CHAPTER 5.2.2.2

Medicago truncatula–pea aphid interaction in the context of global climate change

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5.2.2.2.1 Introduction

Since the industrial revolution, because of anthropogenic effects, i.e. deforestation and fossil fuel combustion, atmospheric CO_2 concentrations have increased from 280 to 409 ppm in April 2017 (Mauna Loa Observatory: NOAA-ESRL), and are expected to reach at least 550 ppm by the year 2050. These increases in atmospheric CO_2 concentrations have serious implications for global warming and climate change (Stocker et al. 2013). Although changes in climate have been anticipated to greatly affect agricultural ecosystems (Fuhrer 2003), increases in atmospheric CO_2 concentration alone can also be very important because it can directly affect plant physiology and indirectly alter the associations between plants and herbivorous insects or plant pathogens (Robinson et al. 2012). These altered interactions may then lead to more severe and frequent outbreaks of pest insects and plant diseases in agricultural ecosystems (Percy et al. 2002).

The effects of elevated CO_2 on C3 plants are generally characterized by increased photosynthetic rate, biomass, and carbon : nitrogen (C : N) ratios in plant tissues (Ainsworth and Long 2005; Ainsworth and Rogers 2007). The assimilation and re-assignment of C and N resources within plant tissues under elevated CO_2 would therefore alter the nutritional quality and palatability of host plants (Couture et al. 2010). For example, C3 crop plants grown under elevated CO_2 exhibit decreases in nitrogen (N) and other trace elements, i.e. zinc and iron (Bloom et al. 2010). These decreases reduce the nutritional value for herbivorous insects and negatively affect their performance (Myers et al. 2014). For those insects that chew whole leaves, a reduction in the N concentration in crop tissue and the resulting increase in the carbon/nitrogen ratio (C : N ratio) under elevated CO_2 cause these insect pests to consume more leaves to meet their N needs (Bezemer and Jones 1998; Sun and Ge 2011). Besides, soybean plants grown under elevated CO_2 decrease their ability to produce jasmonic acid (JA), a hormone that contributes to plant defenses against chewing insects (Zavala et al. 2008). Thus, the effects of elevated CO_2 on herbivorous insects are notable through the cascading effect of host plants.

Elevated CO_2 may also increase the damage to crops caused by phloem-sucking insects including aphids, which is considered as the only feeding guild that has a positive response to elevated CO_2 (see also Chapter 5.2.2.1). Aphids feed exclusively on the phloem sap and are very sensitive to changes in plant quality caused by climate change (Pritchard et al. 2007). Recent meta-analysis results show that on average aphids tend to perform better under elevated CO_2 (Robinson et al. 2012). The conclusions from many statistically significant experiments, however, exhibit idiosyncratic responses of aphids in terms of population abundance, fecundity as well as survival. Although predictions are difficult, it is nevertheless useful to determine why some aphids are more fit while others are less fit under elevated CO_2. A mechanistic understanding can help make sense of these contradictory results. Previous studies demonstrate that the effect of elevated CO_2 on the plant, which includes C and N assimilation, secondary metabolism, plant stomatal conductance, as well as leaf temperature, could in turn affect aphid population numbers and growth (Ainsworth et al. 2006; May et al. 2013).

In this review, using *Medicago truncatula* and the pea aphid *Acyrthosiphon pisum* as examples, we considered how elevated CO_2 modifies host defense, nutrients, and water-use efficiency by altering concentrations of the phytohormones JA, salicylic acid (SA), ethylene (ET), and abscisic acid (ABA). We describe how these elevated CO_2-induced changes in defense, nutrients, and water status facilitate specific stages of aphid feeding, including penetration, phloem-feeding, and xylem absorption in the *M. truncatula*–*A. pisum* interaction.

The Model Legume Medicago truncatula, First Edition. Edited by Frans J. de Bruijn.

