energy relationships could show up as a deficiency in the uptake of elements essential for growth.

After 6 days of aphid infestation, absolute nitrogen and phosphorus accumulation was not significantly different between control and infested plants (Fig. 3-2). This was probably a result of root and shoot respiration not being significantly different between treatments (Figs. 4-1 and 5-2). Even though translocation of photoassimilate to roots of aphid-infested plants was reduced (Figs. 6-2 and 6-3) this reduction had not altered potential root energy production. This view is supported by the fact that the roots of both control and aphid-infested plants had non energy producing alternative respiratory pathway engagement on day 5 (Table 4-3), except for pea aphid-infested pea seedlings, indicating that at this time, there was an adequate supply of substrate to the roots (Lambers 1985) to

maintain the uptake of nitrogen and phosphorus. Absolute nitrogen and phosphorus accumulation was significantly reduced in aphid-infested plants by day 10 (Fig. 3-2). Aphid-infested plant root respiration was also significantly reduced by day 10 (Fig. 4-1) with no alternative pathway engagement (Table 4-3), except for pea seedlings infested with pea aphids. Aphid-infested plant shoot respiration was significantly increased with no alternative respiratory pathway activity (Fig. 5-2) and the flux of photoassimilate to the roots of infested plants was significantly reduced (Fig. 6-2). This collection of data indicates that the roots of aphid-infested plants were not receiving an adequate supply of energy to maintain nitrogen and phosphorus accumulation, either on an absolute (Fig. 3-2) or on a relative (Fig. 3-1) basis, suggesting that the plants were starting to incur nitrogen and phosphorus debts.

Even though the individual leaves of the aphid-infested plants are capable of significantly increasing photosynthetic rates (Table 7-1), implying increased energy supplies, the concomitant increase in leaf (Table 7-3) and shoot (Fig. 5-2) respiration and aphid ingestion of translocate apparently prevent an adequate flux of photoassimilate to the roots of these plants, leaving them unable to maintain the active uptake of nitrogen and phosphorus at the levels observed in control plants. The increase in respiration and the decrease in the carbon economy of aphid-infested shoots probably results from increased cellular maintenance costs (Hawkins *et al.* submitted 1986a) and increased energy costs associated with enhanced phloem loading in response to increased rates of photosynthesis (Hawkins *et al.* submitted 1986c). A decreased carbon economy of aphid-infested shoots along with the imbibition of translocate by aphids resulted in the reduced flux of translocate to the roots (Fig. 6-2). This resulted in lowered substrate supplies to maintain root respiration and caused a decreased uptake of nitrogen and phosphorus from the soil solution. This scenario ultimately, in concert with other aphid-affected processes, resulted in reduced plant growth.

10.2.1 Timing of plant response to aphid feeding

For all the physiological parameters examined on both days 5 and 10 (Hawkins *et al.* 1985, 1986, in press 1986, submitted 1985, submitted 1986a,b,c), the perturbation to the aphid-infested plant was usually not significant on day 5. However, for all plant-aphid systems, by day 10 the aphid-induced change to the physiological system under investigation was usually significant. A similar phenomenon was observed for *Aphis craccivora* feeding on *Vigna sesquipedalis* (Wu and Thrower 1981) suggesting that this may be a general legume response to aphid feeding.

Possibly, a critical amount of translocate has to be lost from the plant before the delayed response of the physiological parameter, induced by aphid feeding, can be perceived. If with time, insufficient energy was available to maintain the infested plant's processes at 'normal' levels, the delayed response could occur. This explanation would be analogous to the direct (aphid removal of translocate from the phloem) and indirect (loss of production that would have resulted from the consumed translocate) effects of aphid feeding on plant growth proposed by Barlow *et al.* (1977). If the hypothesis is correct, it would be the indirect effect which alters the physiology of the aphid-infested plant.

