

Can the plant-mediated impacts on aphids of elevated CO₂ and drought be predicted?

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Abstract

The effects of drought and elevated CO₂ on the performance of sap-feeding aphids is considered. It is assumed that, for these stressors, the major influence will act through altering host plant composition and therefore diet. Changes in the plant may affect the ability to locate phloem tissues, while changes in composition of the sieve element forming the aphid diet may have more direct effects. It is concluded that plant response to conditions where carbon is present in excess (elevated CO₂) or its consumption is exceeded by its availability (drought) is heterogeneous at the cellular level. The complexities of the response of the plant to components of climate change are paralleled by the diversity of the responses of aphids to drought and elevated CO₂. Potential control points are discussed and it is concluded that current knowledge, both descriptive and mechanistic, supports the view that it is unreasonable to expect that a single plant component can predict the general response of aphids to climate change. Instead, it is more likely that aphids use a variety of cues when interacting with their host plants, and individual species respond to changes in their diet differently. Further work examining the response of both plant and aphid transcriptome and metabolome will support or contradict this hypothesis.

Keywords: aphid, carbon partitioning, cell, drought, elevated CO₂, growth, nitrogen, nutrition, osmoregulation, phloem, sieve element

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Introduction

Climate change is potentially the most important factor affecting natural and agricultural ecosystems. Its impact on the performance of herbivores is central to informing ameliorative or adaptive strategies. Climate change constitutes three dominant variables: elevated CO₂, altered rainfall patterns, and temperature. The effects of these on herbivores can be through a direct influence of the stressor, or indirectly through effects on the host plant. Temperature affects herbivorous insects directly (Bale, 2002), and it is argued that these effects will dominate the response of insects to climate change, for example, the distribution of many insects is moving polewards, partly because of increased winter survival (Bale *et al.*, 2002).

The aim of this paper is to more narrowly examine how the components of climate change of elevated CO₂ and water availability affect herbivores indirectly through

their host plants. Altered rainfall patterns will lead to both increased and decreased water availability, imposing stresses of waterlogging and drought, respectively. Excess water can impose anaerobic conditions around plant roots and may result in altered growth and nutrient status (Dennis *et al.*, 2000). However, successful plant response is restricted to a small number of specialists and, on a global scale, will probably not be a significant component of aphid response to climate change. Thus, the major impact of changes in water availability is considered to affect aphids through the effect of drought on their host plants. Elevated CO₂ has little direct effect on insects (Bale, 2002) but does have significant effects on plant growth and composition (e.g. Hartley *et al.*, 2000; Bae & Sicher, 2004; Seneweera & Conroy, 2005), as does drought (Johnson *et al.*, 1996). Many studies have examined the effect of elevated CO₂ on the performance of a diverse range of herbivores, including snails (Bezemer & Knight, 2001), leaf miners (Johns & Hughes, 2002), lepidopteran larvae (Goverde & Erhardt, 2003) and aphids (Awmack *et al.*, 2004). An emerging conclusion is that there is no

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general response to elevated CO₂, with different feeding guilds showing qualitatively different responses (Bezemer & Jones, 1998). This is matched by variability in plant species response to elevated CO₂ (Morgan *et al.*, 2004), which might explain some of the variation in herbivore response. Alternatively, (or additionally), variability in herbivore performance might result from a differential change in the composition of various plant parts in response to elevated CO₂. While chewing insects consume a whole plant organ, herbivores such as aphids feed almost exclusively from one cell type; the sieve element of the phloem. Focussing on the response of plant and sieve element composition to elevated CO₂ and drought, and alterations in the cues that enable aphids to locate it, may allow a clearer prediction of the effects of climate change on this sap-feeding guild.

Effects of elevated CO₂ and drought on plants

Plant response to both drought and elevated CO₂ is complex, both plant growth and composition can be altered. CO₂ is the raw material for the sugars synthesized in photosynthesis and therefore provides the basis for everything else that the plant subsequently does. The effects of elevated CO₂ range from a general increase in plant biomass (Amthor, 1995) to increases in both leaf (Seneweera & Conroy, 2005) and root (Ferris & Taylor, 1994) elongation. In contrast, drought stress has detrimental effects, from reducing productivity (Ciais *et al.*, 2005) to altering expansion of both leaves (Durand *et al.*, 1995) and roots (Johnson *et al.*, 1996). The magnitude of the effect of CO₂ is species specific. For example, C4 plants show little response when compared with C3 plants (Dipperry *et al.*, 1995; Yoder *et al.*, 2000). Stage of plant development introduces further complexity; Experiments in the field using Free Air Carbon Dioxide Enrichment (FACE) indicated that young trees show a greater response to elevated CO₂ than those in a mature canopy (Gielen *et al.*, 2003), leading to the general conclusion that the mature trees that dominate ecosystems may show little or no response to elevated CO₂ (Korner *et al.*, 2005).

An elevation in atmospheric CO₂ is often accompanied by an increase in the relative carbon composition of the plant (Hartley *et al.*, 2000). However, such increases can increase the C:N ratio. As changes in herbivore activity and performance are often restricted by nitrogen, not carbon (Hartley *et al.*, 2000; Awmack & Leather, 2002), this alteration in plant nutrition is considered pivotal in mediating herbivore response to elevated CO₂. Increased availability of carbon under elevated CO₂, as well as the need for more solutes under drought, alters patterns of resource partitioning within the plant. Solute, in particular organics,

generally move through the phloem and so sieve element composition is likely to be particularly affected. In addition, presence of extra carbon can change the distribution of other metabolites (Veteli *et al.*, 2002; Goverde & Erhardt, 2003; Bae & Sicher, 2004). Some of these may have a role in modulating aphid feeding behaviour.

