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

3

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12

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15

16

17 **Abstract**

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

28

## 29 I- Introduction

30

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

36

37 The most popular *Ficus* species is *F.carica*, a deciduous tree that can reach a height of two to five  
38 metres and is frequently referred to as fig tree. *F. carica* is thought to be native to the Middle East  
39 and to be one of the earliest cultivated fruit trees, but it can currently be found in many warm  
40 regions worldwide (Bonamonte et al. 2010). Because its fruits – the common figs – are consumed in  
41 many ways (fresh, dried, and processed) and are rich in minerals, vitamins and fibres, *F. carica* has  
42 become an important crop and is mostly cultivated in the Mediterranean region (Solomon et al.  
43 2006; Oliveira et al. 2009). Indeed, world fig production was approximately one million tons in 2016,  
44 and more than half of this production was in Turkey, Egypt and Algeria (FAO 2016).

45

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

59

60

## 61 II- Physical defences: tough and mineralized leaves

62

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

70

### 71 **Calcium oxalate crystals**

72 Calcium oxalate represents the most common form of plant solid mineralization (Korth et al. 2006;  
73 Bauer et al. 2011). This mineral forms intra- or extracellular crystals in most plant tissues, but its  
74 deposition occurs mostly in the vacuole of crystal idioblasts (Foster 1956; Franceschi and Nakata  
75 2005; Bauer et al. 2011). Idioblasts are isolated cells that differ from their neighbouring tissue  
76 elements in their form, size, content and/or cell wall structure. They can be found in any plant part  
77 and include a wide variety of cells (Foster 1956). In plants, in addition to their calcium regulation  
78 function and their metal detoxification role, calcium oxalate crystals are involved in defence (Finley  
79 1999; Franceschi and Nakata 2005; Pierantoni et al. 2018). These crystals establish a physical barrier  
80 (Ruiz et al. 2002; Hudgins et al. 2003), deter pests and herbivorous mammal feeding (Yoshihara et al.  
81 1980; Ward et al. 1997), and are responsible for abrading the mouthparts of chewing insects (Korth  
82 et al. 2006). These crystals may therefore be complementary to plant toughness in a defence  
83 strategy (Finley 1999). In *Ficus*, a potential reduction in pest attacks has been reported among these  
84 defensive roles (Xiang and Chen 2004; Sosnovsky 2016).

85

86 Calcium oxalate crystals are usually deposited in lines parallel to the major veins of *Ficus* leaves.  
87 These crystals can therefore be found along the vein bundle, the phloem or the ground tissue  
88 supporting the veins (Pierantoni et al. 2018). Depending on the *Ficus* species and the leaf localization,  
89 calcium oxalate crystals are mainly described as prismatic crystals restricted to the bundle sheath  
90 and druses located in the mesophyll and bundle sheath (Wu and Kuo-Huang 1997; Pierantoni et al.  
91 2018). However, this is not a general rule: for example, calcium oxalate crystals are observed in  
92 *F.virgata* in the form of druses located in the bundle sheath and the palisade mesophyll layers but  
93 also as crystal sands in the abaxial epidermis of the leaves. In *F.elastica*, prismatic crystals are  
94 restricted to the bundle sheath, but needle-shaped crystals have also been reported in the epidermis  
95 of leaves (Wu and Kuo-Huang 1997). In *F.carica*, calcium oxalate is mainly found in the phloem in the

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

98

### 99 **Amorphous calcium carbonates (cystoliths)**

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

113

114 Cystoliths are observed in lithocysts, which are specialized idioblastic cells. Lithocysts often contain a  
115 silicified stalk that can remain inside the cell or protrude out of it, and these stalks vary in size (Wu  
116 and Kuo-Huang 1997; Gal et al. 2010; Bauer et al. 2011; Ummu-Hani and Noraini 2013; Pierantoni et  
117 al. 2018). Cystoliths are involved in light harvesting (Gal et al. 2012) and might provide mechanical  
118 properties that are still unclear (Smith and Watt 1986; Bauer et al. 2011; Pierantoni et al. 2018).  
119 Therefore, it might be hypothesized that the calcium content and silica stalk of cystoliths can  
120 contribute to leaf roughness. Several types of lithocysts have been described in the *Ficus* genus: hair-  
121 like lithocysts located in uniseriate epidermis (*F. religiosa*) and papillate lithocysts located in  
122 multiseriate epidermis (*F. virgata*) (Wu and Kuo-Huang 1997). In *F. carica*, spherical lithocysts have  
123 been found adjacent to the abaxial epidermis. Such lithocysts contain a stalk that protrudes outside  
124 of the cell wall and forms a spiked appendage on the surface of the leaf (Fig. 1B) (Mamoucha et al.  
125 2016).

