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Defence mechanisms of *Ficus*: pyramiding strategies to cope with pests and pathogens

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Abstract

Ficus species have adapted to diverse environments and pests by developing physical or chemical protection strategies. Physical defences are based on the accumulation of minerals such as calcium oxalate crystals, amorphous calcium carbonates and silica that lead to tougher plants. Additional cellular structures such as non-glandular trichomes or laticifer cells make the leaves rougher or sticky upon injury. *Ficus* have also established structures that are able to produce specialized metabolites (alkaloids, terpenoids, and phenolics) or proteins (proteases, protease inhibitors, oxidases, and chitinases) that are toxic to predators. All these defence mechanisms are distributed throughout the plant and can differ depending on the genotype, the stage of development or the environment. In this review, we present an overview of these strategies and discuss how these complementary mechanisms enable effective and flexible adaptation to numerous hostile environments.

I- Introduction

Ficus is a large and diverse genus of approximately 850 species belonging to the Moraceae family. The various lifeforms of *Ficus* (trees, stranglers, vines, shrubs, and epiphytes) are adapted to diverse climatic and geographic regions (Janzen 1979; Pierantoni et al. 2018). *Ficus* species can be monoecious or dioecious (Ghana et al. 2017), but this genus is characterized by its peculiar inflorescence, the fig (or syconium), which forms a cavity enclosing tiny flowers (Anstett 2001).

The most popular *Ficus* species is *F.carica*, a deciduous tree that can reach a height of two to five metres and is frequently referred to as fig tree. *F. carica* is thought to be native to the Middle East and to be one of the earliest cultivated fruit trees, but it can currently be found in many warm regions worldwide (Bonamonte et al. 2010). Because its fruits – the common figs – are consumed in many ways (fresh, dried, and processed) and are rich in minerals, vitamins and fibres, *F. carica* has become an important crop and is mostly cultivated in the Mediterranean region (Solomon et al. 2006; Oliveira et al. 2009). Indeed, world fig production was approximately one million tons in 2016, and more than half of this production was in Turkey, Egypt and Algeria (FAO 2016).

F. carica can therefore be regarded as a species of agronomical and economical importance. Similar to other plants, *Ficus* species are subjected to numerous bioaggressors including a large variety of generalist and specialist insects (Basset and Novotny 1999; Novotny et al. 2010; Volf et al. 2018). These species can also be infected by fungi (McKenzie 1986; Hosomi et al. 2012) or by some viruses (Elbeaino et al. 2011; Bayoudh et al. 2017). In Europe, the main pests affecting *F. carica* include the fig wax scale, while the major diseases include fig canker and fig rust (Chamont 2014). Excepting these diseases, which can lead to a significant decrease in yield, *F. carica* and the *Ficus* genus in general seem to be excellent at countering their bioaggressors and especially at resisting their attacks (Chamont 2014). Indeed, to adapt to their hostile environments, *Ficus* species have developed a high diversity of defence strategies including physical structures (mineral deposition, fibre content, and toughness) and chemical constituents (specialized metabolites and particular proteins). This review aims to realize a state of the art of the defence mechanisms developed by *Ficus* plants, especially *F.carica*.

II- Physical defences: tough and mineralized leaves

Leaves are the first targets of herbivores; therefore, their toughness might be considered part of the general plant defence strategy. Toughness is reported to be negatively correlated with herbivore damage (Coley 1983) and this resistance might be related to macromolecular substances such as lignin, fibre and phenolic content (Coley et al. 1985; Xiang and Chen 2004). Toughness can also be reinforced by mineral deposits, such as those of calcium oxalate crystals, amorphous calcium carbonate and silica (Fig. 1). Pierantoni et al. (2018) reported that these three minerals were unequally distributed throughout the tissues of 10 *Ficus* species collected in diverse environments.

Calcium oxalate crystals

Calcium oxalate represents the most common form of plant solid mineralization (Korth et al. 2006; Bauer et al. 2011). This mineral forms intra- or extracellular crystals in most plant tissues, but its deposition occurs mostly in the vacuole of crystal idioblasts (Foster 1956; Franceschi and Nakata 2005; Bauer et al. 2011). Idioblasts are isolated cells that differ from their neighbouring tissue elements in their form, size, content and/or cell wall structure. They can be found in any plant part and include a wide variety of cells (Foster 1956). In plants, in addition to their calcium regulation function and their metal detoxification role, calcium oxalate crystals are involved in defence (Finley 1999; Franceschi and Nakata 2005; Pierantoni et al. 2018). These crystals establish a physical barrier (Ruiz et al. 2002; Hudgins et al. 2003), deter pests and herbivorous mammal feeding (Yoshihara et al. 1980; Ward et al. 1997), and are responsible for abrading the mouthparts of chewing insects (Korth et al. 2006). These crystals may therefore be complementary to plant toughness in a defence strategy (Finley 1999). In *Ficus*, a potential reduction in pest attacks has been reported among these defensive roles (Xiang and Chen 2004; Sosnovsky 2016).

Calcium oxalate crystals are usually deposited in lines parallel to the major veins of *Ficus* leaves. These crystals can therefore be found along the vein bundle, the phloem or the ground tissue supporting the veins (Pierantoni et al. 2018). Depending on the *Ficus* species and the leaf localization, calcium oxalate crystals are mainly described as prismatic crystals restricted to the bundle sheath and druses located in the mesophyll and bundle sheath (Wu and Kuo-Huang 1997; Pierantoni et al. 2018). However, this is not a general rule: for example, calcium oxalate crystals are observed in *F. virgata* in the form of druses located in the bundle sheath and the palisade mesophyll layers but also as crystal sands in the abaxial epidermis of the leaves. In *F. elastica*, prismatic crystals are restricted to the bundle sheath, but needle-shaped crystals have also been reported in the epidermis of leaves (Wu and Kuo-Huang 1997). In *F. carica*, calcium oxalate is mainly found in the phloem in the

form of druses and prismatic crystals. The deposition of calcium oxalate occurs on the inner or outer surface of the vein (Fig. 1A) throughout the development of the leaf (Pierantoni et al. 2018).

Amorphous calcium carbonates (cystoliths)

Cystoliths are mineralized bodies restricted to a few species including some in *Moraceae*; they were first described by Meyen in 1839 (Meyen 1839; Weddell 1854). These calcified structures display concentric layers of cellulose microfibrils associated with pectin and polysaccharides and imbued with calcium carbonate. Depending on the species, these bodies occur in various shapes (oblong-, cigar-, bean-, Y-shaped, etc.), colours (blue, violet and greenish) and sizes (from 25 to 75 μm wide and 15 to 115 μm long) (Ummu-Hani and Noraini 2013). In the *Ficus* genus, the localization of cystoliths depends on their shapes: elongated cystoliths are formed only in the adaxial epidermis and extend into the palisade mesophyll, while spherical cystoliths only appear in the abaxial epidermis and extend into the spongy mesophyll (Pierantoni et al. 2018). Therefore, cystoliths can be found adaxially (*F. benghalensis*), abaxially (*F. religiosa*) or in both epidermises of the leaves (*F. benjamina*) (Ummu-Hani and Noraini 2013; Pierantoni et al. 2018). In contrast to densely packed spherical cystoliths, elongated cystoliths are never located above the veins; therefore, they do not overlap with and are complementary to calcium oxalate crystals (Pierantoni et al. 2018).

Cystoliths are observed in lithocysts, which are specialized idioblastic cells. Lithocysts often contain a silicified stalk that can remain inside the cell or protrude out of it, and these stalks vary in size (Wu and Kuo-Huang 1997; Gal et al. 2010; Bauer et al. 2011; Ummu-Hani and Noraini 2013; Pierantoni et al. 2018). Cystoliths are involved in light harvesting (Gal et al. 2012) and might provide mechanical properties that are still unclear (Smith and Watt 1986; Bauer et al. 2011; Pierantoni et al. 2018). Therefore, it might be hypothesized that the calcium content and silica stalk of cystoliths can contribute to leaf roughness. Several types of lithocysts have been described in the *Ficus* genus: hair-like lithocysts located in uniseriate epidermis (*F. religiosa*) and papillate lithocysts located in multiseriate epidermis (*F. virgata*) (Wu and Kuo-Huang 1997). In *F. carica*, spherical lithocysts have been found adjacent to the abaxial epidermis. Such lithocysts contain a stalk that protrudes outside of the cell wall and forms a spiked appendage on the surface of the leaf (Fig. 1B) (Mamoucha et al. 2016).

Silica

Since plants can easily absorb silicon from soil, it is one of the most common minerals found throughout the plant kingdom (Hodson et al. 2005; Bauer et al. 2011; Strömberg et al. 2016). Silicon

is known to be involved in several physiological processes such as physical support and plant resistance to biotic and abiotic stresses (Bauer et al. 2011; Gautam et al. 2016; Strömberg et al. 2016; Pierantoni et al. 2018). Indeed, since polymerized silica is amongst the hardest materials found in plants, high silica contents are correlated with increased mechanical protection (Hunt et al. 2008). Silica deposits may also deter insect and mammal herbivores by increasing plant abrasiveness (Massey et al. 2007a, b) and abrading herbivore mouthparts (Massey and Hartley 2009; Müller et al. 2014).

In plant leaves, silica is mainly found in the sheath cells of vascular bundles and in epidermal cells (Bauer et al. 2011). It can be deposited within or on cells as particles or microscopic bodies called phytoliths, be incorporated into the cell wall or form silicified cells and tissue sections (Prychid et al. 2003; Strömberg et al. 2016). However, in *Ficus* leaves, silica is mainly located in the cell wall, non-glandular trichomes and stalks of the cystoliths, but this localization is not restricted since the abundance and exact form of silica are variable among species (Pierantoni et al. 2018). Therefore, any cell of the epidermis or the mesophyll might be partially or completely impregnated with silica, such as the guard cells in *F. prasinicarpa* (Chantarasuwan et al. 2014). In *F. carica*, silica is abundant in the adaxial side of the leaf. It is deposited in cell walls and conic trichomes (Pierantoni et al. 2018).

Crystal-containing idioblasts

In *F. carica* leaves, numerous idioblasts that contain spiny crystals have also been observed along the first layer of palisade cells (Fig. 1C). These crystals are different in structure from the cystoliths, but their nature has not been identified (Mamoucha et al. 2016). However, these spiny crystals might contribute to leaf roughness and abrasiveness.

III- Specialized tissues as essential contributors to *Ficus* defences

Non-glandular trichomes

Trichomes are interesting structures that can be found in almost all plant species, and their role in plant defence has long been clearly established (Levin 1973). These structures are also considered idioblasts. They are divided into glandular and non-glandular types, and the latter are involved in physical defence through increasing the resistance to herbivores and the roughness of *Ficus* leaves (Sosnovsky 2016). In the *Ficus* genus, straight and long trichomes seem to be predominant and more abundant on the abaxial side than on the adaxial side of the leaf, but their exact shape, size and

density show strong inter- and intraspecific variations (Klimko and Truchan 2006; Sosnovsky 2016). In *F. carica*, non-glandular trichomes are mainly straight and long and can be curved at the apex (Klimko and Truchan 2006). They appear as spiny unicellular hairs that can be silicified and are present on both surfaces of the leaf but are more numerous on the adaxial epidermis (Fig. 1D-E) (Mamoucha et al. 2016; Pierantoni et al. 2018). Short peltate non-glandular trichomes can also occur on the abaxial side (Klimko and Truchan 2006). In *F. carica* leaves, final-sized non-glandular trichomes appear at early developmental stages and give the leaflets a very aggressive appearance before they gradually appear to scatter as the leaf expands (Mamoucha et al. 2016).

Glandular trichomes

Glandular trichomes can be defined as pluricellular structures accumulating and secreting substances such as defense-specialized metabolites in a compartment that is virtually “outside” the plant body. The morphology and abundance of these structures vary among species (Wagner 1991). In *Ficus*, glandular trichomes can be capitate, uniseriate or filamentous. Capitate trichomes consist of a unicellular short stalk and a 1-5 celled head that may be elliptic in species such as *F. erecta* or spherical in species such as *F. sagittata* (Klimko and Truchan 2006). In *Ficus* leaves, glandular trichomes have been reported on both epidermises, but they seem to occur mainly on the lower one (Sosnovsky 2016). In *F. carica*, glandular trichomes are small capitate trichomes with a 4-celled head; they are formed only under the nerves, on the abaxial epidermis (Fig. 1F). These trichomes develop when the leaf expands, and at maturity, the head cells produce and accumulate metabolites in the form of dark granules (Mamoucha et al. 2016).

