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Effects of *Tetranychus lintearius* (Acari: Tetranychidae) on the structure and water potential in the foliage of the invasive *Ulex europaeus* (Fabaceae) in Australia

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Ulex europaeus, a native of Western Europe and the Mediterranean coast, is a serious agricultural and environmental weed in many parts of the earth, including Australia. *Tetranychus lintearius* attacks *U. europaeus* in its natural range and is one of the four biological-control agents introduced into Australia to regulate populations of *U. europaeus*. *Tetranychus lintearius* is specific to different species of *Ulex*, which enhances its status as a key biological-control agent. Little is known on the physiological effects and structural changes induced by *T. lintearius* on *U. europaeus*. This paper describes anatomical changes and evaluates the physiological effects in *U. europaeus* consequent to feeding by *T. lintearius* for more than 100 d. This study demonstrates that feeding by *T. lintearius* reduces the quantity of photosynthetic tissue in *U. europaeus*, as a result of feeding on mesophyll parenchyma by inserting its stylets through the stomatal aperture. Unfed parenchyma cells display signs of stress, either exhibiting deformed cell organelles aligned along the walls of vacant cells or with cells with no well-defined cell organelles. Subsequent to *T. lintearius* feeding, leaves externally manifest bleaching. After feeding by a population of *T. lintearius*, leaf-water potential is decreases by 30% and tender shoots lose their turgor, indicating water-related stress. *Tetranychus lintearius* has the potential to decelerate vigour and retard the competitiveness of *U. europaeus* by inflicting stress. Our findings reinforce use of *T. lintearius* in the biological management of populations of *U. europaeus* in temperate Australia.

Keywords: Australia, biological control, efficacy, gorse, spider mite, stress physiology

Page identification caption: Marriott et al.

Introduction

Ulex europaeus (commonly known as ‘gorse’) is an aggressive perennial. As a native of Western Europe and the Mediterranean Coast, it is one of the key floral elements of acidic heath lands (Leary et al. 2006). Today, it has spread widely to become a serious agricultural and environmental weed in regions including Australia. *Ulex europaeus* is one of the 20 weeds of national significance in Australia, because of its economic and social impacts in agriculture and environment (Ireson and Davies 2012). *Ulex europaeus* was deliberately introduced into Australasia a little before 1835 as an inexpensive, quick-growing hedge for stock containment and shelter; due to favourable climate and lack of predators, it has turned out to be an invasive (Moss 1960).

Exapion ulicis (Coleoptera: Curculionidae) introduced in 1939, *Tetranychus lintearius* introduced in 1998, *Sericothrips staphylinus* (Thysanoptera: Thripidae) introduced in 2001 (Davies et al. 2007; Ireson et al. 2008), and *Agaonopterix umbellana* (Lepidoptera: Oecophoridae) introduced in 2007 (Tasmanian Institute of Agricultural Research 2008) are the arthropods of choice to biologically manage populations of *U. europaeus* in Australia. Trials using *T. lintearius* were made first in New Zealand (1989–1991) and later in Chile (1996); success levels varied from little impact in New Zealand (Hill et al. 2000) to significantly reducing plant biomass and affecting flowering in Chile (Norambuena et al. 2000). Australian trials found impressive reductions in plant biomass (Davies et al. 2007). Based on these, *T. lintearius* is considered an effective biological-control agent for *U. europaeus* (Davies et al. 2007). *Exapion ulicis* and *T. lintearius* are the most extensively trialled arthropod taxa in *U. europaeus* management in Australia (Ireson and Davies, 2012). Davies et al. (2007) measured the impacts of *T. lintearius* on its establishment as a biological-control agent and the changes it induced on the gross morphology of *U. europaeus*.

As biological-control agents, the Tetranychidae, in general, show many preferred attributes: for example, they have a high rate of fecundity and they produce several generations in a year; they usually attack a plant during its growing season and by the enormity of its populations can damage it severely (Hill and Stone, 1985). Tetranychidae have sucking mouthparts, with which they puncture plant cells and suck cell contents (Tomczyk and Kropczynska 1985), which alters plant metabolism, eventually reducing plant growth and in the long term, affecting flowering and seed production, (Saito 2010). Nonetheless, several Tetranychidae (e.g., *T. urticae*) are also well-known nuisance organisms in horticulture (Silva et al. 2009).

