To tolerate drought or resist aphids? A new challenge to plant science is on the horizon

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Aphids are important herbivorous insects that can cause significant crop damage, leading to yield reduction and economic loss. One avenue being explored to reduce aphid impacts is the development of aphid-resistant plants. Under projected climate scenarios, it is expected that plants will be exposed to greater biotic and abiotic stress, including increased herbivorous insect infestation and exposure to prolonged periods of environmental stress, particularly drought. In response to these projections, plant-aphid interactions under drought conditions have been a subject of growing interest; however, few studies have looked at the impact of drought stress on plant resistance to aphids despite the potential importance for plant breeding. Here, we examine the latest scientific advances regarding variation in plant resistance to aphids under drought, emphasizing underlying mechanisms and functional trade-offs. We conclude that plant tolerance to drought should be incorporated into aphid resistance studies, and that possible cross-tolerance between aphid resistance and drought tolerance conferred by these traits should be examined.

Keywords: Aphid, crop, drought, resistance, susceptible, tolerance, trade-off.

Plants are simultaneously subjected to multiple biotic and abiotic threats. Understanding how plants respond to these factors is essential for predicting the performance of crops, especially in response to climate change (Bellard *et al.*, 2012). In nature, plant populations are shaped by environmental conditions that select for resistance to specific factors. Additionally, strong selection for resistance to one factor can be associated with susceptibility to another (i.e., trade-offs) (Herms and Mattson, 1992). Similar outcomes occur during plant breeding (Denison, 2012), where selection for high yields can come at costs of increased susceptibility to environmental stressors, or selection for resistance traits compromises plant tolerance of other stressors. A better understanding of these phenomena is needed to predict the consequences of stress-driven trait selection in natural vegetation or crops by examining potential trade-offs in selecting traits for biotic stress (e.g., pest and disease resistance) versus those conferring climate resilience (e.g., drought tolerance).

Aphids (Hemiptera: Aphididae) are phytophagous insects with worldwide distribution, representing an important agricultural pest of many crops (Dixon, 1998). Aphids cause plant damage both directly and indirectly. Direct damage results from sap removal during aphid feeding. Indirect damage is caused by the transmission of plant viruses and reduced quality due to build-up of aphid honeydew which favours the growth of microbes such as sooty moulds. Climate projections have estimated both positive and negative effects of climate change on herbivorous species, although most scenarios predict that proliferation of herbivorous insects will increase worldwide (Schneider *et al.*, 2022). One potential consequence of climate change is increased drought. Prolonged periods of drought affect plant homeostasis and the interaction with other organisms and, consequently, the plant response to herbivorous insects like aphids (Luo and Gilbert, 2022). Because there is usually a trade-off between traits, plant breeding programs may encounter difficulties in simultaneously improving drought tolerance and pest resistance.

A conceptual model recently proposed by Leybourne et al. (2021), and supported by experimental results in cereals (Kansman *et al.*, 2022; Leybourne *et al.*, 2022), suggests that plant resistance to aphids increases as water availability decreases. However, the model lacks explicit consideration of how plants differing in tolerance to drought (and thus, in susceptibility to water availability) might also vary in resistance to aphids. Here, we advance this model by incorporating an evolutionary perspective which considers the variation among plant genotypes in intrinsic tolerance to drought, which has been investigated by only a few studies (Quandahor *et al.*, 2019).

Towards an Aphid-Plant Resistance Hypothesis

A recent meta-analysis by Leybourne *et al*. (2021) focusses on aphid responses to drought and identified significant knowledge gaps in our understanding of the effect of drought stress on aphid-susceptible and aphid-resistant plants: only four studies compared the effect of reduced water availability on plants that are resistant and susceptible to aphids. These studies suggest that aphid performance is reduced by drought on both aphid-susceptible and aphid-resistant plants but with a stronger effect on the former (Leybourne *et al.*, 2021). To explain this, Leybourne *et al*. (2021) proposed the "Plant Resistance Hypothesis" (Fig. 1A), which predicts that lower water availability causes a differential change in chemical and molecular defences between susceptible and resistant plants. In other words, susceptible plants display a more distinctive change in plant defences along a water availability gradient than resistant plants, since resistant plants have higher basal levels of defences and a narrower range of responses (Leybourne *et al.*, 2021). This hypothesis focuses on the variation in the concentration of plant defences due to water availability, whereas water availability also affects other plant traits (Kansman *et al.*, 2022). More importantly, this hypothesis assumes that either aphid-susceptible or aphid-resistant plants do not vary in their level of drought tolerance. Plant genetic variation in ability to resist or recover from drought might alter plant responses to short term changes in water availability. Note that while water availability is an environmental condition, drought tolerance describes the ability of plants to resist and be resilient to (recover from) low water conditions (Tardieu, 2022). Surprisingly little is known about how plants with different levels of tolerance to drought differ also in their ability to resist aphids.

