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1 **Slipping through the cracks: Challenges and prospects for investigating fungal plant disease**
2 **complexes**

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11 [epidemiology-and-management-of-plant-disease-complexes](https://www.sciencedirect.com/journal/crop-protection/about/call-for-papers#detection-epidemiology-and-management-of-plant-disease-complexes)

12 **Highlights**

13 1- Disease caused by complexes of fungal phytopathogens are common but often unsuspected

14 2- Symptoms are often insufficient to discriminate between members of a complex

15 3- Complexes often encompass one dominant species and a variable cohort of accompanying species

16 4- Disease complex composition and dynamics are driven by intrinsic and extrinsic factors

17 5- Interactions between members of the complex govern severity and are understudied

18 **Abstract**

19 Plant pathogens frequently do not operate alone when causing diseases. Since the infection process
20 is affected by the interaction between members of the parasite community, this question asks about
21 the efficiency of disease control strategies which are usually tailored to manage only one microbial
22 pathogen at a time. Fungi and oomycetes are among plant disease's most prevalent and damaging
23 causal agents. With the increasing ability to distinguish individual species, thanks to new high-

24 throughput molecular tools, numerous diseases once attributed to a single species are now recognized
25 as being caused by complexes of fungal phytopathogens. How should we approach the study of these
26 complexes? What tools and methodologies are needed to characterize them and decipher their
27 functional interactions? How can we understand and master the drivers of these coinfections and their
28 dynamics under field conditions? Here, we review the current literature on fungal disease complexes
29 to define their commonalities and address some of the current challenges regarding the identification
30 of preferential associations among fungal species indicative of a disease complex and its community
31 dynamics and regulation. This review highlights that fungal species complexes are highly dynamic at
32 geographic and temporal scales and that human actions contributed to the dissemination of new
33 members of species complexes worldwide and to disequilibrium within species complexes, often
34 resulting in more damaging diseases. This review also points to the generally insufficient (or lacking)
35 knowledge of the diversity, dynamics, and functioning of fungal disease complexes, and the risks linked
36 with inappropriate management strategies focusing on only one dominant member of the complex.

37 **Keywords:** plant disease complexes, microbial cohorts, fungal taxonomy, epidemiology, diagnostic,
38 plant-fungal interactions

39

40 Introduction

41 *In natura*, plants are subjected to multiple biotic stresses including interactions with other
42 organisms (microbes, other plants, insects, mammals) that may have beneficial or detrimental effects.
43 They face these multiple interactions sequentially or concomitantly (Atkinson and Urwin, 2012). Many
44 plant diseases, long considered as single disease caused by a single pathogen are now recognized as
45 syndromes, where members of a complex act together, but sometimes also in sequence. This is
46 particularly the case when the disease is caused by closely related species and/or when their individual
47 symptoms may be easily confused in the field (Fitt et al., 2006; Chang et al., 2016; **Table 1**).

48 In the literature, "species complex" may have different meanings ranging from the complexity
49 of taxonomic dissection of what a species name encompasses (taxonomic species complex) to the so-
50 called "complex diseases" in which many species are found associated with the disease (co-
51 occurrence) without knowing which are the causative agent(s) and which are the species which are
52 always present without a role in the disease, or which take advantage of the symptoms as a secondary
53 invader. Other cases, such as the banana Sigatoka disease complex or the wheat leaf blotches, regard
54 a series of species that are associated with a disease with dynamics of replacement occurring with
55 time or as a function of geographic expansion of the host or/and pathogen. Thus, species complexes
56 associated with plant diseases may be broadly categorized into three types: (i) disease complexes in
57 which species co-exist on the plant with different ecological niches and thus are unlikely to interact
58 physically, (ii) disease complexes in which association between different species is the rule, but the
59 participation of each species to disease symptoms and severity is unclear, and (iii) actual disease
60 complexes, in which at least minimal evidence exists on the implication of more than one species to
61 the disease severity. This paper will focus on species complexes of fungi or/and oomycetes belonging
62 to the third category in which the association between species exists or is systematic (but may vary
63 according to environmental conditions) and for which the involvement of each species in the
64 development of the symptoms has been demonstrated. We will not consider the cases of random

65 coinfections or coinfections with multiple genotypes of the same species, which have been reviewed
66 in detail elsewhere (Lamichhane and Venturi, 2015; Tollenaere et al., 2016; Dutt et al., 2022).

67 Despite – or because of – these difficulties, until now, the study of plants and their biotic environment,
68 including epidemiological investigations, is usually done on a strictly pairwise basis (one plant/one
69 pathogen species). In the most extreme cases each member of the pair is restricted to a single
70 genotype, pathotype, biotype, or even individual (strain), overlooking both intraspecific diversity but
71 also all possible interactions between multiple coexisting species. This is (slowly) changing with the
72 knowledge that one plant may often be simultaneously infected by more than one pathogen
73 (Tollenaere et al., 2016; Dutt et al., 2022), but considering multiple interactions in plant pathology
74 remains in its infancy. In the case of microbes that may either be neutral (e.g., endophytes),
75 commensals, or pathogens, the succession or concomitant infections and their effect on the
76 elaboration of the disease begin to be studied at a broader scale with the tools of ecology, each
77 compartment, from the leaf to the field being studied as a whole ecosystem (Borer et al., 2016;
78 Halliday et al., 2017). With these approaches, it is possible to disentangle how different co-infecting
79 pathogens with different trophic modes will share (or compete for) trophic resources (O'Keeffe et al.,
80 2021), how the sequence of infection will favor or be detrimental to other pathogens (Halliday et al.,
81 2020) and how the plant will respond to multiple infections and have its defense reactions either
82 mobilized or suppressed to favor other pathogens (Newton et al., 2010). While the succession of
83 individual diseases with different trophic modes may be dissected experimentally, because each
84 pathogen can be individually manipulated, co-infections, if systematic in the wild, do not mean a
85 constant association between species to cause the disease. This imposes a close monitoring of the
86 timing of infections and requires sophisticated experimental protocols.

87 In this review, we will try to identify the challenges firstly linked to the identification of species
88 complexes and the contribution of each species to the disease. Knowledge of epidemiology and its
89 variation with changing contexts, disease symptomatology, and disease incidence/severity in different

90 contexts stresses the need to develop accurate tools to identify species of disease complexes. Such
91 tools are instrumental in characterizing inter-organism interactions *in vitro* and *in planta* to eventually
92 have a broader understanding of the interactions and the disease outcome. In the last part of the
93 paper, we will then try to define what is a fungal disease complex and what commonalities between
94 them highlight missing information that must be investigated in the future for better control of
95 diseases due to complexes.

96 **1- Recognizing fungal species complexes: from species to symptoms and back again**

97 **1.1. Species complexes: What are we talking about?**

98 For a long time, most plant diseases were believed to be caused by a single species, mainly when
99 the causal agents were complex to discriminate morphologically or through symptoms. The advent of
100 sophisticated molecular tools, notably derived from genomic sequences, (sometimes) allowed to
101 refine and elaborate a more precise fungal taxonomy, which in turn allowed the recognition that many
102 plant diseases are caused by species complexes acting together and including a wide range of
103 fungal/oomycete plant pathogens. **Table 1** and **Supplementary Table S1**, which update and expand on
104 the work published by Lamichhane and Venturi (2015) list a non-exhaustive list of plant diseases due
105 to fungal species complex.

106 **1.2. Are symptoms good enough?**

107 Disease assessment usually begins with the traditional visual scouting for symptoms.
108 Symptoms are host expressions of infection by a pathogen, along with the development of pathogen
109 structures following the success of infection. Symptoms are naturally shaped by environmental
110 conditions, pathogen genotypes, plant genotypes and their physiological status. Thus, identifying
111 pathogenic species has always been a challenge for plant pathologists with observations of a
112 symptomatic state in the host that can range between typical to atypical and undiagnosable.
113 Historically, when the existence of species complexes was unsuspected, variation in symptoms, or

114 atypical symptoms, was not only attributed to environmental factors and/or plant genotypes but also
115 to different pathogenicity groups in the fungal species. For example, in the case of stem canker of
116 crucifers, before the recognition and formal renaming of (at least) two species causing the disease, the
117 discrimination was between “highly virulent” and “slightly virulent” strains of *Leptosphaeria maculans*,
118 or between “aggressive” and “non-aggressive” strains (Pound, 1947; Koch et al., 1989). Similarly, the
119 major grapevine trunk disease, Esca, has over the years been classified in the literature as either a
120 complex single disease (single syndrome or multiple syndromes; Surico, 2009) or a complex of
121 diseases. More recent papers suggest that five different syndromes known should be considered as
122 different symptoms of the same disease (Lecomte et al., 2024).

123 Another issue in recognizing the existence of disease complexes is the difficulty in
124 discriminating symptoms that may be due to different species, especially when they occur on the same
125 infected host. Among the multiple examples listed in **Table 1**, symptoms caused by different *Fusarium*
126 species, *Colletotrichum* species, or *Erysiphe* species are often indistinguishable. An extreme case is the
127 sooty blotch and fly-speck complex of apple, in which more than 100 species cause similar symptoms
128 due to their very peculiar ectophytic lifestyle (Gleason et al., 2019). In some cases, such as the mango
129 malformation disease, different *Fusarium* species cause identical symptoms in different parts of the
130 world, and the disease's principal (or unique) causal agent differs between continents. In contrast, the
131 disease name is unchanged (Freeman et al., 2014) (**Supplementary Table S1**). In some instances, one
132 of the members of the complex may cause only mild symptoms (or have a mostly endophytic behavior)
133 that may be overlooked when a more aggressive species expresses more extensive symptoms. This is
134 the case between two members of the oak powdery mildew complex, with *Erysiphe alphitoides*
135 causing highly sporulating lesions. At the same time, symptoms and sporulation of *E. hypophylla* are
136 subtler, leading to a likely underestimation of this species in the complex (Desprez-Lousteau et al.,
137 2018). Sampling the most typical or pronounced symptoms in the field can thus lead to a bias in
138 estimating the frequency of the different species, notably cryptic ones, associated with a given disease.