5.2.2.2.2 The effects of elevated CO_2 on nitrogen-fixation ability of *M. truncatula*

One of the most commonly seen phenotypes in non-legume C3 plants is a decrease in N content on the average of 10–15% (Taub et al. 2008). There are several hypotheses to explain this phenomenon (Taub and Wang 2008). For example, the hypothesis of decreased N uptake argues that, due to decreases in stomatal conductance and transpiration under elevated CO_2, plant roots reduce the nutrient uptake from soil including the N resource (McDonald et al. 2002). The hypothesis of elevated CO_2-induced N loss refers to increases in N loss by increasing the volatilization of NH_3 and/or by root exudation of organic N (Pang et al. 2006). The dilution hypothesis, the most frequently mentioned, considers that N is diluted by accumulation of more total non-structural carbohydrates (TNC) which results in a greater biomass under elevated CO_2 (Ainsworth and Long 2005). Despite this phenomenon, considering species-specific responses, this hypothesis may possibly be partially or largely responsible for the effects of elevated CO_2 on N content in leaves. By contrast, elevated CO_2 has little effect on the N content in legume plants (only −1.4%), which may possibly be attributed to the interacting with rhizobia (i.e. N-fixing bacteria).

Typically, there are three major pathways involved in N acquisition in legume plants. First, legume plants take up NH_4^+ from soil and incorporate it into organic compounds (Waring and Bremner 1964). Second, plants acquires N through NO_3^- uptake and reduction to NH_4^+ (Keeney and Bremner 1966). Third, legume plants have a unique ability to obtain N from atmospheric N_2 and utilize it through the biological process of N fixation (Keeney and Bremner 1966; see Chapter 6.1). Among these three pathways, N fixation is most costly in terms of energy and resources and legume plants preferentially obtain N via uptake from the soil rather than via N-fixation from the atmosphere. Furthermore, Guo et al. (2013a) have found that the enzyme activity of nitrate reductase (NR) and the expression of *NT* and *NR* genes of *M. truncatula* are downregulated by elevated CO_2. In fact, elevated CO_2 suppresses N uptake ability of *M. truncatula* from soil. Elevated CO_2 reduces stomatal conductance (g_s) of plants, which causes decreases in water use and the decrease in water use translates to higher soil moisture (Leakey et al. 2009). In addition, elevated oxygen consumption by the increased microbial activity (Jongen et al. 1995), may lower the oxygen partia1 pressure in the soil and therefore favor denitrification activity (Zanetti et al. 1996).

Insufficient soil N availability is compensated by increased atmospheric N fixation under elevated CO_2 (Rogers et al. 2009). After reviewing 127 studies, Lam et al. (2012) concluded that the amount of N fixed from the atmosphere by legumes increased 38% under elevated CO_2, which was accompanied by increases in whole plant nodule number (+33%), nodule mass (+39%), and nitrogenase activity (+37%). Indeed, enhancement of N fixation in legumes is essential for overcoming the N limitation under elevated CO_2 (Rogers et al. 2006).

Legumes are very flexible in their utilization of N from the soil and atmosphere under ambient CO_2. For example, deficient in nitrogen fixation (*dnf1*) mutants could not fix atmospheric N_2, or assimilate N via GS/GOGAT, but N concentration in leaves and roots did not differ from that of the wild-type under ambient CO_2. As indicated by increased gene expression and enzyme activities of essential components of the soil N acquisition pathways than wild-type *M. truncatula* Jemalong under ambient CO_2, the *dnf1* mutants compensated for the loss of N fixation by enhancing their uptake of N from soil under ambient CO_2. However, under elevated $CO_{2,}$ the N concentration in *dnf1* plants was lower than in wild-type plants, thus indicating that nitrogen fixation mutants show limited N availability under elevated CO_2 (Guo et al. 2013a).

Integrated measurements of N fixation using the $\delta^{15}N$ isotope dilution method will ultimately lead to a better understanding of the percentage of N derived from atmosphere (% Ndf) and N derived from N-fixation per gram plant dry weight (fixed N). Guo et al. (2013a) found that % Ndf and fixed N were both increased for the wild-type, but were not significantly different for the deficiency in nitrogen fixation mutants under elevated CO_2. Soil N, however, decreased for all genotypes under elevated CO_2. Regardless of CO_2 levels, wild-type plants fix more N_2 from the atmosphere but take up less N from soil than nitrogen fixation mutants. Furthermore, Lüscher et al. (2000) found that even in the high N treatment, ineffectively nodulating lucerne were unable to increase the N concentration and biomass under elevated CO_2. Thus, the amount of N in the soil is insufficient to meet the increasing demand of *M. truncatula* for N in order to fully transform the increased CO_2 fixation into biomass (Zanetti et al. 1997).

