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Negative impact of drought stress on a generalist leaf chewer and a phloem feeder is associated with, but not explained by an increase in herbivore-induced indole glucosinolates

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ABSTRACT

Plants are constantly exposed to multiple biotic and abiotic stresses, such as drought and herbivory. However, plant responses to these stresses have usually been studied in isolation. Here, we take a multidisciplinary approach addressing ecological and chemical aspects of plant responses to generalist herbivores and several intensities of drought. We hypothesize that in brassicaceous plants, the effects of drought stress on herbivores can be explained by an increase in indole glucosinolates. Four-week-old *Arabidopsis thaliana* plants were drought stressed for one week or watered as normal. Three types of drought stress were compared: (1) no watering for 1 week and then rewatered to saturation (low drought); (2) no watering for 1 week and then rewatered to 60% of soil water content (high drought); (3) watering every other day to 60% of soil water content (continuous drought). All three types of drought stress negatively affected both the larval mass of the leaf chewer *Mamestra brassicae* and the population growth of the phloem feeder *Myzus persicae*. This was associated with increased levels of herbivore-induced indole glucosinolates compared to infested control plants. Interestingly, the levels of total indole glucosinolates did not change in uninfested plants, except for the indole 4-methoxy-glucobrassicin that was induced by continuous drought. Two-choice experiments also showed that caterpillars of *M. brassicae*, but not aphids, avoided drought-stressed plants only after feeding on them, but not by visual/olfactory cues. However, on a knockout mutant blocked in the production of indole glucosinolates (*cyp79B2 cyp79B3*), the effect of drought on herbivore performance was similar to that on wild-type plants. The results of this study show that drought stress induced higher levels of indole glucosinolates; however, these levels were not responsible for higher resistance to generalist herbivores in drought-stressed plants.

1. Introduction

Plants live in complex environments where they are exposed to biotic stresses such as insect herbivory, and abiotic stresses such as drought. Despite their constant exposure to simultaneous multiple stresses in nature, plant responses to biotic and abiotic stresses have usually been studied separately for single stress factors (Atkinson and Urwin, 2012; Holopainen and Gershenzon, 2010). There is, therefore, a need for studies integrating plant responses to multiple stresses and ecological interactions (Pineda et al., 2013a, b; Stam et al., 2014). Among all biotic and abiotic stresses, insect herbivory and drought are two of the most important factors limiting productivity in natural and agricultural ecosystems worldwide, and predictions indicate that with current global climatic change the frequency of drought and insect outbreaks will increase (Kurz et al., 2008; Zhao and Running, 2010). However, the effects of drought on plant-insect interactions and the underlying mechanisms remain largely unexplored.

During the last four decades, field and laboratory studies have shown that drought can affect herbivorous insects via their food plants (Copolovici et al., 2014; Hale et al., 2003; Inbar et al., 2001;

Abbreviations: Col-0, Columbia-0; GLS, glucosinolates.

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Khan et al., 2010; Mody et al., 2009; White, 1974). To explain such effects, several hypotheses have been proposed. Based on field observations of increased pest outbreaks after drought periods, the "plant stress hypothesis" proposes that drought has a positive effect on herbivorous insects due to increased nitrogen availability (Mattson and Haack, 1987; White, 1974). On the other side of the full spectrum, the "plant vigour hypothesis" suggests that vigorous plants (i.e. bigger and healthier) are more nutritious for herbivores (Price, 1991). Later, experimental studies demonstrated that drought-stressed host plants can have either negative or positive effects on insect herbivores (Hale et al., 2003; Inbar et al., 2001; Khan et al., 2010; Mody et al., 2009), depending on factors such as insect feeding mode (phloem feeders or chewing insects) or stress temporal pattern (pulsed or continuous) (Huberty and Denno, 2004; Koricheva et al., 1998; Larsson, 1989). More specifically, the "pulsed stress hypothesis" proposed that intermittent drought stress would have a positive effect on phloem feeders through an increase in the availability of nitrogen in the phloem sap with relatively low levels of secondary metabolites, whereas a continuous stress would have a negative effect (Huberty and Denno, 2004). Physiological responses to drought include stomatal closure, reduced photosynthesis and changes in both primary and secondary metabolites (Krasensky and Jonak, 2012). Some of these responses may favor herbivore development through an increase in the available nutritional compounds, such as soluble sugars and free amino acids (Krasensky and Jonak, 2012; Mewis et al., 2012). In contrast, drought can negatively affect herbivorous insects through decreases in plant growth, turgor pressure and water content, as well as through an increase of allelochemicals such as phenolics or glucosinolates (del Carmen Martínez-Ballesta et al., 2013; Inbar et al., 2001). Glucosinolates are the main defensive compounds in the Brassicaceae (Hopkins et al., 2009), and it has been proposed that they may have an important function in avoiding water loss by closing the stomata (Zhao et al., 2008).

