LEGUME PASTURE PEST RESPONSES TO GLOBAL CLIMATE CHANGE

James M.W. Ryalls[1*] and Scott N. Johnson[1]

Hawkesbury Institute for the Environment, Western Sydney University, NSW, Australia

*Corresponding author: J.Ryalls@westernsydney.edu.au

Keywords: amino acid, aphid, legume, lucerne, weevil, white clover

Introduction

Legumes are an integral part of plant communities in pasture ecosystems. They possess many ecologically important characteristics, including the ability to fix atmospheric nitrogen via their association with nitrogen-fixing bacteria that form nodules on the roots. This distinctive feature means that legumes often differ markedly from other plants in their response to elevated concentrations of atmospheric carbon dioxide e[CO$_2$] and elevated air temperatures (eT) (Jensen et al., 2012; Robinson et al., 2012). This makes extrapolation from current meta-analyses difficult, and possibly erroneous when hypothesising how insect pests of legumes will respond to climate change (Newman, 2004; Rogers et al., 2009). Given the importance of nitrogen in insect herbivore diets, it seems intuitive that changes in nitrogen fixation in legumes will affect herbivores feeding on the plant. Sap-feeding herbivores (e.g. aphids), in particular, often respond to changes in organic nitrogen (mainly amino acid concentrations of the phloem). They are consistently identified as net beneficiaries of e[CO$_2$], although few studies have incorporated the combined effects of e[CO$_2$] and eT, which will occur in tandem in the future. Moreover, the mechanistic basis for these effects remains largely unknown (Ryalls et al., 2015).

This research assesses the impacts of e[CO$_2$] and eT on belowground and aboveground insect pests of white clover (*Trifolium repens*) and lucerne (*Medicago sativa*), including the clover root (*Sitona obsoletus*) and lucerne (*Sitona discoideus*) weevils and the pea aphid (*Acyrthosiphon pisum*). We also examine the underlying plant mediated mechanisms linking insect responses to climate change across multiple legume cultivars.

Methods

Plant growth chambers, maintained at either 375 or 700 μmol mol^{-1}, were used to determine the impacts of e[CO$_2$] on *S. obsoletus* feeding on *T. repens*, which were grown in long vertical rhizotubes enclosed with mesh cages to prevent insect movement between plants. Half of the rhizotubes were inoculated with 28 *S. obsoletus* adult egg-laying females seven weeks after sowing. Plants were harvested after a further four weeks, which involved counting and measuring root nodules, and all insects were removed and counted (full details in Johnson and McNicol, 2010).

The combined effects of eT and e[CO$_2$] were assessed using glasshouse compartments with a 2 × 2 factorial design of ambient CO$_2$ (400 μmol mol^{-1}; a[CO$_2$]) and e[CO$_2$] (640 μmol mol^{-1}) at ambient temperatures (26°C daytime temperature) and eT (ambient + 4°C). *Sitona discoideus* eggs were inoculated onto lucerne plants and the numbers of adults that emerged from the soil were recorded. Plants were harvested after eight weeks and the numbers of nodules were recorded (full details in Ryalls et al., 2013).

Pea aphid performance was assessed by recording the life-history characteristics (e.g. fecundity, longevity and reproductive period) of a single aphid feeding on an individual plant from birth to death (~40 days). Aphids were fed on five different genotypes of varying pea aphid resistance ranging from susceptible (cultivar Hunter River) to moderately resistant (cultivars Sequel and Trifecta) to resistant (cultivars Aurora and Genesis) in the same four glasshouse compartments as above. Amino acid concentrations were analysed by reverse-phase high performance liquid chromatography (HPLC) in an Agilent 1260 Infinity HPLC system after pre-column derivatization using phenylisothiocyanate (PITC) (full details in Ryalls, 2016).

Results and Discussion

White clover root nodule abundance and length increased by 114% and 18%, respectively, at 700 μmol mol^{-1} compared with 375 μmol mol^{-1} (Figures 1A and B). 38% more larvae were also recovered (Figure 1C) at 700 μmol mol^{-1} suggesting that larval survival was much better under e[CO$_2$]. Increased larval abundance at e[CO$_2$] was positively correlated with the number of nodules available (Figure 2). Therefore, the widely assumed benefits of e[CO$_2$] on legumes may be effectively wiped out if root herbivory increases simultaneously.

Figure 1.

Effects of eCO$_2$ on (A) the abundance and (B) length of *Trifolium repens* nodules and (C) the number of *Sitona obsoletus* larvae recovered from the soil.