If sufficient CO₂ is fixed, stomata often close to minimize further water loss. Broad leaved trees exposed to elevated CO₂ had reduced transpiration and therefore improved water status (Cech *et al.*, 2003). As a consequence, plants in elevated CO₂ may perform better under drought (Poorter & Perez-Soba, 2001). If the effect varies among different species, as it does between C3 and C4 plants (Yoder *et al.*, 2000), the subsequent change in species composition could affect the distribution of herbivores that feed on them (Hartley & Jones, 2003). Such indirect effects on herbivores could also occur if increases in plant biomass under elevated CO₂ result in an increase in leaf litter. For example, an increase in leaf litter was shown to enhance the nutritional quality of plants through the increased activity of soil microbes and earthworms. As a consequence, performance of the herbivores feeding on those plants also increased (Newington *et al.*, 2004; Wurst *et al.*, 2004). Furthermore, performance of aphids could be affected if climate change affects distribution of their natural enemies (Awmack *et al.*, 1997b).

Heterogeneity of the response of individual plant cells to drought and elevated CO₂

Plants must maintain a lower water potential than the environment to maintain turgor pressure that is essential to their function. Under drought this is achieved through increased accumulation of solutes within cells. Solute used in this process of osmoregulation are diverse and may be organic or inorganic (Iannucci *et al.*, 2002). Growing cells tend to accumulate organic solutes (Johnson *et al.*, 1996), whereas mature tissues can accumulate a range of solutes including inorganic ions (Patakas *et al.*, 2002). Different cells can have very different compositions, within roots (Pritchard *et al.*, 1996) and within a single tissue such as the epidermis (Fricke *et al.*, 1996). Many plant cells have a large vacuole so that organic and inorganic solutes accumulating here will dominate the whole cell complement. As there is little significant metabolism in the vacuole, many different solutes can be utilized, ranging from sugars such as glucose and fructose, through a range of potassium salts and in some circumstances, even relatively toxic solutes such as NaCl (Glenn *et al.*, 1999). The degree of osmoregulation undergone by a tissue will also depend on both tissue age and level of drought

stress imposed (Pritchard *et al.*, 1991). Cytoplasmic enzymes require a tightly regulated environment, so only solutes that do not interfere with their function can be accumulated in this compartment. These so-called compatible solutes include a range of sugars and nitrogen-containing compounds such as proline and betaine (e.g. Rasanen *et al.*, 2004). In addition, individual cell types may vary in composition around the plant, for example, sieve element amino acid composition was higher in young growing leaves than in more mature tissue (Merriitt, 1996).

Plants operate on limited resources and these are partitioned to the most appropriate process. Thus, increased carbon fixation under elevated CO₂ does not necessarily increase the availability of carbon throughout the plant. Drought may result in an uneven solute distribution. For example, if extra carbon becomes available, this is often partitioned to the roots through the phloem. The resulting increased root mass is thought to be an adaptive response to maximize water gain. Root extension can be stimulated by drought, accentuating the increase in root: shoot ratio (Egilla *et al.*, 2001). Thus, both cellular and whole plant adaptive responses can change the local composition of the plant. The impact of climate change on herbivores will, therefore, depend on the change in composition of the particular cell type fed upon, and the effect of diet on the performance of the herbivore feeding guild under consideration (Bezemer & Jones, 1998; Awmack & Leather, 2002).

A variety of different effects of elevated CO₂ on aphid feeding performance have been described. Plants grown under elevated CO₂ had an increased C : N ratio, which reduced the performance of chewing insects but had variable effects on aphid herbivores (Holopainen, 2002). An improvement in aphid performance feeding on plants grown under elevated CO₂ was noted by Awmack *et al.* (1997a), who speculated that this feeding guild might display compensatory feeding responses when faced with altered diet. *Myzus persicae* (Sulzer) had an increased reproductive rate when feeding on plants grown under elevated CO₂, despite no alteration in composition of leaf tissue, highlighting the cell-to-cell heterogeneity that can be masked by a whole tissue approach. In contrast, infestation by the aphid, *Rhopalosiphum padi* (L.) was greatly decreased on the grass, *Festuca arundinacea* (Schreb.), grown at elevated CO₂, despite a significant increase in biomass production by the plant (Newman *et al.*, 1999). Aphids feeding on sycamore and beech grown under elevated CO₂ had slightly decreased performance but unchanged population size (Docherty *et al.*, 1997). A decrease in fecundity of the aphid *Cepegilletta betulaeifoliae* (Granovsky) was observed on aspen trees grown under elevated CO₂ but

this did not correlate with changes at the population level (Awmack *et al.*, 2004). Qualitatively different feeding behaviour can occur within a feeding guild; *M. persicae* showed no difference in performance on *Brassica oleracea* (L.) under elevated CO₂, whereas the cabbage aphid, *Brevicoryne brassicae* (L.), had reduced performance (Bezemer *et al.*, 1999). As the plant material in this experiment was identical, the results imply that the different response of the two aphid species must be due to differences in the way they respond to the range of chemical cues presented by the plant, or species specific differences in their physiology that facilitate feeding at different sites on the plant.

Will drought or elevated CO₂ affect sieve element localization by aphids?

It seems that current data sets do not allow generalizations of aphid performance under climate change. The subtlety of the plant–aphid interaction and its potential diversification under evolutionary pressure may mean that we can only predict changes in performance at the species level; a reductionist approach can provide important data. In contrast to chewing insects, understanding the interactions of plants and phloem feeders is considerably more difficult given the relative inaccessibility of the phloem tissue in plants. In addition, aphids can alter feeding behavior and the way they metabolize the diet and so modify any direct associations between performance and plant composition.

Aphids face many obstacles before they can feed successfully from a sieve element. Some of the major obstacles encountered *en route* from the surface of the plant to the sieve element are shown in Fig. 1. Whether these are structural barriers, such as cuticles and cell walls, or compounds of secondary defence, they can all be modified by the components of climate change.

The outside of the plant is usually covered with a wax cuticle of complex chemistry that can provide cues to stimulate or deter feeding. These potential cues can be altered by elevated CO₂, for example, *Opuntia ficus-indica* (L.), had a thicker cuticle containing more wax following exposure to elevated CO₂ (North *et al.*, 1995). Once it has penetrated a tissue, the aphid attempts to locate the sieve element by puncturing and sampling individual cells (Tjallingii & Esch, 1993). Alteration in the composition of these cells may change the cues used by the aphid to navigate their stylets and thus affect the time taken in 'pathway mode'. As the aphid stylet is of a finite length, changes in the depth to which the insect must penetrate to reach the phloem will also modify performance. The aphid *Tuberolachnus salignus* (Gmelin) feeding on 1 year old *Salix alba* (L.)