Laticifers

In addition to trichomes, laticifers are other idioblasts that are specialized secretory structures producing defensive metabolites and enzymes. These structures have been reported in more than 20000 species from 40 phylogenetically distant families of angiosperms but seem to be most common in tropical plants (Lewinsohn 1991; Konno 2011). Laticifers have been widely studied and reviewed (Hagel et al. 2008; Konno 2011), partly for their involvement in plant defence. Laticifers are elongated living cells that form a complex ramified network that follows the veins throughout the plant and contain a fluid called latex (Dussourd and Eisner 1987; Farrell et al. 1990). The anatomy and features of laticifers are highly variable among species: laticifers can be articulated or non-articulated (Fig. 1G) and secrete latex to a greater or lesser extent (Konno 2011). In the genus *Ficus*, laticifers are non-articulated and multinucleated. These structures appear during the embryonic stage from single cells that elongate and branch without cell division during plant growth (Dussourd and Denno 1991; Hagel et al. 2008; Konno 2011). In the leaves of *F. carica*, the laticifers are located in the mesophyll,

where they form a non-articulated network that follows the veins. More specifically, these structures are located in the nerves, just above the xylem. It should therefore be noted that the secretive apparatus of *F. carica* that includes capitate trichomes and laticifers is almost totally confined to areas around the veins (Mamoucha et al. 2016).

Latex is a cytoplasmic fluid that contains normal plant cell organelles (Kim et al. 2003) and numerous chemical compounds, but its exact composition is highly variable among species (Hagel et al. 2008) and plant parts (Kitajima et al. 2018). As latex is stored under pressure in the laticifers, it constitutes an economic mobile defence mechanism. Due to the internal pressure of laticifers, any rupture leads to the quick mobilization and delivery of a large amount of latex at the site of the injury. As a consequence, even if the total amount of latex produced by the plant is low, the herbivores that attack the plant are immediately exposed to a high quantity of defensive fluid, which is vastly greater than the very small amount initially present at this location (Dussourd and Eisner 1987; Farrell et al. 1990; Dussourd and Denno 1991; Konno et al. 2004; Agrawal and Konno 2009; Konno 2011). In some plants such as *Cryptostegia grandiflora*, latex can even be transported over a distance of 70 cm (Buttery and Boatman 1976).

When exposed to air, latex becomes sticky and acts as a physical defence by forming a barrier, closing wounds, immobilizing insects' mouthparts and even gluing and trapping small insects (Farrell et al. 1990; Dussourd and Denno 1994; Konno et al. 2004; Konno 2011). This stickiness is due to rubber, a terpenoid commonly synthesized in laticifers that is also responsible for the colour of the latex (Kim et al. 2003; Konno 2011). Latex is also involved in chemical defence and has toxic effects: it contains a high diversity of compounds such as irritant proteins and toxic secondary metabolites, the average concentration of which is often higher than that in the rest of the leaf. As a striking example of the defensive role of latex, it has been shown that the leaves of *F. virgata* exhibit strong toxic effects against some lepidopteran larvae but lose their toxicity when the latex is washed off (Konno et al. 2004). For *F. carica*, hundreds of metabolites and more than fifty proteins have been found in latexes from different parts of the plant, with many of these compounds having defensive effects (Kitajima et al. 2018).

IV- *Ficus* defensive metabolites and proteins

The *Ficus* genus contains a high diversity of specialized compounds (such as alkaloids, terpenoids, and phenolics) and proteins that vary among species, cultivars, plant parts and environmental factors. These chemicals confer various biological activities including defensive ones such as antimicrobial (Jeong et al. 2009), antipyretic (Patil Vikas et al. 2010), nematocidal (Liu et al. 2011) and irritant properties (Saeed and Sabir 2002).

Specialized metabolites

Many studies have shown that *Ficus* species contain hundreds of metabolites belonging to various chemical classes (Sirisha et al. 2010; Jun et al. 2012; Oliveira et al. 2012; Marrelli et al. 2014). Among these metabolites, some primary ones have biological activities such as antioxidant properties (Oliveira et al. 2009), but the molecules responsible for defence are generally specialized metabolites belonging to three families: alkaloids, terpenoids and phenolics.

Alkaloids

Among the alkaloid subfamilies already described in the *Ficus* genus, the most studied are the phenanthroindolizidines (Table 1, Fig. 2), which are mainly restricted to the Asclepiadaceae and Moraceae families (Burtoloso et al. 2013; Al-Khdhairawi et al. 2017). A few dozen of these molecules have been reported in *F. septica* stems, roots and sometimes leaves (Baumgartner et al. 1990; Wu et al. 2002; Damu et al. 2005, 2009). Some phenanthroindolizidine alkaloids were also reported in *F. fistulosa* (Yap et al. 2016) and *F. hispida* (Peraza-Sánchez et al. 2002; Yap et al. 2015). These molecules display antiamoebic (Bhutani et al. 1987), antiviral (Li et al. 2006), antifungal and antibacterial activities (Baumgartner et al. 1990), which strongly suggests that they are involved in plant defence against pests. This suggestion of involvement in defence is reinforced by the finding of Konno et al. (2011), who reported that the latex of some *Ficus* species contains phenanthroindolizidine alkaloids in concentrations high enough to kill generalist herbivores. It is also noteworthy that these alkaloids exhibit strong cytotoxicity against cancer cell lines (Peraza-Sánchez et al. 2002; Damu et al. 2005, 2009; Yap et al. 2015, 2016; Al-Khdhairawi et al. 2017). The other alkaloid subfamilies have been much less described in *Ficus*, but some of them also display cytotoxic effects (Yap et al. 2016; Al-Khdhairawi et al. 2017) and antifungal and antibacterial activities (Baumgartner et al. 1990).

Terpenoids

In *Ficus* species, terpenoids belonging to monoterpenoids, triterpenoids, sesquiterpenoids, norisoprenoids, steroids and esters have been reported, and some of them exhibit known bioactivities (Table 1, Fig. 2). Sesquiterpenes are the most abundant subfamily in the leaves of *F.*

carica, whereas monoterpenes accumulate almost exclusively in fruits (Oliveira et al. 2010b). The most striking example of a *Ficus* defensive terpenoid is rubber, a cis-1,4-isoprene polymer responsible for latex stickiness (Konno 2011). *Ficus* steroids might play a role in defence, since they have antiproliferative properties. For example, 6-O-acyl- β -D-glucosyl- β -sitosterol, isolated from *F. carica* latex, showed *in vitro* inhibitory effects against the proliferation of several cancer cell lines (Rubnov et al. 2001). Ojo et al. (2014) reported the presence of cardenolides, a subclass of cardiac steroids (Roberts et al. 2016), in the leaves of *F. asperifolia*. Although the activity of *Ficus*-specific cardenolides has not yet been tested, cardiac steroids are well known for their high toxicity to animals (Roberts et al. 2016). Additionally, cardenolides from the milkweed latexes cause toxic symptoms in caterpillars such as regurgitation, convulsions and hours to days of unresponsiveness (Dussourd and Hoyle 2000; Konno 2011). Triterpenoids such as calotropenyl acetate, methylmaslinate and lupeol acetate are additional examples of molecules involved in defence. These triterpenoids have been described in *F. carica* leaves and display irritant properties when applied to mouse ears (Saeed and Sabir 2002). Several volatile terpenoids found in *Ficus* were shown to have defensive bioactivities in other plants. For instance, Oliveira et al. (2010b) showed that the major sesquiterpenes present in *F. carica* leaves were germacrene D, β -caryophyllene and τ -elemene, while the main monoterpenes in *F. carica* fruits were limonene and menthol. β -caryophyllene has antibacterial properties (Kim et al. 2008) while limonene is known for its insecticidal (Hebeish et al. 2008) and antibacterial activities (Skalicka-Wozniak et al. 2009; Oliveira et al. 2010b).

Phenolics

Phenolic compounds such as phenolic acids, flavonoids, tannins, coumarins and their derivatives such as pyranocoumarins and furanocoumarins have been extensively studied in the *Ficus* genus (Table 1, Fig. 2). Phenolics are well known for their redox and radical scavenging activities, which lead to antioxidant effects (Oliveira et al. 2009; Marrelli et al. 2012; Debib et al. 2014). However, some *Ficus* phenolics also exhibit defensive properties against biotic stresses. Flavonoids and tannins might be responsible for the antimicrobial activity exhibited by some fig extracts (Debib et al. 2014). Flavonoids and pyranocoumarins, such as genistein and 3-hydroxyxanthyletin, respectively, were found in the roots of *F. nervosa* and exhibited antimycobacterial activity (Chen et al. 2010). Many *Ficus* species also contain large amounts of various furanocoumarins, which have already been described in other plants as effective defence molecules against plants, herbivores and pathogens (Beier and Oertli 1983; Zumwalt and Neal 1993; Ojala et al. 2000; Berenbaum 2002; Li et al. 2003; Guo et al. 2016). The large range of organisms affected by the toxicity of furanocoumarins is in part underlain by their mode of action: furanocoumarins can lead to the formation of toxic reactive

oxygen species (Berenbaum 2002), can inhibit cytochrome P450s (Neal and Wu 1994; He et al. 1998; Koenigs and Trager 1998) and, when excited by UV, can bind to macromolecules and cause various reactions leading to mutations or cell death (Dardalhon et al. 1998). Psoralen and bergapten are the main furanocoumarins reported in *F. carica* (Takahashi et al. 2014, 2017; Wang et al. 2017) but they are also among the major phenolics reported in *Ficus* leaves (Oliveira et al. 2012). According to Mamoucha et al. (2016), these metabolites are mainly located around the nerves containing laticifer cells and the glandular trichomes. The defensive effects of furanocoumarins have rarely been studied in *Ficus*, but two studies described the nematicidal effect of psoralen and bergapten extracted from *F. carica* leaves (Liu et al. 2011; Guo et al. 2016) characterized by inhibition of the activities of two important digestive enzymes (amylase and cellulase) in the pine wood nematode (Guo et al. 2016). Finally, it has long been known that fig trees have photosensitizing action and can cause contact dermatitis (Pathak et al. 1962) because of the furanocoumarins they contain (Bonamonte et al. 2010). However, despite this knowledge, various *Ficus* parts are still used in traditional medicine in attempts to cure skin diseases, which regularly cause severe injuries (Bonamonte et al. 2010; Son et al. 2017). Recently, psoralic acid-glucoside (PAG, a glycosylated derivative of psoralen) was also detected in large amounts in fig (Takahashi et al. 2014, 2017; Wang et al. 2017), while its biosynthesis and physiological functions are still unknown.

Defensive proteins

Several proteins such as proteases, protease inhibitors, oxidases and chitinases have been widely reported in the latex of numerous *Ficus* species (Konno 2011). Some of these proteins were described as being very stable, and their activity was not impacted by herbivore digestive juice (Zhu-Salzman et al. 2008; Konno 2011). Such proteins might be involved in defence against herbivores, fungi, viruses and bacteria.

Proteases

Cystein proteases such as ficins were reported in *Ficus* latexes in 1964 by Sgarbieri and collaborators. Plant proteases are considered a very effective defence system against a wide range of insects that act on these insects by deterring them from feeding, interfering with their digestion, reducing their growth rate and increasing their larval mortality (Konno et al. 2004; Konno 2011). Protease activity has also been demonstrated to be negatively correlated with herbivory (Volf et al. 2018). Among the proteases, ficins are assumed to play a major role in plant defence; for instance, the leaves of *F. virgata* exhibit strong toxicity to some lepidopteran larvae but lose this toxicity when their ficins are neutralized with specific inhibitors (Konno et al. 2004). The mechanisms of ficin toxicity have not been deeply investigated (Konno 2011) but their essential function might explain their highly

conserved evolution (Volf et al. 2018). Indeed, the number of ficin isoforms and their amounts vary among species and varieties (Kramer and Whitaker 1964). In the case of *F. carica*, latexes contain large amounts of at least six isoforms of ficins (Kitajima et al. 2018).