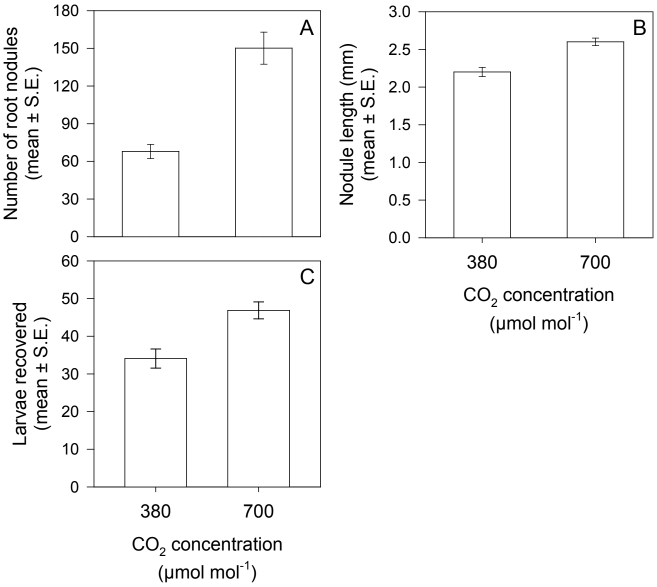
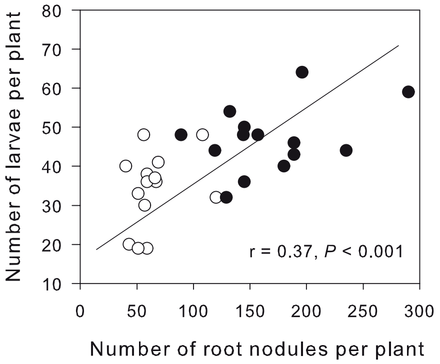


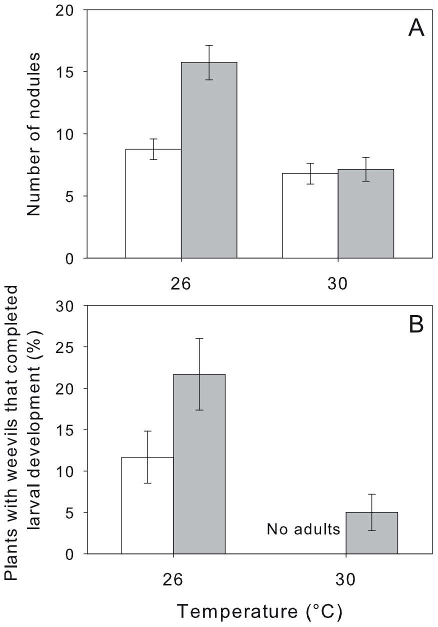
Figure 2.

Correlation between larval abundance and nodule abundance per plant. Significance ($P < 0.05$) indicated by linear regression, including CO_2 as a group factor.



Lucerne showed similar responses to white clover, with nodulation and *S. discoideus* egg to adult development increasing by 56% and 83%, respectively, under e[CO_2], but only at 26°C (Figures 3A and B). Increasing temperatures by 4°C negated the positive effects of e[CO_2] on nodulation and weevil development (i.e. adult emergence from the soil) and significantly reduced nodulation by 43% overall. The contrasting effects of eT and e[CO_2] on weevils potentially occurred through changes in root nodulation patterns, likely associated with a low tolerance of rhizobial bacteria to increased temperatures (Whittington et al., 2013).

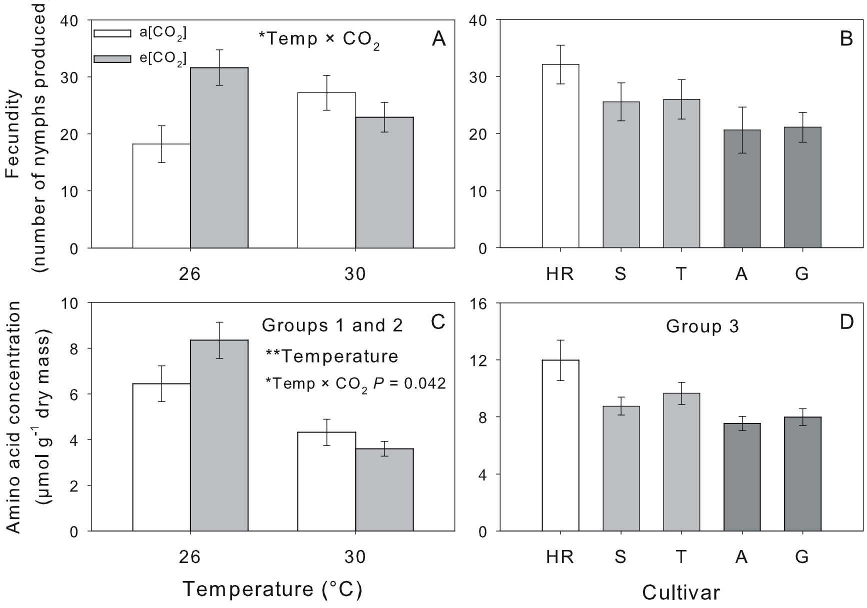
Figure 3.
Effects of temperature on (A) the number of root nodules per plant and (B) the proportion of plants containing weevils that reached adulthood and emerged from the soil under ambient (open bars) and elevated (closed bars) CO_2. Mean values (±SE) shown.