126

### 127 **Silica**

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

130 is known to be involved in several physiological processes such as physical support and plant  
131 resistance to biotic and abiotic stresses (Bauer et al. 2011; Gautam et al. 2016; Strömberg et al. 2016;  
132 Pierantoni et al. 2018). Indeed, since polymerized silica is amongst the hardest materials found in  
133 plants, high silica contents are correlated with increased mechanical protection (Hunt et al. 2008).  
134 Silica deposits may also deter insect and mammal herbivores by increasing plant abrasiveness  
135 (Massey et al. 2007a, b) and abrading herbivore mouthparts (Massey and Hartley 2009; Müller et al.  
136 2014).

137

138 In plant leaves, silica is mainly found in the sheath cells of vascular bundles and in epidermal cells  
139 (Bauer et al. 2011). It can be deposited within or on cells as particles or microscopic bodies called  
140 phytoliths, be incorporated into the cell wall or form silicified cells and tissue sections (Prychid et al.  
141 2003; Strömberg et al. 2016). However, in *Ficus* leaves, silica is mainly located in the cell wall, non-  
142 glandular trichomes and stalks of the cystoliths, but this localization is not restricted since the  
143 abundance and exact form of silica are variable among species (Pierantoni et al. 2018). Therefore,  
144 any cell of the epidermis or the mesophyll might be partially or completely impregnated with silica,  
145 such as the guard cells in *F. prasinicarpa* (Chantarasuwan et al. 2014). In *F. carica*, silica is abundant  
146 in the adaxial side of the leaf. It is deposited in cell walls and conic trichomes (Pierantoni et al. 2018).

147

#### 148 **Crystal-containing idioblasts**

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

153

154

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

156

#### 157 **Non-glandular trichomes**

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

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

172

### 173 **Glandular trichomes**

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

185

### 186 **Laticifers**

187 In addition to trichomes, laticifers are other idioblasts that are specialized secretory structures  
188 producing defensive metabolites and enzymes. These structures have been reported in more than  
189 20000 species from 40 phylogenetically distant families of angiosperms but seem to be most  
190 common in tropical plants (Lewinsohn 1991; Konno 2011). Laticifers have been widely studied and  
191 reviewed (Hagel et al. 2008; Konno 2011), partly for their involvement in plant defence. Laticifers are  
192 elongated living cells that form a complex ramified network that follows the veins throughout the  
193 plant and contain a fluid called latex (Dussourd and Eisner 1987; Farrell et al. 1990). The anatomy and  
194 features of laticifers are highly variable among species: laticifers can be articulated or non-articulated  
195 (Fig. 1G) and secrete latex to a greater or lesser extent (Konno 2011). In the genus *Ficus*, laticifers are  
196 non-articulated and multinucleated. These structures appear during the embryonic stage from single  
197 cells that elongate and branch without cell division during plant growth (Dussourd and Denno 1991;  
198 Hagel et al. 2008; Konno 2011). In the leaves of *F. carica*, the laticifers are located in the mesophyll,

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

203  
204 Latex is a cytoplasmic fluid that contains normal plant cell organelles (Kim et al. 2003) and numerous  
205 chemical compounds, but its exact composition is highly variable among species (Hagel et al. 2008)  
206 and plant parts (Kitajima et al. 2018). As latex is stored under pressure in the laticifers, it constitutes  
207 an economic mobile defence mechanism. Due to the internal pressure of laticifers, any rupture leads  
208 to the quick mobilization and delivery of a large amount of latex at the site of the injury. As a  
209 consequence, even if the total amount of latex produced by the plant is low, the herbivores that  
210 attack the plant are immediately exposed to a high quantity of defensive fluid, which is vastly greater  
211 than the very small amount initially present at this location (Dussourd and Eisner 1987; Farrell et al.  
212 1990; Dussourd and Denno 1991; Konno et al. 2004; Agrawal and Konno 2009; Konno 2011). In some  
213 plants such as *Cryptostegia grandiflora*, latex can even be transported over a distance of 70 cm  
214 (Buttery and Boatman 1976).