In conclusion, elevated CO_2 decreases N uptake from soil but increases N fixation in wild-type *M. truncatula*. This suggests that as atmospheric CO_2 levels continue to rise, legumes may rely more on N fixation and less on N uptake from soil. Although these studies have important implications for agriculture and for regional and global N budgets under predicted CO_2 conditions, the enhancement of leguminous N fixation by elevated CO_2 is environment dependent (West et al. 2005). N fixation can be limited by the availability of other soil nutrients (i.e. molybdenum, phosphorus, potassium) or by abiotic stresses (van Groenigen et al. 2006). Thus, environmental variables in addition to atmospheric CO_2 concentrations should be considered when predicting future N dynamics of legumes.

5.2.2.2.3 The enhanced nitrogen fixation ability of *M. truncatula* could facilitate aphid amino acid metabolism and performance under elevated CO_2

Very different from the leaf-chewing insects, the sap-sucking insects (i.e. aphids) with stylet-like mouthparts feed exclusively upon phloem sap of plants for their nutrient intake (Douglas 2003). Phloem sap, however, provides a highly imbalanced food resource, containing as low as a 0.1 protein/carbohydrate ratio (mainly amino acid/sugar in phloem sap, w/w) (Nowak and Komo 2010). The fact that aphids can grow well is attributed to dozens of specialized cells named bacteriocytes in the haemocoel, which harbor a primary endosymbiont *Buchnera* (Nakabachi et al. 2005). *Buchnera* utilizes non-essential amino acids in aphids to provide up to 90% of essential amino acids (Douglas et al. 2006). Conversely, aphid metabolism supports a substantial flux of non-essential amino acids from the insect to *Buchnera*, mostly via phloem sap of host plants (Wilson et al. 2010). Presumably, this is why aphids prefer host plants with relatively higher N and amino acid concentrations (Nowak and Komo 2010). Thus, even though aphids could develop without one or even several kinds of amino acids (Gündüz and Douglas 2009), the total N concentration especially the amino acid concentration of the host plant is the most important determinant to aphid population fitness.

The mathematic model constructed by Newman et al. (2003) predicts that aphid populations tend to be larger under elevated CO_2 if the host plants have higher N supplementation. Similarly to our previous study, elevated CO_2 enhances biological nitrogen fixation (BNF) to compensate for the N decrease in Jemalong, but had no effect on BNF by the N-fixation deficient mutant *dnf1* (see Chapter 9.2.1), resulting in decreases of N assimilation and N concentration in *dnf1* under elevated CO_2 (Guo et al. 2013a). Furthermore, elevated CO_2 levels tend to increase the concentration of total amino acid in leaves and most individual amino acids in the phloem by enhancing the enzyme activities of N transamination. It seems that the changes of amino acid concentration can be manipulated more easily by pea aphids due to more nodule numbers and stronger BNF under elevated CO_2. In contrast, elevated CO_2 reduces amino acid concentration in *dnf1* plants endowed with less BNF by decreasing enzyme activities of N assimilation and transamination to support a lower population abundance of aphids.

Interacting with *Buchnera* supported the aphids' strong ability to adapt to extreme low N diets (Degnan et al. 2011). By transforming non-essential amino acids into essential amino acids, *Buchnera* provides the aphid with essential amino acids for their growth (Nikoh et al. 2010). Differing from cereal aphids which induce increases of essential amino acids in the phloem which can be used directly for growth (Sandström et al. 2000), Guo et al. (2013b) show that pea aphids largely increase non-essential amino acids (mainly Asn), the predominant composition in amino acids of phloem in *M. truncatula* Jemalong under elevated CO_2. Meanwhile, elevated CO_2 significantly increased Glu and Gln in pea aphid associated with Jemalong plants, which are a key source of nitrogen fueling the integrated amino acid metabolism of the aphid–*Buchnera* partnership (Hansen and Moran 2011). Thus, the imbalanced increases of amino acids in Jemalong plants (mainly Asn) and pea aphids (mainly Glu, Asn, and Gln) suggested that *Buchnera* is involved in amino acid metabolism of pea aphids in response to elevated CO_2. Our results support the view that amino acid imbalances increase under elevated CO_2 through the legume–aphid interaction, which also involves the aphid endosymbionts.