To a lesser extent, *F. carica* latexes also contain other proteases such as subtilases (Kitajima et al. 2018). To the best of our knowledge, the roles of the subtilases found in *Ficus* have never been studied but plant subtilases are involved in many processes, including defence; see Schaller et al. (2018) for a review of plant subtilase activities.

Protease inhibitors

F. carica latexes contain several types of protease inhibitors such as serine and cysteine protease inhibitors (Kitajima et al. 2018). Plant protease inhibitors constitute a well-known defensive tool against a wide range of pathogens and pests. For example, trypsin (a serine protease) inhibitors are reported to have antifungal (Huynh et al. 1992; Terras et al. 1993) and insecticidal activities (Broadway and Duffey 1986; Hilder et al. 1987; Ryan 1990). Indeed, these inhibitors can target proteases present in the insect gut, leading to anti-nutritional effects (Felton and Gatehouse 1996). The main protease inhibitors in *F. carica* latexes are trypsin inhibitors: they are present in large amounts, and no fewer than 10 isoforms have been found within just the latex of the leaves, which makes them the major defensive proteins in *F. carica* latexes, along with ficins (Kitajima et al. 2018).

Oxidases

Peroxidases and polyphenol oxidases are common enzymes present in several plant families, including Moraceae (Konno 2011). When ingested by insects, they can oxidize compounds present in the insect gut, leading to the production of reactive species that can disrupt the arthropod redox status, damage some molecules, or make some amino acids unavailable as nutrients (Felton et al. 1992; Felton and Gatehouse 1996; Zhu-Salzman et al. 2008; Konno 2011). Some plant oxidases are therefore considered defence proteins with potential antinutritive effects, but direct evidence for antiherbivore effects is still scarce (Zhu-Salzman et al. 2008; Sethi et al. 2009; Konno 2011). Peroxidases have been reported in the latex of several *Ficus* species, such as *F. sycomorus* (Mohamed et al. 2011), *F. glabrata* (Kon and Whitaker 1965) and *F. carica*. In *F. carica*, peroxidases are associated with rubber particles (Kim et al. 2003). Polyphenol oxidases have been reported only in the latex of *F. elastica*, but their activity was correlated with the rate of latex coagulation (Wahler et al. 2009). *Ficus* oxidases might therefore impact latex stickiness.

Chitinases

Chitinases have been detected in the latex of *F. carica* (Kitajima et al. 2018) and *F. microcarpa* (Taira et al. 2005). Plant chitinases are well known for their antifungal effects because of their ability to degrade fungal cell walls and subsequently inhibit fungal growth (Schlumbaum et al. 1986; Mauch et al. 1988; Roberts and Selitrennikoff 1988; Melchers et al. 1994). These enzymes may also display insecticidal activities, as exemplified by the study of Lawrence and Novak, who reported that a poplar chitinase delayed insect development (Lawrence and Novak 2006). In *Ficus*, the only defensive effect of chitinases that has been described is a strong antifungal activity in *F. microcarpa* latex (Taira et al. 2005).

Other pathogenesis-related proteins (PRs)

PRs have been widely studied, and many of them exhibit antifungal, antibacterial, insecticidal, nematocidal or antiviral properties (Edreva 2005). These proteins are classified into several groups. The previously described chitinases and peroxidases belong to PR-3 and PR-8, respectively (Stintzi et al. 1993; Hoffmann-Sommergruber 2002; Edreva 2005), but other PRs belonging to PR-1, PR-4 and PR-5 (osmotin) have also been detected in *F. carica* latexes (Kitajima et al. 2018). These proteins are likely to be considered fungicides and therefore involved in plant defence, as demonstrated in the latex of *Calotropis procera* (Niderman et al. 1995; de Freitas et al. 2011; Bai et al. 2013).

Other protein families

In addition to these proteins, other miscellaneous enzymes such as lectins and phosphatases have been detected in *F. carica* latexes (Kitajima et al. 2018) and might be involved in plant defence. Lectins are carbohydrate-binding proteins present in the latexes of plants from several families (Konno 2011). These enzymes have deleterious effects against herbivores and might interfere with viruses, bacteria or fungi (Peumans and Van Damme 1995; Van Damme et al. 1998; Macedo et al. 2015). Phosphatases are less studied (Lynn and Clevette-Radford 1987; Konno 2011), but phosphatase activity has been correlated with retarded development and mortality in several insect species (Liu et al. 2005). The study of these enzymes might therefore be relevant.

V- Modulation of *Ficus* defence mechanisms

Genotype

Ficus defence mechanisms vary depending on numerous factors including the species, variety and individual. The genotype is probably the most relevant factor for explaining the diversity of *Ficus*

minerals (abundance, location, shape, size, and colour), trichomes (morphology, abundance, and location), latex (colour and amount) and chemical content (type, amount, relative amount and location). Genetic factors are also predominant determinants of leaf mineral features and result in a unique mineral system in each species (Pierantoni et al. 2018). For example, the size, shape and location of phytoliths can be used as tools for phylogenetic identification (Prychid et al. 2003).

Seasonal variations and plant age

In *Ficus*, organs are unequally defended throughout the year, but it is sometimes difficult to distinguish the effects of season from the effects of the age and developmental stage of the plant. For example, it has been shown that in *F. carica* leaves and latex, the furanocoumarin content is higher in spring and summer than in autumn (Zaynoun et al. 1984), suggesting that these variations are linked to seasonal effects. On their side, Marrelli and collaborators compared the chemical content of *F. carica* fruits harvested in June, July and September, which clearly corresponded to different fig phenological stages. These authors found that the total amount of phenolics increased with fig ripening, while the furanocoumarin and pyranocoumarin content decreased (Marrelli et al. 2012). These observations were consistent with those in the older study of Zaynoun et al., but Marrelli et al. assumed that the difference was due to ripening. In another study, the chemical composition of *F. carica* leaves, bark and woody parts was shown to vary across months, and these variations were found to be specific to each organ and compound (Marrelli et al. 2014). In addition to chemical differences, differences in the amount of latex occur: more latex is exuded from young *Ficus* organs (shoots, leaves and petioles) than from older ones (Konno 2011). Finally, it is likely that both seasonality and plant development contribute to the modulation of *Ficus* defence and that their actions are combined.

Inducible defence mechanisms

It is widely known that plant defences can be triggered by pest attacks. This induction involves physical and chemical mechanisms. Regarding *Ficus* physical defences, long-term herbivory seems to reinforce leaf toughness and leads to an active increase in the calcium oxalate crystal density in a few *Ficus* species (Xiang and Chen 2004). In some plants, herbivore damage can also induce silicification (McNaughton and Tarrants 1983; Massey et al. 2007b; Strömberg et al. 2016) or an increase in trichome number and density (Baur et al. 1991; Traw and Dawson 2002; Liu et al. 2018). However, to the best of our knowledge, silica and trichome inductions have not been tested amongst species in the *Ficus* genus. Regarding chemical defence mechanisms, it has been reported that the expression of a chitinase, a peroxidase and a trypsin inhibitor was highly induced in *F. carica* latex several hours

after a wounding treatment (Kim et al. 2003). Concerning the induction of specialized metabolites, less is known for *Ficus* species. However, some studies performed on other plants enable us to hypothesize that the induction of the synthesis of some *Ficus* defence molecules may be affected by herbivory or pathogen infection. For example, the production of some furanocoumarins was demonstrated to be elicited in parsnip by mechanical wounding and pathogen infection (Zangerl and Berenbaum, 1990, Roselli et al., 2017 ; Krieger et al. 2018). It is therefore possible that the synthesis of these defence molecules is also enhanced in *Ficus*.

Other factors might also modulate *Ficus* defences, but data covering this topic are scarce. To the best of our knowledge, the only relevant study on this topic reported the impact of phytohormone application (jasmonic acid, salicylic acid or abscisic acid), drought and cold on the expression levels of a chitinase, a peroxidase and a trypsin inhibitor in *F. carica* leaves (Kim et al. 2003). Jasmonic acid enhanced the expression of all three enzymes, whereas salicylic acid and abscisic acid elicited only the trypsin inhibitor and peroxidase, respectively.

In addition, drought induced the expression of peroxidase but decreased the expression of the others two proteins while cold treatment decreased the expression of the three proteins (Kim et al. 2003).

VI- Evolution of *Ficus* defence mechanisms

Evolution and coevolution

Members of *Ficus* are the targets of a number of generalist and specialist insects (Basset and Novotny 1999; Novotny et al. 2010; Volf et al. 2018). Basset and Novotny identified more than 300 leaf-chewing insect species and more than 400 sap-sucking insect species on 15 *Ficus* species from Papua New Guinea (Basset and Novotny 1999). It has been proposed that the defence types affecting the generalist insects are often toxic chemicals leading to their increased mortality (Koricheva et al. 2004; Volf et al. 2015). This assumption is reasonable, especially for *Ficus* polyphenols, which are negatively correlated with the generalist herbivore communities (Volf et al. 2018). However, in this warfare between insects and plants, some specialist insects have developed novel enzymatic mechanisms to disable these chemical weapons (Koricheva et al. 2004; Volf et al. 2015, 2018). In this context, plants adopt different strategies to protect themselves, such as pyramiding chemical diversity and mechanical defences. In this way, reducing the feeding efficiency of insects and increasing the time needed for them to feed result in a longer exposure to toxic metabolites and

natural enemies and, in turn increased insect mortality (Koricheva et al. 2004; Volf et al. 2015, 2018). In 2018, Volf and collaborators investigated the relationships between *Ficus* pests and various defence traits of *Ficus*, i.e., trichomes (length and density), chemical content and diversity (alkaloids, polyphenols and triterpenes) and polyphenol oxidative / cysteine protease activities using 19 Papua New Guinean *Ficus* species. The authors discovered significant correlations between several *Ficus* traits and insect community structures. For instance, they showed that the trichome length, alkaloid diversity, and protease and polyphenol oxidative activities were correlated with the overall community structure, while trichome density was only correlated with choreutids. The selective pressure exerted by some pest attacks might therefore explain why some *Ficus* traits were phylogenetically conserved and others escalated or diverged (Volf et al. 2018). However, in this arms race, plants are not the only ones that have established alternative strategies. To overcome the toxicity of latex, some insects found that when cutting some canal branches, they induced latex emission and freed the distal tissues from a certain amount of the toxic liquid (Dussourd and Denno 1991; Konno 2011), making those distal tissues safer for consumption. Such a strategy has not been studied in detail in *Ficus* but has been deeply discussed for other latex-producing plants (Dussourd 2017).

Defence mechanism distribution on *Ficus* plants

Pests are not the same for leaves, fruits and lignified tissues; therefore, defence strategies are not equally distributed throughout the plant. Each organ has its own histology with different defensive structures, and the abundance of defensive chemicals is highly variable from one organ to another. For instance, the main enzymes of *Ficus* petiole latex are trypsin inhibitors, but the main proteins of fruits and trunk latexes are ficins (Kitajima et al. 2018). Leaves are the main targets of herbivores and consequently display adapted defences. *F. carica* leaves are well protected by structures that prevent herbivory (trichomes and minerals) and produce a wide diversity of active chemicals. The biosynthesis of some defensive compounds, such as furanocoumarins, is more active in leaves than in other organs (Kitajima et al. 2018). The main proteins found in the latex of leaf petioles are trypsin inhibitors, which are effective against fungi and numerous insects (Kitajima et al. 2018). Fig inflorescences display a different set of defences that are directly related to the function of these organs. First, fig pollination relies on agaonid wasps (Hymenoptera: Chalcidoidea: Agaonidae). Interestingly, these insects grow inside the ripening fruits. Second, fruits are essential for seed dissemination, which is realized after consumption by vertebrate species (Cruaud et al. 2012). Because of these two ecological functions, fruits need to be protected but must not be too toxic to these partners. Therefore, even if *F. carica* fruit latex contains high amounts of ficins and trypsin inhibitors, some toxic chemicals are produced in smaller amounts than in leaves (Kitajima et al.