139 Finally, the last level of complexity is when too many species are associated with a disease,
140 can be re-isolated from the diseased part of the plant, and induce symptoms in controlled conditions.
141 This may be partly due to taxonomic complexity and difficulty in discriminating the different species
142 in some genera (see below), but this is common in soilborne diseases such as root rots in many plant
143 species with numerous *Fusarium*, *Pythium*, and/or *Rhizoctonia* species involved (**Supplementary Table**
144 **S1**). This is also observed with *Colletotrichum* diseases such as the coffee berry disease (Nguyen et al.,
145 2010). Also, the description of many species associated with the disease may be a bias for
146 understudied pathosystems, or for those that only received recent attention, such as cranberry fruit
147 rot (Wood et al., 2023) (**Supplementary Table S1**). In these cases, more studies might be needed to
148 reduce the number of candidate pathogens to those actually causing the disease. This is also important
149 for wood diseases, such as trunk grapevine diseases, in which several endophytic/latent species live
150 for months or years within plant tissues and may turn pathogens under certain stress conditions
151 (Bertsch et al., 2013). The long persistence and slow development of the symptoms favor such
152 cooccurrences, which make the detection of the primary pathogen elusive; indeed, no significant
153 difference is observed between the fungal communities that inhabit apparently healthy and visibly
154 diseased individual plants, and the primary pathogen may have remained latent for years before
155 causing the actual symptoms. In the case of Esca disease, mature vineyards (10 years or more) are
156 generally affected by white rot caused by Basidiomycetes such as *Fomitiporia mediterranea*, which
157 modifies the disease expression (Fisher and Gonzales Garcia, 2015). A last level of complexity is when
158 a single species may cause different symptoms depending on the plant organs infected (Larignon,
159 2016).

160 **1.3. Fungal species: What's in a name?**

161 Fungal taxonomy has always been complex and confusing due to few differentiating morphological
162 characters, upon which the differentiation used to be made, the large proportion of asexual species,
163 and the existence of different names for sexual vs. asexual stage (Jayawardena et al., 2021). Massive

164 taxonomical revision led to numerous species name changes in fungi due to reinforcement in species
165 circumscriptions, along with increased complexity uncovered within some genera with the advent of
166 genomics and sophisticated identification tools (for a review on the incidence of taxonomic confusion
167 on disease control, see Jayawardena et al., 2021). In the case of fungal phytopathogens, control and
168 management strategies may vary for the fungal species, and accurate identification and nomenclature
169 for each species are essential prerequisites. The changes of names, most often associated with a
170 reclassification of the target species through the use of more appropriate molecular markers, lead to
171 changes in familiar names of plant pathogenic fungi, complicating the work of plant pathologists.
172 These nomenclature changes are often not discussed with the community, building up an additional
173 level of confusion when a new name is proposed by taxonomists, but not used by the applied or
174 research community (for example, see the *Leptosphaeria/Plenodomus* dispute) or even strongly
175 rejected by the research community (for example, see the *Fusarium solani/Neocosmospora* dispute;
176 Geiser et al., 2021). Older naming of *Colletotrichum* spp. became obsolete with sequencing data,
177 resulting in decades of publications to which it is now difficult to refer. This definitely can introduce
178 confusion in both the appropriate taxonomic uses and the understanding of pathosystems. One
179 striking example is the *Ascochyta* blight complex of pea, which, until 2007, was referred to as
180 anthracnose instead of *Ascochyta* due to a translation problem. One species of the complex has
181 furthermore undergone two name changes over three years: the sexual form, named *Mycosphaerella*
182 *pinodes* until 2007, has seen its name changed to *Didymella pinodes* (Peever et al., 2007) and later to
183 *Peyronellae pinodes* (Aveskamp et al., 2010; Farr et al., 2010). Other prominent examples include: (i)
184 *Ganoderma* species causing root-rot disease of *Acacia*, for which a large number of synonyms exist
185 due to narrow or poorly defined species concepts, that led to propose a 10-year moratorium on the
186 description of new *Ganoderma* species (Glen et al., 2009); (ii) *Teratosphaeria* on Eucalyptus and the
187 lack of consensus on the most appropriate anamorph genus, due the confused phylogeny of the genus
188 *Coniothyrium* (Crous et al., 2009); (iii) *Phoma* with more than 3,000 described species, which is

189 polyphyletic with unclear species boundaries in terms of morphological and molecular identification
190 (Deb et al., 2020).

191 Three more examples, largely present in **Supplementary Table S1**, illustrate the various issues
192 linked to taxonomic assignment of fungal species and complexes. The genus *Colletotrichum* has been
193 adequately termed “a catalog of confusion” due to its perpetually changing taxonomy and the
194 similarity of symptoms between different species (Hyde et al., 2009a, 2009b; Cannon et al., 2012;
195 Dowling et al., 2020). The advent of sequencing and molecular identification revealed that these
196 species, along with other species of *Colletotrichum*, are actually “taxonomic species complexes”
197 composed of numerous diverse and distinct species. As of 2021, 257 species are listed within
198 *Colletotrichum* and grouped into 15 species complexes (Talhinhas and Baroncelli, 2021).

199 The genus *Rhizoctonia* is a highly divergent group of sterile basidiomycetes, which includes
200 several economically crucial root-infecting pathogens. Discrimination between 'groups' was first based
201 on the characterization of the cell nuclear condition (multinucleate, binucleate, and uninucleate) and
202 the ability of hyphae to anastomose with tester isolates of designated anastomosis groups (AGs). One
203 of the most common and damaging group is *R. solani*, which is distributed worldwide, has an extensive
204 host range and induces a large diversity of symptoms. It exhibits a large variability in morphology,
205 pathogenicity, and biology, leading to discriminations into 14 somatically AGs, which are non-
206 interbreeding populations with distinct evolutionary origins, and more and more studies suggest
207 *Rhizoctonia* (particularly *R. solani*) are indeed large fungal complexes composed of numerous
208 genetically distinct groups with disparate life histories (Ajayi-Oyetunde and Bradley, 2018).

209 *Fusarium* comprises a large number (estimated to be more than 300 species) of phylogenetically
210 diverse and ubiquitous fungi with a complex and unsettled taxonomy. With the advent of molecular
211 phylogenies, phytopathogenic *Fusarium* were roughly grouped into four taxonomic species
212 complexes : (i) the *Fusarium fujikuroi* species complex inducing Bakanae disease of rice, ear rot of
213 maize, pitch canker of pine, etc.; (ii) the *Fusarium graminearum* species complex inducing Fusarium

214 head blight of wheat and barley, comprising at least 16 phylogenetically distinct species; (iii) the *F.*
215 *oxysporum* species complex causing vascular wilts; and (iv) the *F. solani* species complex causing foot
216 and root rot of diverse hosts and estimated to comprise at least 60 phylogenetically distinct species
217 (Aoki et al., 2014).

218 Taken together, these examples illustrate that confusion within some taxonomic species
219 complexes is due to (i) different levels of taxonomic resolution, (ii) difficulties in naming the actual
220 species causing the disease, (iii) difficulties in mining the literature or the sequences databases with
221 incorrect naming's (or incorrect assignation of sequence data to one or several species).

222 **1.4. Identification and detection tools**

223 Notwithstanding taxonomic complexities, accurate diagnosis of a plant disease requires precise
224 knowledge of the pedoclimatic conditions under which it develops, the stage of the plant at which it
225 can be observed, knowledge of the resistance present in the host plant, and information on the
226 sources of inoculum. However, a cultivated plant has to cope with several infections and multiple
227 infections can blur the whole picture and compromise disease identifications.

228 The development of image-based web and mobile apps to help diagnose plant diseases such
229 as those present on the INRAE portal e-phytia (<https://ephytia.inra.fr/en/Home/index>) or
230 complementary innovative participatory sensing platforms, as done for automated plant identification
231 through the PI@ntNet experience; Joly et al. (2016), constitutes a significant breakthrough but remains
232 limited in the case of symptom similarities between distinct diseases or multiple infections.
233 Experienced experts with field knowledge of the disease, able to decide on equivocal symptoms or on
234 photographs that may be challenging to interpret, is an invaluable resource, but on the verge of
235 extinction. All of these tools rely on access to accurate sources : while it is relatively easy to access
236 photo banks and descriptions of the biological and epidemiological cycles of the causal agents for
237 widespread or severe diseases (e.g., the American Phytopathological Society website;

238 <https://www.apsnet.org/Pages/default.aspx>), it is incomparably more difficult for diseases of minor
239 importance, of limited distribution, or emerging threats.

240 The development of molecular techniques often led scientists to use DNA barcode sequences
241 as a new way to circumvent the taxonomic expertise needed to identify fungal species morphologically
242 (Schenk et al., 2012; Crandal et al., 2020; Massart et al., 2022). However, studies in which causal agents
243 are inferred from sequence data alone may hamper the ability of the research community to properly
244 identify causative agents and produce confusion or debatable outcomes lasting for decades (Somera
245 and Mazzola, 2022). Ideally, symptom recognition should be coupled with isolation of the associated
246 species and, whenever possible, fulfillment of Koch's postulates. Unfortunately, classical isolation
247 techniques on selective or semi-selective media may often not yield any causal agents due to
248 intractability or (much) higher growth rate of other fungi/oomycetes (typically *Fusarium* or *Pythium*
249 species).