5.2.2.2.4 Pea aphids benefit from the modification of chemical induced resistance by elevated CO_2 in *M. truncatula*, associated with enhanced nitrogen fixation

Elevated CO_2 tends to alter the plant chemical defenses that are induced by herbivorous insects (Casteel et al. 2008). For example, elevated CO_2 increases soybean susceptibility to two chewing insects, the Japanese beetle and the western corn rootworm, by suppressing the expression of the proteinase inhibitor gene and other downstream genes of the JA pathway (Zavala et al. 2009). For piercing-sucking insects, Sun et al. (2013) found that JA-regulated defense is more effective against green peach aphids than SA-regulated defense in *Arabidopsis thaliana*, and that elevated CO_2 tends to enhance the ineffective SA signaling pathway and reduce the effective JA signaling pathway against aphids. Unlike the non-legume *A. thaliana* plants, *M. truncatula* is a legume with BNF ability. The enhanced BNF can compensate for decreases in plant N concentration and subsequently transform elevated CO_2-induced increase in photosynthesis into larger biomass. When the BNF was inactivated, the biomass of *dnf1* plants was not significantly affected by elevated CO_2. It is reasonable to suspect that differences in N availability and growth traits of the two genotypes under elevated CO_2 may cause differences in N and photosynthate allocation into structural and chemical defenses against herbivores.

Guo et al. (2014a) found that wild-type plants increase non-glandular and glandular trichome density under elevated CO_2, resulting in prolonged aphid probing. In contrast, *dnf1* plants tend to decrease foliar trichome density under elevated CO_2, resulting in less surface and epidermal resistance to aphids. On the other hand, elevated CO_2 enhances the ineffective SA-dependent defense pathway but decreases the effective JA/ethylene-dependent defense pathway in aphid-infested Jemalong plants. Therefore, probing time decreases and the duration of phloem sap ingestion of aphid increases on Jemalong plants under elevated CO_2, which in turn increased aphid feeding efficiency. The results associated with wild-type and *dnf1* suggest that a functional BNF system is crucial to suppress efficient chemical defense for aphids to obtain efficient feeding under elevated CO_2.

5.2.2.2.5 Elevated CO_2 decreases the response of the ethylene signaling pathway in *M. truncatula* to increase nitrogen fixation and decrease plant resistance against pea aphids

In legumes, BNF is regulated by several hormone signaling pathways including the ethylene signaling pathway (see

Chapters 10.1 and 10.4). The involvement of the phytohormone ethylene in nodulation was initially proposed based on studies showing that the application of exogenous ethylene or its biosynthetic precursor ACC (1-aminocyclopropane-1-carboxylic acid) suppresses nodulation (Penmetsa et al. 2008; Ma et al. 2002; see Chapter 10.4). Once the key gene Mtskl in the ethylene-perception pathway was mutated in *M. truncatula*, the resulting ethylene-insensitive mutant, *sickle*, produced more nodules than the wild-type, and its nitrogenase activity was increased about two times (Penmetsa and Cook 1997).

Furthermore, ethylene is the most important hormone involved in plant resistance against pathogens and pests. The expression of genes involved in ethylene production and ethylene signaling (ACC oxidase and ethylene-responsive elements) are upregulated in response to aphid infestation (Moran et al. 2002; Divol et al. 2005). Ethylene is also responsible for the regulation of reactive oxygen species (ROS) and downstream defensive enzymes against aphids (Jung et al. 2009). Guo et al. (2014b) found that the ethylene-insensitive mutant sickle, which produces more nodules and has enhanced BNF as well as reduced resistance to aphids relative to the wild-type, will supply more N nutrition to aphids and be less resistant to aphids. Thus, the ethylene signaling pathway could affect the aphid performance by altering both nutrition and resistance capacity of host plants.