The potential of *T. lintearius* in the biological management of *U. europaeus* was flagged by Helmut Zwölfer in 1963 based on naturally occurring large populations of *T. lintearius* on *U. europaeus* in Europe. Zwölfer (1963) reported that large-scale infestations lead to the death of, usually, the branches and, occasionally, the whole plant. Subsequent trials on *T. lintearius* based on Zwölfer’s remark were challenged, since a majority of the Tetranychidae are polyphagous; moreover, uncertainty prevailed on the taxonomy of *Tetranychus* (Hill and Stone 1985). Host-specificity trials testing nearly 70 plants indicated that *T. lintearius* usually remains confined to genetic borders of *Ulex* (Hill and O’Donnell, 1991; Ireson et al. 2003). Tests verifying whether *T. lintearius* was reproductively isolated and whether this taxon can readily hybridize (Hill and Stone, 1985) showed that *T. lintearius* possesses preferred traits of a biological-control agent, such as being relatively restricted to the generic limits of the host plant and building its populations rapidly. Populations of *T. lintearius* have been shown to reduce biomass and shoot growth of *U. europaeus* (Rice 2004; Davies et al. 2007); and have a strong potential to induce modest–severe chlorosis in *U. europeaus* (Stone 1986). These findings have narrowed it down as a candidate-of-choice in contemporary biological management of *U. europaeus*.

Because the tetranychids are established mesophyll feeders, in this study we aimed to characterize structural changes and water relations in the needle-like foliage of *U. europaeus* consequent to feeding by *T. lintearius*. We also aimed to verify whether feeding actions of *T. lintearius* would adequately stress *U. europeaus* foliage in the context of biological management. Therefore, we evaluated structural changes and water relations in *U. europaeus* consequent to feeding by *T.*

linterarius, the nature of cellular dynamics in the foliage, and assays of leaf-water potential and gas exchange.

Natural history of *Ulex europaeus* and *Tetranychus linterarius*

Ulex europaeus, an invasive in Australia, occurs extensively in agricultural and urban landscapes of temperate Australia (e.g., the Australian Capital Territory, New South Wales, South Australia, Tasmania, Victoria, and the southern Mediterranean parts of Western Australia). *Ulex europaeus* lives up to 30 y and grows to 4 m (Lee et al. 1986). Seeds develop in small pods that are produced in late spring (November) and through summer (December–February); about 600 seeds/m² develop and remain viable for more than 25 y (Ireson and Davies, 2012). Management of *U. europaeus* has been a daunting task because of its long-lived seed bank.

Colonies of 100s–1000s of *T. linterarius* (Figs. 1, 2) live on *U. europaeus*, spinning large webs. *Tetranychus linterarius* spin webs as they spread on branches of *U. europaeus*. Such webs by *T. linterarius* could be an adaptation to exclude competitors from ‘profiting from down-regulated plant defences’, as shown in the infestations of *Tetranychus evansi* on glasshouse-raised *Solanum lycopersicum* var. Santa Clara I-5300 (Solanaceae) (Sarmiento et al. 2011). Populations of *T. linterarius* move among branches of *U. europaeus* and between neighbouring plants, enabled either by wind or by sticking on to the strings of web swaying by wind action (Fig. 1). Development from eggs to adults is c. 30 d at 20°C and populations build up with rising temperature (Stone, 1986). They feed on the young as well as mature needle-like foliage of *U. europaeus*. Intensely fed areas on *U. europaeus* foliage appear as bleached chlorotic patches (Fig. 3).

Materials and methods

Plant and acarine materials

In the first–second weeks of February 2009, 50 approximately 3-y old *U. europaeus* plants grown in commercial-grade potting mix in a glasshouse at the Keith Turnbull Research Institute (Frankston, Victoria, Australia) were transported to the University-of-Ballarat glasshouse (37°37’S; 143°53’E; hereafter ‘glasshouse’). Twenty plants were maintained in mite-proof cages in the glasshouse (16–25°C; 12:12 L–D; RH 70–80%). Active populations of *T. linterarius* obtained from natural areas in Learmonth, Victoria (37°24’S; 143°41’E) were introduced on to shoots of *U. europaeus* in the glasshouse. Adults of *T. linterarius*, estimated at clusters of 750–1000, usually lived proximal to the web they had spun, whereas the nymphs and eggs remained in the web. The introduced *T. linterarius* colonies and their effects on host plants were monitored with a hand-held 10x lens three times every 24 h for the first 7–10 d. This monitoring was critical to determine the establishment of the introduced *T. linterarius* populations on the foliage of *U. europaeus*. Observations were made to assess the appearance of chlorotic spots, which later coalesced into large patches. In the first week of March and May 2009 (21 d and 105 d respectively), 2-cm long leaves were excised from *U. europaeus* raised in the glasshouse for microscopic analyses: five leaf samples were obtained with *T. linterarius* actively feeding on them (21 d), five leaf samples bearing chlorotic patches (105 d), and five uninfested leaf samples were also taken from plants (21 d, 105 d, respectively).