250 While identification and detection are essential, there is still a need to check that the isolated
251 species are involved in the disease, alone or collectively. This is not trivial, since identification of
252 species associated with a disease using molecular techniques does not mean that the species is a
253 causal agent. For example, in the case of the Apple Replant Disease, DNA sequencing technologies
254 indicated the importance of the association of *Fusarium* species to the disease. However, this was
255 debated, and a recent review stated that "the claim that *Fusarium* spp. are key pathogens associated
256 with Apple Replant Disease in China is a recurring inaccurate statement reported in many of these
257 studies. The statement regarding *Fusarium* functioning as an ARD pathogen is primarily based upon
258 sequence-based studies in which relative abundances of *Fusarium* spp. in replant soils from the Bohai
259 Bay and Loess Plateau region were positively correlated with disease severity in growth tests with
260 apple seedlings. Thus far, however, the evidence in support of the role of *Fusarium* as an ARD-causing
261 agent in China remains inconclusive" (Somera and Mazzola, 2022). In the case of disease complexes,
262 the role of each species as a causative agent is often challenging to disentangle and fulfilling Koch's

263 postulates¹ is often elusive for example when some species of the complex cannot be cultured or set
264 the disease in controlled conditions (Evans, 1976). This has been widely investigated in medical
265 sciences where cohorts of microbes are commonly found to cause disease (Balmer and Tanner, 2011)
266 and for which other routes to demonstrate the involvement of one species in the disease have been
267 proposed, including (i) community sequencing to identify all members of the complex; (ii)
268 development of computational models of microbial interactions to assess if pathogens are sufficient
269 or necessary for disease development; (iii) target culturing of putative causative agents, and (iv)
270 pathological tests of both primary pathogens and mixes of species (Byrd and Segre, 2016).

271 This underscores the importance of coupling these approaches with molecular diagnostics-
272 based on barcode sequences or whole genome sequencing (Schneider and Collmer, 2010), but also to
273 set up tools to monitor the dynamics of disease establishment, particularly in the case of simultaneous
274 infections (e.g., combining spore traps with real-time qPCR to identify the potential pathogens at play
275 or using the Droplet digital PCR (ddPCR) technique in the case of low microbial inoculum levels; see
276 Smiley et al., 2016 and Chatterton et al., 2023 for examples regarding soilborne pathogen
277 communities). Expanding this line of approach requires a systematic update of sequence and
278 taxonomic databases, to ensure that reference sequences are attributed to the correct current
279 species. In many cases (and mainly, but not exclusively, taxonomic species complex), the naming
280 corresponding to the sequence was initially incorrect or has become obsolete because of taxonomic
281 changes (Hofstetter et al., 2019). As mentioned by Hofstetter et al. (2019): "Apart from the fact that
282 poor taxon coverage in public depositories remains the principal impediment for successful species
283 identification, it can be deplored that even very recent fungal sequence deposits in GenBank involve
284 an uncomfortably high number of misidentifications or errors with associated metadata."

¹ (i) the organism must be consistently associated with the lesions of the disease; (ii) the organism must be isolated from the lesions and grown in pure culture; (iii) the organism from pure culture must be re-inoculated into the healthy host and must cause the same disease as was initially observed; (iv) the organism must be re-isolated into culture and shown to be identical to the organism isolated initially (Ross and Woodward, 2016).

285 **2- Dynamics of species complexes**

286 **2.1. Dominant species**

287 Species complexes are usually highly dynamic at all spatial and temporal scales, as can be shown
288 by differences in the composition and prevalence of species in many parasitic complexes
289 **(Supplementary Table S1)**. At the geographic scale, species complexes can range from those in which
290 the disease is caused by different cohorts of pathogens in different environments (e.g., the postharvest
291 kiwifruit decay; Huang et al., 2023), to diseases in which the causal agents are only episodically found
292 together (e.g., the stem canker of Eucalyptus for which the *Teratosphaeria gauchensis* and *T. zuluensis*
293 species complex are only associated in Uganda; Aylward et al., 2019), to those for which the same
294 species are always associated whichever the location (e.g. *Ascochyta blight* on pea, caused by a
295 complex involving at least three related pathogen species found associated together worldwide;
296 Punithalingam and Gibson, 1976; Le May et al., 2009). In all cases, species complexes are characterized
297 by the existence of one (more rarely two or three) dominant species **(Table 1)**. This is the case for *F.*
298 *graminearum* in Fusarium head blight of wheat (Backhouse, 2014), *Pseudocercospora musae* for
299 Sigatoka disease of banana (Esguerra et al., 2014), or *P. pinodes* for Ascochyta blight (Le May et al.,
300 2009). However, the dominant species may vary in different parts of the world, as found for the *L.*
301 *maculans-L. biglobosa* species complex dominated by *L. maculans* everywhere except in China (Deng
302 et al., 2023), the leaf blotch diseases of wheat with *Zymoseptoria tritici* dominating in many parts of
303 the world except Northern Europe, Australia or part of Asia where *Parastagonospora nodorum* is the
304 dominant species (Justesen et al., 2016), or Purple leaf blotch of leek with *Stemphylium vesicarium*
305 dominating in most parts of the world, but absent in some parts of Europe where *Alternaria porri*
306 dominates (Suheri and Price, 2001).

307 **2.2. A highly variable cohort of accompanying species**

308 While dominant species are generally identical in different parts of the world, this is less true
309 for the cohort of species making up the species complex with strong regional and continental
310 variability (**Supplementary Table S1**). This is observed for species complexes in which tens of
311 associated species are included and for simpler examples in which only two species are involved. The
312 *L. maculans*-*L. biglobosa* species complex is a textbook case since *L. maculans* is always associated with
313 *L. biglobosa*, but the (sub)species involved varies with the continent, likely due to an ancient and
314 independent geographic separation between the diverse *L. biglobosa* subspecies, that are generally
315 able to attack, though with minor damages, more crucifer species than *L. maculans* (Dilmaghani *et al.*,
316 2009; 2012). *L. maculans* being a species that expanded more recently along with the increased
317 success of oilseed rape as a cash crop, gets associated independently with the naïve *L. biglobosa* sub-
318 species (Dilmaghani *et al.*, 2012). Another example is the Ascochyta blight of pea, previously known to
319 be caused by three fungal pathogens, *A. pisi*, *P. pinodes* and *P. pinodella* (Le May *et al.*, 2009). However,
320 four additional species have recently been described associated with this disease, *P. koolunga*
321 (Davidson *et al.*, 2009) and *P. glomerata* (Tran *et al.*, 2014a) in Australia, and *P. herbarum* (Li *et al.*,
322 2011), and *Boerema exigua* var. *exigua* (Li *et al.*, 2012) in eastern Asia. All these fungi are necrotrophic,
323 generalist, and polyphagous species. In this complex, *P. koolunga* has only been observed in certain
324 plots in southern Australia (Davidson *et al.*, 2009), while *A. pisi* has rarely been detected in European
325 pea plots. In Western Australia, the pathogen population associated with the Ascochyta blight has
326 been dynamic across time and geographic location (Tran *et al.*, 2014b) to the extent that *P. koolunga*
327 can be even more virulent than *D. pinodes* and *P. pinodella* on stems and more virulent than *D. pinodes*
328 on leaves of field pea (Tran *et al.*, 2014b). In the case of Mango Malformation disease, the ubiquitous
329 dominant species, *Fusarium mangiferae*, is accompanied by diverse additional *Fusarium* species such
330 as *F. sterilihyphosum* in Brazil and South Africa, *F. mexicanum* in Mexico, *F. proliferatum* in China and
331 *F. tuiense* in Brazil (Freeman *et al.*, 2014). Regional and continental variation is the rule for species
332 complexes involving many species (e.g., up to 30). For example, in the apple replant disease, variation
333 of the complex is evident among geographic regions, between orchards within the same region, and

334 even between sites or years within the same orchard (Somera and Mazzola, 2022). At a larger
335 geographic scale, specificity also exists with, in particular, *Pythium* (now *Phytophthium*) *vexans* and
336 *Cylindrocarpon macrodidymum* that cause extensive growth reductions in apple seedlings in South
337 Africa but are rarely present associated with apple roots in North America or Europe (Somera and
338 Mazzola, 2022). In comparison, *R. solani*, *P. ultimum*, and *P. intermedium*, which are frequently
339 associated with replant disease in North America and Europe, have not yet been isolated from apple
340 roots in South Africa (Tewoldemedhin et al., 2011; Somera and Mazzola, 2022). *Alternaria japonica*
341 and *A. ethzedia* are significant components of Alternaria leaf spot of rapeseed in Australia, *A.*
342 *arborescens*, *A. hordeicola*, *A. infectoria*, *A. malvae*, *A. metachromatica*, *A. tenuissima* can also be
343 found on rapeseed (Al-Lami et al., 2020), while only four species, *Alternaria brassicae*, *A. brassicicola*,
344 *A. japonica*, *A. alternata* are found in Serbia (Blagojević et al., 2020). Eleven fungal pathogens have
345 been shown to cause fruit rot of cranberries in British Columbia (Wood et al., 2023), while only five
346 species were prevalent in Quebec fields (Conti et al., 2022). In the case of Botryosphaeria dieback of
347 grapevine (but, in general, of all grape trunk diseases; Larignon, 2016), regional differences in co-
348 occurrence patterns, among the 26 species that have been reported to be associated with this disease
349 worldwide, are also evident with *Diplodia seriata* isolated in at least ten countries, *Lasiodiplodia*
350 *theobromae* and *L. viticola* found in USA vineyards, *Botryosphaeria dothidea* and *Neofusicoccum*
351 *parvum* in Portuguese vineyards, *Diplodia mutila* and *Neofusicoccum luteum* in New Zealand vineyards
352 and *Neofusicoccum australe* in Australian vineyards (Bellée et al., 2017). Regardless of this overall
353 variability, even in diseases whose causal agents are uneasy to identify, such as the Apple Replant
354 Disease, there is often a “surprisingly consistent assemblage of pathogens” comprising of fungi,
355 oomycetes, and occasionally nematodes acting as primary causal agents (Mazzola et al., 2015).