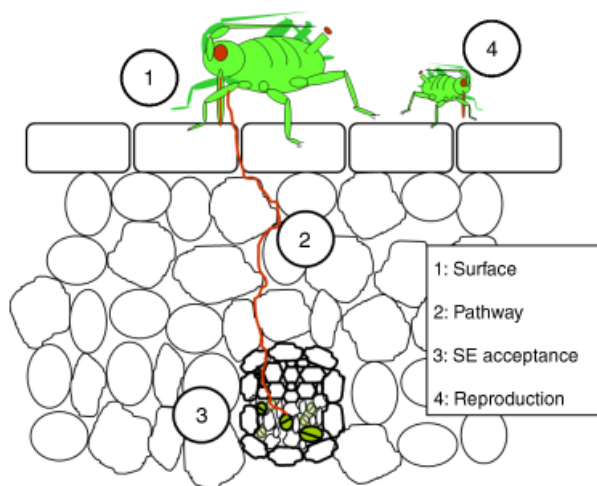


Fig. 1 Main barriers encountered by phloem feeding aphid herbivores preventing optimal performance during feeding. 1, *Surface effects*: once the plant is located the aphid must receive appropriate cues to commence probing the tissue. 2, *Pathway*: the stylet takes a mainly intercellular route but cells are pierced and 'tasted' on route, intra- or inter-cell mechanical and chemical changes can directly impeded progress or interfere with final sieve element location. 3, *Sieve element acceptance*: Once the sieve element is penetrated specific plant defence responses or unsuitable sap composition can prevent significant phloem sap ingestion. 4, *Reproduction*: once ingestion commences the sieve element sap must be of correct nutritional balance to permit maximal aphid growth and reproduction. Note that cells and aphid are not drawn to scale.

only needed to insert the distal end of its stylet, but had to insert the full 1.8 mm stylet length in trees aged 5–6 years, as secondary thickening had increased the depth of the cambium (Mittler, 1957). Young aphids have shorter stylets than adults and are therefore restricted to younger tissues of the host plant where the sieve element is nearer the surface (Elliott & Hodgson, 1996). The components of climate change of both elevated CO_2 and drought can have significant effects on leaf structure. In *Populus* species, long-term exposure to elevated CO_2 significantly increased leaf thickness (Tricker *et al.*, 2004). Similarly, leaves of the tropical tree, *Alternanthera crucis* (Moq.), increased in thickness following drought (Rengifo *et al.*, 2002). In *Olea europea* (L.), drought decreased leaf cell size and density, as well as the number of leaf scales (Bosabalidis & Kofidis, 2002). The grass, *Elymus repens* (L.), increased leaf thickness following long-term exposure to elevated CO_2 and there was also an increase in sclerenchymatous tissue between the vascular bundles (Engloner *et al.*, 2003). All these changes in leaf anatomy and structure may potentially affect the ability of phloem feeding insects to locate the sieve element.

In addition to physical features, alteration of carbon partitioning within the plant can lead to changes in

nondietary factors that can influence insect feeding, including increases in levels of secondary plant compounds (Veteli *et al.*, 2002). For example, phenolic concentration increased in tissues of *Dactylis* and *Bromus* species growing at elevated CO_2 (Castells *et al.*, 2002). The aphid, *R. padi* performed better under high CO_2 , despite an increase in the tannin content of whole leaves (Zhang *et al.*, 2003) and an increase in plant phenolic compounds (Bezemer & Jones, 1998). Presumably, the precise feeding method of the aphid allows it to avoid some of these potential defence compounds. Attempts to predict the impact on aphid performance through surface or pathway effects may be confounded by the different responses of aphid species to the same cues. Comparing *M. persicae* and *B. brassicae* feeding on a range of host plants revealed the different responses of the two aphid species. For example, from an analysis of cell probing before phloem ingestion it was concluded that, in contrast to *M. persicae*, *B. brassicae* feeding behaviour was not affected by the presence of phagostimulants such as glucosinolates (Cole, 1997). Supporting this conclusion, analysis of probing behaviour of *B. brassicae* on leaves and stems of *Sinapis alba* (L.) were unable to correlate differences in feeding with glucosinolate composition of the tissue (Gabrys *et al.*, 1997). These qualitative differences in behaviour between aphid species were further exemplified by differences observed in the effects of elevated CO_2 on reproduction; *M. persicae* produced more offspring at elevated CO_2 , whereas *B. brassicae* reproductive rates were depressed (Bezemer *et al.*, 1999).

The effect of climate change on phloem composition

Once the aphid locates the vascular bundle, it pierces and samples sap from several sieve tube elements before selecting one to feed from (Tjallingii & Esch, 1993). The composition of phloem sap not only influences the aphid's decision of whether to accept or reject a host, but it will also determine subsequent growth and reproduction. Partly because of practical difficulties in obtaining pure sieve element sap, and partly because analytical techniques have until recently lacked sensitivity, there is relatively little information about phloem composition. It is generally accepted that phloem sap consists of water, carbohydrate (often as sucrose), amino acids, inorganic ions, proteins, hormones, mRNA, lipids and various secondary compounds (Van Bel, 2003). Advances in molecular and analytical techniques will begin to identify the range of compounds present in the sieve element and, thus, highlight previously uninvestigated targets, including sieve element proteins, which may influence aphid feeding (Walz *et al.*, 2004; Kehr, 2006).

