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Methodological Advances

Quantifying water use in a plant–fungal interaction

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ABSTRACT

Fungal rust galls are parasitic fungi on *Acacia* host plants. The amount of water required by fungal parasites has yet to be widely measured. Sap flow instrumentation was installed on branches of *Acacia implexa* to measure water use of a fungal rust gall. Host water use was also measured. Partial regression analysis, using gall sap flow against environmental variables, was performed to determine a possible driving mechanism of gall sap flow. Following the removal of galls from branches, sap flow immediately returned to, and stayed at, zero flow. Gall sap flow was 4.63 % of total host tree sap flow on a daily and cumulative basis. Vapour pressure deficit explained the greatest amount of variation in gall sap flow, suggesting gradients of water potential was the driving mechanism of water use. This study demonstrated that instruments which can measure low sap flow can be used to quantify fungal water use.

Introduction

The acquisition of water is critical for all living organisms. Direct uptake of water from the environment is common, however many organisms have evolutionary adaptations and ecological interactions to acquire water from other organisms. In interactions with plants, many organisms are dependent on plant hosts for most of their critical resources, especially water and nutrients. In particular, a number of diverse organisms, such as fungi, insects, bacteria and nematodes, can become completely reliant on their host plant for nutrition (e.g. Janzen 1966; Gullan & Kosztarab 1997; Smith *et al.* 1997; Williamson & Gleason 2003). The evolutionary life history of these organisms means they are obligate feeders on their host plant. How these organisms interact with their host to gain nutrients, such as carbon or nitrogen, has long been studied. How they directly acquire water, on the other hand, has yet to be widely quantified.

Galling organisms are an example of parasites closely associated with their host plant. A gall is plant tissue that has been deformed during growth and can exist on roots, stems, leaves or buds (Redfern 2011). Galls are visible protrusions on the plant, tumour– or wart–like, that may be small or large, woody masses. Galls are induced by various organisms with

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the most common being insects, nematodes and fungi. The gall acts as a habitat for the parasite in which resources are sequestered and, depending on the species, a part or all of a life cycle is carried out. Galling insects have been particularly well studied (e.g. Shorthouse & Rohfritsch 1992; Williams 1994; Crespi *et al.* 1997; Wool 2004), with fungal-induced galls less so.

There is evidence that some insects are dependent on plant water for biological and ecological activities (Kennedy *et al.* 1958; Sumner *et al.* 1983, 1986; Connor 1988; Holtzer *et al.* 1988; Hanks & Denno 1993; Schowalter *et al.* 1999). A meta-analysis by Huberty & Denno (2004) found that sap-feeding insects, including xylem feeders, were negatively affected by host plant water stress. The same review, and another by Koricheva *et al.* (1998), found insect-formed galls were also negatively affected by host plant water stress. Gall-forming insects increase in abundance with precipitation (Price & Clancy 1986; Schowalter *et al.* 1999) due to the need for healthy tissue growth for gall colonisation and development (Larsson 1989; Price 1991, 1994; Preszler & Price 1995). There is less evidence for mutualistic or parasitic fungi being directly affected by host plant water activity. Much of the work has centred on symbiotic mycorrhizal fungi on plant roots (Augé 2001; Lehto & Zwiazek 2011). For example, host trees can mine water deep in the soil profile and, through the process of hydraulic lift, can translocate water to mycorrhizal fungi or sporocarps at the soil surface (Querejeta *et al.* 2003; Querejeta *et al.* 2007; Lilleskov *et al.* 2009). As far as can be ascertained, there is no evidence for fungi-formed galls on host plants being affected by plant water status, let alone whether fungi-formed galls are dependent on water from the plant host at all.