356 **2.3. Historical shifts in dominant species**

357 Species complexes are far from static over time. There are many examples of shifts between
358 dominant species within a species complex due to the invasion of new territories, following the success

359 and dissemination of a host plant with a narrow genetic basis, changes in agronomic practices, or other
360 unresolved factors (**Table 1**). A well-documented example includes the successive shifts between *P.*
361 *nodorum* and *Z. tritici* as dominant species in the wheat blotch complex in Europe. Until the 1960s, *P.*
362 *nodorum* was the main wheat disease in north-western Europe and *Z. tritici* was considered a minor
363 pathogen unworthy of being combatted with fungicides (Fitt et al., 2006). PCR analysis of the
364 Rothamsted archived wheat samples over 160 years showed that *Z. tritici* dominated the leaf tissues
365 in the mid-19th century and after 1985, and *P. nodorum* was predominant in the mid-20th century
366 (Bearchell et al., 2005). Nowadays, *Z. tritici* has become the main pathogen in many countries (e.g.,
367 except Norway in Europe), and *P. nodorum* is often a minor component of the disease (Justesen et al.,
368 2016). The *L. maculans*-*L. biglobosa* complex is also illustrative of such temporal shifts in dominant
369 species. While there is no record of when *L. maculans* and *L. biglobosa* 'brassicae' began to co-exist in
370 Western Europe, the global spread of *L. maculans* has been documented (Fitt et al., 2006; Dilmaghani
371 et al., 2012). In Canada, only *L. biglobosa* 'canadensis' was reported before 1970. From an initial
372 establishment in Saskatchewan during the mid-1970s, *L. maculans* spread throughout Canada by the
373 late 1980s and became predominant while co-existing with *L. biglobosa*. Similarly, in Europe, there has
374 been a global eastward move of *L. maculans* to countries such as Poland, where *L. biglobosa* was the
375 dominant member of the complex. There are also some documented shifts in the Fusarium head blight
376 of wheat across the wheat-growing areas worldwide from populations dominated by *F. culmorum* and
377 *F. graminearum* to complexes dominated by *F. avenaceum* and *F. poae* (Audenaert et al., 2011).
378 Similarly, in Western Australia, the two new species identified between 1984 to 2012 to be associated
379 with the Ascochyta blight complex became prevalent over the usually dominant *P. pinodes* in specific
380 years at certain locations (Tran et al., 2014b). Finally, anthropogenic and/or long-distance
381 transportation of inoculum also contributes to changes in the prevalence of species within species
382 complexes. One prominent example is the ongoing invasion by a damaging pathogen is the Shigatoka
383 disease of banana in which three species are involved: *Pseudocercospora musae* (yellow Sigatoka), *P.*
384 *eumusae* (eumusae leaf spot), and *P. fijiensis* (black Sigatoka). *P. musae* was the most important foliar

385 fungal disease of banana until its replacement by *P. fijiensis*, adapted to more banana genotypes and
386 more aggressive and competitive than *P. musae* (Esgerra et al., 2024). *P. fijiensis* was reported for the
387 first time in Fiji in 1963 but was probably widespread in Southeast Asia and the South Pacific by that
388 time. It had spread more widely across Asia and began to appear in Africa by 1980. It spread to the
389 South American continent and the Caribbean, and it expanded more into Africa by 1999 (Esgerra et
390 al., 2024). It is postulated that the three species co-existed on bananas until changes related to the
391 genetics of the pathogens or/and exogenous factors led to the over-dominance of one species over
392 the others. A similar trend of worldwide infection was observed for stem canker of Eucalyptus, where
393 constant introductions in new countries due to global trade favored the dissemination of the
394 destructive *T. gauchensis* and *T. zuluensis* species complexes (Aylard et al., 2019). Oak and tanoak
395 mortality (Sudden oak death) which was due to invasion by a highly aggressive species, *Phytophthora*
396 *ramorum*, while sympatric species *P. nemorosa* and *P. pseudosyringae* cause similar but milder
397 symptoms (Kozonitas et al., 2017). Symptoms of *P. ramorum* infection were first noticed in 1994–1995
398 in Californian forests and European nurseries (Kozonitas et al., 2017).

399 **2.4. Yearly changes in species complex composition**

400 Dynamics of species complex composition are often apparent at the time of cropping over one
401 growing season or, for perennial plants at the scale of the plant's life. The order in which different
402 pathogens infect a plant may condition the type of interactions that will be established. Ecological
403 theory suggests that community assemblages are determined by deterministic processes, such as
404 those based on niche occupancy involving competitive interactions and more stochastic processes
405 based on dispersal processes. An extreme form of the effect of this first arrival is its postulated impact
406 on the host plant's receptivity (local/systemic increased resistance or local/systemic suppression of
407 resistance responses) through changes in the expression of its immune system (Menge and
408 Sutherland, 1987). While they may help structure the intra-host species complexes, and subsequent
409 epidemiology of the components of the disease, to date, such 'priority effects' (Fukami, 2015; Fukami

410 et al., 2016) have rarely been investigated for fungal disease complexes in the frame of differences in
411 the timing of infection within a growing season (Karvonen et al., 2009; Hoverman et al., 2013). For
412 annual crops, differences in epidemiology between different members of a species complex are likely
413 to influence the composition of the species complex at different times of cropping or on different plant
414 organs. Currently, studies mainly address the epidemiology of different diseases (e.g., Halliday et al.,
415 2017; Barbetti et al., 2021) but rarely the epidemiology of the different species complex members,
416 which may be very difficult to disentangle in many examples. Such studies also often compare
417 pathogens with different trophic modes (e.g., Halliday et al., (2017) analyzed priority effects by
418 comparing four symbionts of tall fescue: the non-pathogenic endophyte, *Epichloe coenophialum*, and
419 three pathogens: the biotroph *Puccinia coronata*, the hemibiotroph *Colletotrichum cereale* and the
420 necrotroph *Rhizoctonia solani*) while, in many cases, the different species complex members have
421 similar trophic behavior (**Supplementary Table S1**). Last, soil-dwelling pathogens are often much less
422 tractable to comparative epidemiological studies compared to air-borne pathogens.

423 In the case of the oak powdery mildew complex, the two most prevalent species, *Erysiphe*
424 *alphitoides*, and *E. quercicola* have opposite relative abundance patterns early and late in the season.
425 It is thus postulated that the coexistence of these two species on the same host may result from a
426 trade-off between overwintering (flag-shoots vs. chasmothecia) and late-season performance (Feau
427 et al., 2012). In the case of Ascochyta blight, various studies have shown that *P. pinodes* and *P.*
428 *pinodella* can develop on the same host plant while apparently occupying the same ecological niche
429 (Davidson et al., 2012). Indeed, in a study on naturally infested winter pea plots, Le May et al. (2018)
430 showed that these two pathogenic species can co-exist throughout the growing season, albeit with
431 changes in their relative prevalence. In the UK, it is suggested that *O. yallundae* infections initiated in
432 autumn may progress more quickly than infections through leaf sheaths to reach the stem in spring.
433 Fitt et al. (2006) thus suggested that epidemiological differences lead to colonizing different tissues
434 and intra-plant niche separation. The same authors made the same postulate for the *L. maculans-L.*
435 *biglobosa* complex: differences in epidemiology due to more extended maturation of pseudothecia in

436 *L. biglobosa* than *L. maculans* were suggested to be responsible for different symptoms (lower vs.
437 upper stem lesions) or ecological niches within the plants (Fitt et al., 2006). This later case was debated
438 when finding that epidemiological differences in terms of maturation and ejection of ascospores may
439 not exist in other parts of the world, such as continental Europe, or may change from one season to
440 the other. For example, Jacques et al. (2021) found no difference in the epidemiology of the two
441 species but high amounts of both *L. maculans* and *L. biglobosa* in the stem tissues at the end of the
442 growing season. In the *Alternaria* early blight/brown spot of potatoes, shifts in species prevalence can
443 be observed during the growing season: small-spored *Alternaria* species (mainly *A. arborescens* but
444 also *A. alternata*) were the most prevalent species on potato leaves over time, but the proportion of
445 the highly virulent *A. solani* increased with the progression of the disease. The authors speculated that
446 pioneer species favor subsequent infection by damaging species (Vandecasteele et al., 2018). This
447 model illustrates that even opportunists can be considered part of a disease complex whenever
448 primary infections facilitate the development of secondary infections, thus increasing the damages.

449 For perennial plants, one documented example of changes in the composition of a species
450 complex is the secondary attack by Basidiomycete white rot fungi years after the Esca disease is
451 expressed, with these fungi being an integral part of the disease (Fisher and Gonzales Garcia, 2015).

452 **2.5. Drivers of dynamics**

453 In general, two drivers explain the dynamics of species complex: (i) extrinsic factors linked to
454 the action of man on the agroecosystem (worldwide trade, use of genetic resistance in the host,
455 fungicide use), which are likely to have a long-term effect to restructure the species complexes and to
456 favor the replacement of species by others, and (ii) intrinsic factors (environmental requirements,
457 biological traits of the species, host plant receptivity, and the parasitic community composition in the
458 geographic area of cropping) which will structure local composition of the species complex and its
459 dynamics over the cropping season.