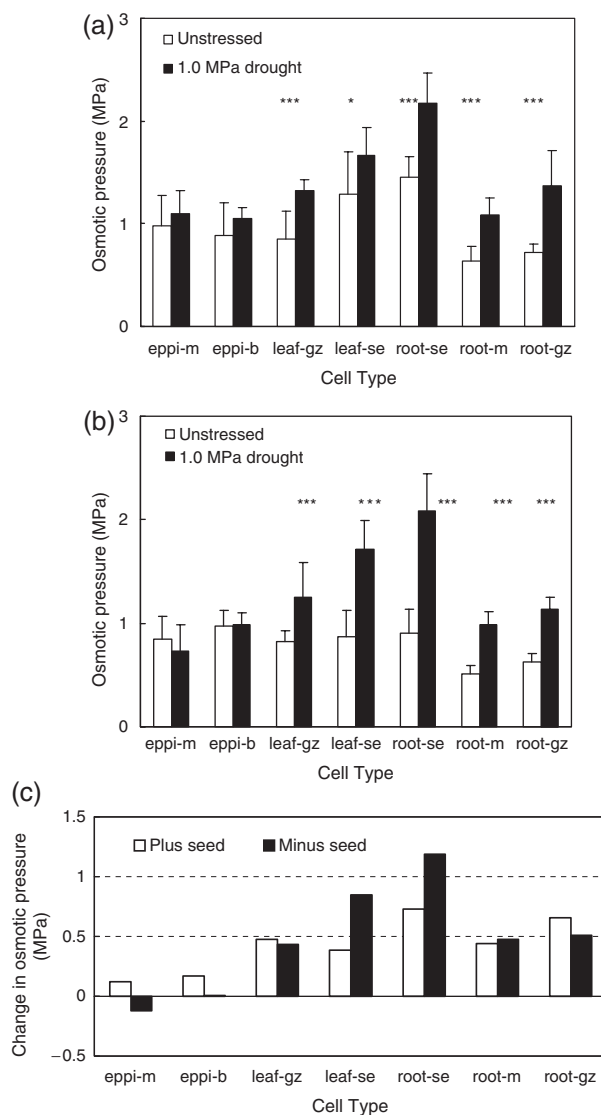
Despite the lack of detailed knowledge, it is clear that phloem solute composition is not constant and varies between different plant species, during development and following environmental perturbation (e.g. Smith & Milburn, 1980; Merritt, 1996; Gould *et al.*, 2004). Both the total number of solutes and the relative amounts of each component can change, making the diet more or less suitable. In maize, variation in root extension over the day–night cycle was interpreted as an increase in translocation of carbohydrate through the phloem to the root (Muller *et al.*, 1998). However, diurnal variation in sieve element composition is not common to all plants, as absence of photoassimilates during the night can be buffered by uptake from storage tissues (Weatherly *et al.*, 1959). Reduced levels of light reduced sieve element sucrose in *Salix alba* from over 300 mM to below 30 mM (Mittler, 1958). Large changes in sieve element sucrose occurred in *Tilia americana* (L.) around bud break, correlating with changes the feeding rate of the aphid *Longistigma caryae* (Harris) (Hill, 1962).

Response to drought can vary between different cell types. Exposure of barley seedlings to drought increased the osmotic pressure of sieve element cells to a greater extent than other cell types (Fig. 2). In plants with the seed present, cell osmotic pressure ranged from 0.63 to 1.45 MPa (Fig. 2a). The growing points of both leaves and roots had high osmotic pressure, reflecting their need for high turgor to drive growth. However, the greatest osmotic pressures were seen in phloem sieve elements. Following a 24 h exposure to 1.0 MPa drought stress, sieve elements and cells at growing points osmoregulated to a greater extent than the older tissues, (Fig. 2a).

As osmotic pressure of the sieve element is not usually iso-osmotic with aphid haemolymph, changes in the osmotic pressure of the sieve element increase the cost of osmoregulation. Under unstressed conditions

barley sieve element sap was 1.2–1.4 MPa, similar to that of aphid haemolymph (Pritchard, 1996). However, both honey dew and aphid haemolymph remain unchanged even when faced with diet osmotic pressures of up to 4 MPa (Wilkinson *et al.*, 1997). The difference in water potential between aphid and diet represents a large driving force, potentially dehydrating the aphid. That aphids can osmoregulate is well known (Weatherly *et al.*, 1959; Fisher *et al.*, 1984; Douglas, 2003). Aphids are able to raise the water potential of their diet by polymerizing the incoming sucrose into oligosaccharides with gut-localized enzymes (Wilkinson *et al.*, 1997; Karley *et al.*, 2005). Presumably, the alteration in metabolism required for osmoregulation constitutes a cost to the aphid, reducing performance. However, drought does not always affect phloem-feeding herbivores; whitefly feeding on water-stressed melon did not show

Fig. 2 Response to drought of intact and seedless barley cells. Six-day-old barley seedlings were grown hydroponically in the presence or absence of the external osmoticum, 6000 polyethylene glycol to achieve a water potential of 1.0 MPa. After 24 h exposure the osmotic pressure of individual cells was measured. Cell sap was extracted from leaf and root cells using the pressure probe, osmotic pressure was measured using melting point depression of picolitre samples, phloem sap was obtained using aphid styletomy (Pritchard, 1996). Each bar represents the mean of between 15 and 38 measurements \pm SD. eppl-m, mature leaf epidermis; eppl-b, young leaf epidermis; leaf-gz, leaf growing zone; leaf-se, leaf sieve element; root-se, root sieve element; root-m mature root cortical cells; root-gz, root growing zone. Significant differences at $P < 0.05$ or $P < 0.01$ shown as * and *** (J. Pritchard, unpublished results). (a) Intact plant. (b) Seed removed. (c) Change in osmotic pressure following drought.



any change in performance despite an increase in phloem sugars (Isaacs *et al.*, 1998). As it is difficult to separate the effects of osmotic stress from changes in individual dietary components, the precise costs of aphid osmoregulation are difficult to determine.

The heterogeneous pattern of solute accumulation and osmoregulation, illustrated in Fig. 2a, depends on the availability of carbon within the plant. Carbon availability depends on environmental conditions that affect its production (e.g. light, elevated CO₂) or its consumption (e.g. drought) and can be manipulated experimentally in a number of ways such as root pruning, imposition of drought, alteration of light levels or, in young seedlings, by removal of the seed. Plant response to drought is moderated by the availability of carbon, illustrated by the alteration in pattern of solute distribution following seed removal. In unstressed barley seedlings, removal of the seed lowered sieve element osmotic pressure in comparison with intact plants (compare Fig. 2a and b). However, sieve element osmotic pressure was increased following drought by over 1.0 MPa in seedless plants. Thus, in carbon sufficient plants, sieve element cells showed the greatest increase in osmotic pressure following drought (Fig. 2c). While in these experiments the carbon resource was manipulated by removing the seed, during climate change a similar interaction may occur if elevated CO₂ increases carbon availability and alters the cellular response. Thus, the interaction between resource availability and partitioning makes it difficult to model the response of the plant to climate change at the cellular scale.

