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Temperature and light drive manganese accumulation and stress in crops across three major plant families

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ABSTRACT

Environmental change is giving rise to conditions that exacerbate the solubilisation of manganese (Mn) in soil, and genetics underpins plant response to Mn oversupply. Here, Mn accumulation in foliage was investigated at the cellular level via controlled studies and fieldwork. Manganese sensitive and tolerant wheat (*Triticum aestivum* L.) and soybean (*Glycine max* Merr.) lines were given heat and Mn stress treatments exclusive of solar radiation, while the field study focused on a canola (*Brassica napus* L.) cultivation affected by seasonal Mn toxicity. Foliar Mn concentrations in wheat and soybean increased with Mn treatment, a response augmented by heat. Wheat under maximum stress exhibited a phenotypic difference in cellular Mn accumulation well before visible symptoms were manifested. Large cells peripheral to major vascular bundles of the sensitive line contained highly localised Mn. Excess foliar Mn in soybean was located primarily in the dermal apoplastic tissues of both cultivars, with symplastic localisation only in the Mn-sensitive cultivar. In soybean leaves, the dark patches formed due to Mn treatment had greatly elevated concentrations of co-localised Mn, calcium (Ca), phosphorus (P) and carbon (C) and were depleted in oxygen (O) and potassium (K), compared to adjacent tissues. In stressed field canola, Mn was localised most highly in the cell layers closest to the leaf upper surfaces directly exposed to solar radiation, and worse affected by chlorosis compared to the undersides. These chlorotic tissues had Mn concentrations several-fold those of green tissues. Although the spatial distribution patterns of Mn deposited in leaf cells showed some phenotypic variation, environmental factors contributed to Mn accumulation and stress. The key findings of this study highlight that climate and plant Mn toxicity are linked inextricably, and in so doing add meaningful commentary to discussion around apoplastic vs symplastic Mn toxicity mechanisms in plants.

1. Introduction

Manganese stress affects plant productivity on dry, acidic, or waterlogged soils. It is a geographically and seasonally heterogeneous problem underpinned by climate, soil chemistry,plant genetics and physiology. Early research linking environmental effects to Mn stress in crop plants, coupled with recent improved understanding about Mn phytotoxicity,and suggests that changing global climatic variables (IPCC, 2014) will exacerbate Mn phytotoxicity (Fernando and Lynch, 2015). Among these, rising atmospheric temperatures, increasing frequencies of extreme flooding events, drought, extended periods of solar radiation, elevated atmospheric CO_2 and ozone levels are potent activators both individually and interactively (Bromfield et al., 1983; Dias, 2009; Fernando et al., 2009; Fernando and Lynch, 2015; Hayes et al., 2012; Heenan and Carter, 1977; Lynch and StClair, 2004; Rufty et al., 1979; Scott et al., 1987; StClair and Lynch, 2010). Although the impact of Mn toxicity on plant systems is documented in at least six decades of scientific literature, the potential compounding effects of climate change has drawn little interest.

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Manganese in soil commonly occurs in three interchangeable oxidation states, i.e., as insoluble Mn(III) and Mn(IV) oxides, and in its soluble form, as loosely-bound Mn(II) in the soil solution (Clarkson, 1988; Gilkes and McKenzie, 1988). Its bioavailability increases when insoluble Mn oxides are reduced to form Mn(II) ions. The release of Mn(II) in soil is favoured by acidification, high temperatures, extreme wetting and drying cycles, continuous waterlogging; processes that overexpose plants to bioavailable Mn (Grasmanis and Leeper, 1966; Hayes et al., 2012; Heenan and Carter, 1977; Siman et al., 1974). Manganese is a micronutrient essential to plant function including photosynthesis. It acts as an enzyme co-factor in the key hydrolysis reaction of photosystem II (PSII), facilitates homeostatic redox chemistry, and is incorporated into several important enzymes such as superoxide dismutase and certain acid phosphatases (Burnell, 1988). Mangnanese concentrations often exceed normal nutritional requirements without apparent physiological stress (Foulds, 2003; Marschner, 2002). Recent appraisal of the literature draws attention to the over-accumulation of foliar Mn previously interpreted as Mn tolerance, particularly in crop species (Fernando and Lynch, 2015). Re-evaluation is warranted on the basis of new findings showing that excessively accumulated symplastic Mn can become phytotoxic upon interaction with environmental triggers such as solar radiation and atmospheric ozone (González and Lynch, 1999b; González et al., 1998; StClair et al., 2005; StClair and Lynch, 2004). Excess symplastic Mn can effect physiological stress mechanisms including photobleaching under solar radiation, and oxidative stress due to Mn antagonism against metal co-factors integral to stress-mitigating enzyme activities (Fernando and Lynch, 2015; González et al., 1998; Horiguchi, 1988; StClair et al., 2005; StClair and Lynch, 2004, 2005).