Twenty plants of *U. europaeus* were used in obtaining water-potential measurements. They were placed in 10 insect-proof cages (two in each cage) in the glasshouse, hand watered with measured quantities of water, and fertilized with Seasol™ (40 ml/9 L) twice over 2 weeks through to the new emergence of new shoots to ensure establishment in the glasshouse. *Tetranychus linterarius* colonies were obtained from mature *U. europaeus* plants growing along the Midland Highway approximately 20 km south of Ballarat (37°42’S, 143°59’E) and in the Buninyong region (37°38’S, 143°51’E). Shoots of *U. europaeus* bearing neonate colonies of *T. linterarius* aggregated at the tips were collected and rapidly transported to glasshouse. Ten plants were infested with colonies of 750–1000 individuals of *T. linterarius*, whereas 10 other plants were retained as control. The introduced *T. linterarius* colonies were observed using a hand-held 10x lens three times in the first 24 h and then

every day after introduction. Observations of the introduced colonies were necessary to determine how long the colonies persisted and fed on the host plants.

Microscopy

Leaves of *U. europaeus* with actively feeding populations of *T. lintearius* excised from the glasshouse populations, stored in plastic containers containing moist-cotton balls, were transported in an ice chest to the Department of Zoology, University of Melbourne, by road. These leaves were stored at <10°C for 12 h before fixing in FAA to stun *T. lintearius* nymphs and adults feeding on *U. europaeus* foliage and then in situ embedded them in paraffin wax. The leaf samples thus fixed were dehydrated through an ascending-alcohol series and transferred to paraffin wax following Feder and O'Brien (1968). Cross and longitudinal sections of the leaf materials were made at 8 µm thickness in a Hyrax M25 automated rotary microtome (Carl Zeiss™, Jena, Germany) and stained with haematoxylin–eosin and Mallory's–aniline blue combinations. Photographs were made using a Nikon™ DSL camera (Nikon Corporation, Tokyo, Japan) attached to a stereo-binocular microscope and a Leica™ DM1000 camera (Leica Microsystems, Wetzlar, Germany) attached to a compound microscope. Stained micropreparations were observed with a BH–2 Olympus compound microscope (Olympus, Tokyo, Japan).

Portions of excised leaves (2 mm²) and individuals of *T. lintearius* were fixed in 2.5% gluteraldehyde in 0.1 M PO₄ buffer (pH 7.4) for 4 h at room temperature (21°C) and processed for transmission- and scanning-electron microscopy.

For transmission-electron microscopy, the chosen materials were rinsed three times in freshly prepared PO₄ buffer (pH 7.4; 0.1 M) for 30 min before post-fixing in 1% OsO₄ (0.1 M; PO₄ buffer pH 7.4) for 2 h. They were dehydrated in 10, 30, 50, 70, 90, 100% ethanol for 30 min in each concentration. Samples were infiltrated with increasing concentrations of LR–White resin in ethanol (1:2) for 8 h followed by 2:1 resin–ethanol treatment overnight. The samples were then infiltrated in 100% resin with two changes of 24 h each. The tissues were subsequently embedded in the resin and polymerized at 60°C for 24 h. Embedded tissues were sectioned using a diamond-edge knife in an Ultracut R™ ultramicrotome (Leica Microsystems, Wetzlar, Germany) and sections of 90 nm thickness were collected onto pioloform-coated 200 mesh copper grids. They were contrasted using 2% uranyl acetate for 10 min and lead citrate for 5 min, and viewed in a TEM CM120 Biotwin™ TEM (Phillips, Eindhoven, The Netherlands) at 120 kV acceleration voltage. Images were captured with a Multiscan 600CW Gatan™ camera (California, USA).