Plant tolerance to aphids under drought stress

In their relationship with aphids, plants may not only evolve resistance as antagonistic response mechanisms, but also develop tolerance to aphids. This is another missing link within the proposed Plant Resistance Hypothesis (Leybourne *et al.*, 2021) resulting from a lack of available research. Unlike resistance, tolerance is the ability of plants to recover from herbivore damage through growth and compensatory physiological processes (Strauss and Agrawal, 1999). Most of the evidence suggests that tolerance of and resistance to herbivores represent independent plant defence strategies (Pearse *et al.*, 2017). The evolution of cardenolides and regrowth ability in milkweeds is a good example (Agrawal and Fishbein, 2008) and resistance and tolerance tend to be positively correlated in crops (Leimu and Koricheva, 2006). However, plant tolerance as a defence mechanism has received little attention in aphid-plant interactions (Peterson *et al.*, 2017), and much less in relation to drought (Mitchell *et al.*, 2016). Further research is needed to assess whether plants can display cross-tolerance to drought and aphid attacks (Foyer *et al.*, 2016).

Examining interactions between drought tolerance and aphid resistance from a trade-off perspective

Plants often show trade-offs between different functions that can be explained by resource limitations and by developmental constraints at the molecular level that regulate those trade-offs (Herms and Mattson, 1992). Limited resource availability can lead to conflicting demands among different fitness-related traits, preventing plants from investing simultaneously in growth, reproduction, and defence. A negative correlation between resistance to aphids and the ability to tolerate drought among a set of plant genotypes would indicate that tolerance to drought requires the allocation of resources for an improved water economy at the expense of defence against aphids (Fig. 1B, Model 1).

Plant genotypes could differ in the resistance level to aphids based on their intrinsic level of drought tolerance. This raises questions about the predicted responses of drought-tolerant and drought-susceptible plant genotypes to aphid attack when exposed to a water availability gradient (as in Leybourne et al. 2021). Under drought conditions, do drought-susceptible plants show relatively larger increases in aphid resistance than drought-tolerant plants because the latter invest more in tolerating drought? (Fig. 1C). We propose that future studies dealing with drought and aphid attack should focus on drought tolerance traits that reduce aphid fitness and aphid resistance traits that reduce water loss, particularly when trait expression is elevated under reduced water availability (Fig. 1C). Alternatively, if resistance to aphids is independent of drought tolerance, aphid resistance might not vary under drought conditions. Disentangling these relations is key to guiding plant domestication programs in the context of developing climate-resilient crops.

A mechanistic approach to understand the relationship between aphid resistance and drought tolerance

Plant resistance to aphids can be conferred by chemical deterrence traits, physical barriers to aphid settling and feeding, and traits that reduce plant quality for feeding (Mitchell *et al.,* 2016). Plant traits conferring tolerance to drought include the accumulation of metabolites that maintain turgor and tissue functionality under water scarcity (Benkeblia, 2022), mechanisms to regulate stomatal aperture and tissue relative water content (Buckley, 2019), and changes to root and leaf tissue structure (Fang and Xiong, 2015). Although the relation between these drought tolerance and aphid resistance mechanisms has seldom been explored (Kansman *et al.*, 2022) , from a crop breeding perspective it is important to understand the potential for traits to confer cross-tolerance between these two stressors. The mechanisms underpinning effects of water availability on aphid resistance proposed by Leybourne et al. (2021) could be examined further for their potential to confer drought tolerance: in Box 1, we illustrate how drought tolerance and aphid resistance traits might interact, and the plant signalling pathways that could communicate cross-tolerance, highlighting potential breeding targets for cross-tolerance.

Conclusions

We highlight that studying the ability of plants to resist aphids under conditions of water restrictions requires consideration that the outcome might be affected by plant genotypic variation in tolerance of drought. Plants may evolve (or be selected through breeding) to express greater drought tolerance, and these traits might also respond to water availability within a generation. The traits and mechanisms underlying aphid resistance and drought tolerance functions may or may not be related but could be subject to trade-offs; understanding their genetic and environmental control is crucial for breeding crops for future climates. Importantly, plant traits that confer aphid tolerance (i.e., compensatory response by plants to damage inflicted by aphids) should be explored for any potential role in plant drought tolerance. As with resistance, both drought and aphid tolerance may have a common molecular and physiological basis and generate cross-tolerance. These views should guide future research in this area.

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Author contributions

CCR, PG, AJK, DJL: conceived and wrote the manuscript; CCR and DJL designed the figures.

Conflict of interest

The authors declare no competing interests.