460 2.5.1. *Extrinsic factors: global trade*

461 Worldwide distribution of highly productive plant genotypes is often accompanied by
462 establishment of a more damaging species that will eventually co-exist or replace the indigenous ones.
463 This has been observed for the banana Sigatoka disease: high susceptibility and narrow genetic bases
464 of existing Cavendish cultivars towards *C. fijiensis* (along with its ability to infect all types of banana
465 genotypes) favored dramatic expansion of *C. fijiensis* to the detriment of *C. musae*, less aggressive and
466 with a restricted host range. In this model, resistant cultivars that could be used in subsistence
467 situations are available but are usually less productive or desirable than susceptible ones (Ploetz,
468 2001). Teratosphaeria stem canker has also become a significant disease of Eucalyptus species planted
469 in tropical and subtropical areas, outside their native range, for the production of wood (Aylward et
470 al., 2019). The movement of Eucalyptus germplasm from Australia to and between other countries
471 favored the multiple introductions of Eucalyptus or leaf pathogens that naturally co-exist with their
472 host in their native regions, which may have facilitated the emergence of damaging species and species
473 complexes causing canker (Aylward et al., 2019). Two *Phytophthora* species are responsible for sweet
474 chestnut ink disease, namely *Phytophthora × cambivora* and *P. cinnamomi*. Both species originate from
475 Asia and have been widely dispersed worldwide by human activity. One last example is the sudden
476 oak death that occurred in South Western America in the late 20th century, following multiple
477 introductions of *Phytophthora ramorum* (Kozonitas et al., 2017). This led to the establishment of a
478 short-term species complex with indigenous *Phytophthora* species, causing similar symptoms on oak
479 or California Bay Laurel, *P. nemorosa*, and *P. pseudosyringae* (Kozonitas et al., 2017).

480 2.5.2. *Extrinsic factors: Genetic resistance and host susceptibility*

481 Plant genetic resistance is bred to control one pathogen species and, if consisting of major-gene
482 complete resistance, will be highly efficient, at least in the short/medium term, to eradicate the target
483 species if the species' population is primarily avirulent. Resistance will be bred toward the dominant,
484 most damaging species when addressing species complexes. If obeying the ecological dogmas, one

485 expectancy is that eradicating one species will free an ecological niche for other associated fungal
486 species that will take over. In the case of disease complexes, the theory has rarely been challenged by
487 experiments in the field. One such experiment was done on *L. maculans*-*L. biglobosa* system by
488 growing two near-isogenic rapeseed genotypes differing by the presence of a novel resistance gene,
489 *Rlm11*, known to be efficient against ca. 95% of the *L. maculans* populations in France (and inefficient
490 towards *L. biglobosa*; Balesdent et al., 2013). The *L. maculans*-*L. biglobosa* system has a complex
491 parasitic cycle with leaf spotting followed by an endophytic asymptomatic colonization of the stem
492 tissues until causing a stem canker at the end of the growing season many months after the initial leaf
493 spotting occurred. Both *L. maculans* and *L. biglobosa* are present at the leaf level and colonize together
494 the tissues to eventually develop their sexual stage on stem residues (Gay et al., 2021, 2023). The
495 authors combined a field experiment with qPCR follow-up of the colonization of tissues for the whole
496 growing season. As expected, the *Rlm11* gene was efficient at the leaf level: it prevented *L. maculans*
497 from leaf spotting, the colonization of stem tissues by the fungus, and the eventual development of
498 stem canker (Jacques et al., 2021). The systematic colonization of the *Rlm11* isogenic line by *L.*
499 *biglobosa* further confirmed that the absence of *L. maculans* freed up an ecological or trophic niche to
500 benefit *L. biglobosa*. While this example is based on experimental data, there are a series of other
501 studies in which it is postulated that higher resistance (or susceptibility to one of the species of the
502 species complex) influences its composition, acting in conjunction with other environmental factors or
503 agronomical practices. For example, it is hypothesized that the historical shift between *P. nodorum*
504 and *Z. tritici* is partly attributable to increased host susceptibility to *Z. tritici* (Shaw et al., 2008). In the
505 *Ascochyta* blight model, *P. pinodes* can be favored and rendered dominant by using resistance genes.
506 For example, in the mid-1960s in Canada, *P. pinodes* became the dominant species on field pea seed
507 after introducing resistance to *A. pisi* (Tivoli and Banniza, 2007).

508 Host tissue susceptibility varies with host genotype and age (Kus et al., 2002; Al-Naimi et al.,
509 2005). This increase in resistance or susceptibility according to the age of the plant or host tissue has
510 been demonstrated for several pathosystems (Miller, 1983; Reuveni et al., 1986; Pretorius et al., 1988;

511 Roumen et al., 1992; Kus et al., 2002) and may differentially affect members of a species complex. For
512 example, Le May et al. (2009) showed that *P. pinodella* species developed more readily on senescent
513 or aged organs. Thus, early attack of plants by *P. pinodes* weakens plant defenses and accelerates
514 senescence processes, enabling *P. pinodella* to establish itself more rapidly.

515 2.5.3. Extrinsic factors: sensitivity to fungicides

516 While there are very few examples of the disequilibrium that a major R gene will induce in a
517 species complex, there are a plethora of examples of how fungicides affect the composition of the
518 species complex and contribute, or contributed to changes in species prevalence or global composition
519 of the complex (**Table 1**). This may be due to intrinsic differences in susceptibility towards one
520 molecule between species of the complex or the rise of resistant strains in one species following
521 massive treatment with fungicides. Among the examples of intrinsic susceptibility to fungicides, the
522 relative abundance of the two eyespot pathogens, *Oculimacula acuformis*, and *O. yallundae* has
523 changed on multiple occasions from 1986 to 2000 in Western Europe, which was attributed to
524 differential fungicide selection (Parnell et al., 2008). *O. acuformis* was found to be less susceptible than
525 *O. yallundae* to most DMI fungicides in France and naturally resistant to triazoles, leading to a shift of
526 prevalence of *O. yallundae* to *O. acuformis* within the disease complex (Leroux et al., 2013). In contrast,
527 *O. acuformis* was more sensitive to cyprodinil than *O. yallundae*. With the registration of cyprodinil in
528 the mid-1990s, the relative abundance of *O. acuformis* began to decline in all French regions to benefit
529 *O. yallundae*. However, the authors also indicated that this phenomenon was not a general trend in
530 Europe and that factors other than fungicide use may influence the population dynamics of cereal
531 eyespot fungi (e.g., variety, crop rotation, and soil management; Leroux et al., 2012). Similarly, in the
532 case of the replacement of *P. nodorum* by *Z. tritici*, the introduction of foliar fungicides in the late
533 1970s indicated that fungicide use was associated with increased amounts of *Z. tritici*, with a very
534 small, though significant, effect (Shaw et al., 2008). In the Fusarium head blight of wheat, it is
535 documented that *F. langsethiae*, *F. avenaceum*, and *F. poae* are more resistant to the ergosterol

536 biosynthesis-inhibiting azole fungicides than *F. graminearum*, thus contributing to shifts across the
537 wheat-growing areas worldwide from populations dominated by *F. culmorum* and *F. graminearum* to
538 *F. avenaceum* and *F. poae*-dominated complexes (Audenaert et al., 2011; Tini et al., 2020). In the case
539 of leaf blotch of onion, *Stemphylium vesicarium* was considered of minor importance in the complex
540 of foliar diseases affecting onion in North America until its re-emergence around 2010 as the dominant
541 member of the complex affecting large-scale onion production (Hay et al., 2019). In these regions, the
542 insensitivity of *S. vesicarium* towards QoI, carboxamide, and anilinopyrimidine fungicides is
543 widespread and accounts for its re-emergence (Hay et al., 2019).

544 One interesting point that reflects what may be obtained with resistance genes is when one
545 species has been eradicated by fungicide use in conventional farms and thus is not recognized as a
546 member of a disease complex. At the same time, it is a complete component of the disease complex
547 in organic farming: this has been reported by Conti et al. (2022) when comparing the cranberry fruit
548 rot species complex pathogens between organic and conventional farming. The authors found that
549 one species, *Monilia oxycocci*, was exclusively found in organic farms, indicating that fungicides had
550 an intense and persistent effect on this species among all others of the species complex. Similarly,
551 *Botrytis pseudocinerea* is hypersensitive to most fungicides but naturally resistant to fenhexamid; it
552 thus may dominate over *B. cinerea* in organic farms but not under conventional cropping systems
553 (Plesken et al., 2015). While it may be a significant cause of grey mold in some crop species, it often
554 appears to be replaced by *B. cinerea* after fungicide treatments. For example, it was frequently found
555 in spring on cultivated strawberries but was primarily replaced by *B. cinerea* following fungicide
556 applications (Plesken et al., 2015). While less studied, different species assemblages are also
557 encountered for the Sooty blotch and fly-speck complex of apple between organic and conventional
558 orchards (Gleason et al., 2019).