10.2.2 Energy consumption and production in infested plants

Aphid infestation resulted in an increased respiratory sink for the whole plant as postulated by Hawkins *et al.* (1985) when changes in root respiration, shoot respiration and plant growth are considered (Figs. 4-1, 5-2 and 5-1). Calculated from the values in these figures (and from the typical case of control broad bean respiration), the percentage reduction in growth of infested plant was 23 and 49 for pea aphid-infested pea and broad bean, and 24 and 50 for cowpea aphid-infested cowpea and broad bean; while the percentage decrease in whole, aphid-infested, plant respiration with respect to controls was 1 and 27 for pea aphid-infested pea and broad bean, and 19 and 20 for cowpea aphid-infested cowpea and broad bean. Therefore, plant growth decreased proportionally more than respiration did when aphid-infested plants were compared to control plants, indicating a larger respiratory

sink in the infested plants. The postulate of a decreased photosynthetic energy supply in aphid-infested plants is apparently incorrect as judged by the net daily carbon gain for leaves of control and infested plants (Table 7-5). However, when aphid consumption of photoassimilate and decreased flux of translocate to the roots are considered, there is a decreased supply of energy available for plant growth in parts of the plant remote from the source of the substrate in the infested plants. Therefore, aphid-infested plants have net increased respiratory sinks and net decreased substrate sources and this results in decreased plant growth with respect to controls.

10.3 General versus specific plant response to aphid feeding

The suggestion that the plant response to aphid feeding is specific to a particular plant-aphid system (Dixon 1971b; Galecka 1977; Wu and Thrower 1981) did not apply to many of the physiological parameters examined in this study. The physiological responses of aphid-infested tissue were very similar regardless of the plant-aphid combinations utilized: decreased plant growth, decreased nitrogen and phosphorus accumulation, decreased root and increased shoot respiration, decreased translocation to the roots, and increased leaf photosynthesis and respiration. Rather, the responses are general for combinations of these legumes and the cowpea and pea aphid species, and may possibly be general for aphid-infested legumes. Even the application of 6-Benzyl-aminopurine (BAP) to legume tissue appears to result in a general rather than a species specific response (Hawkins *et al.* submitted 1986d).

Some species specific responses were, however, observed. On a percentage basis, nitrogen and phosphorus accumulation was plant species specific (Fig. 3-1) and, except for aphid-infested pea plants, the infestation did not alter the plant species' nitrogen and phosphorus accumulation patterns. Other than the general decrease in the flux of translocate to the roots, the effect of aphid feeding on whole plant translocation patterns appeared to be somewhat plant-aphid species specific (Fig. 6-3). Regulation of the cytochrome respiratory pathway in aphid-infested

shoots was different between the plant species but the difference did not appear to be attributable to aphid feeding *per se* (Hawkins *et al.* submitted 1986a). The effect of a short-term aphid infestation on long-term plant growth was also a species specific phenomenon: the cowpea aphid cowpea plant combination was not significantly different in biomass from controls at final harvest even though infested plants had been significantly smaller at the time of aphid removal (Hawkins *et al.* in press 1986), but the pea aphid broad bean plant combination did not recover after aphid removal, formerly infested plants being still significantly smaller than controls at final harvest (Appendix B, Table 1).

The response of long-term plant growth to the short-term aphid infestation could, however, also vary with the aphid species utilized. The growth rates of the two aphid species were often quite different on different host plants (Fig. 2-2 and Table 3-3) suggesting species specific aphid-plant interactions. It is possible that very different effects would be observed for cowpea and broad bean long-term growth if the reciprocal aphid-plant combinations were used. If this were the case, the long-term plant growth response would be specific to the plant-aphid combination under investigation.

When a response being measured has resulted from the integration of many other physiological processes, the response may be seen to be plant-aphid species specific. This could be true e.g., for long-term plant growth and whole plant translocation patterns. However, when a single or less complex physiological process is examined, the differences observed between plant-aphid combinations may be so slight that the response appears to be general to all legume-aphid systems being investigated. In short, the more basic the process contributing to an overall physiological response, the less likely that the measured process will be specific to the plant-aphid system being investigated.

10.4 Physiological significance of the alternative respiratory pathway

It was proposed by Lambers (1980, 1982, 1985) that the physiological significance of the non-phosphorylating alternative respiratory pathway was to act as an 'energy overflow' when substrate (carbohydrate) supply was in excess of the energy requirements of the phosphorylating cytochrome pathway. Some of the evidence presented in this thesis lends support to the hypothesis and certainly none contradicts it.