215  
216 When exposed to air, latex becomes sticky and acts as a physical defence by forming a barrier,  
217 closing wounds, immobilizing insects' mouthparts and even gluing and trapping small insects (Farrell  
218 et al. 1990; Dussourd and Denno 1994; Konno et al. 2004; Konno 2011). This stickiness is due to  
219 rubber, a terpenoid commonly synthesized in laticifers that is also responsible for the colour of the  
220 latex (Kim et al. 2003; Konno 2011). Latex is also involved in chemical defence and has toxic effects: it  
221 contains a high diversity of compounds such as irritant proteins and toxic secondary metabolites, the  
222 average concentration of which is often higher than that in the rest of the leaf. As a striking example  
223 of the defensive role of latex, it has been shown that the leaves of *F. virgata* exhibit strong toxic  
224 effects against some lepidopteran larvae but lose their toxicity when the latex is washed off (Konno  
225 et al. 2004). For *F. carica*, hundreds of metabolites and more than fifty proteins have been found in  
226 latexes from different parts of the plant, with many of these compounds having defensive effects  
227 (Kitajima et al. 2018).

228  
229

#### 230 **IV- *Ficus* defensive metabolites and proteins**

231



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

237

### 238 **Specialized metabolites**

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

244

### 245 **Alkaloids**

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

262

### 263 **Terpenoids**

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

267 *carica*, whereas monoterpenes accumulate almost exclusively in fruits (Oliveira et al. 2010b). The  
268 most striking example of a *Ficus* defensive terpenoid is rubber, a cis-1,4-isoprene polymer  
269 responsible for latex stickiness (Konno 2011). *Ficus* steroids might play a role in defence, since they  
270 have antiproliferative properties. For example, 6-O-acyl- $\beta$ -D-glucosyl- $\beta$ -sitosterol, isolated from *F.*  
271 *carica* latex, showed *in vitro* inhibitory effects against the proliferation of several cancer cell lines  
272 (Rubnov et al. 2001). Ojo et al. (2014) reported the presence of cardenolides, a subclass of cardiac  
273 steroids (Roberts et al. 2016), in the leaves of *F. asperifolia*. Although the activity of *Ficus*-specific  
274 cardenolides has not yet been tested, cardiac steroids are well known for their high toxicity to  
275 animals (Roberts et al. 2016). Additionally, cardenolides from the milkweed latexes cause toxic  
276 symptoms in caterpillars such as regurgitation, convulsions and hours to days of unresponsiveness  
277 (Dussourd and Hoyle 2000; Konno 2011). Triterpenoids such as calotropenyl acetate,  
278 methylmaslinate and lupeol acetate are additional examples of molecules involved in defence. These  
279 triterpenoids have been described in *F. carica* leaves and display irritant properties when applied to  
280 mouse ears (Saeed and Sabir 2002). Several volatile terpenoids found in *Ficus* were shown to have  
281 defensive bioactivities in other plants. For instance, Oliveira et al. (2010b) showed that the major  
282 sesquiterpenes present in *F. carica* leaves were germacrene D,  $\beta$ -caryophyllene and  $\tau$ -elemene, while  
283 the main monoterpenes in *F. carica* fruits were limonene and menthol.  $\beta$ -caryophyllene has  
284 antibacterial properties (Kim et al. 2008) while limonene is known for its insecticidal (Hebeish et al.  
285 2008) and antibacterial activities (Skalicka-Wozniak et al. 2009; Oliveira et al. 2010b).

286

## 287 **Phenolics**

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

301 oxygen species (Berenbaum 2002), can inhibit cytochrome P450s (Neal and Wu 1994; He et al. 1998;  
302 Koenigs and Trager 1998) and, when excited by UV, can bind to macromolecules and cause various  
303 reactions leading to mutations or cell death (Dardalhon et al. 1998). Psoralen and bergapten are the  
304 main furanocoumarins reported in *F. carica* (Takahashi et al. 2014, 2017; Wang et al. 2017) but they  
305 are also among the major phenolics reported in *Ficus* leaves (Oliveira et al. 2012). According to  
306 Mamoucha et al. (2016), these metabolites are mainly located around the nerves containing laticifer  
307 cells and the glandular trichomes. The defensive effects of furanocoumarins have rarely been studied  
308 in *Ficus*, but two studies described the nematicidal effect of psoralen and bergapten extracted from  
309 *F. carica* leaves (Liu et al. 2011; Guo et al. 2016) characterized by inhibition of the activities of two  
310 important digestive enzymes (amylase and cellulase) in the pine wood nematode (Guo et al. 2016).  
311 Finally, it has long been known that fig trees have photosensitizing action and can cause contact  
312 dermatitis (Pathak et al. 1962) because of the furanocoumarins they contain (Bonamonte et al.  
313 2010). However, despite this knowledge, various *Ficus* parts are still used in traditional medicine in  
314 attempts to cure skin diseases, which regularly cause severe injuries (Bonamonte et al. 2010; Son et  
315 al. 2017). Recently, psoralic acid-glucoside (PAG, a glycosylated derivative of psoralen) was also  
316 detected in large amounts in fig (Takahashi et al. 2014, 2017; Wang et al. 2017), while its biosynthesis  
317 and physiological functions are still unknown.