Wheat (*Triticum aestivum* L.), soybean (*Glycine max*) Merr.), and canola (*Brassica napus* L.) are crop species widely reported as accumulating high concentrations of foliar Mn and/or affected by toxicity (Burke et al., 1990; Heenan and Campbell, 1980, 1990; Heenan and Carter, 1976; Keisling et al., 1984; McVittie, 2012; Moroni et al., 2012; Reddy et al., 1991; Scott and Wratten, 1997; Sparrow and Uren, 1987); yet, it is unknown how they mediate excessively accumulated foliar Mn. Scott et al. (1987) have attempted, albeit inconclusively, to examine Mn accumulation at the cellular level in wheat leaves. They observed that warm to hot ambient field conditions often overrode the effects of soil liming in mitigating Mn toxicity. StClair and Co-workers (StClair and Lynch, 2004; StClair et al., 2005) and González et al. (1998) described Mn stress in North American forest trees and in common bean (*Phaseolus vulgaris* L.) as primarily manifesting in free radical damage. In examining a Mn tolerant variety, González and Lynch (1999a) used cell fractionation to demonstrate disposal in the vacuoles of dermal cells, a test that indicated excess Mn in the symplast and a distinct phenotypic tolerance trait. Two decades prior, research (Horst and Marschner, 1978; Wissemeier and Horst, 1987) concluded that the rapid oxidation of Mn(II) accumulated in tissues was a significant Mn toxicity mechanism manifested in dark foliar spots comprising higher Mn oxides and phenols, observations thus far not commonly observed in the field. These studies hypothesised Mn phytotoxicity as being restricted to the apoplast, a result that contradicts findings that free radical damage, whilst initially less obvious, is a primary Mn stress mechanism restricted to the symplast (González et al., 1998; StClair et al., 2005; StClair and Lynch, 2004). This study employed microscopy and microanalysis to examine at the cellular level, a) the effects of temperature on Mn treatment on sensitive and tolerant wheat and soybean varieties under controlled conditions, and, b) seasonal Mn stress in canola grown in the field.

2. Materials and methods

2.1. Plant propagation and treatment

Seeds of wheat (*Triticum aestivum* L. (Poaceae)) cultivars Teal (AUS 12330; Mn sensitive) and Carazinho (AUS 90151; Mn tolerant) denoted here as 'T' and 'C', were surface-sterilised, rinsed, and water-imbibed overnight. Otherwise identical experiments were conducted under two different ambient temperature regimes of normal (temp 1) and elevated temperature (temp 2) conditions of 22 °C/17 °C (12 h day/night) and 32 °C/27 °C (12 h day/night). The latter was imposed on four-day old seedlings raised at normal temperature, i.e. on day 1 of Mn treatment. A flotation format similar to that of (Moroni et al., 2012) was used for germination. Seeds were placed crease down on plastic mesh (grid holes ~5-mm square) floating on aerated distilled water held in 2-l solution culture pots and left overnight in a darkened growth chamber set at 22 °C/17 °C (12 h day/night). Seedlings were thinned to four per each of 18 pots, with water replaced by a nutrient solution of pH ~4.9 containing (μM) Ca 1000, Mg 300, NO_3 2900 and NH_4 300. Prior ambient conditions were maintained for a further 24 h after which incandescent illumination was commenced on a 12-h day/night cycle. Two days later, on day 1 of the experiment, halogen illumination was added to provide photosynthetically active radiation (PAR) of 200 μmol m^{-2} s^{-1}, and Mn treatments commenced as follows. Control (0 μM), medium (300 μM) and high (1000 μM) Mn treatments were administered via a basal nutrient solution containing (μM) Ca 1000, Mg 300, K 800, NO_3 3600, NH_4 600, PO_4 (added as H_2PO_4) 100, SO_4 100, Cl 34, Na 20, Fe 10, B 6, Mn 2, Zn 0.5, Cu 0.15, Mg 0.1 (Moroni et al., 2012). Throughout the study, the pH of the growth medium was maintained at just below 5 units by adding 0.1 M solutions of acid (HCl) or base (NaOH), dropwise. The three levels of Mn exposure, i.e. the basal solution containing 2 μM Mn, and the Mn-amended basal solutions containing 302 and 1002 μM Mn will henceforth be referred to as Mn1, Mn2, and Mn3 respectively.

Soybean *Glycine max* Merr. (Fabaceae) cultivars Bragg (Aus TRCF 309844) and Lee (AusTRCF 310199), respectively Mn-sensitive and tolerant, are referred to here as cultivars "B" and "L". Seeds were surface sterilised and germinated in the dark, sandwiched between the inner wall of a glass beaker and several layers of (10 mM) $CaSO_4$-moistened filter paper. In three days, they were shallow-planted into 10 mM $CaSO_4$-moistened pearlite under a closed canopy of clear plastic film, and kept in a growth chamber (12 h day-night 25°/20 °C). One week later, seedlings were placed in an aerated basal nutrient solution used for the wheat experiment above, in a growth chamber set at 22 °C/17 °C (12 h day/night) with incandescent lighting. Halogen lighting was commenced five days later, as described above, prior to beginning Mn treatments identical to those administered in the wheat experiment. As for the wheat experiments, this soybean study was repeated at the higher temperature regime of 32 °C/27 °C (12 h day/night). The growth medium pH was maintained at 5.5.

2.2. Field sampling of canola leaves and host soil

The site was a commercially grown crop of canola (*Brassica napus* L. cultivar Pioneer 44Y89 (Brassicaceae)) on a farm in southern New South Wales, Australia (34°54′17.64″S; 147°0′10.1″E), an agricultural region regularly affected by Mn toxicity in winter. Plants exhibited foliar toxicity symptoms interpreted by farmers as Mn stress in field canola, i.e., marginal and interveinal chlorosis, some deformity as cupping, with no evidence of black spotting reported in Mn crop experiments (Fig. 1e and f). Leaf chlorosis was more severe on the upper surfaces