When translocate supply was significantly reduced after 10 days of aphid infestation (Fig. 6-2), the alternative pathway was still engaged in the roots of all control plants while it was only engaged in one instance in the roots of infested plants (Table 4-3). This indicates that restriction of substrate supply can limit alternative pathway engagement in roots. Shoot respiration of aphid-infested plants was significantly increased after 10 days of feeding but none of the increase was attributable to the alternative respiratory pathway, even though it was engaged in some control shoots (Fig. 5-2). This suggests that the aphid removal of substrate from the phloem induces an increased shoot respiration without 'wasteful' alternative pathway activity, perhaps indicating that alternative pathway activity is dependent upon an excess supply of substrate. The application of BAP [which is known to promote photosynthetic unit formation (Buschmann and Lichtenthaler 1977) and to enhance photosynthesis (Li and Proctor 1984)] to broad bean and pea seedlings resulted in increased alternative pathway respiration in the shoots of treated plants (Fig. 9-2B and Table 9-2), whereas roots of all treatments displayed alternative pathway activity (Fig. 9-2A). Since growth was not significantly enhanced by BAP treatment (Fig. 9-2A) and photosynthesis likely was, and there were no aphids to imbibe the excess photosynthate, this suggests that the alternative pathway was operating because of the higher levels of substrate available to the cytochrome pathway.

It appears that when potential substrate supply is limited, such as in aphid-infested plant roots or shoots, there is no activity of the alternative pathway even though its potential capacity is very large. Conversely, when substrate supply does

not appear to be limited, such as in control plants and BAP treated shoots and possibly roots, the alternative pathway is engaged. Therefore, *in vivo* there is a good possibility that one of the functions of the alternative respiratory pathway is to act as an energy overflow when substrate supply is in excess of the energy needs of the cytochrome pathway.

10.5 Compensatory carbon gain and plant growth

The question was presented as to whether the enhanced net daily carbon gain of aphid-infested leaves (Table 7-5) meant there was a net carbon gain for the whole plant (Hawkins *et al.* submitted 1986c). Clearly, there was not because of the significant reductions observed in infested plant growth after 10 days (Fig. 2-1). The enhanced production of photosynthate was not being utilized in plant growth, but was being imbibed by the aphids for their growth. This contributed to the significant reduction in plant growth but does not wholly account for it (Hawkins *et al.* 1985).

However, a 'catch up' or compensatory growth response was evident in the experiment on long-term growth effects of a short-term aphid infestation (Hawkins *et al.* in press 1986). The formerly infested plants exhibited an enhanced growth with respect to controls after aphid removal. If the leaves of the formerly infested plants had maintained their higher carbon gain after the removal of the aphids, this would account for their ability to grow at an increased rate compared to controls. In theory, if this enhanced growth rate of formerly infested plants were to proceed without restriction, it would allow their biomass to catch up and even to surpass that of control plants. The catch up was observed for cowpea plants that had been infested with cowpea aphids (Hawkins *et al.* in press 1986). However, when pea aphids fed on broad bean, the formerly infested seedlings remained significantly smaller than controls at the final harvest, even though their biomass was ultimately increasing at a greater rate than that of controls (Appendix B, Table 1). This result is somewhat perplexing because the infested broad bean leaves displayed a similar enhanced net daily carbon gain (Table 7-5) to that of cowpea,

but the 'catch up' was insufficient to overcome the original discrepancy by harvest time. Perhaps, in comparing the growth of cowpea and broad bean, the recovery after aphid removal was qualitatively similar, but the significant quantitative differences reflect the geographic origins (Cubero 1974; Steele 1976) and subsequent evolutionary history of the two species.

It is not known how respiration and photosynthesis change in the leaf or shoot after aphid removal. Perhaps, in the case of cowpea enhanced growth, respiration rates decreased and photosynthetic rates were unchanged, while in broad bean, possibly, there was a delay in the decrease of the respiration rate while photosynthesis, as in cowpea, was unchanged. If the mechanism(s) underlying enhanced growth after aphid removal were delineated, it would provide much basic and important information about the interaction between respiration, photosynthesis and ultimate plant growth.