318

### 319 **Defensive proteins**

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

325

### 326 **Proteases**

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

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

339 To a lesser extent, *F. carica* latexes also contain other proteases such as subtilases (Kitajima et al.  
340 2018). To the best of our knowledge, the roles of the subtilases found in *Ficus* have never been  
341 studied but plant subtilases are involved in many processes, including defence; see Schaller et al.  
342 (2018) for a review of plant subtilase activities.

343

#### 344 **Protease inhibitors**

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

354

#### 355 **Oxidases**

356 Peroxidases and polyphenol oxidases are common enzymes present in several plant families,  
357 including Moraceae (Konno 2011). When ingested by insects, they can oxidize compounds present in  
358 the insect gut, leading to the production of reactive species that can disrupt the arthropod redox  
359 status, damage some molecules, or make some amino acids unavailable as nutrients (Felton et al.  
360 1992; Felton and Gatehouse 1996; Zhu-Salzman et al. 2008; Konno 2011). Some plant oxidases are  
361 therefore considered defence proteins with potential antinutritive effects, but direct evidence for  
362 antiherbivore effects is still scarce (Zhu-Salzman et al. 2008; Sethi et al. 2009; Konno 2011).  
363 Peroxidases have been reported in the latex of several *Ficus* species, such as *F. sycomorus* (Mohamed  
364 et al. 2011), *F. glabrata* (Kon and Whitaker 1965) and *F. carica*. In *F. carica*, peroxidases are  
365 associated with rubber particles (Kim et al. 2003). Polyphenol oxidases have been reported only in  
366 the latex of *F. elastica*, but their activity was correlated with the rate of latex coagulation (Wahler et  
367 al. 2009). *Ficus* oxidases might therefore impact latex stickiness.

368

#### 369 **Chitinases**

370 Chitinases have been detected in the latex of *F. carica* (Kitajima et al. 2018) and *F. microcarpa* (Taira  
371 et al. 2005). Plant chitinases are well known for their antifungal effects because of their ability to  
372 degrade fungal cell walls and subsequently inhibit fungal growth (Schlumbaum et al. 1986; Mauch et  
373 al. 1988; Roberts and Selitrennikoff 1988; Melchers et al. 1994). These enzymes may also display  
374 insecticidal activities, as exemplified by the study of Lawrence and Novak, who reported that a poplar  
375 chitinase delayed insect development (Lawrence and Novak 2006). In *Ficus*, the only defensive effect  
376 of chitinases that has been described is a strong antifungal activity in *F. microcarpa* latex (Taira et al.  
377 2005).

378

### 379 **Other pathogenesis-related proteins (PRs)**

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

387

### 388 **Other protein families**

389 In addition to these proteins, other miscellaneous enzymes such as lectins and phosphatases have  
390 been detected in *F. carica* latexes (Kitajima et al. 2018) and might be involved in plant defence.  
391 Lectins are carbohydrate-binding proteins present in the latexes of plants from several families  
392 (Konno 2011). These enzymes have deleterious effects against herbivores and might interfere with  
393 viruses, bacteria or fungi (Peumans and Van Damme 1995; Van Damme et al. 1998; Macedo et al.  
394 2015). Phosphatases are less studied (Lynn and Clevette-Radford 1987; Konno 2011), but  
395 phosphatase activity has been correlated with retarded development and mortality in several insect  
396 species (Liu et al. 2005). The study of these enzymes might therefore be relevant.