The emerging data suggest that elevated CO_2 tends to modulate the ethylene signaling pathway that affects responses to insect herbivores (Zavala et al. 2013). Furthermore, Guo et al. (2014b) found that elevated CO_2 downregulated the expression of the ethylene signaling pathway genes *ACC*, *SKL*, and *Ethylene Responsive Factor* (ERF) in A17 plants. This result suggests that *M. truncatula* suppresses the ethylene signaling pathway under elevated CO_2 so as to increase nodulation and BNF and thereby satisfy the increased demand for N. The ethylene signaling pathway in *M. truncatula*, however, has also been found to provide resistance against the pea aphid (Gao et al. 2008). Elevated CO_2 decreases the activities of defensive enzymes superoxide dismutase (SOD), peroxidase (POD), and polyphenol oxidase (PPO) in infested *M. truncatula* plants. Although the transcriptomics and functional genomics indicate that piercing-sucking insects mainly trigger the SA signaling pathway in plants (Thompson and Goggin 2006), many studies have demonstrated that ethylene and JA signaling is more effective than SA signaling in the plant defense against aphids (Walling 2008; Zhu-Salzman et al. 2004). Guo et al. (2014b) found that the suppression of JA/ethylene signaling pathway defensive genes by elevated CO_2 would shorten the penetration stage of aphids and helped aphids obtain nutrition from phloem sap as soon as possible.

In summary, elevated CO_2 decreased the ethylene-dependent resistance of wild-type *M. truncatula* against the pea aphid. On the other hand, the decrease in the ethylene signaling pathway increases the nodulation and BNF and thereby increases the phloem amino acids supporting aphid reproduction. The two effects of the ethylene signaling pathway would synergistically increase the fitness of pea aphids under elevated CO_2 (Figure 5.2.2.2.1).

5.2.2.2.6 Stomatal closure of *M. truncatula* improves aphid feeding under elevated CO_2

Because CO_2 is the substrate for plant photosynthesis, elevated CO_2 typically increases the photosynthetic rate of C3 plants (Ainsworth and Long 2005). Furthermore, to avoid the uptake of excess carbon under elevated CO_2, plants partially close stomatal

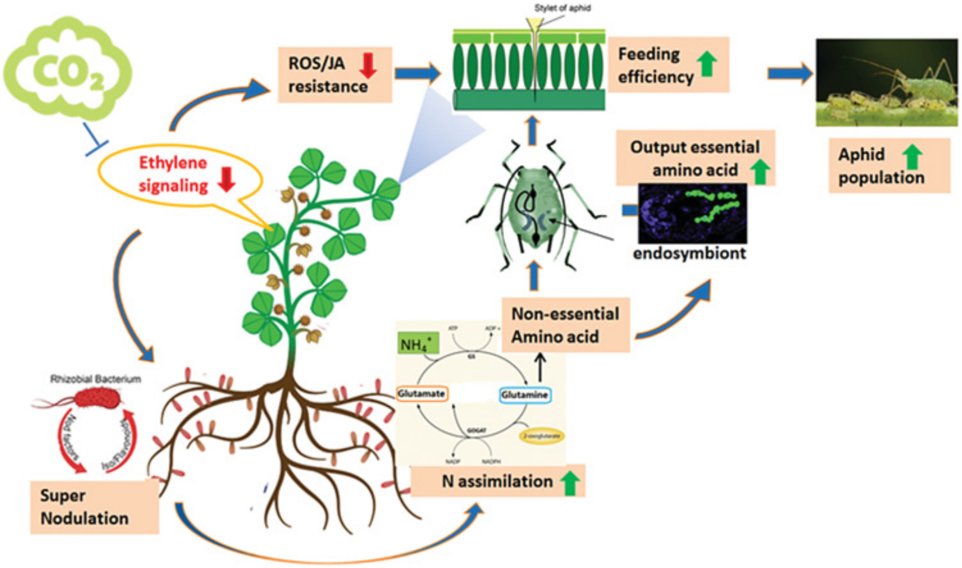

Figure 5.2.2.2.1 Elevated CO_2 affects both nutritional quality and resistance of *M. truncatula* against aphids by downregulating the ethylene signaling pathway.