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Figure legends

Figure 1. A) Original model proposed by Leybourne et al. (2021) that relates the performance of aphids on aphid-resistant and aphid-susceptible plants as a function of the water availability. B) Model 1 proposed herein relating the resistance to aphids as a function of plant drought tolerance. C) Model 2 proposed herein results from subjecting and not subjecting different plant genotypes of a crop to drought. Each dot (blue or white) on panels B and C represents hypothetical different plant genotypes or intraspecific plant variants (e.g., accessions, cultivars, varieties) for which aphid resistance and drought tolerance is estimated. For example, the herbivory resistance level of a given plant genotype is estimated as plant biomass in aphid-challenged plants versus plant biomass in control plants (not challenged by aphids), all of them grown under no water restriction. By contrast, the level of drought tolerance for a given plant genotype is estimated as plant biomass in water-stressed plants versus plant biomass in control plants (with no water restriction). Traits for future focus include drought tolerance traits that reduce aphid fitness (1) and aphid resistance traits that reduce water loss (2), particularly when trait expression is elevated under reduced water availability (3).

Box 1: Potential mechanisms underpinning the interactions between drought tolerance and aphid resistance.

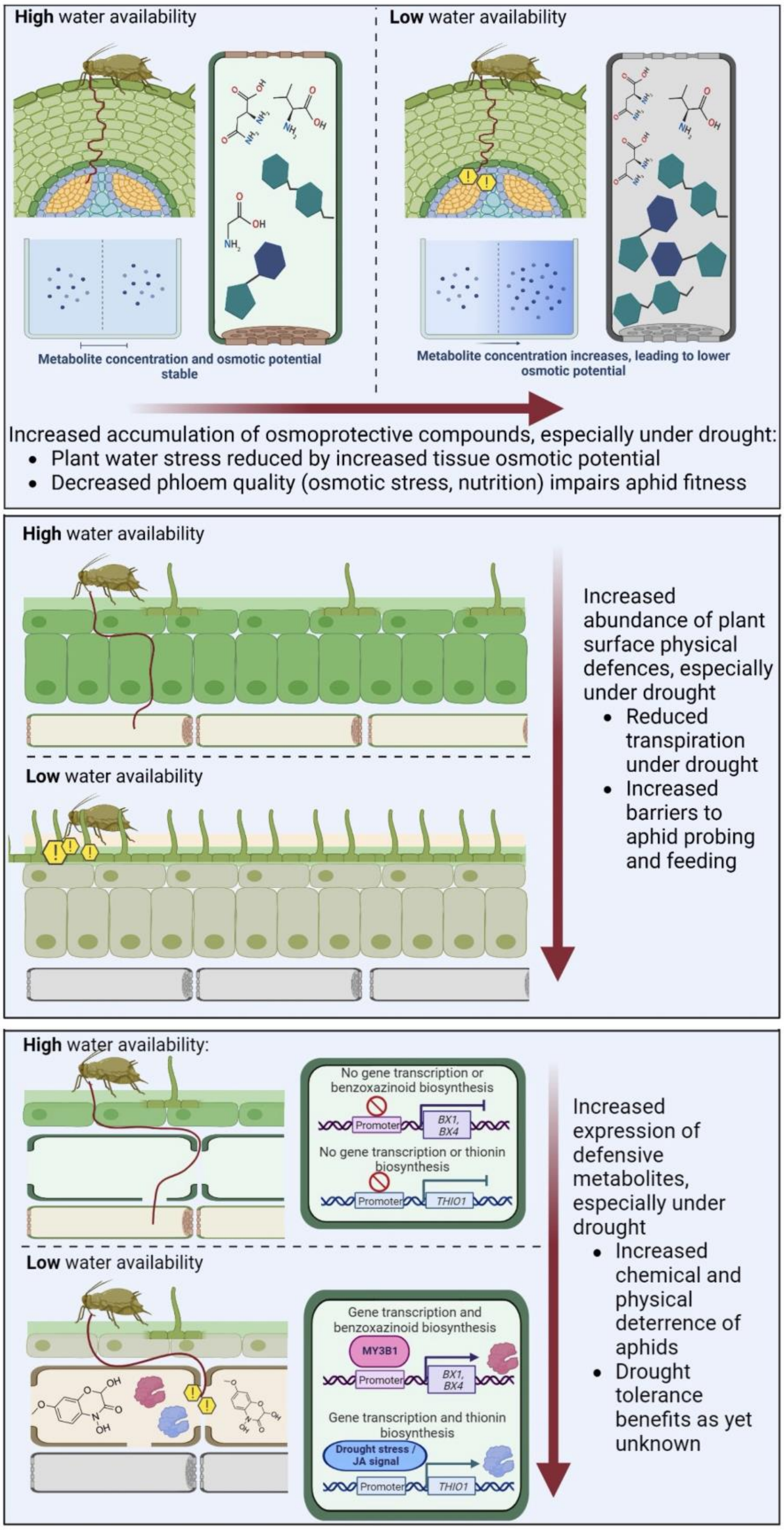
Plant traits conferring drought tolerance can also confer aphid resistance, and vice versa. Additionally, there is growing evidence for cross-talk between molecular signalling pathways responding to these two stressors that may explain their interaction. Cross-tolerance could result, therefore, from biochemical responses that influence osmotic potential and nutritional quality (A), physical characteristics that alter water loss and aphid infestation (B), and elevated molecular defences (C). These can also be involved in cross-tolerance and cross-talk with aphid resistance traits.