559 *2.5.4. Intrinsic factors: environmental requirements*

560 Thermal (and other environment-related) biology in fungi requires complex experiments to
561 identify conducive temperature/humidity/light conditions for growth, sporulation, and sexual
562 reproduction (e.g., Boixel et al., 2022). Such experiments have rarely been done to compare the
563 environmental requirements of members of disease complexes, and when this information is
564 available, it mainly derives from general field observations (**Supplementary Table S1**). In the case of
565 sudden oak death caused by a complex of three *Phytophthora* species, field surveys showed that, while
566 they occupy the same niche in plants, and despite the broader geographic distribution of *P. nemorosa*
567 compared to the destructive *P. ramorum*, *P. nemorosa* exhibits a narrower ecological amplitude than
568 *P. ramorum*. *P. nemorosa* can persist in a *P. ramorum*-dominated region but only in some sites when
569 overall conditions are dry (Kozanitas et al., 2017). When conditions became more humid and favorable
570 to sporulation of the asexual *P. ramorum*, an increase in *P. ramorum* and a decrease in *P. nemorosa*
571 were observed, even in fields where *P. nemorosa* was previously dominant (Kozanitas et al., 2017). In
572 the case of post flowering stalk rot of maize, temperature may be one factor that determines the
573 composition of the species complex: hot and dry growing conditions promote systemic infection of
574 maize by *F. verticillioides*, especially before or during pollination and predispose the plants to infection
575 by *F. moniliforme* and *F. proliferatum* at maize silking stage (Khokar et al., 2014). For ear rot of maize,
576 hot and dry weather conditions are optimal for *F. verticillioides* and *F. temperatum*, while *F.*
577 *graminearum* prefers cool and wet conditions (Pfordt et al., 2020). In the case of Apple Replant
578 disease, two critical environmental factors affect the composition of the species complex and their
579 interactions and activity: soil temperature and moisture. Contrasting seasonal patterns have been
580 shown to influence the relative dominance of *Rhizoctonia* vs. *Pythium* spp. (Somera and Mazzola,
581 2021). In cooler, water-logged soil conditions, *Phytophthora* spp. significantly contributes to disease
582 development. Fluctuations in the abundance and species composition of *Pythium* spp. also depend on
583 the season: in experiments on alfalfa, root isolation frequencies of *P. ultimum* and *P. irregulare* were
584 significantly positively correlated with rainfall. In contrast, the frequency of isolation of *P. sylvaticum*
585 from root tissue was negatively correlated with rainfall and positively correlated with soil temperature.

586 These analyses were done in alfalfa fields, but the three *Pythium* species investigated are also highly
587 relevant to the Apple replant disease complex (Somera and Mazzola, 2021). One unusual effect of
588 human-modified environment is found with the historical replacement of *P. nodorum* by *Z. tritici* as a
589 significant wheat disease. While numerous factors have been postulated (fungicide sensitivity, host
590 susceptibility) to be responsible for the shift (which is likely multi factorial), Chandramohan and Shaw
591 (2013) correlated it with a significant increase in sulphate and sulphurous acid in the atmosphere
592 during the industrial era. To go further, statistical analyses based on field disease observation or fungal
593 biomass data (qPCR or ddPCR) could be used to explore the patterns of occurrence of the different
594 species associated with a disease complex and, in particular, the dominance of one or other group of
595 fungi as a function of the agro-climatic variables (extrapolated to any point in space from
596 meteorological data via spatial interpolation) that best predict abundances to be explained through
597 random forest machine learning algorithms (Breiman, 2001). In the case of Fusarium head blight of
598 wheat, this method could be used to explore the ratio between the species associated with the disease
599 (*Fusarium* spp. and *Microdochium* spp.) in particular concerning thermal conditions, cumulative
600 rainfall and global radiation over flowering as the presence of *Microdochium* spp. on wheat ears is
601 generally associated with low global radiation (*Microdochium nivale* being sensitive to UV-A radiation,
602 which corresponds to 95% of UV radiation; Newsham et al., 1997), relatively cool temperatures, and
603 frequent short rain showers (Xu et al., 2008).

604 2.5.5. Intrinsic factors: life history traits

605 Life history traits are defined as the biological characteristics of organisms that structure their
606 life histories (Begon et al., 2006). Assessing the life-history traits of the different species involved in
607 disease complexes is essential for understanding the biological and epidemiological differences at the
608 origin of their (possible) niche differentiation and coexistence. Coexistence implies the mutual
609 exploitation of at least one shared limited resource necessary for development and reproduction by
610 at least two individuals, often leading to the establishment of competitive interactions (Amarasekare

611 et al., 2004; Mideo et al., 2008). The exploitation of the shared resource by one individual leads to a
612 reduction in the availability of this resource for the other individuals, which may result in a change in
613 the host's exploitation strategy and affect epidemic dynamics, but also act on the evolution of the life
614 history traits of the individuals (Susi et al., 2015; Tollenaere et al., 2016; Abdullah et al., 2017). For
615 example, Montarry et al. (2008) used the theory of 'r' (productivity: individuals develop in an
616 environment with high availability of resources and little competition) and 'K' strategies (efficiency:
617 individuals develop in an environment with limited resource availability and intense competition) to
618 assess the coexistence of different groups of strains of *Erysiphe necator*, the agent responsible for
619 grapevine downy mildew. Dutt et al. (2020) showed that this may also apply to species complexes, and
620 observed three different life history strategies in cryptic species of the pea Ascochyta blight complex:
621 pioneer colonisers, scavengers, and intermediates (renamed opportunists in Dutt et al., 2021). Most
622 *P. pinodes* isolates behaved as "pioneer colonizers," developing first and producing pycnidiospores
623 faster but in lower amounts than in the other two strategies. Part of *P. medicagenis* isolates and a few
624 *P. pinodes* isolates developed a "scavenger" strategy: they developed later and more slowly but
625 produced more pycnidiospores with fewer resources. The remaining *P. medicagenis* isolates and one
626 *P. pinodes* isolate developed an "intermediate" strategy with intermediate levels of lesion expansion
627 and spore production. This illustrates that, for species with the same trophic behavior and depending
628 on the same resources within the species complex, the life history traits are only partially species-
629 specific and that individuals within species may have different strategies. However, the existence of
630 these different strategies suggests that intermediate and scavenger strains are promoted when plant
631 tissues are already infected by *P. pinodes* strains. This was already observed in co-inoculation
632 experiments where a *P. medicagenis* lesion size increase was observed when *P. pinodes* was inoculated
633 six days earlier on the same host (Le May et al., 2009). They used the theory of "r" and "K" strategies
634 to understand how these three strategies allow the coexistence of these strains. The pioneer
635 colonizers would present an "r" type strategy and would be good colonizers of empty ecological niches,
636 with the capacity to persist in their niche over time and with weak responses to competition. By

637 contrast, scavengers would present a "K" strategy and use their strong competitive ability to develop
638 on old tissue already colonized by strains presenting one of the other strategies. In this model, it is
639 interesting to note the plasticity within the *P. pinodes* species, which may adopt the three different
640 strategies and was thus suggested by Dutt et al. (2020) to have a wider diversity of ecological niches.
641 The existence of "pioneer colonizer" species, paving the route for following ones, has also been found
642 in other models such as the Brown Apical Necrosis of walnut in which *Fusarium semitectum* and *F.*
643 *graminearum* are the most aggressive species with a pioneering role (Scotton et al., 2015) or *Alternaria*
644 diseases of Brassica or potatoes for which it is speculated that pioneer species favor subsequent
645 infection by damaging species or by opportunistic pathogens that concur to increase disease severity
646 (Vandecasteele et al., 2017; Al-Lami et al., 2020; Budde-Rodriguez et al., 2022).

647 The coexistence of two species with the same trophic mode occurs in other species complexes
648 (**Supplementary Table S1**). For example, co-occurrences of *O. yallundae* and *O. aciformis* on up to
649 56% of the stems in a survey of wheat crops in France suggested that the two-eyespot species do not
650 prevent each other from colonizing the stem, implying niche differences between them (Fitt et al.,
651 2006). However, only some systems have been investigated with this epidemiological dissection of life
652 history traits as done in the Ascochyta blight of pea species complex.

653 2.5.6. Intrinsic factors: genetic exchanges

654 Horizontal gene transfer between unrelated species (e.g., between bacteria and fungi, between
655 fungi and oomycetes, or between different fungal species) has been suggested to be important in the
656 evolution of plant-infecting fungal pathogens and to allow the interspecific transfer of pathogenicity
657 genes (Richards et al., 2011). Consequently, it is suggested to allow the generation of more damaging
658 pathogens. The mechanisms include anastomosis of cellular structures, conjugation-like transfer
659 between bacteria and yeasts, and dispensable chromosome transfer (Richards et al., 2011).
660 Theoretically, species complex of fungal species adapted on the same host species, sharing the same
661 space and ecological niche, and generally taxonomically related would expect to be situations of choice

662 to favor the rise of damaging species via genetic exchange. To date, the only case of undisputed
663 documented genetic exchange between two fungal species is the transposon-mediated transfer of
664 virulence factors (*ToxA* gene encoding a host selective toxin) between *P. nodorum* and *Pyrenophora*
665 *tritici-repentis* (Friesen et al., 2006). *P. tritici-repentis* was formerly a negligible disease of wheat, but
666 this transfer led to the rise of a damaging species that is now part of the leaf blotch diseases of wheat
667 (Justesen et al., 2016). In the case of Fusarium crown rot of wheat in Australia, *Fusarium*
668 *pseudograminearum* is the species most commonly recovered from diseased plants. Still, the three
669 species, *F. pseudograminearum*, *F. graminearum*, and *F. culmorum*, are present throughout the
670 Australian wheat growing regions and can all cause Fusarium crown rot. Comparative genomic analysis
671 indicated that the *F. pseudograminearum* genome contains pathogenicity genes homologous to genes
672 that are unevenly distributed across cereal pathogens while being absent in other fungal genomes.
673 Phylogenetic analysis supported the hypothesis of horizontal gene transfer between diverse cereal
674 pathogens (Gardiner et al., 2012). Apart from these exemplary cases, such demonstrations have rarely
675 been done between two fungal species, but are postulated for other complexes. In the case of Sigatoka
676 disease, more than 20 *Mycosphaerella* species, often only mildly virulent, are described on banana,
677 many of which can co-exist with the members of the Sigatoka disease complex on the same leaf or
678 even the same lesion (Arzanlou et al., 2007). As observed for the *P. nodorum*-*P. tritici-repentis*
679 example, it is possible that niche sharing by multiple closely related species on the same host could
680 facilitate inter-species exchange of genetic material and result in new species with altered virulence
681 patterns (Chang et al., 2016). The richness of the *C. fijiensis* genome in transposable elements would
682 favor such events (Santana et al., 2012). Similar horizontal gene transfers between related species are
683 also postulated for *Alternaria* diseases, with *A. alternata* being known to be prone to such events of
684 dispensable chromosome transfer, similar to what is known in *F. oxysporum*, increasing its
685 pathogenesis or modifying its host range (Akagi et al., 2009).