Osmoregulation requires changes in total solute concentration, however, the response of individual solutes will not necessarily change in parallel. In *Ricinus communis* (L.) while there is high phloem sucrose and low potassium concentration during the day when carbon is readily available, total solute concentration remains unchanged despite a decrease in carbon during the night, as potassium salts make up the shortfall during this period (Smith & Milburn, 1980). Such reciprocity of individual solutes makes it difficult to predict changes in specific phloem composition under the different environmental conditions imposed by climate change.

While the increase in phloem osmotic pressure following drought represents an increase in the total number of solutes, it does not indicate which solutes are changing. Direct effects of elevated CO₂ on phloem composition have not been well studied. Circumstantial evidence for changes in sieve element solutes is provided by the observation that root growth can be increased under elevated CO₂, reflecting an increased amount of photoassimilate moving to the root tip

through the phloem (Ferris & Taylor, 1994). Different solutes can generate the same osmotic pressure. In knockout mutants of *Arabidopsis thaliana* (L.) lacking an amino acid transporter, phloem osmotic pressure was the same as wild type plants. Despite this constancy, the contribution to osmotic pressure by amino acids was 62% in the wild type, but only 16% in the AAP6 mutant. Some other solute(s), perhaps sucrose, make up the shortfall (Hunt *et al.*, 2006). Thus, phloem composition is also strongly affected by plant genotype.

Gradients of solutes away from sieve elements may be used by aphids to locate their food source. Any alteration in the amount of solute in the phloem may affect rates of leakage. Thus, any impact of climate change on sieve element solute levels may alter the efficiency of sieve tube localization.

Phloem solute concentrations change following drought (e.g. Fig. 2). If a solute such as sucrose is an important cue in sieve element location, alteration of its phloem concentration will change the sucrose gradient that the aphid stylet may follow in locating its feeding site. The hypothesis that phloem sucrose is a cue for phloem location was tested using *Arabidopsis* plants mutant in the AKT 2/3 potassium channel, resulting in a lowered phloem sucrose concentration (Deeken *et al.*, 2002). The electronic penetration graph (EPG) technique was used to measure feeding behaviour on these plants (Tjallingii & Esch, 1993). *M. persicae* feeding on the mutant took significantly longer to attain sustained phloem ingestion than on wild-type plants (Fig. 3). While sucrose levels are affected by drought and elevated CO₂, thereby potentially reducing aphid ability to locate the sieve element, other solutes may also be used as cues by aphids. More information is needed on both the regulation of phloem composition and the plant cues used in its localization by aphids.

Sieve element sap as diet

Once the sieve element has been located, two factors affect subsequent aphid performance. Firstly, appropriate cues must be present to allow sustained feeding and secondly, sap composition should permit optimal growth and reproduction. Many substances have been proposed as phagostimulants. For example, decreasing the levels of sucrose, one of the suggested phagostimulants, in artificial diets decreased aphid feeding rate (Klingauf, 1987). Furthermore, variations in growth, reproduction and behaviour of aphids, together with their tendency to migrate between host plants, has been attributed to the large fluctuations in sucrose content that occur during various times of the year as a result of changes in levels of photosynthesis (Auclair, 1963). Many aphids preferentially colonize the growing tips

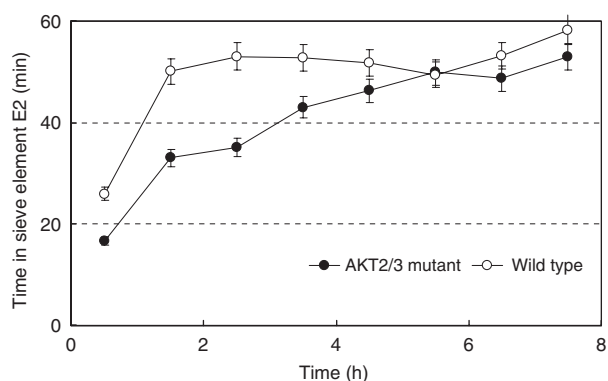


Fig. 3 Effect of reduced phloem sucrose on aphid feeding behaviour. Time spent in sustained ingestion of phloem sap (E2 phase) for each hour for adult *Myzus persicae* feeding on wild type and AKT 2/3 knockout mutants with reduced sucrose phloem concentration. Each point represents the mean of 23 electronic penetration graph determinations \pm SE. Scheirer-Ray-Hare test showed that effects of both time ($P = 0.002$) and genotype ($P = 0.003$) were significant. Mann-Whitney test indicated that time in E2 phase was significantly lower in the mutant in hours 2, 3 and 4 at a probability of $P < 0.05$. (B. Griffiths, unpublished results).

and shoots of plants, a tendency that may be due to young tissue being physically easier to penetrate, or perhaps because these growing sinks contain the highest concentration of carbohydrates and amino acids, as observed in *Brassica nigra* (L.) (Merritt, 1996).

Despite, or perhaps because of, the dominance of carbohydrate as a phloem solute, nitrogen levels are predicted to have the greatest influence on aphid performance (Mittler, 1967a; Jansson & Smilowitz, 1985; Dixon, 1998). Changes in phloem nitrogen often occur in tandem with changes in overall phloem carbohydrate, illustrating that changes in phloem solutes are often linked. It is therefore difficult to separate the effects of individual solutes. Aphid species sometimes migrate from their winter woody plant hosts, when levels of nitrogen decrease during the summer time, to annual crops. In the autumn, levels of nitrogen increase again in senescing leaves of the woody plants and aphids migrate back to these hosts, at a time when annual plants are no longer favourable hosts. Studies of aphids feeding on artificial diets have shown that the concentration and composition of dietary amino acids influence their feeding behaviour and performance (Mittler, 1967b, 1970; Srivastava & Auclair, 1975). In fact, aphid performance on plants could be affected not only by the overall amino acid concentration, but by the relative concentrations of different amino acids (Mittler, 1967a, b). Most importantly, a subset of 10 so-called essential amino acids must be present in the diet to facilitate growth and reproduction. A decrease in the

level, or omission, of individual amino acids from a mixture of 20 in a synthetic diet affected the feeding and growth of *M. persicae*, this aphid species also preferred a mixture of sucrose and amino acids to pure sucrose (Mittler, 1967a).