10.6 Possible mechanisms to account for aphid-induced changes in the physiology of their host plants

Several mechanisms were presented in the thesis to explain the observed physiological responses of the plant to aphid feeding. The principal ones were:

(a), that the increased shoot respiration in aphid-infested plants was the result of a delayed shoot senescence caused by changes in the levels of endogenous plant growth substances (Hawkins *et al.* submitted 1986a). However, it has been reported that aphid infestation of other plant species resulted in enhanced plant senescence (Kennedy and Stroyan 1959);

(b), that aphid-induced increases in leaf photosynthesis and possibly respiration resulted from changes in the normal plant source-sink relationship caused by increased concentrations of cytokinins and auxins and the removal of translocate by the aphids (Hawkins *et al.* submitted 1986c, submitted 1986b);

(c), that the compensatory growth displayed by cowpea plants after aphid removal resulted from an interaction between endogenous plant hormones and hormone

regulators (hormonal and non-hormonal) injected by the aphids (Hawkins *et al.* in press 1986);

(d), that aphid-induced lateral branching in broad bean (Table 6-1) resulted from an increase in the cytokinin to auxin ratio (Hawkins *et al.* submitted 1986d);

(e), that plants had increased shoot respiration, decreased root respiration, and increased lateral branching induced by aphid feeding and the effects could be mimicked in uninfested plants by BAP treatment (Hawkins *et al.* submitted (1986d).

All of these proposals intimate that aphid feeding (probably via substances injected in saliva) caused changes in the endogenous levels of plant growth regulators, but the mechanism(s) for the interaction between aphid and plant growth substances remains to be described. Such a fine tuning in the relationship between plants and aphids is neither unlikely nor surprising when their long period of coevolution (Southwood 1973) is considered.

10.6.1 A proposal concerning the aphid-induced reduction in plant growth

Kain *et al.* (1977) proposed that the reduction of plant biomass caused by aphid feeding resulted from a combination of the interaction of aphid saliva upon host plant physiological processes (without any elaboration on where the saliva was interacting) and the removal of translocate by the aphids. To date, this appears to be the most accurate proposal presented to account for the aphid-induced reductions in plant growth.

I would like to refine this proposal in the following form.

Potential plant biomass is not achieved in aphid-infested plants because of the culmination of the effects of (i) hormonal and non-hormonal substances present in the aphid's saliva interacting with and altering the levels of endogenous plant growth substances and (ii) translocate removal by the aphid, and both of these facilitate the modification of host plant physiological processes.

10.7 Future experiments and economic implications

The proposal that aphid infestation may result in a delay of shoot senescence (Hawkins *et al.* 1986a) could be of major importance both biologically and agriculturally if the exact mechanism can be elucidated. Growth could be enhanced if shoot senescence were able to be slowed or delayed and the increased growth could be translated into a commensurate increased yield, provided that the harvest could still be instituted efficiently and economically.

In order to investigate this possibility it is necessary to conduct a series of experiments to ascertain the effects of aphids, cytokinins and auxins on mitochondrial development and respiratory expression. Also, the effects of the hormone *in vitro* on mitochondrial action would have to be examined to determine if the mitochondria are involved in the delay of senescence.

The aphid enhancement of the photosynthetic process and apparent induction of compensatory growth also has agricultural advantages along the same lines as those outlined for the delaying of plant senescence. First it would be important to determine if aphid-infested whole plant compensatory growth resulted from continued increased rates of photosynthesis with the respiration rate being decreased, or from other permutations of these processes. The next step would be an attempt to simulate respiratory and photosynthetic responses using a 'cocktail' of plant growth substances. If these experiments proved successful in producing a net daily carbon gain, experiments similar to those proposed for mitochondria could be conducted on leaves and chloroplasts to determine their *in vivo* and *in vitro* regulatory mechanisms.

The above studies, with the potential of significantly enhancing plant growth and production, may need to be linked with detailed translocation mapping experiments because it may be very difficult to separate enhanced respiration and photosynthesis from the altered translocation patterns. This is a very tedious, costly and labour intensive proposition. However, the series of respiratory and photosynthetic experiments may provide sufficient information because all three physiological processes appear to be regulated by similar plant growth substance reactions.