686 **3- Interactions between fungal species in species complexes**

687 3.1. Generalities

688 Pathogen species exploiting the same resource may affect each other through antagonism (one
689 pathogen has a negative effect on the development of the other, leading to rarer co-occurrence than
690 expected from individual species frequencies, for example via induction of plant disease responses,
691 competition for space or nutrients, or toxin production), or synergism (one pathogen promotes the
692 development of the other leading to more frequent co-occurrence than expected from individual
693 species frequencies due to effect on the establishment and development of later infesting species by
694 early infecting pathogens, for instance by altering host susceptibility via suppression of resistance
695 (Begon et al., 2006; Le May et al., 2009; Abdullah et al., 2017). These theoretical views, however, must
696 not ignore cases where one species is unaffected by the presence of the other, as exemplified below
697 in the *L. maculans*-*L. biglobosa* species complex.

698 Coexistence implies the exploitation by at least two individuals of at least one common limited
699 resource necessary for their development and reproduction. It necessitates that resources are shared,
700 so that local coexistence is the rule (synchronicity of production of infectious organs, massive amount
701 of inoculum, small size of plant infected organs). In this case, the presence of each organism modifies
702 the selective value and/or the size of the equilibrium population of the other (Pianka, 1981). The
703 concepts of coexistence and competition are, therefore, closely linked and can be interpreted through
704 the filter of niche theory, with competitive interactions (either exploitative, apparent, or interfering)
705 between individuals being dependent on the degree of niche breadth and overlap (MacArthur, 1972;
706 Pianka, 1981; Putman, 1986; Begon et al., 2006). Several studies have shown that the division of
707 resources imposed by the coexistence of different pathogenic individuals in multiple infections can
708 modify their host exploitation strategy and affect epidemic dynamics notably through the evolution of
709 the life history traits of individuals (Fitt et al., 2006; Susi et al., 2015; Tollenaere et al., 2016).

710 3.2. Evidence from field surveys

711 Field surveys can be carried out to define the prevalence of each species making up the complex
712 and to note the simultaneous or sequential presence of these species. However, this approach is only
713 possible for pathogenic species that are distinguishable using diagnostic visual (no confusion between
714 symptoms) and/or molecular (correct assignment of sequences) tools. Field surveys allow us to
715 identify thermal/environment preferences, differences in epidemiology, differences in susceptibility
716 to the disease, and the impact of co-occurrence on the severity of the disease. They also indicate when
717 and where the species in a complex will likely interact. Thus, in the case of disease complexes, surveys
718 allow us to determine the co-occurrence at the region, field, plant organ, and symptom levels. Co-
719 occurrence studies also must consider plant architecture (organ distribution and size), to predict if the
720 architecture (and timing of production of inoculum) will reduce the probability that pathogens with
721 the same ecological niches may share the same resources locally.

722 The joint occurrence of species together may provide hints about the existence of unknown or
723 unsuspected species complexes, which can be inferred from association analyses (Severns and Sykes,
724 2020). Severns and Sykes (2020) proposed to use indicator species analysis to estimate the strength of
725 association of one species with *a priori* groups of interests and randomization tests to evaluate the
726 probability of association. This approach was used to identify the causative agent(s) of an unknown
727 squash disease among a pool of 10,000 isolates corresponding to 15 fungal species. Following the
728 identification of seven species with the highest association scores (including pathogenic and
729 nonpathogenic species), pathogenicity tests were done with the five most common species, *F. solani*,
730 *F. oxysporum*, *F. culmorum*, *Plectosphaerella cucumerina* and *Septophoma terrestris*, and showed that
731 symptoms caused by one species alone were far less severe than in mixed pathogenicity trials (Rivedal
732 et al., 2020; Severns and Sykes, 2020). This substantiated the involvement of a species complex in a
733 disease for which no prior information existed. In rare cases, such as the oak downy mildew, detailed
734 analyses of co-occurrence at multiple nested scales have been performed (Desprez-Lousteau et al.,
735 2018).

736 3.3. Evidence from confrontations in axenic culture

737 While some information on the modes of interaction between species within a disease complex
738 could be gained by simple confrontation assays in axenic conditions, this has rarely been done. It is
739 essential to evaluate the interaction between the species under artificial conditions and without the
740 host plant. There, antagonism (direct toxicity, trophic competition) can be first suspected, considering
741 that these are artificial approaches and that the competition may not be expressed at this growth
742 stage or may be plant-mediated. The advantage of these approaches is that they allow testing of
743 various substrates for possible competition for the use of specific nutrients (e.g., iron). One of the few
744 examples is the *L. maculans*-*L. biglobosa* complex where various confrontation analyses were done:
745 analyses of germination rates in a liquid medium when conidia are mixed; confrontations on solid
746 media of GFP- and RFP-labelled isolates of *L. maculans* and *L. biglobosa*, respectively (including RNA-
747 seq analysis of gene expression); confrontation on solid medium of older cultures (Elliott et al., 2007;
748 Gay et al., 2023). When mixing conidia of both species on an axenic medium, *L. biglobosa* growth
749 overran the development of *L. maculans*, and more than 99% of the RNA-seq reads were assigned to
750 *L. biglobosa* seven days after inoculation. However, when lowering the *L. biglobosa* spore
751 concentration, RFP-*L. biglobosa* and GFP-*L. maculans* mycelia grew alongside at the same rate without
752 signs of exclusion. Last, if *L. biglobosa* was added two days after *L. maculans*, only *L. maculans*
753 developed (Gay et al., 2023). Bingol et al. (2024) also found that, during assays in axenic culture, *L.*
754 *biglobosa* inhibited the growth of *L. maculans* if it was inoculated before or simultaneously with *L.*
755 *maculans*, but not if it was inoculated after *L. maculans*. Overlay of *L. biglobosa* conidia suspensions
756 over a 13-day-old *L. maculans* colony completely abolished the germination of *L. biglobosa* (Elliott et
757 al., 2007). Similarly, in confrontation experiments, an exclusion front between the two species was
758 evident, and the hyphae from both species did not merge (Gay et al., 2023). In addition, nutrient
759 requirements were investigated for both species during axenic growth, indicating that *L. biglobosa* can
760 utilize more carbon sources than *L. maculans*. However, it is less efficient than *L. maculans* to use them
761 for its growth (Frac et al., 2022). Furthermore, the *L. biglobosa* catabolic activity peaks four days before

762 that of *L. maculans* suggesting it can deprive *L. maculans* of food if present before it (Frac et al., 2022).
763 This example illustrates that synergism or antagonism is not an intrinsic property of the system and
764 that changes in modes of interaction may occur depending on the timing of inoculation, the amount
765 of inoculum, and the age of the fungal tissues. Here, for example, it is known that *L. maculans* produces
766 a toxin, sirodesmin PL, that is toxic to *L. biglobosa* but is only produced late in the *L. maculans* growth
767 (or plant colonization) (Elliott et al., 2007), leading to a late antagonism after a long coexistence.

768 **3.4. Evidence from *in planta* plant coinfection experiments**

769 *3.4.1 How to analyze?*

770 Pathogen interaction analysis under controlled conditions has been the subject of numerous
771 publications in recent years, but they rarely (or only marginally) addressed the particularities of fungal
772 species complex pathogens of plants (Alizon et al., 2013; Tollenaere et al., 2016; Dutt et al., 2020). The
773 analysis of interactions between pathogens on young plants is an approach that makes it possible to
774 mix different inoculation protocols and conditions of co-occurrence of the pathogens studied. It is also
775 possible to study the impact of the location of competing pathogens on the plant, the sequential arrival
776 of infections, and the age of the host plant. These interactions can be studied quite precisely by
777 measuring either (i) the number of symptoms developed in the case of single/multiple infections and
778 the quantity of offspring produced and (ii) monitoring the life-history traits of pathogens in coinfection
779 (not done to date for fungal species complexes). However, specific and reliable methodologies are
780 needed to assess these components under controlled conditions. Initially developed to screen varietal
781 resistances, inoculated young plants and detached leaf systems have subsequently been adapted to
782 study interactions between several coinfecting pathogens (Andrison et al., 2023).

783 When co-inoculations with two or three species are compared with a single species, all cases
784 (reduced disease severity, increased disease severity, lack of incidence) can be observed. However,

785 the outcome of the co-infection is usually not an intrinsic property of the complex and may be modified
786 by priority effects, timing of infection, or location of infection sites.

787 3.4.2 Increased disease severity

788 One common outcome is increased disease severity when more than one species is inoculated.
789 This was found with the coinfection of *Aglaonema modestum* by *Fusarium aglaonematis* sp. nov. along
790 with *Fusarium elaeidis* (Zhang et al., 2022). In the case of kiwifruit decay, disease severity depends on
791 the relative abundance of the causal pathogenic fungal species: co-occurrence and interaction results
792 showed that the four most prevalent species (*Aspergillus japonicus*, *A. flavus*, *Botryosphaeria*
793 *dothidea*, and *Penicillium oxalium*) exhibit a positive interaction with other fungi and that *A. japonicus*
794 and *B. dothidea* exhibit a positive interaction with each other (Huang et al., 2023). In the case of Apple
795 replant disease, the disease is the combined effect of what happens when several pathogenic
796 soilborne microorganisms act together rather than individually, and the severity of the disease is
797 higher when *Ilyonectria macrodidyma* and *Pythium irregulare* are inoculated together than when each
798 is inoculated alone (Tewoldemedhin et al., 2011). More severe declines were also found when
799 inoculating grapevines with more than one species (compared to single pathogen inoculation) involved
800 in *Botryosphaeria dieback*, black foot, Esca or Petri disease (Probst et al., 2012; Sparapano et al., 2001;
801 Whitelaw-Weckert et al., 2013).