Manipulation of aphid diet *in planta* has also demonstrated the importance of nitrogen on aphid settlement and development (Ponder *et al.*, 2000). Aphids feeding on plants grown under high nitrogen supply had a higher intrinsic rate of increase. The phloem sap had a higher concentration of amino acids and the aphids spent longer plugged into the sieve element. Thus, diet, performance and behaviour were all consistently increased, suggesting a causal link between phloem nitrogen and aphid fitness. In potato, aphid feeding rate varied with the concentration of phloem nitrogen, suggesting that in this case, phloem nitrogen quality is an important cue (Karley *et al.*, 2002).

The effects of CO₂ on nitrogen levels in the plant seem quite different to those caused by drought stress. The literature indicates that when atmospheric CO₂ is increased the concentration of nitrogen decreases, increasing C:N ratios (e.g. Johns & Hughes, 2002; Chen *et al.*, 2005). This is a dilution effect caused by increased growth and/or alteration in resource partitioning (Docherty *et al.*, 1997), but these effects vary in magnitude and nature depending on the species tested (Hartley *et al.*, 2000; Awmack *et al.*, 2004).

An accelerated rate of plant development under high CO₂ can increase the rate of senescence, and is therefore predicted to increase phloem amino acid levels. Such an increase can also occur if drought causes increased proteolysis, which can increase the availability of free soluble amino acids (Bolsinger & Fluckiger, 1984, 1989; Docherty *et al.*, 1997). For example, senescing leaves of *B. nigra* had higher concentrations of phloem amino acids than younger, mature leaves (Merritt, 1996). In *Salix alba*, sieve element amino acids decreased by 50% following bud burst and decreased further to 15% in mature tissue, however, during senescence amino acids increased to 65% of the prebud-burst levels (Mittler, 1958). Given the apparent importance of nitrogen to aphids, these changes would be predicted to have a significant effect on their performance. Indeed, sap-sucking insects may be the more sensitive to such changes than other feeding guilds such as miners and chewers (Koricheva *et al.*, 1998). Drought altered phloem concentrations of the secondary compounds rutin and cyanide in cassava (Calatayud *et al.*, 1994), changes that could partially explain alterations in the performance of the phloem-feeding mealy bug *Phenacoccus manihoti* (Matile-Ferrero).

While there have been some studies on the effects of drought, information regarding the phloem-specific

effects of elevated CO_2 is scarce. However, Wang & Nobel (1995) reported a decrease of 17% in amino acids concentration in the phloem of *O. ficus-indica* (L.) under a doubled CO_2 concentration, consistent with the increase in C:N ratios seen in whole tissues. The majority of studies have examined nitrogen and amino acid content only in whole leaf samples (Docherty *et al.*, 1997; Hughes & Bazzaz, 2001). Interestingly, in potato leaves there was no correlation between leaf C:N ratio and a different measure of the same parameter in the phloem; the sucrose–amino acid ratio (Karley *et al.*, 2002). Thus, at present there is no clear trend between the amounts of nitrogen in foliar tissues and aphid performance.

Aphid compensation

Despite the correlation sometimes apparent between phloem composition and aphid performance, the situa-

tion is usually more complex for two reasons. Firstly, changes in sieve element composition can be complex, with both carbohydrate and nitrogen levels changing independently. Secondly, aphid behaviour and metabolism can be modified to compensate for nonideal diets.

In droughted *Dactylis glomerata* (L.), osmotic stress increased sieve element osmotic pressure from 1.1 to 2.5 MPa, with 6% of the increase in osmotic pressure attributable to an increase in amino acids (Hale *et al.*, 2003, Fig. 4a and c). It may be assumed that the majority of the osmotic adjustment was due to increases in carbohydrate, probably sucrose. Despite this increase in phloem carbohydrates there was no difference in the time taken by aphids to reach the phloem, suggesting that sucrose was not a major factor of aphid performance in this study (data not shown). Interestingly, in a second grass species, *Arrhenatherum elatius* (L.), there was a similar change in both osmotic pressure and phloem amino acids following drought stress. Despite