Information gained from such experiments would prove useful to plant breeders, geneticists and molecular biologists in their quest for improved plant growth and production, particularly if key regulatory mechanisms at the protein level were identified.

10.8 Conclusion

Clearly, the overall effect of short-term aphid infestation on their legume host plants was the significant reduction of plant growth via important alterations to central physiological processes, such as respiration, photosynthesis and translocation. These were apparently caused by aphid-induced changes in the levels of endogenous plant growth substances. However, there is the possibility that short-term aphid feeding also results in potentially beneficial alterations in legume host plant physiology. The study of aphid-host plant physiology has the potential to provide basic information regarding plant performance and could become a very important and an exciting field of research.

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APPENDIX A

COWPEA PLANT WATER RELATIONS

Cowpea plants were raised from seed in the glasshouse at a density of one seed per pot. 14 days after planting, the plants were divided according to height into 5 blocks each of 20 plants and each block was randomly divided into 10 pairs of plants; each pair comprised a control plant, and an aphid-infested plant with 10, 8-day-old adult cowpea aphids placed on it. This was repeated for the other four blocks. See Hawkins *et al.* (1985) for further detail and explanation regarding the design.

After 10 days of aphid infestation, plant water potential [measured using a pressure chamber (Scholander *et al.* 1965)] and stomatal conductance [measured using a steady state diffusive porometer (Licor 1600, Lambda Instrument Corporation, Lincoln, NB, U.S.A.)] were determined on one pair of plants that was randomly selected from each block (Figs. 1 & 2) for each sample time period (5 control and 5 infested plants for each sample).

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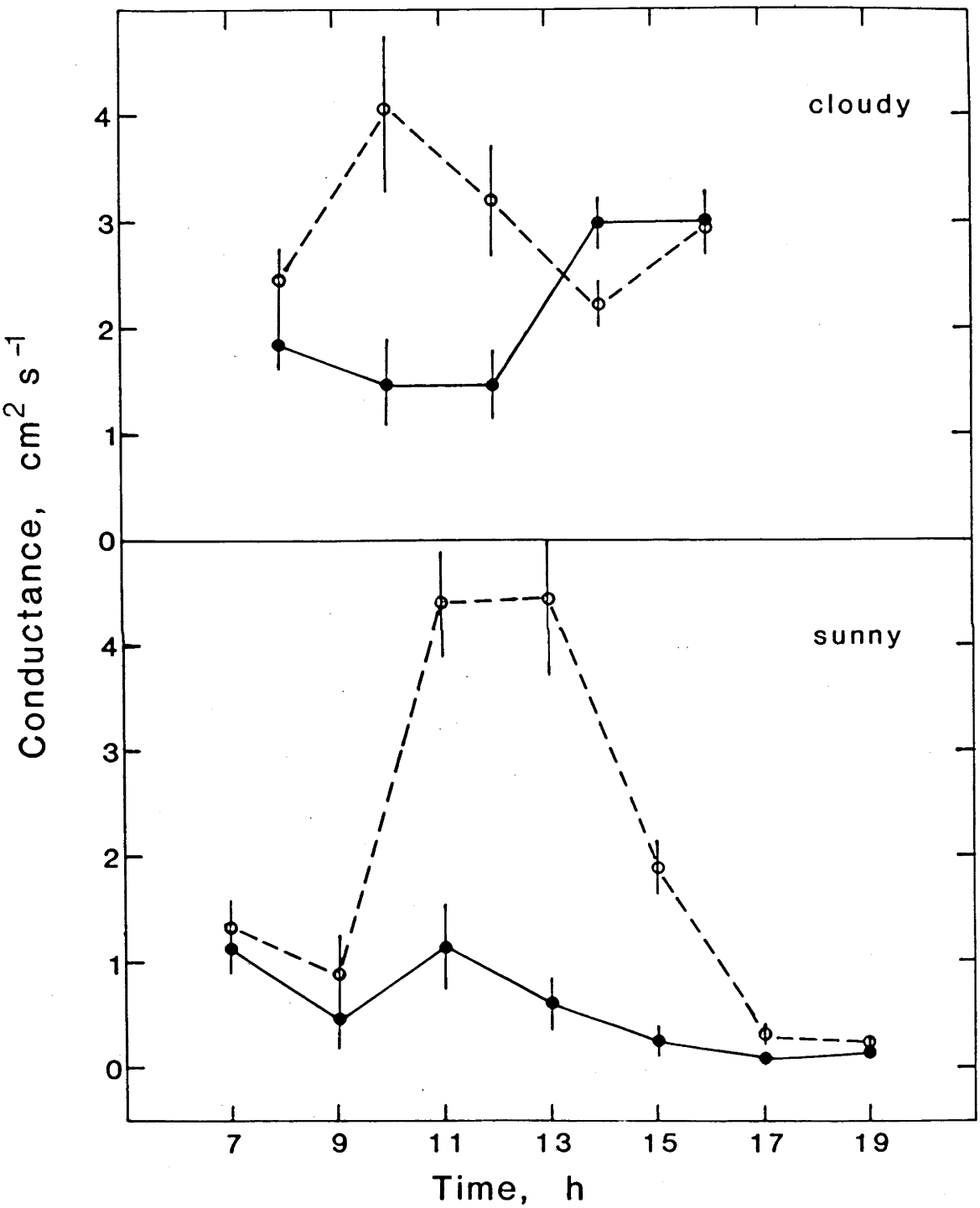
Figure 2. Cowpea stomatal conductances (conducted on plants just prior to harvesting for water potential determinations) after 10 days of aphid infestation on a cloudy day (as in Fig. 1) and a sunny day (as in Fig. 1). Vertical bar for each mean is the SE, n = 5 plants. Control plants, ●——●; aphid-infested plants, O- -O.