397

398

## 399 **V- Modulation of *Ficus* defence mechanisms**

400

### 401 **Genotype**

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

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

409

#### 410 **Seasonal variations and plant age**

411 In *Ficus*, organs are unequally defended throughout the year, but it is sometimes difficult to  
412 distinguish the effects of season from the effects of the age and developmental stage of the plant.  
413 For example, it has been shown that in *F. carica* leaves and latex, the furanocoumarin content is  
414 higher in spring and summer than in autumn (Zaynoun et al. 1984), suggesting that these variations  
415 are linked to seasonal effects. On their side, Marrelli and collaborators compared the chemical  
416 content of *F. carica* fruits harvested in June, July and September, which clearly corresponded to  
417 different fig phenological stages. These authors found that the total amount of phenolics increased  
418 with fig ripening, while the furanocoumarin and pyranocoumarin content decreased (Marrelli et al.  
419 2012). These observations were consistent with those in the older study of Zaynoun et al., but  
420 Marrelli et al. assumed that the difference was due to ripening. In another study, the chemical  
421 composition of *F. carica* leaves, bark and woody parts was shown to vary across months, and these  
422 variations were found to be specific to each organ and compound (Marrelli et al. 2014). In addition to  
423 chemical differences, differences in the amount of latex occur: more latex is exuded from young *Ficus*  
424 organs (shoots, leaves and petioles) than from older ones (Konno 2011). Finally, it is likely that both  
425 seasonality and plant development contribute to the modulation of *Ficus* defence and that their  
426 actions are combined.

427

#### 428 **Inducible defence mechanisms**

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

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

445  
446 Other factors might also modulate *Ficus* defences, but data covering this topic are scarce. To the best  
447 of our knowledge, the only relevant study on this topic reported the impact of phytohormone  
448 application (jasmonic acid, salicylic acid or abscisic acid), drought and cold on the expression levels of  
449 a chitinase, a peroxidase and a trypsin inhibitor in *F. carica* leaves (Kim et al. 2003). Jasmonic acid  
450 enhanced the expression of all three enzymes, whereas salicylic acid and abscisic acid elicited only  
451 the trypsin inhibitor and peroxidase, respectively.

452  
453 In addition, drought induced the expression of peroxidase but decreased the expression of the others  
454 two proteins while cold treatment decreased the expression of the three proteins (Kim et al. 2003).

455

456

## 457 **VI- Evolution of *Ficus* defence mechanisms**

458

### 459 **Evolution and coevolution**

460 Members of *Ficus* are the targets of a number of generalist and specialist insects (Basset and  
461 Novotny 1999; Novotny et al. 2010; Volf et al. 2018). Basset and Novotny identified more than 300  
462 leaf-chewing insect species and more than 400 sap-sucking insect species on 15 *Ficus* species from  
463 Papua New Guinea (Basset and Novotny 1999). It has been proposed that the defence types affecting  
464 the generalist insects are often toxic chemicals leading to their increased mortality (Koricheva et al.  
465 2004; Volf et al. 2015). This assumption is reasonable, especially for *Ficus* polyphenols, which are  
466 negatively correlated with the generalist herbivore communities (Volf et al. 2018). However, in this  
467 warfare between insects and plants, some specialist insects have developed novel enzymatic  
468 mechanisms to disable these chemical weapons (Koricheva et al. 2004; Volf et al. 2015, 2018). In this  
469 context, plants adopt different strategies to protect themselves, such as pyramiding chemical  
470 diversity and mechanical defences. In this way, reducing the feeding efficiency of insects and  
471 increasing the time needed for them to feed result in a longer exposure to toxic metabolites and

472 natural enemies and, in turn increased insect mortality (Koricheva et al. 2004; Volf et al. 2015, 2018).  
473 In 2018, Volf and collaborators investigated the relationships between *Ficus* pests and various  
474 defence traits of *Ficus*, *i.e.*, trichomes (length and density), chemical content and diversity (alkaloids,  
475 polyphenols and triterpenes) and polyphenol oxidative / cysteine protease activities using 19 Papua  
476 New Guinean *Ficus* species. The authors discovered significant correlations between several *Ficus*  
477 traits and insect community structures. For instance, they showed that the trichome length, alkaloid  
478 diversity, and protease and polyphenol oxidative activities were correlated with the overall  
479 community structure, while trichome density was only correlated with choreutids. The selective  
480 pressure exerted by some pest attacks might therefore explain why some *Ficus* traits were  
481 phylogenetically conserved and others escalated or diverged (Volf et al. 2018). However, in this arms  
482 race, plants are not the only ones that have established alternative strategies. To overcome the  
483 toxicity of latex, some insects found that when cutting some canal branches, they induced latex  
484 emission and freed the distal tissues from a certain amount of the toxic liquid (Dussourd and Denno  
485 1991; Konno 2011), making those distal tissues safer for consumption. Such a strategy has not been  
486 studied in detail in *Ficus* but has been deeply discussed for other latex-producing plants (Dussourd  
487 2017).