The rust gall is a fungal-induced gall commonly found on trees and shrubs of Australian acacias (Fig 1). *Acacia implexa* is a host for a specialised genotype of the fungus, *Uromycladium tepperianum* (Morris 1987). The rust gall is initially formed by the fungus but is subsequently colonised by a community of invertebrates including mites, mealy bugs and predatory beetles (Eisen 2001; personal observation). Little is known about the physiology of acacia rust galls and the interaction between the fungus and invasive invertebrate community. It is expected that the rust gall is dependent on vascular supply from the host plant for growth and development. Therefore, it was hypothesised that gall water use will mimic host plant water use with diurnal peaks and nocturnal troughs. Additionally, as invertebrate activity increases with temperature (Huey & Kingsolver 1989; Angilletta *et al.* 2002), it was hypothesised that water use within the gall would be positively correlated with temperature. That is, the hypothesis is an increase in temperature will increase activity within the gall which will lead to greater demand for water.

The principal aim of this study was to determine whether fungal-induced galls on *A. implexa* parasitised water from the tree host. Water use, *via* sap flow instrumentation, was established on stems infected with rust galls and the hypothesis was tested that the removal of galls would lead to a cessation of sap flow. The proportion of rust gall water use, as a share of total tree water use, was determined. Meteorological variables including solar radiation, temperature and vapour pressure deficit were measured to determine the principal driving mechanism of sap movement and to test the hypothesis that host tree sap flow or temperature was the main driver of sap flow in gall-infected stems.

Methods and materials

Study site and tree selection

The study site was located approximately 30 km south of Armidale, northern New South Wales, Australia (30°42′20″S, 151°46′35″E). The area is regenerating grazing land on the edge

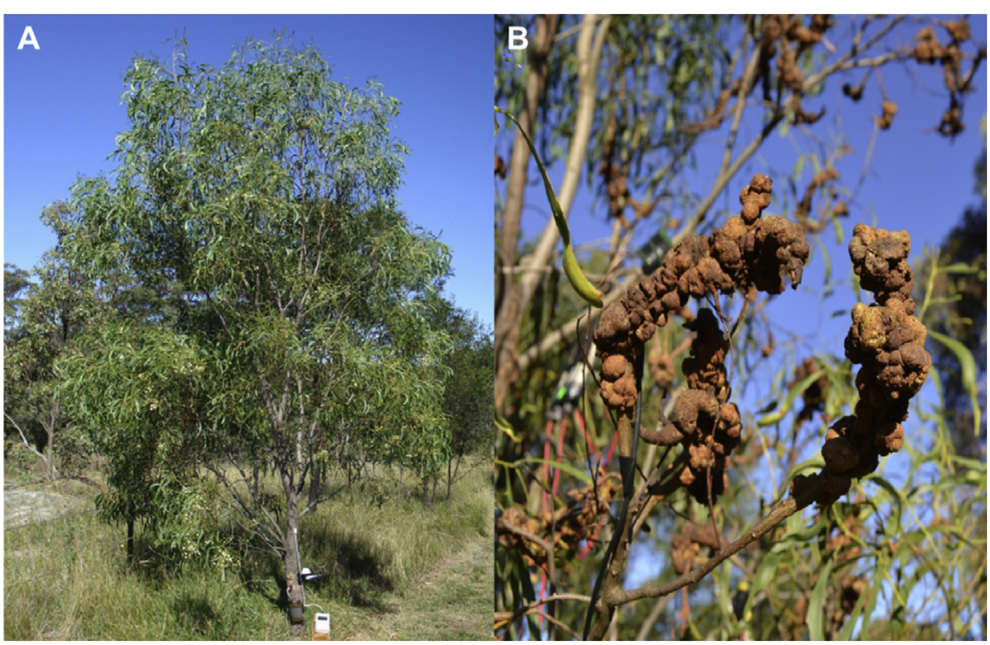

Fig 1 – (A) The host tree sampled in the study, a 4.26 m *Acacia implexa*. (B) An example of the rust gall fungus (*Uromycladium tepperianum*) growing on the *A. implexa* host tree.