A) Biochemical traits: Osmoprotective mechanisms include changes in the composition and concentrations of secondary metabolites, soluble proteins, amino acids, and carbohydrates (Osakabe et al., 2014). Concentrations of non-structural carbohydrates (NSC) are modulated by drought and act as a carbohydrate reserve for stress (Sadras et al., 2021); recently, NSC were also reported to contribute towards plant resistance to aphids in cereals (Sadras et al., 2020). Other osmoprotective metabolites, such as essential amino acids, have also been associated with aphid resistance (Leybourne et al., 2019). The potential mechanism(s) through which these metabolites provide cross-tolerance against aphids could be through the low osmotic potential generated by high metabolite concentration, and reduced phloem nitrogen quality, which can limit aphid performance (Sadras et al., 2020, 2021).

B) Morphological traits*:* Morphological traits: Trichomes and epicuticular waxes, can provide drought tolerance by limiting transpiration. Recent research has indicated that drought stress can stimulate the production of these physical traits (Saska et al., 2021, 2022), which have also been associated with increased aphid resistance (Valim et al., 2016).

C) Defence signalling pathways: Benzoxazinoids represent a key example of cross-talk between drought tolerance and resistance to aphids. The role of benzoxazinoids as defensive metabolites in aphid resistance in cereals has been well documented (Niemeyer, 2009), and linked to resistance mechanisms such as induction of callose deposition (Zhou et al., 2018). Recent research has shown that benzoxazinoid biosynthesis is regulated by the drought-induced transcription factor MYB31 (Batyrshina et al., 2022), indicating that it could also respond to drought. The regulation of thionin gene expression is another example of cross-talk since its expression was greater in aphid resistant than susceptible plants (Leybourne et al., 2019; Escudero-Martinez et al., 2017) and did not change in response to drought, whereas expression was upregulated in susceptible plants (Leybourne et al., 2022). The role of these metabolites in drought tolerance have yet to be established.

Box 1



Increased accumulation of osmoprotective compounds, especially under drought:

- Plant water stress reduced by increased tissue osmotic potential
- Decreased phloem quality (osmotic stress, nutrition) impairs aphid fitness

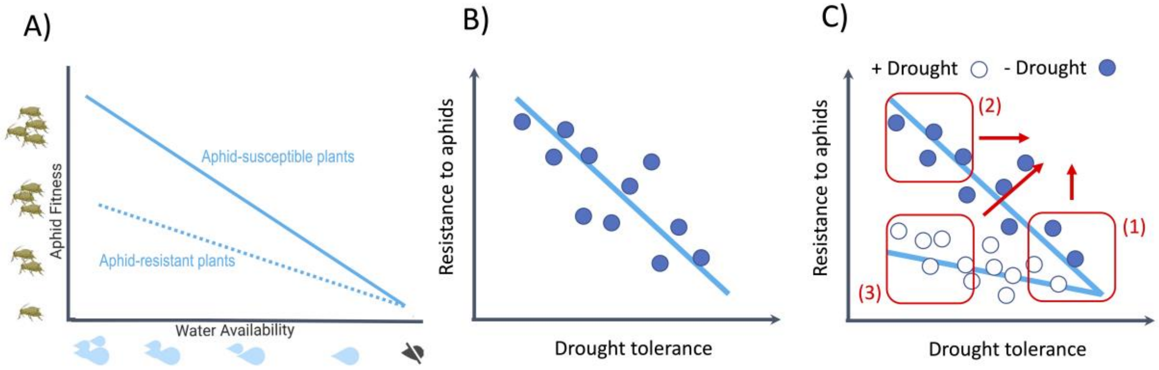
Increased abundance of plant surface physical defences, especially under drought

- Reduced transpiration under drought
- Increased barriers to aphid probing and feeding

Increased expression of defensive metabolites, especially under drought

- Increased chemical and physical deterrence of aphids
- Drought tolerance benefits as yet unknown

Figure 1


A)
Aphid Fitness
Aphid-susceptible plants
Aphid-resistant plants
Water Availability
B)
Resistance to aphids
Drought tolerance
C)
+ Drought ○
- Drought ●
(2)
(1)
(3)
Resistance to aphids
Drought tolerance