2018). For furanocoumarin, the levels are 500 to 1000 times lower in the inflorescences than in leaves (Oliveira et al. 2009). Lignification is a physical defence structure that provides mechanical protection. Therefore, trunk, bark and other woody parts are less likely than other parts to be damaged by herbivorous insects. However, lignified parts are defended by chemical mechanisms that seem to cover a broad spectrum of bioaggressors. For example, the main defensive protein found in *F. carica* trunk latex is ficin, which is effective against insects and fungi. Moreover, trunk latex contains the largest amounts of chitinases, acid phosphatases and PR proteins (Kitajima et al. 2018), which are described as antifungal, antibacterial, insecticidal, nematocidal and antiviral molecules.

VII- Conclusion

In this review, we have shown that there is not a single defence mechanism that is used in all members of *Ficus*. The strategies are highly diverse and are adapted to each organ of the plant. Some are constitutive, whereas others are inducible. These mechanisms have evolved over time as a function of the environment, and the synergistic action of different strategies has made *Ficus* resistant to a large set of pathogens. Such a pyramiding strategy is illustrated by latex, which is both a chemical and mechanical defence mechanism. This strategy is, however, not universal, and one of its limitations is the size of the aggressor that can be defended against. Indeed, tiny insects that sever a laticifer while feeding on *Ficus* can be confronted with an amount of latex that is large compared to their small size and the quantity of leaf they ingest. In contrast, large mammal herbivores that feed on whole leaves are confronted with the average concentration of latex diluted in the leaf and are therefore unaffected by its accumulation at the site of the injury (Konno 2011). However, as shown by the emergence of canal-cutting insects in response to plant latex, the story is not at the end and evolution continues. This fascinating arms race makes this plant an ideal model for further study.

534 **Table 1: Secondary metabolites found in *Ficus* (ND = No data in *Ficus*)**

535 ¹ Other interesting defensive activities have been highlighted in non-*Ficus* species for similar compounds.

	Type of compound	Compounds	<i>Ficus</i> species	Bioactivities	References
Alkaloids	Phenanthroindolizidine alkaloids	Ficuseptines A & B, 13aR-tylophorine, 13aR-tylocrebrine, 13aR-isotylocrebrine, 10S,13aR-tylocrebrine N-oxide	<i>F. septica</i>	Cytotoxicity	(Wu et al. 2002; Damu et al. 2005, 2009)
		Antofine	<i>F. septica</i>	Antifungal, antibacterial	(Baumgartner et al. 1990; Wu et al. 2002; Damu et al. 2005, 2009)
		O-methyltylophorinidine, hispilosine	<i>F. hispida</i>	Cytotoxicity	(Peraza-Sánchez et al. 2002; Yap et al. 2015)
		Others	<i>F. septica</i> , <i>F. fistulosa</i>	ND ¹	(Damu et al. 2005, 2009; Yap et al. 2016)
	Chlorophenanthroindolizidine alkaloids	Tengechlorenine	<i>F. fistulosa</i>	Cytotoxicity	(Al-Khdhairawi et al. 2017)
	Indolizidine alkaloids	4,6-bis-(4-methoxyphenyl)-1,2,3-trihydroindolizidinium chloride	<i>F. septica</i>	Antifungal, antibacterial	(Baumgartner et al. 1990)
	Septicine-type alkaloids	Fistulopsine A & B	<i>F. fistulosa</i>	Cytotoxicity	(Yap et al. 2016)
	8,4'-oxyneolignan-alkaloids	Hispidacine	<i>F. hispida</i>	Vasorelaxant	(Yap et al. 2015)
	Bis-benzopyrroloisoquinoline alkaloids	Tengerensine	<i>F. fistulosa</i>	ND ¹	(Al-Khdhairawi et al. 2017)
	Furoquinoline alkaloids	Skimmianine	<i>F. coronata</i>	ND ¹	(Smyth et al. 2012)
Terpenoids	Triterpenoids and pentacyclitriterpenes	Baueranol, calotropenyl acetate, lupeol acetate, methyl maslinate, oleanolic acid	<i>F. carica</i> , <i>F. nervosa</i>	Irritant	(Saeed and Sabir 2002; Chen et al. 2010; Oliveira et al. 2012; Jaina et al. 2013)
		Others	<i>F. benghalensis</i> , <i>F. carica</i>	ND ¹	(Ahmad et al. 2011; Oliveira et al. 2012; Jaina et al. 2013)
	Steroids	6-O-acyl- β -D-glucosyl- β -sitosterol	<i>F. carica</i>	Cytotoxicity	(Rubnov et al. 2001)
		Others	<i>F. benghalensis</i> , <i>F. carica</i> , <i>F. nervosa</i> , <i>F. septica</i>	ND ¹	(Athnasios et al. 1962; Chen et al. 2010; Oliveira et al. 2012; Marrelli et al. 2012)
	Cardiac steroids (cardenolides)		<i>F. asperifolia</i>	ND ¹	(Konno 2011; Ojo et al. 2014)

	Rubber (cis-1,4-isoprene polymer)		<i>Ficus</i>	Latex stickiness	(Konno 2011)
	Monoterpenes, sesquiterpenes, norisoprenoids, esters, etc.		<i>F. asperifolia</i> , <i>F. benghalensis</i> , <i>F. carica</i> , <i>F. hispida</i>	ND ¹	(Peraza-Sánchez et al. 2002; Oliveira et al. 2010a, b; Ahmad et al. 2011; Marrelli et al. 2012; Ojo et al. 2014; Marrelli et al. 2014)
Phenolics	Phenolic acids	Chlorogenic acid	<i>F. carica</i> , <i>F. elastica</i>	Antioxidant	(Teixeira et al. 2006; Oliveira et al. 2009; Teixeira et al. 2009; Lazreg Aref et al. 2011; Oliveira et al. 2012; Takahashi et al. 2014)
		Others	<i>F. carica</i>	ND ¹	
	Flavonoids (Flavones, flavonols, flavonones, bengalensides, catechin, anthocyanidins)	Genistein, prunetin, (2S)-naringenin	<i>F. nervosa</i>	Antimycobacterial	(Chen et al. 2010)
		Rutin and others	<i>F. benghalensis</i> , <i>F. carica</i> , <i>F. nervosa</i> , <i>F. ruficaulis</i>	Antioxidant	(Chang et al. 2005; Vaya and Mahmood 2006; Oliveira et al. 2009; Chen et al. 2010; Lazreg Aref et al. 2011; Ahmad et al. 2011; Takahashi et al. 2014)
	Tannins and phlobatannins		<i>F. asperifolia</i>	ND ¹	(Ojo et al., 2014)
	Simple coumarins		<i>F. coronata</i> , <i>F. nervosa</i> , <i>F. ramentacea</i>	ND ¹	(Yarosh and Nikonov 1973; Chen et al. 2010; Smyth et al. 2012)
	Pyranocoumarins	3-hydroxyxanthyletin	<i>F. nervosa</i>	Antimycobacterial	(Chen et al. 2010)
		Others	<i>F. carica</i> , <i>F. nervosa</i>	ND ¹	(Chen et al. 2010; Marrelli et al. 2012)
	Furanocoumarins	Psoralen Bergapten	<i>F. carica</i> , <i>F. coronata</i> , <i>F. palmata</i> , <i>F. salicifolia</i> , <i>F. sycomorus</i>	Nematicidal ¹	(Abu-Mustafa et al. 1963; Zaynoun et al. 1984; Oliveira et al. 2009, 2010a; Liu et al. 2011; Ahmad et al. 2011; Smyth et al. 2012; Jaina et al. 2013; Marrelli et al. 2014; Alam et al. 2015; Guo et al. 2016)
		Angelicin, bergaptol, chalepin, marmesin, PAG, pimpinellin, rutamarin, rutaretin, xanthotoxin, xanthotoxol and others	<i>F. capensis</i> , <i>F. carica</i> , <i>F. coronata</i> , <i>F. cyathistipula</i> , <i>F. nervosa</i> , <i>F. ruficaulis</i>	ND ¹	(Caporale et al. 1970; Gibernau et al. 1997; Chen et al. 2010; Smyth et al. 2012; Marrelli et al. 2012; Jaina et al. 2013; Takahashi et al. 2014; El-Sakhawy et al. 2016; Mamoucha et al. 2016; Takahashi et al. 2017; Wang et al. 2017)

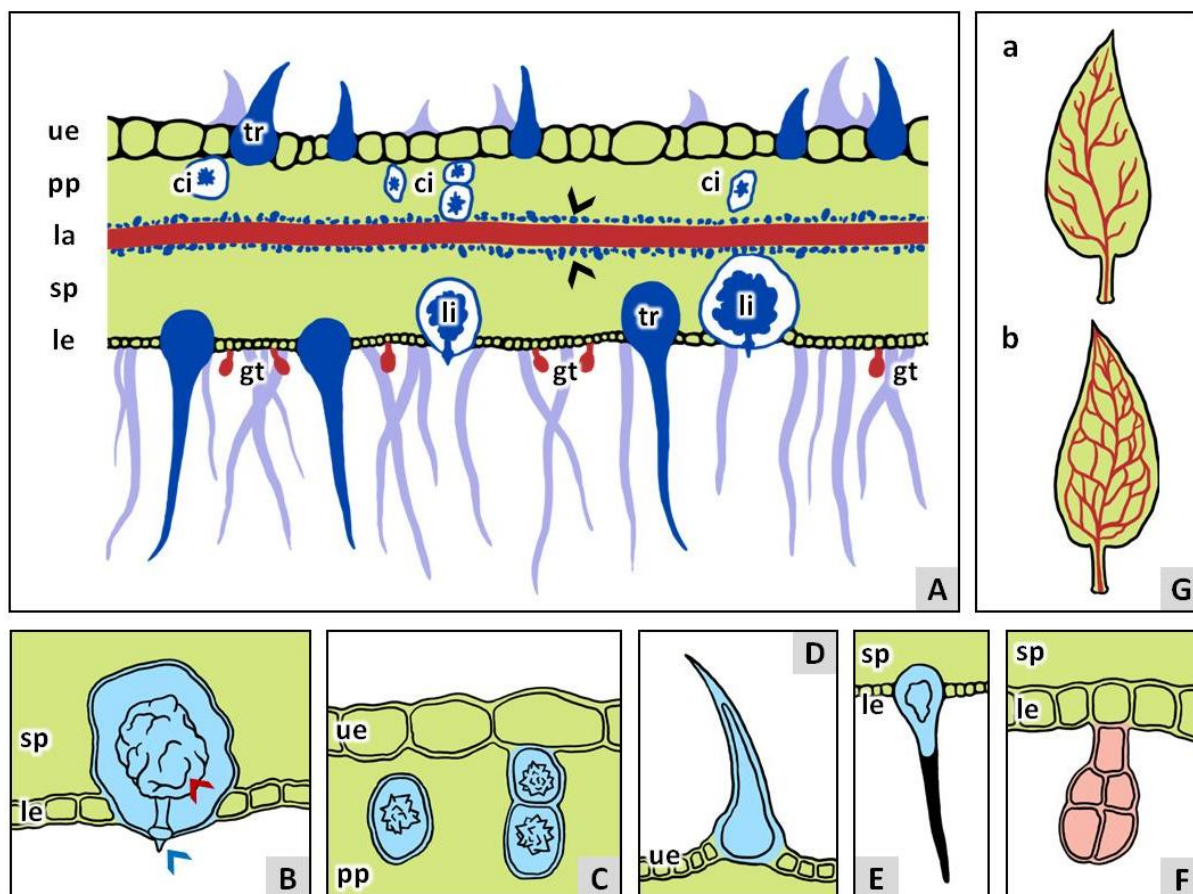


Fig. 1A-G Schematic representation of some defensive structures described in *F. carica* leaves (Mamoucha et al. 2016; Pierantoni et al. 2018). The mineralized structures are in blue, and the secretory structures are in red. **(A)** Cross-section from a *F. carica* leaf. Druses and prismatic crystals of calcium oxalate (black arrowheads) are deposited parallel to a vein that hosts a laticifer (la). Spherical lithocysts (li) appear in the lower epidermis (le) and extend into the spongy parenchyma (sp). Crystal idioblasts (ci) containing spiny crystals appear in the palisade parenchyma (pp). Non-glandular trichomes (tr) can be silicified and form on surface of both the upper (ue) and lower (le) epidermises. Glandular trichomes (gt) form only under the nerves. **(B)** Detail of a lithocyst that contains a cystolith (red arrowhead) and a spike (blue arrowhead) that protrudes outside the cell wall of the leaf. **(C)** Detail of some crystal idioblasts located in the palisade parenchyma; they contain spiny crystals of indeterminate nature and might appear in pairs. **(D)** Detail of a conic silicified trichome (non-glandular) that forms on the upper epidermis. **(E)** Detail of a long hairy silicified trichome (non-glandular) that forms on the lower epidermis. **(F)** Detail of a capitate secretory trichome (glandular) that forms under the nerves; it has one single-celled stalk and a four-celled head that accumulates metabolites in the form of dark granules. **(G)** Anatomy of the laticifers. Non-articulated laticifers (a) have tree-like shapes. Articulated laticifers (b) merge to form a structure with (anastomosing laticifers) or without loops (non-anastomosing laticifers). *Ficus* laticifers are non-articulated.