488

#### 489 **Defence mechanism distribution on *Ficus* plants**

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



507 2018). For furanocoumarin, the levels are 500 to 1000 times lower in the inflorescences than in  
508 leaves (Oliveira et al. 2009). Lignification is a physical defence structure that provides mechanical  
509 protection. Therefore, trunk, bark and other woody parts are less likely than other parts to be  
510 damaged by herbivorous insects. However, lignified parts are defended by chemical mechanisms that  
511 seem to cover a broad spectrum of bioaggressors. For example, the main defensive protein found in  
512 *F. carica* trunk latex is ficin, which is effective against insects and fungi. Moreover, trunk latex  
513 contains the largest amounts of chitinases, acid phosphatases and PR proteins (Kitajima et al. 2018),  
514 which are described as antifungal, antibacterial, insecticidal, nematocidal and antiviral molecules.

515

516

## 517 **VII- Conclusion**

518

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

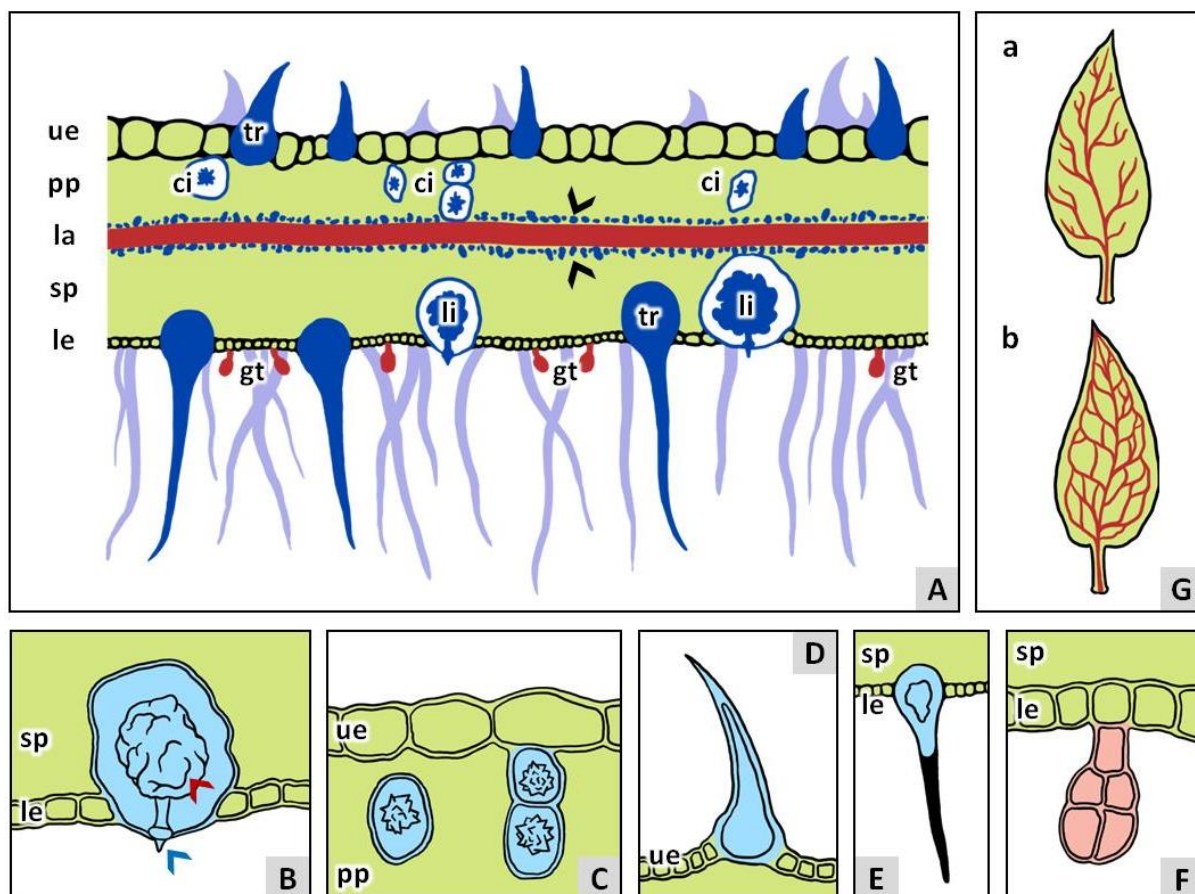
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533