For scanning-electron microscopy, the samples dehydrated in ascending ethanol series were critical-point dried in CPD 010 (Balzers™, New Hampshire, USA) and mounted on to 25 mm aluminium stubs using double-side carbon tabs. The leaf edges were initially coated with silver liquid, dried, and gold coated in an Edwards™ S150B sputter coater (New York, USA). Materials were imaged in a field-emission scanning electron microscope (XL30, Phillips™, Eindhoven, The Netherlands) at an acceleration voltage of 2 kV.

Water-potential measurements

The shoots from the glasshouse raised populations of *U. europaeus* were excised and placed in airtight-plastic containers and swiftly (<1 minute) transported to the University of Ballarat's Seed-Ecology Laboratory. Leaf-water potential was measured using a Water Status Console (Series 3000™, Soil Moisture Equipment Corp, Santa Barbara, USA) following Scholander et al. (1965). Two measurements were obtained from each shoot: one at 0500–0700 h (pre-dawn) and the other at 1200–1400 h (midday). The uninfested-plant foliage of comparable age was measured to determine whether they showed similar water potentials. The temperature in the glasshouse at the time of obtaining leaf-water potential measurements was also recorded.

Data analysis

Water-potential measurement data were analysed by applying two-way ANOVA using the SuperANOVA software program (Abacus Concepts™, Berkeley, CA). Residual plots of each ANOVA were obtained to examine the homogeneity of variance.

Results

Mouth parts of *Tetranychus lintearius*

The gnathosoma includes elongate and sharply pointed pedipalps, a prominent rostrum, and stylets (Fig. 4). The movable segment of the chelicerae is modified into an elongate stylet. Similar to that known in most other Tetranychoidae (e.g., André and Remacle 1984), the cheliceral bases fuse to form a stylophore that can be retracted into the rostrum. The stylophore includes the stylets, which slide along the rostral gutter. We estimate the length of the extended stylets at 80–90 μm , similar to those shown in several Tetranychoidae (see Beard et al. 2012).

Structural changes in *Ulex europaeus*

Populations of *T. lintearius* usually aggregate in the furrows on the needle-like foliage of *U. europaeus*, where the stomata are more numerous than on the ridges (Fig. 5, 6), which has been observed both in 21 d and 105 d samples. *Tetranychus lintearius* insert their stylets through the stomatal aperture and feed on the mesophyll cells that occur immediately below (Fig. 7). They suck nearly the entire cell contents so that only hyaline layer of cytoplasm remains along the periphery of cells; chloroplasts tend to remain closely adpressed to walls (Fig. 8). A mesophyll cell from uninfested needle of *U. europaeus* of similar age (Fig. 9) shows that feeding by *T. lintearius* alters the subcellular structure of *U. europaeus* leaf cells. They include degenerated cytoplasm (evident as debris) and cell organelles; due to sucking of cell fluid, the mesophyll cells lose their turgor and the cells appear flaccid with wavy and unevenly thickened cell walls (Fig. 10). *Tetranychus lintearius* feeds on the mesophyll parenchyma; however, the phloem elements and phloem parenchyma display signs of hyperplasia and dynamic cell profiles. A modest level of hypertrophy occurs in consequence, which appears more due to the stress transmitted from the neighbouring cells rather than due to direct feeding effect of *T. lintearius* (Figs. 11, 12).

The feeding-puncture point is evident with a distinct 'cone' shaped callosic response (21 d; Fig. 13; callose confirmed: aniline-blue stain reaction in light-microscopy, Peterson et al. 2008). The immediately adjoining point to this response site includes distorted cell membrane and exhibits several microbodies gathered here. Abundant cytoplasm occurs, interspersed with numerous small and large vacuoles. Chloroplasts, nucleus, and other cell organelles occur clustered close to the puncture point. Along the puncture point rough endoplasmic reticulum occurs in a distinct pattern: as concentric rings. Prominent plasmodesmatic strands characterize intercellular junctions between this cell and neighbouring cells. Chloroplasts occurring close to the puncture point include limited numbers of plastoglobuli; thylakoids appear irregularly and are abnormally vesiculated. Chloroplasts occurring away from the puncture site appear inflated and include large starch granules. The nucleus is relatively small, but displays an evenly dispersed chromatin and a few isolated patches of condensed chromatin. Walls are unevenly thickened and appear undulated.