Alkaloids	<div data-bbox="263 219 470 414"> <p>Antofine (Phenanthroindolizidine alkaloid)</p> </div> <div data-bbox="542 219 750 414"> <p>Tengechlorenine (Chlorophenanthroindolizidine alkaloid)</p> </div> <div data-bbox="869 219 1109 436"> <p>Hispidacine (8,4'-oxyneolignan-alkaloid)</p> </div> <div data-bbox="263 504 630 698"> <p>Tengerensine (Bis-benzopyrroloisoquinoline alkaloid)</p> </div> <div data-bbox="694 504 869 698"> <p>Fistulopsine A (Septicine-type alkaloid)</p> </div> <div data-bbox="949 526 1109 676"> <p>Skimmianine (Furoquinoline alkaloid)</p> </div> <div data-bbox="1157 336 1364 683"> <p>4,6-bis-(4-methoxyphenyl)-1,2,3-trihydroindolizidinium chloride (Indolizidine alkaloid)</p> </div>
Terpenoids	<div data-bbox="263 817 518 974"> <p>Bauerenol (Triterpenoid)</p> </div> <div data-bbox="662 817 997 974"> <p>6-O-acyl-β-D-glucosyl-β-sitosterol (Steroid)</p> </div> <div data-bbox="1157 817 1364 974"> <p>Germacrene D (Sesquiterpene)</p> </div>
Phenolics	<div data-bbox="383 1086 614 1220"> <p>Chlorogenic acid (Phenolic acid)</p> </div> <div data-bbox="718 1108 949 1220"> <p>Genistein (Flavonoid)</p> </div> <div data-bbox="263 1288 470 1377"> <p>3-hydroxyxanthyletin (Pyranocoumarin)</p> </div> <div data-bbox="598 1310 774 1377"> <p>Psoralen (Linear furanocoumarin)</p> </div> <div data-bbox="949 1288 1109 1377"> <p>Angelicin (Angular furanocoumarin)</p> </div> <div data-bbox="1117 1108 1364 1377"> <p>Rutin (Flavonoid)</p> </div>

Fig. 2: Structure of some *Ficus* secondary metabolites

References

- Abu-Mustafa EA, El-Tawil BAH, Fayez MBE (1963) Constituents of local plants - IV. *Ficus carica* L., *F. sycomorus* L. and *F. salicifolia* L. leaves. *Phytochemistry* 3:701–703. doi: [https://doi.org/10.1016/S0031-9422\(00\)82968-4](https://doi.org/10.1016/S0031-9422(00)82968-4)
- Agrawal AA, Konno K (2009) Latex: A model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. *Annu Rev Ecol Evol Syst* 40:311–331. doi: <https://doi.org/10.1146/annurev.ecolsys.110308.120307>
- Ahmad S, Rao H, Akhtar M, et al (2011) Phytochemical composition and pharmacological prospectus of *Ficus bengalensis* Linn. (Moraceae)- A review. *J Med Plants Res* 5:6393–3400. doi: <https://doi.org/10.5897/JMPR11.455>
- Alam P, Siddiqui N, Basudan O, et al (2015) Comparative profiling of biomarker psoralen in antioxidant active extracts of different species of genus *Ficus* by validated HPTLC method. *Afr J Tradit Complement Altern Med* 12:57. doi: <https://doi.org/10.4314/ajtcam.v12i1.9>
- Al-Khdhairawi AAQ, Krishnan P, Mai C-W, et al (2017) A bis-benzopyrroloisoquinoline alkaloid incorporating a cyclobutane core and a chlorophenanthroindolizidine alkaloid with cytotoxic activity from *Ficus fistulosa* var. *tengerensis*. *J Nat Prod* 80:2734–2740. doi: <https://doi.org/10.1021/acs.jnatprod.7b00500>
- Anstett MC (2001) Unbeatable strategy, constraint and coevolution, or how to resolve evolutionary conflicts: the case of the fig/wasp mutualism. *Oikos* 95:476–484. doi: 10.1034/j.1600-0706.2001.950313.x
- Athnasios AK, El-Kholy IE-S, Soliman G, Shaban M (1962) Constituents of leaves of *Ficus carica*, I .1. Isolation of psoralen, bergapten, psi-taraxasterol, and beta-sitosterol. *J Chem Soc Resumed* 4253–4264. doi: <https://doi.org/10.1039/jr9620004253>
- Bai S, Dong C, Li B, Dai H (2013) A PR-4 gene identified from *Malus domestica* is involved in the defense responses against *Botryosphaeria dothidea*. *Plant Physiol Biochem* 62:23–32. doi: <https://doi.org/10.1016/j.plaphy.2012.10.016>
- Basset Y, Novotny V (1999) Species richness of insect herbivore communities on *Ficus* in Papua New Guinea. *Biol J Linn Soc* 67:477–499. doi: <https://doi.org/10.1111/j.1095-8312.1999.tb01943.x>
- Bauer P, Elbaum R, Weiss IM (2011) Calcium and silicon mineralization in land plants: Transport, structure and function. *Plant Sci* 180:746–756. doi: <https://doi.org/10.1016/j.plantsci.2011.01.019>
- Baumgartner B, Erdelmeier CAJ, Wright AD, et al (1990) An antimicrobial alkaloid from *Ficus septica*. *Phytochemistry* 29:3327–3330. doi: [https://doi.org/10.1016/0031-9422\(90\)80209-Y](https://doi.org/10.1016/0031-9422(90)80209-Y)
- Baur R, Binder S, Benz G (1991) Nonglandular leaf trichomes as short-term inducible defense of the grey alder, *Alnus incana* (L.), against the chrysomelid beetle, *Agelastica alni* L. *Oecologia* 87:219–226. doi: <https://doi.org/10.1007/BF00325259>

596 Bayoudh C, Elair M, Labidi R, et al (2017) Efficacy of tissue culture in virus elimination from caprifig
 597 and female fig varieties (*Ficus carica* L.). Plant Pathol J 33:288–295. doi:
 598 <https://doi.org/10.5423/PPJ.OA.10.2016.0205>

599 Beier RC, Oertli EH (1983) Psoralen and other linear furocoumarins as phytoalexins in celery.
 600 Phytochemistry 22:2595–2597. doi: [https://doi.org/10.1016/0031-9422\(83\)80173-3](https://doi.org/10.1016/0031-9422(83)80173-3)

601 Berenbaum MR (2002) Postgenomic chemical ecology: from genetic code to ecological interactions. J
 602 Chem Ecol 28:873–896. doi: <https://doi.org/10.1023/A:1015260931034>

603 Bhutani K, Sharma G, Ali M (1987) Plant based antiamoebic drugs; Part I. Antiamoebic activity of
 604 phenanthroindolizidine alkaloids; Common structural determinants of activity with emetine.
 605 Planta Med 53:532–536. doi: <https://doi.org/10.1055/s-2006-962803>

606 Bonamonte D, Foti C, Lionetti N, et al (2010) Photoallergic contact dermatitis to 8-methoxypsoralen
 607 in *Ficus carica*. Contact Dermatitis 62:343–348. doi: <https://doi.org/10.1111/j.1600-0536.2010.01713.x>

609 Broadway RM, Duffey SS (1986) Plant proteinase inhibitors: Mechanism of action and effect on the
 610 growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exiqua*. J Insect
 611 Physiol 32:827–833. doi: [https://doi.org/10.1016/0022-1910\(86\)90097-1](https://doi.org/10.1016/0022-1910(86)90097-1)

612 Burtoloso A, Bertonha A, Rosset I (2013) Synthesis of alkaloids: Recent advances in the synthesis of
 613 phenanthroindolizidine alkaloids. Curr Top Med Chem 14:191–199. doi:
 614 <https://doi.org/10.2174/1568026613666131213154633>

615 Buttery BR, Boatman SG (1976) Water deficits and flow of latex. In: Soil Water Measurement, Plant
 616 Responses, and Breeding for Drought Resistance, United Kingdom Edition. Academic Press,
 617 London, pp 233–289

618 Caporale G, Dall’Acqua F, Marciani S, Capozzi A (1970) Studies on the Biosynthesis of Psoralen and
 619 Bergapten in the Leaves of *Ficus carica*. Z Für Naturforschung B 25:700–703. doi:
 620 <https://doi.org/10.1515/znb-1970-0709>

621 Chamont S (2014) *Ficus carica* . Les maladies et ravageurs. In: Ephytia.
 622 [http://ephytia.inra.fr/fr/C/18200/Hypp-encyclopedie-en-protection-des-plantes-Les-](http://ephytia.inra.fr/fr/C/18200/Hypp-encyclopedie-en-protection-des-plantes-Les-maladies-et-ravageurs)
 623 [maladies-et-ravageurs](http://ephytia.inra.fr/fr/C/18200/Hypp-encyclopedie-en-protection-des-plantes-Les-maladies-et-ravageurs). Accessed 17 Sep 2018

624 Chang M-S, Yang Y-C, Kuo Y-C, et al (2005) Furocoumarin glycosides from the leaves of *Ficus ruficaulis*
 625 merr. var. antaoensis. J Nat Prod 68:11–13. doi: <https://doi.org/10.1021/np0401056>

626 Chantarasuwan B, Baas P, van Heuven B-J, et al (2014) Leaf anatomy of *Ficus* subsection *Urostigma*
 627 (Moraceae). Bot J Linn Soc 175:259–281. doi: <https://doi.org/10.1111/boj.12165>

628 Chen L-W, Cheng M-J, Peng C-F, Chen I-S (2010) Secondary metabolites and antimycobacterial
 629 activities from the roots of *Ficus nervosa*. Chem Biodivers 7:1814–1821. doi:
 630 <https://doi.org/10.1002/cbdv.200900227>

631 Coley PD (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest.
 632 Ecol Monogr 53:209–234. doi: <https://doi.org/10.2307/1942495>

633 Coley PD, Bryant JP, Chapin FS (1985) Resource Availability and Plant Antiherbivore Defense. Science
 634 230:895–899. doi: <https://doi.org/10.1126/science.230.4728.895>