534 **Table 1: Secondary metabolites found in *Ficus* (ND = No data in *Ficus*)**535 <sup>1</sup> 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 <sup>1</sup>	(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 <sup>1</sup>	(Al-Khdhairawi et al. 2017)
Furoquinoline alkaloids	Skimmianine	<i>F. coronata</i>	ND <sup>1</sup>	(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 <sup>1</sup>	(Ahmad et al. 2011; Oliveira et al. 2012; Jaina et al. 2013)
	Steroids	6-O-acyl- $\beta$ -D-glucosyl- $\beta$ -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 <sup>1</sup>	(Athnacios et al. 1962; Chen et al. 2010; Oliveira et al. 2012; Marrelli et al. 2012)
Cardiac steroids (cardenolides)		<i>F. asperifolia</i>	ND <sup>1</sup>	(Konno 2011; Ojo et al. 2014)	

	<b>Rubber</b> (cis-1,4-isoprene polymer)		<i>Ficus</i>	Latex stickiness	(Konno 2011)
	<b>Monoterpenes, sesquiterpenes, norisoprenoids, esters, etc.</b>		<i>F. asperifolia</i> , <i>F. benghalensis</i> , <i>F. carica</i> , <i>F. hispida</i>	ND <sup>1</sup>	(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	<b>Phenolic acids</b>	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 <sup>1</sup>	
	<b>Flavonoids (Flavones, flavonols, flavonones, bengalenosides, catechin, anthocyanidins)</b>	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)
	<b>Tannins and phlobatannins</b>		<i>F. asperifolia</i>	ND <sup>1</sup>	(Ojo et al., 2014)
	<b>Simple coumarins</b>		<i>F. coronata</i> , <i>F. nervosa</i> , <i>F. ramentacea</i>	ND <sup>1</sup>	(Yarosh and Nikonov 1973; Chen et al. 2010; Smyth et al. 2012)
	<b>Pyranocoumarins</b>	3-hydroxyxanthyletin	<i>F. nervosa</i>	Antimycobacterial	(Chen et al. 2010)
		Others	<i>F. carica</i> , <i>F. nervosa</i>	ND <sup>1</sup>	(Chen et al. 2010; Marrelli et al. 2012)
	<b>Furanocoumarins</b>	Psoralen Bergapten	<i>F. carica</i> , <i>F. coronata</i> , <i>F. palmata</i> , <i>F. salicifolia</i> , <i>F. sycomorus</i>	Nematicidal <sup>1</sup>	(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 <sup>1</sup>	(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)



536

537 **Fig. 1A-G Schematic representation of some defensive structures described in *F. carica* leaves**

538 **(Mamoucha et al. 2016; Pierantoni et al. 2018).** The mineralized structures are in blue, and the

539 secretory structures are in red. **(A)** Cross-section from a *F. carica* leaf. Druses and prismatic crystals of

540 calcium oxalate (black arrowheads) are deposited parallel to a vein that hosts a laticifer (la). Spherical

541 lithocysts (li) appear in the lower epidermis (le) and extend into the spongy parenchyma (sp). Crystal

542 idioblasts (ci) containing spiny crystals appear in the palisade parenchyma (pp). Non-glandular

543 trichomes (tr) can be silicified and form on surface of both the upper (ue) and lower (le) epidermises.