The cells occurring near the cell with the feeding puncture include a dynamic nucleus, which is, however, enlarged (Fig. 14). The nucleus includes an evenly dispersed chromatin and several but small isolated patches of condensed chromatin. Cytoplasm is abundant and interspersed by many small and large vacuoles. Some of the chloroplasts appear distorted and present a branched contour. Mitochondria are stretched and they occur closely around the nucleus (Fig. 14). Chloroplasts are numerous and inflated, and include vesiculated thylakoids (Fig. 15), large starch granules (Fig. 15), and numerous plastoglobuli (Fig. 16). Several microbodies occur dispersed throughout the

cytoplasm, especially along the wall (Fig. 16). Walls are unevenly thickened, presenting an undulated contour (Fig. 17).

In 105 d, the mesophyll tissue beneath the furrow degenerates, and the cells turn necrosed (Fig. 18). Remnant parenchyma cells accumulate abundant darkly stained inclusions, which we suspect represented stored phenolic materials. Lower portions of the needle-like foliage with no apparent mite feeding damage display numerous parenchyma cells with darkly staining inclusions and loss of the star-like shape (as in cross sectional views) (Fig. 19).

Water potential

Tetranychus lintearius feeding significantly reduced water potential in the foliage of *U. europaeus* ($p < 0.001$). Prior to infesting *U. europaeus* with *T. lintearius*, the average-water potential (ψ) was -522 kPa and -598 kPa at predawn and midday, respectively (Fig. 20a). The temperatures in the glasshouse were 18°C (predawn) and 23°C (midday).

Twenty-two d after infestation by *T. lintearius* the ψ of the control plants was -527 kPa at predawn and -614 kPa at midday. The ψ for infested plants were -662 kPa predawn and -834 kPa at midday (Fig. 20b). The recorded temperatures in the glasshouse were 19°C predawn and 25°C midday. The average difference for predawn and midday water potential between control and infested plants was at 31% negative reduction in ψ . The reduction of ψ between control plants and infested plants at predawn was 25% and at midday it was 36%. The average reduction ψ in between pre-dawn and midday measurements was significant ($p = 0.001$), **as was the reduction in ψ between infested and infested plants. The interaction between infestation level and diurnal period was not significant** (Table 1).

Discussion

This paper describes time-related structural changes recorded and water-potential measurements made in the needle-like foliage of *U. europaeus* infested by *T. lintearius* populations, comparing them with the uninfested foliage of similar age. *Tetranychus lintearius* is the arthropod of choice to manage exploding populations of *U. europaeus* in temperate Australia.

In this paper we demonstrate that *T. lintearius* feeds on the mesophyll parenchyma cells of *U. europaeus*. One critical effect that manifests itself is the emptying of host-mesophyll cells before those cells collapse. Cells in the vicinity of the cell coming under the direct-feeding pressure in *U. europaeus* show active but varying subcellular profiles in 21 d after infestation. Concentric arrangement of rough endoplasmic reticulum (RER) usually signifies a low cell-energy status (Smith et al. 2002); concentric rings of RER in conjunction with mitochondria showing degenerated inner-membrane system indicate stressed subcellular conditions prevailing in that environment. These cells show dense plasmodesmatic strands at the intercellular junctions (Baluška et al. 2004) and this is active communication with the stressed cell due to feeding action of *T. lintearius*. Cells adjacent to the cell on which *T. lintearius* feeds mobilize primary metabolites to the stressed cell to neutralize stress and to enable it to restore its normal metabolism (Shabala 2010). Mobilization of nutrients *via* short-distance transport (Schulz 1995, Botha and Cross 2000) is advantageous to populations of *T. lintearius*, since the mobilized primary metabolites nourish them. Cells adjoining the fed cell show degenerating cytoplasm before they become necrosed. Localized necrosis, occurring as a rapid response to feeding action (< 20 min) (Westphal et al. 1981), has been demonstrated among many species of the Eriophyoidea (Westphal 1977; Lindquist et al. 1996) and the Tetranychioidea (Kielkiewicz 1985; Rilling and Düring 1990).