- 635 Cruaud A, Rønsted N, Chantarasuwan B, et al (2012) An extreme case of plant–insect
636 codiversification: Figs and fig-Pollinating wasps. *Syst Biol* 61:1029–1047. doi:
637 <https://doi.org/10.1093/sysbio/sys068>
- 638 Damu A, Kuo P-C, Shi L-S, et al (2009) Cytotoxic phenanthroindolizidine alkaloids from the roots of
639 *Ficus septica*. *Planta Med* 75:1152–1156. doi: <https://doi.org/10.1055/s-0029-1185483>
- 640 Damu AG, Kuo P-C, Shi L-S, et al (2005) Phenanthroindolizidine alkaloids from the stems of *Ficus*
641 *septica*. *J Nat Prod* 68:1071–1075. doi: <https://doi.org/10.1021/np050095o>
- 642 Dardalhon M, de Massy B, Nicolas A, Averbek D (1998) Mitotic recombination and localized DNA
643 double-strand breaks are induced after 8-methoxypsoralen and UVA irradiation in
644 *Saccharomyces cerevisiae*. *Curr Genet* 34:30–42. doi:
645 <https://doi.org/10.1007/s002940050363>
- 646 de Freitas CDT, Sousa Nogueira FC, Vasconcelos IM, et al (2011) Osmotin purified from the latex of
647 *Calotropis procera*: Biochemical characterization, biological activity and role in plant defense.
648 *Plant Physiol Biochem* 49:738–743. doi: <https://doi.org/10.1016/j.plaphy.2011.01.027>
- 649 Debib A, Tir-Touil A, Mothana RA, et al (2014) Phenolic content, antioxidant and antimicrobial
650 activities of two fruit varieties of Algerian *Ficus carica* L: Antioxidant and antimicrobial activity
651 of Algerian figs. *J Food Biochem* 38:207–215. doi: <https://doi.org/10.1111/jfbc.12039>
- 652 Dussourd DE (2017) Behavioral sabotage of plant defenses by insect folivores. *Annu Rev Entomol*
653 62:15–34. doi: <https://doi.org/10.1146/annurev-ento-031616-035030>
- 654 Dussourd DE, Denno RF (1991) Deactivation of plant defense: correspondence between insect
655 behavior and secretory canal architecture. *Ecology* 72:1383–1396. doi:
656 <https://doi.org/10.2307/1941110>
- 657 Dussourd DE, Denno RF (1994) Host range of generalist caterpillars: trenching permits feeding on
658 plants with secretory canals. *Ecology* 75:69–78. doi: <https://doi.org/10.2307/1939383>
- 659 Dussourd DE, Eisner T (1987) Vein-cutting behavior: insect counterploy to the latex defense of plants.
660 *Science* 237:898–901. doi: <https://doi.org/10.1126/science.3616620>
- 661 Dussourd DE, Hoyle AM (2000) Poisoned pluriines: toxicity of milkweed latex and cardenolides to
662 some generalist caterpillars: *Chemoecology* 10:11–16. doi:
663 <https://doi.org/10.1007/PL00001810>
- 664 Edreva A (2005) Pathogenesis-related proteins: research progress in the last 15 years. *Gen Appl Plant*
665 *Physiol* 31:105–124
- 666 Elbeaino T, Digiario M, Martelli GP (2011) Complete sequence of Fig fleck-associated virus, a novel
667 member of the family Tymoviridae. *Virus Res* 161:198–202. doi:
668 <https://doi.org/10.1016/j.virusres.2011.07.022>
- 669 El-Sakhawy F, Kassem H, Abou-Hussein D, et al (2016) Phytochemical investigation of the bioactive
670 extracts of the leaves of *Ficus cyathistipula* Warb. *Z Für Naturforschung C* 71:141–154. doi:
671 <https://doi.org/10.1515/znc-2015-0274>
- 672 FAO (2016) FAOSTAT Database. In: Food Agric. Organ. U. N. <http://www.fao.org/faostat/en/#data>

673 Farrell BD, Dussourd DE, Mitter C (1990) Escalation of plant defense: do latex and resin canals spur
674 plant diversification? *Am Nat* 138:881–900. doi: <https://doi.org/10.1086/285258>

675 Felton GW, Donato KK, Broadway RM, Duffey SS (1992) Impact of oxidized plant phenolics on the
676 nutritional quality of dietary protein to a noctuid herbivore, *Spodoptera exigua*. *J Insect*
677 *Physiol* 38:277–285. doi: [https://doi.org/10.1016/0022-1910\(92\)90128-Z](https://doi.org/10.1016/0022-1910(92)90128-Z)

678 Felton GW, Gatehouse JA (1996) Antinutritive plant defence mechanisms. In: *Biology of the Insect*
679 *Midgut*, Lehane MJ, Billingsley PF (edn). Springer, Dordrecht, pp 373–416

680 Finley DS (1999) Patterns of calcium oxalate crystals in young tropical leaves: a possible role as an
681 anti-herbivory defense. *Rev Biol Trop* 47:27–31

682 Foster AS (1956) Plant idioblasts: Remarkable examples of cell specialization. *Protoplasma* 46:184–
683 193. doi: <https://doi.org/10.1007/BF01248877>

684 Franceschi VR, Nakata PA (2005) Calcium oxalate in plants: formation and function. *Annu Rev Plant*
685 *Biol* 56:41–71. doi: <https://doi.org/10.1146/annurev.arplant.56.032604.144106>

686 Gal A, Brumfeld V, Weiner S, et al (2012) Certain biominerals in leaves function as light scatterers.
687 *Adv Mater* 24:OP77–OP83. doi: <https://doi.org/10.1002/adma.201104548>

688 Gal A, Weiner S, Addadi L (2010) The stabilizing effect of silicate on biogenic and synthetic
689 amorphous calcium carbonate. *J Am Chem Soc* 132:13208–13211. doi:
690 <https://doi.org/10.1021/ja106883c>

691 Gautam P, Lal B, Tripathi R, et al (2016) Role of silica and nitrogen interaction in submergence
692 tolerance of rice. *Environ Exp Bot* 125:98–109. doi:
693 <https://doi.org/10.1016/j.envexpbot.2016.02.008>

694 Ghana S, Suleman N, Compton SG (2017) Style length variation in male and female figs: development,
695 inheritance, and control of pollinator oviposition. *Entomol Exp Appl* 162:41–50. doi:
696 <https://doi.org/10.1111/eea.12533>

697 Gibernau M, Buser HR, Frey JE, Hossaert-McKey M (1997) Volatile compounds from extracts of figs of
698 *Ficus carica*. *Phytochemistry* 46:241–244. doi: [https://doi.org/10.1016/S0031-](https://doi.org/10.1016/S0031-9422(97)00292-6)
699 [9422\(97\)00292-6](https://doi.org/10.1016/S0031-9422(97)00292-6)

700 Guo Q, Du G, He H, et al (2016) Two nematicidal furocoumarins from *Ficus carica* L. leaves and their
701 physiological effects on pine wood nematode (*Bursaphelenchus xylophilus*). *Nat Prod Res*
702 30:1969–1973. doi: <https://doi.org/10.1080/14786419.2015.1094804>

703 Hagel J, Yeung E, Facchini P (2008) Got milk? The secret life of laticifers. *Trends Plant Sci* 13:631–639.
704 doi: <https://doi.org/10.1016/j.tplants.2008.09.005>

705 He K, Iyer KR, Hayes RN, et al (1998) Inactivation of cytochrome P450 3A4 by bergamottin, a
706 component of grapefruit juice. *Chem Res Toxicol* 11:252–259. doi:
707 <https://doi.org/10.1021/tx970192k>

708 Hebeish A, Fouda MMG, Hamdy IA, et al (2008) Preparation of durable insect repellent cotton fabric:
709 Limonene as insecticide. *Carbohydr Polym* 74:268–273. doi:
710 <https://doi.org/10.1016/j.carbpol.2008.02.013>

711 Hilder VA, Gatehouse AM, Sheerman SE, et al (1987) A novel mechanism of insect resistance
712 engineered into tobacco. *Nature* 330:160–163. doi: <http://doi.org/10.1038/330160a0>

713 Hodson MJ, White PJ, Mead A, Broadley MR (2005) Phylogenetic variation in the silicon composition
714 of plants. *Ann Bot* 96:1027–1046. doi: <https://doi.org/10.1093/aob/mci255>

715 Hoffmann-Sommergruber K (2002) Pathogenesis-related (PR)-proteins identified as allergens.
716 *Biochem Soc Trans* 30:930–935. doi: <https://doi.org/10.1042/bst0300930>

717 Hosomi A, Miwa Y, Furukawa M, Kawaradani M (2012) Growth of fig varieties resistant to
718 ceratocystis canker following infection with *Ceratocystis fimbriata*. *J Jpn Soc Hortic Sci*
719 81:159–165. doi: <https://doi.org/10.2503/jjshs1.81.159>

720 Hudgins JW, Krekling T, Franceschi VR (2003) Distribution of calcium oxalate crystals in the secondary
721 phloem of conifers: a constitutive defense mechanism? *New Phytol* 159:677–690. doi:
722 <https://doi.org/10.1046/j.1469-8137.2003.00839.x>

723 Hunt JW, Dean AP, Webster RE, et al (2008) A novel mechanism by which silica defends grasses
724 against herbivory. *Ann Bot* 102:653–656. doi: <https://doi.org/10.1093/aob/mcn130>

725 Huynh QK, Borgmeyer JR, Zobel JF (1992) Isolation and characterization of a 22 kDa protein with
726 antifungal properties from maize seeds. *Biochem Biophys Res Commun* 182:1–5. doi:
727 [https://doi.org/10.1016/S0006-291X\(05\)80103-2](https://doi.org/10.1016/S0006-291X(05)80103-2)

728 Jaina R, Jain SC, Bhagchandani T, Yadav N (2013) New furanocoumarins and other chemical
729 constituents from *Ficus carica* root heartwood. *Z Für Naturforschung C* 68:3–7. doi:
730 <https://doi.org/10.1515/znc-2013-1-201>

731 Janzen DH (1979) How to be a fig. *Annu Rev Ecol Syst* 10:13–51. doi:
732 <https://doi.org/10.1146/annurev.es.10.110179.000305>

733 Jeong M-R, Kim H-Y, Cha J-D (2009) Antimicrobial activity of methanol extract from *Ficus carica*
734 leaves against oral bacteria. *J Bacteriol Virol* 39:97–102. doi:
735 <https://doi.org/10.4167/jbv.2009.39.2.97>

736 Jun LI, Tian Y, Sun B, et al (2012) Analysis on volatile constituents in leaves and fruits of *Ficus carica* by
737 GC-MS. *Chin Herb Med* 4:63–69. doi: <https://doi.org/10.3969/j.issn.1674-6384.2012.01.010>

738 Kim JS, Kim YO, Ryu HJ, et al (2003) Isolation of stress-related genes of rubber particles and latex in
739 fig tree (*Ficus carica*) and their expressions by abiotic stress or plant hormone treatments.
740 *Plant Cell Physiol* 44:412–414. doi: <https://doi.org/10.1093/pcp/pcg058>

741 Kim YS, Park SJ, Lee EJ, et al (2008) Antibacterial compounds from rose bengal-sensitized
742 photooxidation of β -caryophyllene. *J Food Sci* 73:C540–C545. doi:
743 <https://doi.org/10.1111/j.1750-3841.2008.00879.x>

744 Kitajima S, Aoki W, Shibata D, et al (2018) Comparative multi-omics analysis reveals diverse latex-
745 based defense strategies against pests among latex-producing organs of the fig tree (*Ficus*
746 *carica*). *Planta* 247:1423–1438. doi: <https://doi.org/10.1007/s00425-018-2880-3>

747 Klimko M, Truchan M (2006) Morphological variability of the leaf epidermis in selected taxa of the
748 genus *Ficus* L. (Moraceae) and its taxonomic implication. *Acta Soc Bot Pol* 75:309–324. doi:
749 <https://doi.org/10.5586/asbp.2006.038>

750 Koenigs LL, Trager WF (1998) Mechanism-based inactivation of P450 2A6 by furanocoumarins.
751 Biochemistry 37:10047–10061. doi: <https://doi.org/10.1021/bi980003c>

752 Kon S, Whitaker JR (1965) Separation and partial characterization of the peroxidases of *Ficus glabrata*
753 Latex. J Food Sci 30:977–985. doi: <https://doi.org/10.1111/j.1365-2621.1965.tb01873.x>

754 Konno K (2011) Plant latex and other exudates as plant defense systems: Roles of various defense
755 chemicals and proteins contained therein. Phytochemistry 72:1510–1530. doi:
756 <https://doi.org/10.1016/j.phytochem.2011.02.016>

757 Konno K, Hirayama C, Nakamura M, et al (2004) Papain protects papaya trees from herbivorous
758 insects: role of cysteine proteases in latex. Plant J 37:370–378. doi:
759 <https://doi.org/10.1046/j.1365-313X.2003.01968.x>

760 Koricheva J, Nykänen H, Gianoli E (2004) Meta-analysis of trade-offs among plant antiherbivore
761 defenses: are plants jacks-of-all-trades, masters of all? Am Nat 163:E64–E75. doi:
762 <https://doi.org/10.1086/382601>