pores, which decreases stomatal conductance (Ainsworth and Rogers 2007). Free-air CO_2 enrichment (FACE) experiments have shown that elevated CO_2 decreased stomatal conductance by an average of 22% for five functional plant groups that included 285 plant species (Ainsworth and Rogers 2007). As noted, the decreased stomatal conductance reduces water loss from plants and increases their water potential and water content (Pritchard et al. 2007; Wullschleger et al. 2002). Furthermore, the reduced stomatal conductance induces decreased evapotranspiration, which increases leaf temperature (up to 2 °C) (Bernacchi et al. 2001). Thus, these effects are likely to alter the fitness of herbivorous insects.

O'Neill et al. (2011) found that soybean leaves grown under elevated levels of CO_2 at SoyFACE have increased leaf temperature caused by reduced stomatal conductance. By increasing the *Glycine max* leaf temperature, elevated CO_2 may increase populations of *Aphis glycines* and their impact on crop productivity. Furthermore, aphids move into soybean fields fairly early in the summer and their population builds slowly with increasing temperatures until it peaks at the end of July and beginning of August (Ragsdale et al. 2004). The increased temperature of plants grown under elevated CO_2 may allow aphid populations to build faster and peak earlier in the season.

Water potential of the host plant plays a crucial role in the feeding phase and hemolymph osmoregulation of aphids (Huberty and Denno 2004; Pompon et al. 2010). Because aphids feed exclusively on sugar-enriched phloem sap, they experience phloem osmotic pressure that is as much as four to five times greater than the osmotic pressure in the aphid's hemolymph; this difference in osmolarity can reduce aphid feeding efficiency and fitness (Douglas et al. 2006). Moreover, to avoid self-dehydration and osmotic stress in the haemolymph caused by high osmolarity of phloem sap, aphids must occasionally consume xylem sap, which has a lower osmolarity than phloem sap (Pompon et al. 2011). Because the passive feeding of aphids depends on a relatively high plant water potential (Jactel et al. 2012), it is reasonable to suspect that decreases in stomatal aperture and increases in plant water potential induced by elevated CO_2 would facilitate aphid feeding.

Sun et al. (2015) used a *sta-1* (the stomatal movement is insensitive to ABA) mutant of *M. truncatula* and its wild-type A17 to determine whether pea aphid infestation induces stomatal closure of *M. truncatula* and whether these interactions between aphid and plant are altered by elevated CO_2. Their study found that stomatal closure of host plants could be induced by both aphid infestation and elevated CO_2. The stomatal closure was beneficial to aphids by increasing the duration of xylem-feeding. Aphids spent less time on the xylem-feeding phase when reared on ABA-insensitive mutant *sta-1* plants than on wild-type *M. truncatula*, which apparently led to a higher osmolarity and lower water content on *sta-1* plants than on wild-type plants. As a result, the aphids reared on *sta-1* plants spent less time in the phloem-feeding phase and had lower numbers than aphids on wild-type plants. Furthermore, regardless of the plant genotype, elevated CO_2 increased the xylem-feeding phase, decreased the hemolymph osmolarity, increased aphid water content, increased the duration of phloem feeding, and increased pea aphid numbers on *M. truncatula*. In summary, pea aphid-induced stomatal closure in *M. truncatula* facilitated xylem-feeding and thereby promoted phloem-feeding and increases in aphid numbers, and these effects were further enhanced by elevated CO_2 (Figure 5.2.2.2.2).