cloudy
sunny
Conductance, cm^2 s^{-1}
0
1
2
3
4
7
9
11
13
15
17
19
Time, h

APPENDIX B

LONG-TERM BROAD BEAN GROWTH

Broad bean plants were raised from seed in the glasshouse at a density of one seed per pot. 14 days after planting, the plants were divided according to height into 5 blocks of 19 plants and each block was randomly divided into 9 pairs of plants; each pair comprised a control plant, and an aphid-infested plant with 10, 8-day-old adult pea aphids placed on it. The remaining plant was harvested on experimental day 0. This procedure was repeated for the other four blocks.

On days 5, 10, 15, 20, and 25 a control and an aphid-infested plant were harvested from each block and their dry weights were determined. On day 25, the 40 plants that remained were sprayed with Rogor^{R} to remove the aphids (see Hawkins *et al* in press 1986). A control and a formerly infested plant were harvested from each block on days 35, 50, 70, and 100 and their dry weights were determined (Table 1).

Reference

HAWKINS, C.D.B., M.I. WHITECROSS and M.J. ASTON. In press 1986. Long-term effects on cowpea plant growth of a short-term aphid infestation. *Can. J. Bot.* **64**:

Table 1. Mean plant dry weights for control and pea aphid-infested broad bean plants on experimental days 0, 5, 10, 15, 20, and 25 and for control and formerly aphid-infested broad bean on days 35, 50, 70, and 100 all with the LSD and the percentage of aphid-infested plant biomass with respect to control plant biomass.

	Plant DW[$] g			A/C
Day	C	A	LSD	%
0	0.448	0.448	ns	100.0
5	0.730	0.697	ns	95.5
10	1.205	0.790	0.152	65.6
15	1.981	0.831	0.182	41.9
20	2.718	0.848	0.836	31.2
25[¢]	5.003	1.521	2.082	30.4
35	11.257	3.087	2.489	27.4
50	17.217	4.870	2.977	28.1
70	29.138	14.766	2.442	50.7
100	77.690	42.360	9.412	54.5

Note: If the means are not significantly different (ns) by ANOVA, the LSD is not presented. ($\alpha = 0.05$).

$, Abbreviations: DW, dry weight; C, control plants; A, aphid-infested or formerly aphid-infested plants.

¢, On day 25 all remaining plants, both control and aphid-infested, were sprayed with Rogor(R), a systemic insecticide, to remove the aphids.