763 Korth KL, Doege SJ, Park S-H, et al (2006) *Medicago truncatula* mutants demonstrate the role of plant
764 calcium oxalate crystals as an effective defense against chewing insects. Plant Physiol
765 141:188–195. doi: <https://doi.org/10.1104/pp.106.076737>

766 Kramer DE, Whitaker JR (1964) *Ficus* enzymes II. Properties of the proteolytic enzymes from the latex
767 of *Ficus carica* variety Kadota. J Biol Chem 239:2178–2183

768 Lawrence SD, Novak NG (2006) Expression of poplar chitinase in tomato leads to inhibition of
769 development in Colorado Potato Beetle. Biotechnol Lett 28:593–599. doi:
770 <https://doi.org/10.1007/s10529-006-0022-7>

771 Lazreg Aref H, Gaaliche B, Fekih A, et al (2011) *In vitro* cytotoxic and antiviral activities of *Ficus carica*
772 latex extracts. Nat Prod Res 25:310–319. doi:
773 <https://doi.org/10.1080/14786419.2010.528758>

774 Levin DA (1973) The Role of Trichomes in Plant Defense. Q Rev Biol 48:3–15. doi:
775 <https://doi.org/10.1086/407484>

776 Lewinsohn TM (1991) The geographical distribution of plant latex. Chemoecology 2:64–68. doi:
777 <https://doi.org/10.1007/BF01240668>

778 Li H, Hu T, Wang K, et al (2006) Total synthesis and antiviral activity of enantioenriched (+)-deoxytylo-
779 phorinine. Lett Org Chemistry 3:806–810. doi:
780 <https://doi.org/10.2174/157017806779117067>

781 Li W, Schuler MA, Berenbaum MR (2003) Diversification of furanocoumarin-metabolizing cytochrome
782 P450 monooxygenases in two papilionids: specificity and substrate encounter rate. Proc Natl
783 Acad Sci 100:14593–14598. doi: <https://doi.org/10.1073/pnas.1934643100>

784 Liu F, Yang Z, Zheng X, et al (2011) Nematicidal coumarin from *Ficus carica* L. J Asia-Pac Entomol
785 14:79–81. doi: <https://doi.org/10.1016/j.aspen.2010.10.006>

786 Liu M, Zhou F, Pan X, et al (2018) Specificity of herbivore-induced responses in an invasive species,
787 *Alternanthera philoxeroides* (alligator weed). Ecol Evol 8:59–70. doi:
788 <https://doi.org/10.1002/ece3.3615>

- 789 Liu Y, Ahn J-E, Datta S, et al (2005) *Arabidopsis* vegetative storage protein is an anti-insect acid
790 phosphatase. *Plant Physiol* 139:1545–1556. doi: <https://doi.org/10.1104/pp.105.066837>
- 791 Lynn KR, Clevette-Radford NA (1987) Acid phosphatases from latices of euphorbiaceae.
792 *Phytochemistry* 26:655–657. doi: [https://doi.org/10.1016/S0031-9422\(00\)84760-3](https://doi.org/10.1016/S0031-9422(00)84760-3)
- 793 Macedo M, Oliveira C, Oliveira C (2015) Insecticidal activity of plant lectins and potential application
794 in crop protection. *Molecules* 20:2014–2033. doi:
795 <https://doi.org/10.3390/molecules20022014>
- 796 Mamoucha S, Fokialakis N, Christodoulakis NS (2016) Leaf structure and histochemistry of *Ficus*
797 *carica* (Moraceae), the fig tree. *Flora - Morphol Distrib Funct Ecol Plants* 218:24–34. doi:
798 <https://doi.org/10.1016/j.flora.2015.11.003>
- 799 Marrelli M, Menichini F, Statti GA, et al (2012) Changes in the phenolic and lipophilic composition, in
800 the enzyme inhibition and antiproliferative activity of *Ficus carica* L. cultivar Dottato fruits
801 during maturation. *Food Chem Toxicol* 50:726–733. doi:
802 <https://doi.org/10.1016/j.fct.2011.12.025>
- 803 Marrelli M, Statti GA, Tundis R, et al (2014) Fatty acids, coumarins and polyphenolic compounds of
804 *Ficus carica* L. cv. Dottato: variation of bioactive compounds and biological activity of aerial
805 parts. *Nat Prod Res* 28:271–274. doi: <https://doi.org/10.1080/14786419.2013.841689>
- 806 Massey FP, Ennos AR, Hartley SE (2007a) Grasses and the resource availability hypothesis: the
807 importance of silica-based defences. *J Ecol* 95:414–424. doi: <https://doi.org/10.1111/j.1365-2745.2007.01223.x>
- 809 Massey FP, Hartley SE (2009) Physical defences wear you down: progressive and irreversible impacts
810 of silica on insect herbivores. *J Anim Ecol* 78:281–291. doi: <https://doi.org/10.1111/j.1365-2656.2008.01472.x>
- 812 Massey FP, Roland Ennos A, Hartley SE (2007b) Herbivore specific induction of silica-based plant
813 defences. *Oecologia* 152:677–683. doi: <https://doi.org/10.1007/s00442-007-0703-5>
- 814 Mauch F, Mauch-Mani B, Boller T (1988) Antifungal hydrolases in pea tissue. *Plant Physiol* 88:936–
815 942. doi: <https://doi.org/10.1104/pp.88.3.936>
- 816 McKenzie EHC (1986) New plant disease record in New Zealand: Fig rust (*Cerotelium fici*) on *Ficus*
817 *carica*. *N Z J Agric Res* 29:707–710. doi: <https://doi.org/10.1080/00288233.1986.10430467>
- 818 McNaughton SJ, Tarrant JL (1983) Grass leaf silicification: Natural selection for an inducible defense
819 against herbivores. *Proc Natl Acad Sci* 80:790–791. doi:
820 <https://doi.org/10.1073/pnas.80.3.790>
- 821 Melchers LS, Apotheker-de Groot M, van der Knaap JA, et al (1994) A new class of tobacco chitinases
822 homologous to bacterial exo-chitinases displays antifungal activity. *Plant J* 5:469–480. doi:
823 <https://doi.org/10.1046/j.1365-313X.1994.05040469.x>
- 824 Meyen J (1839) Matériaux pour servir à l’histoire du développement des diverses parties dans les
825 plantes. In: *Annales des Sciences Naturelles. Botanique*. Crochard, 1869, Paris, pp 257–279
- 826 Mohamed SA, Abdel-Aty AM, Belal Hamed M, et al (2011) *Ficus sycomorus* latex: A thermostable
827 peroxidase. *Afr J Biotechnol* 10:17532–17543. doi: <https://doi.org/10.5897/AJB11.2375>

828 Müller J, Clauss M, Codron D, et al (2014) Growth and wear of incisor and cheek teeth in domestic
829 rabbits (*Oryctolagus cuniculus*) fed diets of different abrasiveness. J Exp Zool Part Ecol Genet
830 Physiol 321:283–298. doi: <https://doi.org/10.1002/jez.1864>

831 Neal JJ, Wu D (1994) Inhibition of insect cytochromes P450 by furanocoumarins. Pestic Biochem
832 Physiol 50:43–50. doi: <https://doi.org/10.1006/pest.1994.1056>

833 Niderman T, Genetet I, Bruyère T, et al (1995) Pathogenesis-Related PR-1 Proteins are antifungal.
834 Plant Physiol 108:17–27. doi: <https://doi.org/10.1104/pp.108.1.17>

835 Novotny V, Miller SE, Baje L, et al (2010) Guild-specific patterns of species richness and host
836 specialization in plant-herbivore food webs from a tropical forest: Plant-herbivore food webs
837 in tropical forest. J Anim Ecol 79:1193–1203. doi: <https://doi.org/10.1111/j.1365-2656.2010.01728.x>

839 Ojala T, Remes S, Haansuu P, et al (2000) Antimicrobial activity of some coumarin containing herbal
840 plants growing in Finland. J Ethnopharmacol 73:299–305. doi:
841 [https://doi.org/10.1016/S0378-8741\(00\)00279-8](https://doi.org/10.1016/S0378-8741(00)00279-8)

842 Ojo OA, Ajiboye BO, Ojo AB, et al (2014) Phytochemical, proximate analysis and mineral composition
843 of aqueous crude extract of *Ficus asperifolia* Miq. J Adv Med Life Sci 1:1–4. doi:
844 <https://doi.org/10.5281/zenodo.1000388>

845 Oliveira AP, Baptista P, Andrade PB, et al (2012) Characterization of *Ficus carica* L. cultivars by DNA
846 and secondary metabolite analysis: Is genetic diversity reflected in the chemical
847 composition? Food Res Int 49:710–719. doi: <https://doi.org/10.1016/j.foodres.2012.09.019>

848 Oliveira AP, Silva LR, Ferreres F, et al (2010a) Chemical assessment and *in Vitro* antioxidant capacity
849 of *Ficus carica* latex. J Agric Food Chem 58:3393–3398. doi:
850 <https://doi.org/10.1021/jf9039759>

851 Oliveira AP, Silva LR, Guedes de Pinho P, et al (2010b) Volatile profiling of *Ficus carica* varieties by HS-
852 SPME and GC–IT-MS. Food Chem 123:548–557. doi:
853 <https://doi.org/10.1016/j.foodchem.2010.04.064>

854 Oliveira AP, Valentão P, Pereira JA, et al (2009) *Ficus carica* L.: Metabolic and biological screening.
855 Food Chem Toxicol 47:2841–2846. doi: <https://doi.org/10.1016/j.fct.2009.09.004>

856 Pathak MA, Daniels Jr F, Fitzpatrick TB (1962) The Presently Known Distribution of Furocoumarins
857 (Psoralens) in Plants. J Invest Dermatol 39:225–239. doi:
858 <https://doi.org/10.1038/jid.1962.106>

859 Patil Vikas V, Bhangale SC, Patil VR (2010) Evaluation of anti-pyretic potential of *Ficus carica* leaves.
860 EVALUATION 2:48–50

861 Peraza-Sánchez SR, Chai H-B, Shin YG, et al (2002) Constituents of the leaves and twigs of *Ficus*
862 *hispida*. Planta Med 68:186–188. doi: <https://doi.org/10.1055/s-2002-20257>

863 Peumans WJ, Van Damme EJM (1995) Lectins as plant defense proteins. Plant Physiol 109:347–352.
864 doi: <https://doi.org/10.1104/pp.109.2.347>

865 Pierantoni M, Tenne R, Rephael B, et al (2018) Mineral deposits in *Ficus* leaves: Morphologies and
866 locations in relation to function. *Plant Physiol* 176:1751–1763. doi:
867 <https://doi.org/10.1104/pp.17.01516>

868 Prychid CJ, Rudall PJ, Gregory M (2003) Systematics and biology of silica bodies in monocotyledons.
869 *Bot Rev* 69:377–440. doi: [https://doi.org/10.1663/0006-](https://doi.org/10.1663/0006-8101(2004)069[0377:SABOSB]2.0.CO;2)
870 [8101\(2004\)069\[0377:SABOSB\]2.0.CO;2](https://doi.org/10.1663/0006-8101(2004)069[0377:SABOSB]2.0.CO;2)

871 Roberts DM, Gallapaththy G, Dunuwille A, Chan BS (2016) Pharmacological treatment of cardiac
872 glycoside poisoning: Cardenolide poisoning. *Br J Clin Pharmacol* 81:488–495. doi:
873 <https://doi.org/10.1111/bcp.12814>

874 Roberts WK, Selitrennikoff CP (1988) Plant and bacterial chitinases differ in antifungal activity. *J Gen*
875 *Microbiol* 134:169–176. doi: <https://doi.org/10.1099/00221287-134-1-169>

876 Rubnov S, Kashman Y, Rabinowitz R, et al (2001) Suppressors of cancer cell proliferation from Fig
877 (*Ficus carica*) resin: Isolation and structure elucidation. *J Nat Prod* 64:993–996. doi:
878 <https://doi.org/10.1021/np000592z>

879 Ruiz N, Ward D, Saltz D (2002) Calcium oxalate crystals in leaves of *Pancratium sickenbergeri*:
880 constitutive or induced defence? *Funct Ecol* 16:99–105. doi: [https://doi.org/10.1046/j.0269-](https://doi.org/10.1046/j.0269-8463.2001.00594.x)
881 [8463.2001.00594.x](https://doi.org/10.1046/j.0269-8463.2001.00594.x)