544 Glandular trichomes (gt) form only under the nerves. **(B)** Detail of a lithocyst that contains a cystolith

545 (red arrowhead) and a spike (blue arrowhead) that protrudes outside the cell wall of the leaf. **(C)**

546 Detail of some crystal idioblasts located in the palisade parenchyma; they contain spiny crystals of

547 indeterminate nature and might appear in pairs. **(D)** Detail of a conic silicified trichome (non-

548 glandular) that forms on the upper epidermis. **(E)** Detail of a long hairy silicified trichome (non-

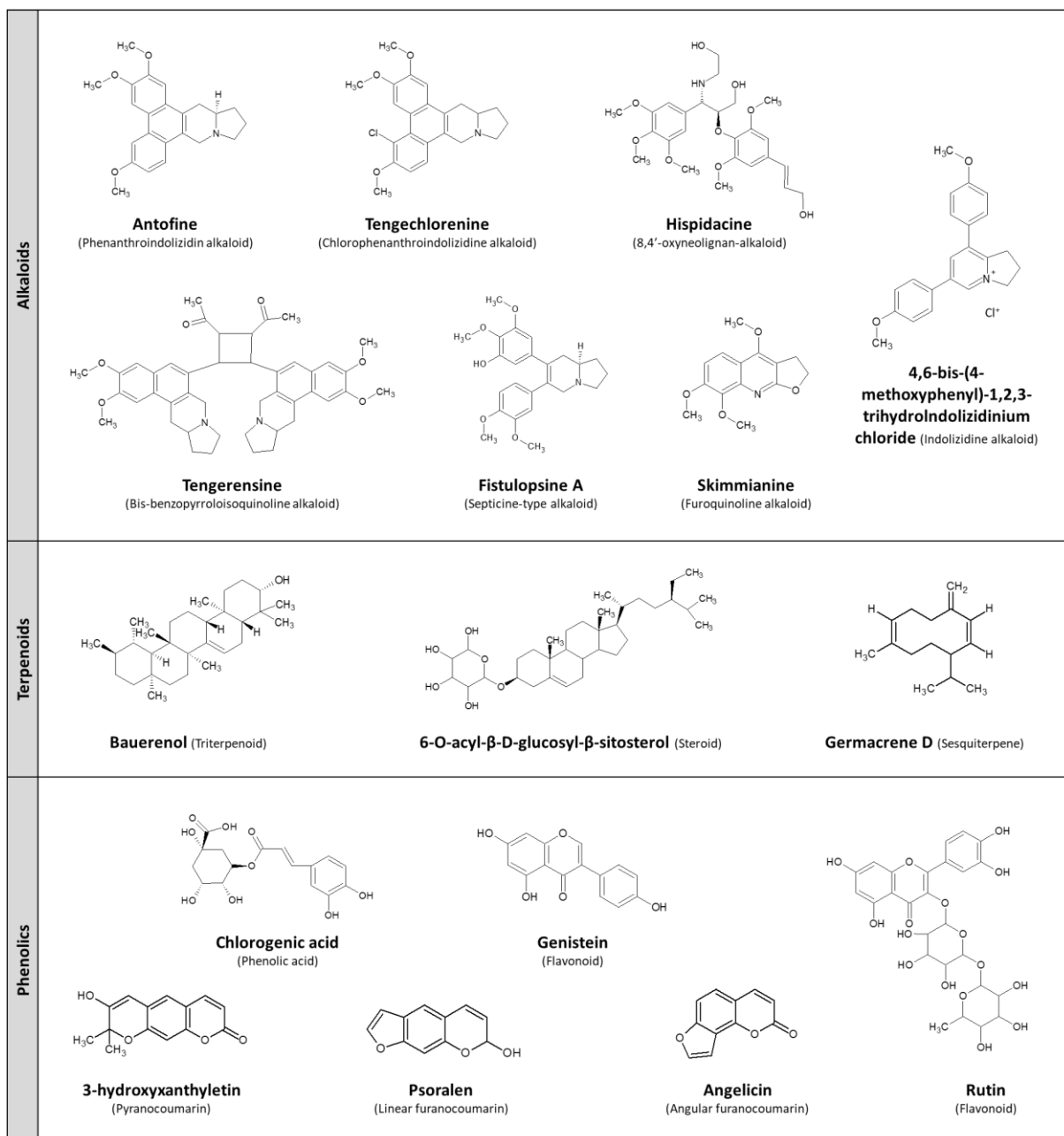
549 glandular) that forms on the lower epidermis. **(F)** Detail of a capitate secretive trichome (glandular)

550 that forms under the nerves; it has one single-celled stalk and a four-celled head that accumulates

551 metabolites in the form of dark granules. **(G)** Anatomy of the laticifers. Non-articulated laticifers (a)

552 have tree-like shapes. Articulated laticifers (b) merge to form a structure with (anastomosing

553 laticifers) or without loops (non-anastomosing laticifers). *Ficus* laticifers are non-articulated.



554

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

556

557

558

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560

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