Interestingly, in recent years several herbivores have each been studied on a range of plant species from the Brassicaceae family (Table 1) (Gutbrodt et al., 2012, 2011; Khan et al., 2010, 2011; Mewis et al., 2012; Prill et al., 2014; Simpson et al., 2012; Tariq et al., 2013a, 2012; Vickers, 2011). However, these studies do not reveal a pattern that can predict the effect of drought on herbivore behaviour or performance. One of the possible reasons, in addition to environmental and genotypic effects, is that there is no standardization in the application of drought stress in terms of intensity and temporal pattern. This compilation of studies with

Table 1
Effects of drought on herbivores feeding on Brassicaceae and associated glucosinolate (GLS) levels.

Plant species	Herbivore species	Feeding behaviour	Specialization	Effect of drought on herbivore performance	Type of drought[a]	Effect of drought on glucosinolates	Reference
Alliaria petiolata	*Pieris brassicae*	Leaf chewer	Specialist	Positive	4 pulsed drought cycles (20–60%)	Decrease in total GLS (constitutive)	Gutbrodt et al. (2011)
	Spodoptera littoralis	Leaf chewer	Generalist	Negative			
Arabidopsis thaliana Col-0	*Brevicoryne brassicae*	Phloem feeder	Specialist	No effect	Continuous drought for 1 week (50%)	Decrease in indole GLS in phloem (constitutive) Increase in aliphatic GLS in leaves (constitutive)	Mewis et al. (2012)
	Myzus persicae	Phloem feeder	Generalist	Positive			
Brassica nigra	*B. brassicae*	Phloem feeder	Specialist	Negative	Continuous drought for 9 days (0%)	Not evaluated	Vickers (2011)
	M. persicae	Phloem feeder	Generalist	Negative			
B. nigra	*B. brassicae*	Phloem feeder	Specialist	Positive	Roots kept separate from the water level (field)	Increase in sinigrin (field)	Prill et al. (2014)
Brassica oleracea var. *capitata*	*M. persicae*	Phloem feeder	Generalist	Negative	Continuous drought (<50%)	Not evaluated	Simpson et al. (2012)
B. oleracea var. *gemmifera*	*P. brassicae*	Leaf chewer	Specialist	Positive	4 pulsed drought cycles (30%)	No effect on total GLS (constitutive)	Gutbrodt et al. (2012)
	S. littoralis	Leaf chewer	Generalist	Positive			
B. oleracea var. *gemmifera*	*B. brassicae*	Phloem feeder	Specialist	Positive	Continuous drought for 4 weeks (50–75%) or pulsed (50%)	Increase in indole GLS (constitutive)	Tariq et al. (2012)
	M. persicae	Phloem feeder	Generalist	Positive			
B. oleracea var. *gemmifera*	*Delia radicum*	Root feeder	Specialist	Negative	Continuous drought for 4 weeks (50%)	Not evaluated	Tariq et al. (2013b)
B. oleracea var. *gemmifera*	*Mamestra brassicae*	Leaf chewer	Generalist	No effect	3 pulsed drought cycles (300 ml)	Not evaluated	Weldegergis et al. (2014)
B. oleracea var. *italica*	*B. brassicae*	Phloem feeder	Specialist	No effect	Continuous drought for 1 week (25%)	Decrease in indole GLS (constitutive)	Khan et al. (2010)
	M. persicae	Phloem feeder	Generalist	Positive		No effect aliphatic (constitutive)	
B. oleracea var. *italica*	*B. brassicae*	Phloem feeder	Specialist	Not evaluated	Continuous drought for 1 week (25%)	No effect on indole GLS (induced by *B. brassicae*)	Khan et al. (2011)
	M. persicae	Phloem feeder	Generalist	Not evaluated		Decrease in indole GLS (induced by *M. persicae*)	

[a] Between brackets, the percentage of water relative to the control, that the drought treatment received.