882 Ryan CA (1990) Protease inhibitors in plants: genes for improving defenses against insects and
883 pathogens. *Annu Rev Phytopathol* 28:425–449. doi:
884 <https://doi.org/10.1146/annurev.py.28.090190.002233>

885 Saeed MA, Sabir AW (2002) Irritant potential of triterpenoids from *Ficus carica* leaves. *Fitoterapia*
886 73:417–420. doi: [https://doi.org/10.1016/S0367-326X\(02\)00127-2](https://doi.org/10.1016/S0367-326X(02)00127-2)

887 Schlumbaum A, Mauch F, Vögeli U, Boller T (1986) Plant chitinases are potent inhibitors of fungal
888 growth. *Nature* 324:365–367. doi: <http://doi.org/10.1038/324365a0>

889 Sethi A, McAuslane HJ, Rathinasabapathi B, et al (2009) Enzyme induction as a possible mechanism
890 for latex-mediated insect resistance in romaine lettuce. *J Chem Ecol* 35:190–200. doi:
891 <https://doi.org/10.1007/s10886-009-9596-6>

892 Sgarbieri VC, Gupte SM, Kramer DE, Whitaker JR (1964) *Ficus* enzymes I. Separation of the proteolytic
893 enzymes of *Ficus carica* and *Ficus glabrata* latices. *J Biol Chem* 239:2170–2177

894 Sirisha N, Sreenivasulu M, Sangeeta K, Chetty CM (2010) Antioxidant properties of *Ficus* species—a
895 review. *Int J PharmTech Res* 2:2174–2182

896 Skalicka-Wozniak K, Los R, Glowinski K, Malm A (2009) Volatile compounds in fruits of *Peucedanum*
897 *cervaria* (Lap.) L. *Chem Biodivers* 6:1087–1092. doi: <https://doi.org/10.1002/cbdv.200800236>

898 Smith DL, Watt WM (1986) Distribution of lithocysts, trichomes, hydathodes and stomata in leaves of
899 *Pilea cadierei* Gagnep. & Guill. (Urticaceae). *Ann Bot* 58:155–166. doi:
900 <https://doi.org/10.1093/oxfordjournals.aob.a087193>

901 Smyth TJP, Ramachandran V, Brooks P, Smyth WF (2012) Investigation of antibacterial
902 phytochemicals in the bark and leaves of *Ficus coronata* by high-performance liquid

903 chromatography-electrospray ionization-ion trap mass spectrometry (HPLC-ESI-MSn) and ESI-
904 MSn. Electrophoresis 33:713–718. doi: <https://doi.org/10.1002/elps.201100302>

905 Solomon A, Golubowicz S, Yablowicz Z, et al (2006) Antioxidant activities and anthocyanin content of
906 fresh fruits of common Fig (*Ficus carica* L.). J Agric Food Chem 54:7717–7723. doi:
907 <https://doi.org/10.1021/jf060497h>

908 Son J-H, Jin H, You H-S, et al (2017) Five cases of phytophotodermatitis caused by fig leaves and
909 relevant literature review. Ann Dermatol 29:86–90. doi:
910 <https://doi.org/10.5021/ad.2017.29.1.86>

911 Sosnovsky Y (2016) Sucking herbivore assemblage composition on greenhouse *Ficus* correlates with
912 host plant leaf architecture. Arthropod-Plant Interact 10:55–69. doi:
913 <https://doi.org/10.1007/s11829-015-9408-6>

914 Stintzi A, Heitz T, Prasad V, et al (1993) Plant ‘pathogenesis-related’ proteins and their role in defense
915 against pathogens. Biochimie 75:687–706. doi: [https://doi.org/10.1016/0300-](https://doi.org/10.1016/0300-9084(93)90100-7)
916 [9084\(93\)90100-7](https://doi.org/10.1016/0300-9084(93)90100-7)

917 Strömberg CAE, Di Stilio VS, Song Z (2016) Functions of phytoliths in vascular plants: an evolutionary
918 perspective. Funct Ecol 30:1286–1297. doi: <https://doi.org/10.1111/1365-2435.12692>

919 Taira T, Ohdomari A, Nakama N, et al (2005) Characterization and antifungal activity of Gazyumaru
920 (*Ficus microcarpa*) latex chitinases: Both the chitin-binding and the antifungal activities of
921 class I chitinase are reinforced with increasing ionic strength. Biosci Biotechnol Biochem
922 69:811–818. doi: <https://doi.org/10.1271/bbb.69.811>

923 Takahashi T, Okiura A, Kohno M (2017) Phenylpropanoid composition in fig (*Ficus carica* L.) leaves. J
924 Nat Med 71:770–775. doi: <https://doi.org/10.1007/s11418-017-1093-6>

925 Takahashi T, Okiura A, Saito K, Kohno M (2014) Identification of phenylpropanoids in fig (*Ficus carica*
926 L.) leaves. J Agric Food Chem 62:10076–10083. doi: <https://doi.org/10.1021/jf5025938>

927 Teixeira DM, Canelas VC, do Canto AM, et al (2009) HPLC-DAD quantification of phenolic compounds
928 contributing to the antioxidant activity of *Maclura pomifera*, *Ficus carica* and *Ficus elastica*
929 extracts. Anal Lett 42:2986–3003. doi: <https://doi.org/10.1080/00032710903276646>

930 Teixeira DM, Patão RF, Coelho AV, da Costa CT (2006) Comparison between sample disruption
931 methods and solid–liquid extraction (SLE) to extract phenolic compounds from *Ficus carica*
932 leaves. J Chromatogr A 1103:22–28. doi: <https://doi.org/10.1016/j.chroma.2005.11.047>

933 Terras F, Schoofs H, Thevissen K, et al (1993) Synergistic enhancement of the antifungal activity of
934 wheat and barley thionins by radish and oilseed rape 2S albumins and by barley trypsin
935 inhibitors. Plant Physiol 103:1311–1319. doi: <https://doi.org/10.1104/pp.103.4.1311>

936 Traw BM, Dawson TE (2002) Differential induction of trichomes by three herbivores of black mustard.
937 Oecologia 131:526–532. doi: <https://doi.org/10.1007/s00442-002-0924-6>

938 Ummu-Hani B, Noraini T (2013) The structure of cystoliths in selected taxa of the genus *Ficus* L.
939 (Moraceae) in Peninsular Malaysia. AIP Conf Proc 1571:372–376. doi:
940 <https://doi.org/10.1063/1.4858686>

941 Van Damme EJM, Peumans WJ, Barre A, Rougé P (1998) Plant lectins: a composite of several distinct
 942 families of structurally and evolutionary related proteins with diverse biological roles. Crit
 943 Rev Plant Sci 17:575–692. doi: <https://doi.org/10.1080/07352689891304276>

944 Vaya J, Mahmood S (2006) Flavonoid content in leaf extracts of the fig (*Ficus carica* L.), carob
 945 (*Ceratonia siliqua* L.) and pistachio (*Pistacia lentiscus* L.). BioFactors 28:169–175. doi:
 946 <https://doi.org/10.1002/biof.5520280303>

947 Volf M, Hrccek J, Julkunen-Tiitto R, Novotny V (2015) To each its own: differential response of
 948 specialist and generalist herbivores to plant defence in willows. J Anim Ecol 84:1123–1132.
 949 doi: <https://doi.org/10.1111/1365-2656.12349>

950 Volf M, Segar ST, Miller SE, et al (2018) Community structure of insect herbivores is driven by
 951 conservatism, escalation and divergence of defensive traits in *Ficus*. Ecol Lett 21:83–92. doi:
 952 <https://doi.org/10.1111/ele.12875>

953 Wagner GJ (1991) Secreting glandular trichomes: more than just hairs. Plant Physiol 96:675–679. doi:
 954 <https://doi.org/10.1104/pp.96.3.675>

955 Wahler D, Gronover CS, Richter C, et al (2009) Polyphenoloxidase silencing affects latex coagulation
 956 in *Taraxacum* species. Plant Physiol 151:334–346. doi:
 957 <https://doi.org/10.1104/pp.109.138743>

958 Wang T, Jiao J, Gai Q-Y, et al (2017) Enhanced and green extraction polyphenols and
 959 furanocoumarins from Fig (*Ficus carica* L.) leaves using deep eutectic solvents. J Pharm
 960 Biomed Anal 145:339–345. doi: <https://doi.org/10.1016/j.jpba.2017.07.002>

961 Ward D, Spiegel M, Saltz D (1997) Gazelle herbivory and interpopulation differences in calcium
 962 oxalate content of leaves of a desert lily. J Chem Ecol 23:333–346. doi:
 963 <https://doi.org/10.1023/B:JOEC.0000006363.34360.9d>

964 Weddell HA (1854) Sur les cystolithes ou concrétions calcaires des Urticées et d'autres végétaux. In:
 965 Annales des Sciences Naturelles. Botanique. Masson, 1854, Paris, pp 267–272

966 Wu CC, Kuo-Huang LL (1997) Calcium crystals in the leaves of some species of Moraceae. Bot Bull
 967 Acad Sin 38:

968 Wu P-L, V. Rao K, Su C-H, et al (2002) Phenanthroindolizidine alkaloids and their cytotoxicity from the
 969 leaves of *Ficus septica*. Heterocycles 57:2401–2408. doi: [https://doi.org/10.3987/COM-02-](https://doi.org/10.3987/COM-02-9615)
 970 9615

971 Xiang H, Chen J (2004) Interspecific variation of plant traits associated with resistance to herbivory
 972 among four species of *Ficus* (Moraceae). Ann Bot 94:377–384. doi:
 973 <https://doi.org/10.1093/aob/mch153>

974 Yap VA, Loong B-J, Ting K-N, et al (2015) Hispidacine, an unusual 8,4'-oxyneolignan-alkaloid with
 975 vasorelaxant activity, and hispiloscine, an antiproliferative phenanthroindolizidine alkaloid,
 976 from *Ficus hispida* Linn. Phytochemistry 109:96–102. doi:
 977 <https://doi.org/10.1016/j.phytochem.2014.10.032>

978 Yap VA, Qazzaz ME, Raja VJ, et al (2016) Fistulopsines A and B antiproliferative septicine-type
 979 alkaloids from *Ficus fistulosa*. Phytochem Lett 15:136–141. doi:
 980 <https://doi.org/10.1016/j.phytol.2015.12.007>

981 Yarosh EA, Nikonov GK (1973) The coumarins of the genus *Ficus*. Chem Nat Compd 9:255–256. doi:
 982 <https://doi.org/10.1007/BF00563357>

983 Yoshihara T, Sogawa K, Pathak MD, et al (1980) Oxalic acid as a sucking inhibitor of the brown
 984 planthopper in rice (Delphacidae, Homoptera). Entomol Exp Appl 27:149–155. doi:
 985 <http://doi.org/10.1111/j.1570-7458.1980.tb02959.x>

986 Zangerl AR, Berenbaum MR (1990) Furanocoumarin induction in wild parsnip - Genetics and
 987 populational variation. Ecology 71:1933–1940. doi: <https://doi.org/10.2307/1937601>

988 Zaynoun ST, Aftimos BG, Ali LA, et al (1984) *Ficus carica*; isolation and quantification of the
 989 photoactive components. Contact Dermatitis 11:21–25. doi: [https://doi.org/10.1111/j.1600-](https://doi.org/10.1111/j.1600-0536.1984.tb00164.x)
 990 [0536.1984.tb00164.x](https://doi.org/10.1111/j.1600-0536.1984.tb00164.x)

991 Zhu-Salzman K, Luthe DS, Felton GW (2008) Arthropod-inducible proteins: broad spectrum defenses
 992 against multiple herbivores. Plant Physiol 146:852–858. doi:
 993 <https://doi.org/10.1104/pp.107.112177>

994 Zumwalt JG, Neal JJ (1993) Cytochromes P450 from *Papilio polyxenes*: adaptations to host plant
 995 allelochemicals. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 106:111–118. doi:
 996 [https://doi.org/10.1016/0742-8413\(93\)90261-I](https://doi.org/10.1016/0742-8413(93)90261-I)

997