All measurements of aphid performance increased under e[CO_2] (e.g. aphid fecundity increased by 42%) at ambient temperatures. However, eT negated the positive effects of e[CO_2] on fecundity, which was similar to when aphids were reared at a[CO_2] (Figure 4A). Such negation of e[CO_2] effects by eT has also been found in other aboveground insect–plant interactions (Murray et al., 2013) and further emphasises the need to consider multiple climate change factors simultaneously when assessing the effects of global environmental change. The effects of eT and e[CO_2] on aphid performance held true across five *M. sativa* cultivars, demonstrating the generality of their effects. As expected, aphids generally performed better (e.g. fecundity increased) on less resistant cultivars (Figure 4B).

The effects of eT and e[CO_2] on aphid fecundity were mirrored by two groups of amino acids (Groups 1 and 2, comprising asparagine[1], aspartate[1], glutamate[1], arginine[2] and histidine[2]) (Figure 4C), which were significantly correlated with aphid fecundity ($F_{1,168} = 6.98$, $P = 0.009$). Another group of amino acids (Group 3, comprising alanine, glutamine, glycine, leucine, lysine, phenylalanine, proline, serine, threonine and valine) mirrored the effects of cultivar on aphid performance (Figure 4D). This suggests that lucerne resistance is driven by functional groups of amino acids, and this may inform selection programmes that target improved aphid resistance of cultivars.

Figure 4.
Effects of eT and e[CO_2], and cultivar, on (A and B) aphid fecundity and (C and D) grouped amino acid concentrations. Mean values (±SE) are shown. Significant terms indicated by * ($P < 0.05$) and ** ($P < 0.01$). Darker bars indicate higher resistance to *Acyrthosiphon pisum* in B and D.



Conclusions

Increasing CO_2 concentrations may enhance biological nitrogen fixation by legumes, but potential benefits (e.g. provision of N without chemical fertilizers) may be undermined by larger populations of weevil larvae belowground. Overall, e[CO_2] increased root nodulation, and amino acid concentrations, which had beneficial effects on weevils and aphids, respectively. However, when we applied eT, this negated the positive impacts of e[CO_2] on root nodulation and caused amino acid concentrations to decline. This, in turn, negatively affected insect herbivores. We identified discrete functional groups of amino acids that underpinned the effects of e[CO_2] and eT, in addition to plant genotype, on aphid performance, which held true across five *M. sativa* genotypes. Combining this knowledge with amino acid profiles of existing cultivars raises the possibility of predicting future susceptibility to aphids and preventing outbreaks of a global pest.

References

Jensen, E.S., Peoples, M.B., Boddey, R.M., Gresshoff, P.M., Hauggaard-Nielsen, H., Alves, B.J.R., and Morrison, M.J. (2012). Legumes for mitigation of climate change and the provision of feedstock for biofuels and biorefineries. A review. *Agron. Sustain. Dev.* 32, 329-364.

Johnson, S.N., and McNicol, J.W. (2010). Elevated CO_2 and aboveground–belowground herbivory by the clover root weevil. *Oecologia* 162, 209-216.

Murray, T.J., Ellsworth, D.S., Tissue, D.T., and Riegler, M. (2013). Interactive direct and plant-mediated effects of elevated atmospheric [CO_2] and temperature on a eucalypt-feeding insect herbivore. *Glob. Change Biol.* 19, 1407-1416.

Newman, J.A. (2004). Climate change and cereal aphids: the relative effects of increasing CO_2 and temperature on aphid population dynamics. *Glob. Change Biol.* 10, 5-15.

Robinson, E.A., Ryan, G.D., and Newman, J.A. (2012). A meta-analytical review of the effects of elevated CO_2 on plant–arthropod interactions highlights the importance of interacting environmental and biological variables. *New Phytol.* 194, 321-336.

Rogers, A., Ainsworth, E.A., and Leakey, A.D.B. (2009). Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? *Plant Physiol.* 151, 1009-1016.

Ryalls, J.M.W. (2016). *The Impacts of Climate Change and Belowground Herbivory on Aphids via Primary Metabolites.* PhD, Western Sydney University.

Ryalls, J.M.W., Moore, B.D., Riegler, M., Gherlenda, A.N., and Johnson, S.N. (2015). Amino acid-mediated impacts of elevated carbon dioxide and simulated root herbivory on aphids are neutralized by increased air temperatures. *J. Exp. Bot.* 66, 613-623.

Ryalls, J.M.W., Riegler, M., Moore, B.D., Lopaticki, G., and Johnson, S.N. (2013). Effects of elevated temperature and CO_2 on aboveground–belowground systems: a case study with plants, their mutualistic bacteria and root/shoot herbivores. *Front. Plant Sci.* 4, 445.

Whittington, H.R., Tilman, D., and Powers, J.S. (2013). Consequences of elevated temperatures on legume biomass and nitrogen cycling in a field warming and biodiversity experiment in a North American prairie. *Funct. Plant Biol.* 40, 1147-1158.