The feeding-puncture site with the cone-shaped callosic deposition is a wound-healing response. Similar responses have been shown at the feeding sites of diverse Eriophyoidea (e.g., Westphal et al. 1981; Petanović and Kielkiewicz 2010). The puncture site on *U. europaeus*-mesophyll cells appears as a dimple on the wall, the shape of which matches with the contour of the stylet tip, as shown in other

Tetranychoida (Beard et al. 2012). As the mite, sucks cell contents, the foliage-parenchyma cells of *U. europaeus* respond by draining their contents to *T. lintearius*. Cells adjoining the fed cell also appear stressed due to the feeding injury and secretion of salivary chemicals by *T. lintearius*. To enable the affected cells to regain their turgor which is lost in fed cells and which is evident by their unevenly thickened and wavy walls and shrunken cytoplasm, callose accumulates at the site of feeding, sealing the puncture on the wall (Shackel et al. 1991). Cell-puncture sealing with callose is demonstrated in susceptible interactions between *Eriophyes cladophthirus* (Acarina: Eriophyoidea) and *Solanum dulcamara* (Solanaceae) (Westphal et al. 1981) and in other arthropod-plant interactions (Dietrich et al. 1994, Kant et al. 2004, Luna et al. 2011).

Subcellular profiles near the puncture site on the mesophyll cells of *U. europaeus* appear closely similar to that which occurs during the feeding action of *Nilaparvata lugens* Biotype 1 (Hemiptera: Delphacidae) on the leaves of *Oryza sativa* var. TN1 and B5 (Poaceae) (Wang et al. 2008). Mesophyll cells of *U. europaeus* under the feeding pressure of *T. lintearius* manifest enlarged and disorganized chloroplasts and mitochondria, and also nuclei with condensed chromatin. In these cells, the numbers of mitochondria are fewer than those found in uninfested cells. The mitochondria in the infested-leaf mesophyll cell appear stretched, with alterations in their membrane configuration indicating that the cells suffer low-oxygen tension, which leads to localized hypoxia (Van Gestel and Verbelen, 2002). Swollen cristae indicate depolarization of the mitochondrial-membrane system. Mitochondrial profiles strongly indicate symptoms of cell stress preceding plant-cell death (Yu et al. 2002). The chloroplasts appear inflated and display altered photosynthetic apparatuses, such as vesiculated thylakoids. The chloroplasts include great numbers of starch granules and plastoglobuli. These modifications denote lowered levels of Rubisco-binding proteins and other proteases (Demirevska et al. 2008). The increase in the numbers of plastoglobuli reflects the level of oxidative stress on the photosynthetic apparatus, due to higher concentrations of CO₂ (Sallas et al. 2003, Raman et al. 2006). Occurrence of several microbodies (either peroxysomes or glyoxisomes), especially in the degenerating cytoplasm in *U. europaeus* mesophyll, is striking. Organelles, either with an intensified oxidizing metabolic activity or with an accelerated rate of electron flow, such as chloroplasts, mitochondria, and microbodies, are the demonstrated reactive-oxygen species (ROS) production sites in plant cells (Bi et al. 2009). Importantly, ROS production is a key response of plant cells to stress (Skopelitis et al. 2006). Plentiful microbodies, especially the peroxisomes, evidently generate H₂O₂ in *U. europaeus* cells, resorting to a photorespiratory pathway consequent to the stress due to *T. lintearius* feeding.

Compared with the data generated from uninfested foliage of similar age, reduction in foliage-water potential is significant in *U. europaeus* when attacked by *T. lintearius*. Drop in the foliage-water potential, in turn entails loss of cell turgor and stomatal closure, leading to decrease in the rate of transpiration and photosynthesis, which subsequently leads to reduced plant growth. Low-water potentials influence stomatal closure in plants, restricting CO₂ entry and thus affecting the rate and efficiency of photosynthesis. Wounding by *T. lintearius* could be another critical factor, which influences the rate of greater ROS evolution, leading to localized anoxia and thus stressing of *U. europaeus* (Cabrera et al. 1994, Kessler and Baldwin 2002).

Previous population-based investigations have shown that *T. lintearius* is an agent of high potential in the biological management of *U. europaeus* in Australia (see Ireson and Davies 2012). These works show that shoots *U. europaeus* suffer bleaching and necrosis. Our work explains how cellular and subcellular changes occur in the mesophyll of *U. europaeus* consequent to infestations of *T. lintearius*. Findings reported in this paper also demonstrate the physiological changes that occur in the infested cells of *U. europaeus*, adding validity for employing populations of *T. lintearius* in the biological management of *U. europaeus* — an invasive in temperate Australia.

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