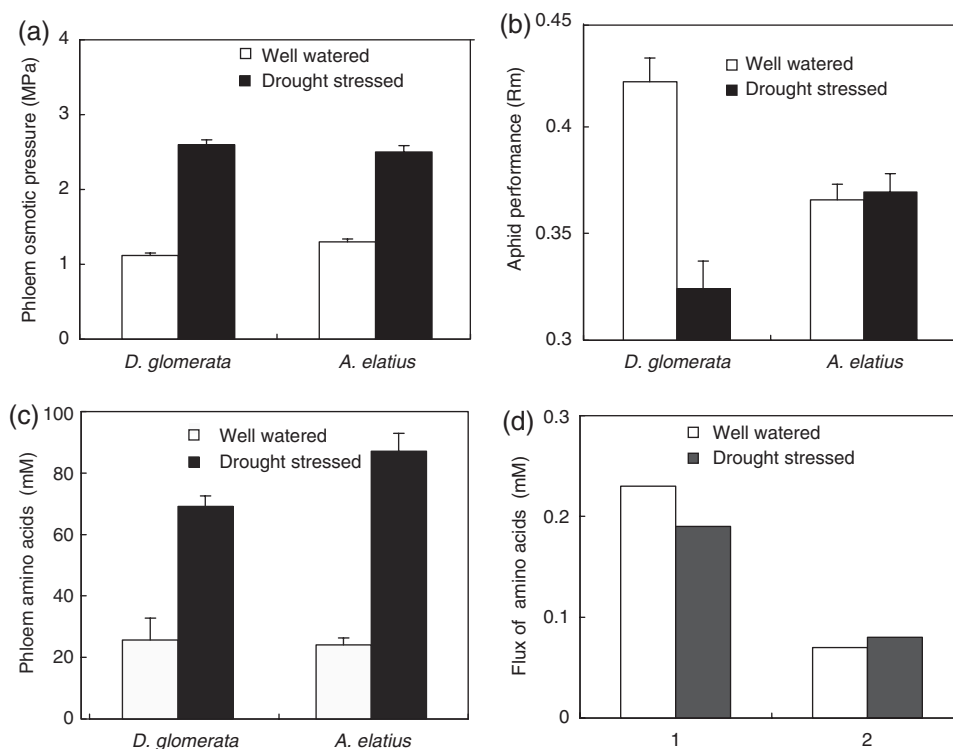


Fig. 4 (a) Total phloem solutes (measured as sap osmotic pressure) on well-watered or droughted native British grasses *Dactylis glomerata* or *Arrhenatherum elatius*. Each point is the mean of at least 13 determinations \pm SE. Increases in osmotic pressure following drought are significantly different ($P = 0.05$). Both species showed the same response. (b) Aphid reproductive performance (measured as Rm) on two well-watered or droughted native British grasses. Each point is the mean of 14 determinations \pm SE. Difference in Rm are significantly different between species and between treatment for *D. glomerata* but not between treatments for *A. elatius*. (c) Total phloem amino acids measured using high-performance liquid chromatography of phloem sap on two well-watered or droughted native British grasses. Each point is the mean of nine to 14 determinations \pm SE. Increases in total amino acids significantly different ($P < 0.05$) but not between species. (d) Flux of amino acids through aphids feeding on two well-watered or droughted native British grasses. All data were calculated from phloem amino acid composition (Fig. 5c) and volume of diet ingested by the aphids (data not shown). All data are redrawn from Hale *et al.* (2003).

the similarity of change in phloem composition, aphid performance was unaffected by drought when feeding on this species (Fig. 4b). In this study, changes in aphid performance could not be explained by changes in the total or individual amino acids. Changes in the feeding rate of aphid feeding and the uptake of solutes from the diet indicated that the flux of essential amino acids through the aphid gave the best correlation with the observed changes in fitness, both between the two species of host plant and following drought (Fig. 4d). As there is little detailed information regarding phloem composition or the chemical cues that affect aphid feeding behaviour, it is currently not possible to predict how aphid compensatory behaviour affects the relationship between diet and performance.

Transgenic technologies can be used to alter phloem nitrogen levels to study the effects on aphid feeding behaviour and performance (Hunt *et al.*, 2006). Analysis of amino acid concentrations in sieve element sap samples extracted from individual transgenic plants has been made possible by the use of laser-induced fluorescent capillary electrophoresis (Zhu *et al.*, 2005). In *Arabidopsis* plants with a nonfunctional AAP6 or ANT1

gene, there were no large changes in total phloem solutes (Fig. 5a). However, the levels of both total amino acids and a subset of ten essential amino acids were either decreased in the AAP6 mutant or increased in the ANT1 mutant (Fig. 5b). As aphid performance is hypothesized to be restricted by the level of phloem amino acids, such alterations would be predicted to respectively decrease and increase the reproductive performance of aphids. While there was a small but significant decrease in the performance of aphids feeding on AAP6, there was no change in the fitness of aphids feeding on a diet with elevated amino acid content (Fig. 5c). Similar to the environmental modification of diet seen in the *Dactylis/Arrhenatherum* example, the lack of a correlation between diet and performance appears to contradict the aphid/nitrogen diet hypothesis (Hale *et al.*, 2003). However, once again the behaviour of the aphid modified the relationship and illustrates that simple extrapolation of performance from diet may not be possible. Measurement of the amino acid concentration in the honey dew of aphids feeding on the different diets in these mutants plant revealed differences in the ability of the aphids to

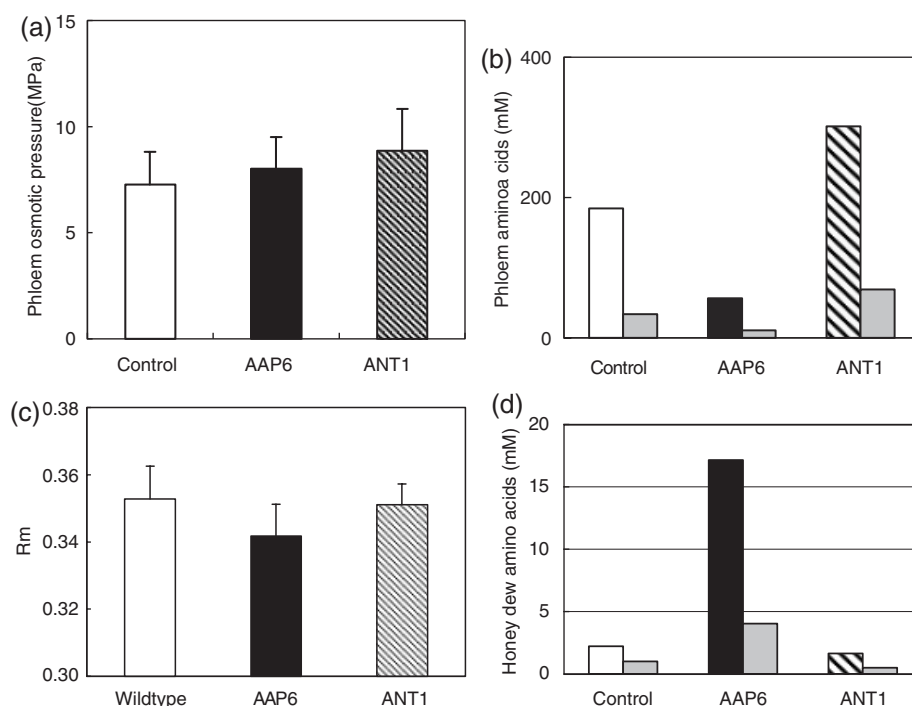


Fig. 5 (a) Osmotic pressure of phloem sap extracted from control *Arabidopsis* plants and those lacking a functional amino acid transporter gene, either AAP6 or ANT1. Each bar represents 11–13 observations \pm SE. (b) Amino acids in phloem sap extracted from control *Arabidopsis* plants and those lacking a functional AAP6 or ANT1 gene. Grey bars represent essential amino acids. Each bar represents eight to 12 observations. (c) Reproductive performance measured as Rm of aphids feeding on control *Arabidopsis* plants and those lacking a functional AAP6 or ANT1 gene. Grey bars represent essential amino acids. Each bar represents 24–26 observations \pm SE. (d) Amino acids concentration of honeydew of aphids feeding on control *Arabidopsis* plants and those lacking a functional AAP6 or ANT1 gene. Grey bars represent essential amino acids. Each bar represents 10–14 observations \pm SE. All data from Hunt *et al.* (2006).