5.2.2.2.7 Conclusion and perspective

These studies have provided evidence that elevated CO_2 alters resistance, nutritional value, and water status of *M. truncatula* and that these changes affect certain feeding stages of pea aphids. The evidence also indicates that such changes and effects could be mediated by the phytohormones JA, SA, ET, and ABA. In these and related studies, elevated CO_2 stimulated the SA signaling pathway and thereby increased the epidermis and mesophyll resistance of *M. truncatula*. However, elevated CO_2 decreased JA and ET signaling pathways, which reduced the total time required by aphids to reach the phloem. The decreased ET signaling pathway also increased the N fixation ability of legumes and thereby increased their synthesis of amino acids, which in turn increased amino acid acquisition by aphids. Moreover, elevated CO_2 decreased stomatal aperture and increased plant water potential, which thereby increased aphid xylem absorption and enhanced aphid osmoregulation. These results provide a comprehensive understanding from an ecological and molecular view to unravel the complex co-evolutionary interaction between aphids and their host plant, and their response to a future elevated CO_2 environment (Sun et al. 2016). Nevertheless, transcriptomic evidence shows that elevated CO_2 has a wide range of effects on plant metabolism (including C and N assimilation, secondary metabolism, and transportation), all of which may affect aphid performance (Ainsworth et al. 2006; May et al. 2013). Thus, the effects of elevated CO_2 on the interaction between plants and aphids cannot be understood by simply relating one aspect of plant quality to a specific feeding phase of the aphid.

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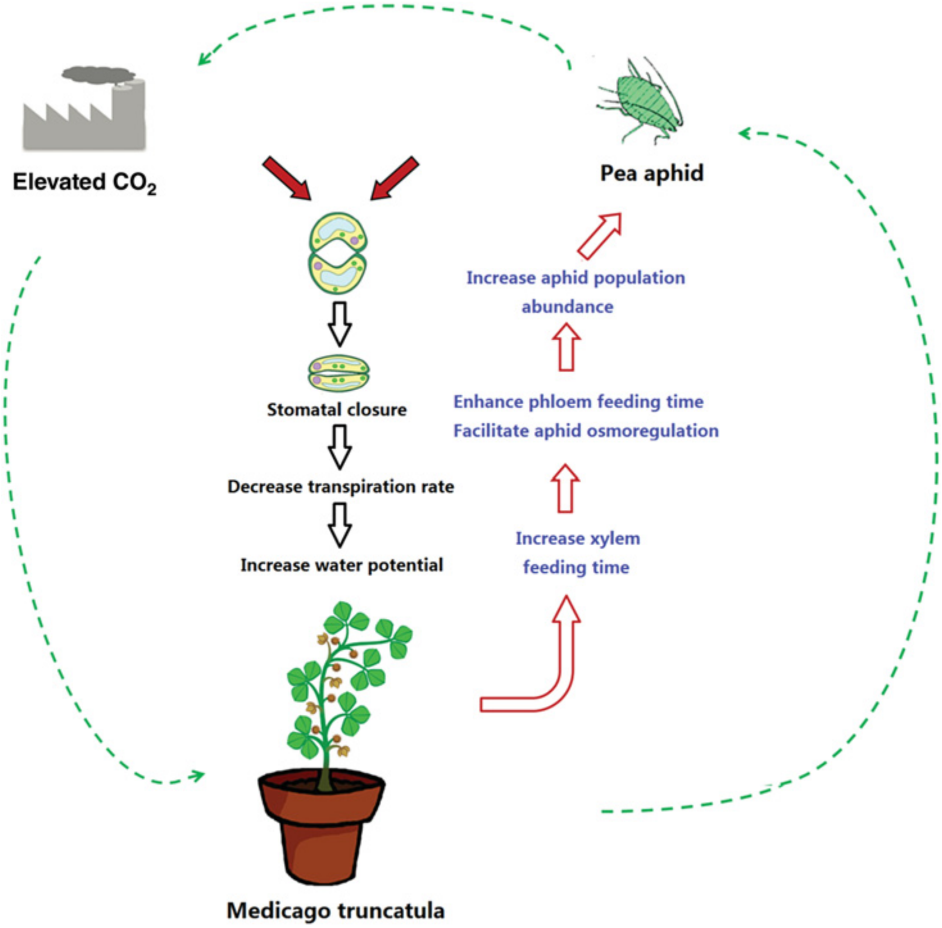

Figure 5.2.2.2.2 Model depicting how changes in stomatal aperture and water status of *M. truncatula* facilitate aphid feeding under elevated CO_2.

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