respond to altered diet as aphids feeding on low amino acid levels had higher honeydew amino acids. In addition, aphids feeding on both mutants had elevated feeding rates as shown by increased levels of honeydew production, reflecting a potential increase in the amount of phloem sap available to the aphid, but there was no increase in aphid performance (Fig. 5c). Thus, modification of diet illustrates a number of compensatory feeding behaviours and metabolic pathways in aphids, including an increase in the time spent in the phloem (e.g. Ponder *et al.*, 2000, Fig. 6), change in feeding rate (e.g. Hale *et al.*, 2003, Fig. 4d) or an alteration in metabolism (Fig. 5d).

Compensatory metabolism can include many biochemical pathways both within the aphid and its bacterial symbiont *Buchnera*. The presence of this symbiont provides yet another way in which the relationship between diet and performance may be difficult to unravel. While different species or clones of aphids may have different responses to the same host diet, the same genotype of aphid may well contain different symbiont genotypes and therefore vary in metabolic efficiency. This area lies outside the scope of this paper and the reader is referred to recent reviews by Douglas (2003, 2006).

Conclusions

The overall conclusion of this reductionist examination of plant response to climate change is that currently, a general prediction of the response of aphid herbivores is not possible. A similar conclusion was drawn from an analysis of current data and model predictions by Newman (2003). Predicting the response of aphids to

climate change would be facilitated by greater knowledge of the response of plants to elevated CO₂ and drought. Unfortunately, plant response is variable both temporally and spatially, and phloem composition is regulated differently from that of other plant compartments. At present, we know very little about the nature or mechanism of phloem composition or its regulation. The relationship between aphid diet, behaviour and ultimately fitness is not well understood, indeed current and emerging data suggests that the response of different aphid species on the same host plant to elevated CO₂ is not consistent (Bezemer *et al.*, 1999) and may (Ponder *et al.*, 2000) or may not (Girousse & Bournoville, 1994) correlate with phloem composition. Under water stress the role of nitrogen nutrition is key to understanding the changes in performance of sap-feeding insects, however, it is not clear how environmental change affects nitrogen availability within the plant (Huberty & Denno, 2004). Aphid species can have different preferred feeding sites on the same plant (Qureshi & Michaud, 2005), with consequences for both phloem localization and composition. Such differences must have evolved as aphid species exploit subtle differences in plant chemistry. Aphid appear to be well adapted to deal with heterogeneity in plant quality and may, thus, be largely unaffected by changes imposed by climate change. If there is a common response of individual aphid species to changes in host plants, it must lie in an as yet undetected universal cue, a conclusion also reached by Newman (2003). The recent discovery of the forisome protein in sieve elements (Will & van Bel, 2006) may provide a single mechanism that regulates all sap-feeding herbivores, however, even if the mechanism is universal, it is already apparent that there is variability in the ability of aphids to overcome this plant defence (Tjallingii, 2006). In any case, the operation of such a cell specific defence mechanism will have no bearing on the complex relationship between plants and aphids during phloem localization. Furthermore, prediction may not even be possible at the level of the species, for example, different genotypes of the pea aphid *Acyrtosiphon pisum* (Harris) showed qualitatively different responses of population size, genotype and phenotype frequencies following exposure to a combination of elevated CO₂ and O₃ (Mondor *et al.*, 2005).

It seems likely that the niche separation seen in the different feeding strategies of aphids has evolved by responding to different plant cues, in both the pathway and phloem. Our model of plant–aphid interactions may become clearer as fuller knowledge of the phloem proteome (Barnes *et al.*, 2004; Walz *et al.*, 2004) and metabolome uncovers more potential cues. General mechanisms underlying plant–insect interactions may

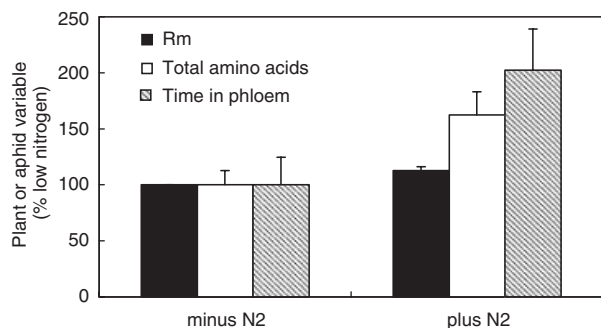


Fig. 6 Relationship between aphid performance (Rm), sustained phloem ingestion and phloem amino acid concentration for *Rhopalosiphum padi* on 7-day-old barley seedlings supplemented with zero or 8 mM nitrogen. Data is the mean of 21 measurements \pm SE expressed as % of the low nitrogen treatments. Mann–Whitney test on Rm and time in phloem, and *t*-test on phloem amino acids indicated that all parameters were significantly higher ($P < 0.05$) in nitrogen sufficient plants. Data redrawn from Ponder *et al.* (2000).

also be revealed as transcriptomic approaches catalogue the responses of plants to different aphids (Thompson & Goggin, 2006). The completion and exploitation of the aphid genome will provide essential complementary information.

The complexities of aphid interactions with the phloem have so far defied generalization. It is possible that the new molecular and analytical technologies will give an increasingly detailed understanding of the interactions of individual aphid species with their host plants, rather than a general prediction of response at the level of the whole sap-feeding guild. However, more accurate modelling, particularly combining factors such as temperature and drought (Zvereva & Kozlov, 2006), may allow prediction, especially in more focussed scenarios (Newman, 2005).

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