802 3.4.3 Reduced disease severity

803 There are also a series of cases where antagonism between species is observed. For example, in
804 the sheath disease of rice, an antagonistic effect of *R. solani* vs. *R. oryzae* and *R. oryzae-sativae* is
805 observed, while *R. oryzae* lowers disease incidence if inoculated before *R. solani* (Latif et al., 2022). In
806 the case of Fusarium head blight of wheat, co inoculation experiments with *F. avenaceum*, *F.*
807 *culmorum*, *F. graminearum* and *F. poae* support the existence of competitive interactions with large
808 reductions in fungal biomass but, in contrast, large increase in the amount of mycotoxins in the plant

809 tissues (Xu et al., 2007). When it comes to the chestnut ink disease, *Phytophthora cambivora*, *P.*
810 *citricola*, and *P. cactorum* have similar ecological requirements and are often found together; it is
811 postulated that they tend to exclude other *Phytophthora* species of the species complex when they
812 are together (Vettrano et al., 2005).

813 3.4.4 Complex or unresolved cases

814 In many cases, however, it is difficult to know the nature of the interaction between the species,
815 like with *Alternaria porri* and *Stemphylium vesicarium*, which often occur together in the same purple
816 leaf blotch lesion on *Allium* species (Suheri and Price, 2001). They may vary between different species
817 of the complex as found for the oak sudden death, in which co-inoculation experiments showed that
818 *P. ramorum* excludes (but only partially) *P. nemorosa*. However, there is a priority effect if *P. nemorosa*
819 arrives first. In this species complex, *P. pseudosyringae* develops a neutral coexistence with the others
820 in case of coinfection (Kozonitas et al., 2017). In the case of maize ear rot, antagonistic interactions
821 were identified between *F. subaglutinans* and *F. verticillioides*, but also a potential synergism between
822 *F. fujikoroii* and *F. verticillioides* (Logrieco et al., 2021).

823 Le May et al. (2009) used young pea seedlings to study the impact of the positioning of the
824 fungal species and their sequential inoculation on different life history traits of interest (incubation,
825 symptom extension, and latency) and showed a significant effect of the least aggressive fungal species,
826 *P. pinodella* on infection efficiency. Coinfection studies have been repeatedly done with the leaf blotch
827 pathogens of wheat, *P. nodorum*, and *Z. tritici* (and also *P. tritici-repentis*), but the results reported are
828 somewhat contradictory. Jenkins and Jones (1981) found no evidence of an interaction between *P.*
829 *nodorum* and *Z. tritici* but identified a non-significant decrease in *P. nodorum* infection on plants
830 previously inoculated with *Z. tritici*. While no interaction could be identified, the authors found a lesser
831 yield reduction when both species were inoculated together compared to individually. In contrast,
832 Harrower (1978) suggested that *Z. tritici* has a weaker colonizing ability but that it is increased when
833 the leaf tissue is already necrotic due to *P. nodorum* infection. Nolan et al. (1999) showed strong

834 interactions between *Z. tritici* and *P. nodorum* leading to increased or decreased spore production on
835 ears of wheat: *P. nodorum* inhibited *Z. tritici* if inoculated first, while *Z. tritici* stimulated *P. nodorum* if
836 inoculated first. Competitive interactions have also been reported with *P. tritici-repentis*, which can
837 antagonize *P. nodorum* in competition trials (Adee et al., 1990) and in fields with a high density of
838 wheat residues (Sutton and Vyn, 1990). Due to differences in latent period (5-8 days for *P. tritici-*
839 *repentis* vs. 3-4 weeks for *Z. tritici*), it was expected that *P. tritici-repentis* could also outcompete *Z.*
840 *tritici*, but this was not found in field conditions with *Z. tritici* constantly more prevalent than *P. tritici-*
841 *repentis* (Justensen et al., 2021). In the case of the *L. maculans-L. biglobosa* species complex,
842 coinfection tests under controlled conditions also produced contradictory data. Some authors
843 reported that pre-inoculations on the same or different leaves by *L. biglobosa* significantly reduced
844 the size of lesions caused by *L. maculans* (Chen and Fernando, 2006; Liu et al., 2006). Without analyzing
845 the interaction between the members of the species complex, the authors suggested the occurrence
846 of indirect, plant-mediated interactions between the two *Leptosphaeria* species. Other coinfection
847 studies resulted in the exclusion of *L. maculans* by *L. biglobosa*, but only if an equal amount of inoculum
848 was provided at the same place and at the same time (Gay et al., 2023). Lower amounts of spores of
849 *L. biglobosa* compared to those of *L. maculans*, or a two-day delay in the inoculation of *L. biglobosa*,
850 did not prevent expression of typical *L. maculans* symptoms, and systemic colonization of the plant by
851 *L. maculans* (Gay et al., 2023; Bingol et al., 2024).

852 3.4.5 Transcriptomic-based approaches to disentangle in planta interactions

853 In the *L. maculans-L. biglobosa* model, extensive RNA-seq studies have been done to relate fungi
854 and plant gene expression to the outcome of the tripartite interaction (Gay et al., 2023). The plant
855 expressed stress- and disease resistance-related genes very early in response to the attack by the
856 necrotroph *L. biglobosa*, while these reactions were suppressed until the development of symptoms
857 by the hemibiotroph *L. maculans* (Gay et al., 2021; 2023). When both species were inoculated
858 together, the following trends were observed: (i) the plant reacted to the mixed inoculation similarly

859 to the way it reacts to the inoculation by *L. biglobosa* alone; (ii) *L. biglobosa* sets up its pathogenicity
860 program seemingly unaffected by the presence of *L. maculans* or the plant reaction; (iii) *L. maculans*
861 is blocked very early in its transcriptomic activity and cannot set up the pathogenicity programs
862 required for endophytic colonization and subsequent tissues necrosis. In this system, as mentioned
863 above, antagonism depended on the timing and location of the inoculation. However, antagonism
864 between the two species at this early stage of colonization was suggested to be multifactorial (but did
865 not include direct toxicity between the two species). Thus, likely factors responsible for *L. maculans*
866 inhibition could include: (i) competition for trophic resources, with *L. biglobosa* being more demanding
867 on resources, (ii) creation, via the necrotrophic behavior of *L. biglobosa*, of an unsuitable plant
868 environment for the growth of *L. maculans*, (iii) direct effect of the plant resistance responses usually
869 inhibited by *L. maculans*. In this system, priority effects are not seen: *L. maculans*, in suppressing plant
870 defense responses, does not favor infection by *L. biglobosa* (Gay et al., 2023).

871 **4- Prospects for management of diseases caused by species complexes**

872 Sustainable management of fungal plant diseases is a highly desirable goal often far from
873 reaching due to conflicting socio-economic interests, scarcity of environment-friendly durable
874 solutions to control the infections (genetic resistance, biocontrol), and the tremendous adaptive
875 potential of fungi. The existence of constant coinfections, specifically of disease complexes, adds
876 additional complexity to reaching sustainable control strategies. As underlined above, species
877 complexes are complicated and sometimes blurred concepts. To date, despite the significant advances
878 in the identification of fungal species - in line with the current emphasis on community assemblages,
879 such as the plant-associated microbiota-, there are still many cases where diseases caused by species
880 complexes are not recognized as such. There are likely numerous new or understudied diseases in
881 which the involvement of a complex is still unsuspected. Also, the focus on the dominant species is
882 misleading in some cases. Scientific literature or extension websites (incl. Wikipedia) will readily
883 identify and publicize one species of the complex (as is the case of *Alternaria brassicicola*, or *A.*

884 *brassicae* for *Alternaria* leaf spot of Brassica, *Fusarium verticillioides* for maize stalk rot and ear rot,
885 *Colletotrichum kahawae* for coffee berry disease, etc.). This leads to focus disease control on the
886 dominant species, and there are numerous historical changes in equilibrium within species complexes
887 due to human actions. As underlined by Tran et al. (2014b) for *Ascochyta* blight of pea, this raises the
888 issue of “the development of effective resistance against the black spot complex, because breeding
889 programs are focused almost exclusively on resistance to *P. pinodes*, largely ignoring other major
890 pathogens in the disease complex. Furthermore, the development and deployment of effective host
891 resistance or fungicides against just one or two of the pathogens in the disease complex could radically
892 shift the makeup of the population toward pathogen species that are least challenged by the host
893 resistance or fungicides, creating an evolving black spot complex that remains ahead of breeding and
894 other management efforts”. The question of the contribution of the different members of a complex
895 to overall epidemic development and the level of crop losses observed at the end of the cycle has
896 never been fully raised. It is questionable whether it is worth targeting one member of a complex at
897 the expense of establishing an ecological imbalance between the different parasitic species that make
898 up the complex and threatening the durability of control methods.

899 For all of these reasons, there is a crucial need to identify better, dissect, and solve interactions
900 within species complexes whenever the biology of the fungi and/or the host plant allows us to do so
901 (**Fig. 1**). The first step is to identify the existence of species complexes and be able to review their
902 spatiotemporal variability, along with the intrinsically more damaging species (if any). Large-scale field
903 surveys and the use of DNA barcoding, with metagenomics tools, followed by indicator species analysis
904 (Severns and Sykes, 2020) are likely to identify the existence of unsuspected species complexes and
905 the association of species making up a given species complex in different agricultural contexts. There
906 is also a need to “predict” the relative importance of species within species complex using modeling
907 approaches as developed in medical sciences (Buffié et al., 2007; Byrd and Segre, 2016) or reduction
908 tools for studies of microbiome dynamics (Shahin et al., 2023). This will enable us to identify the
909 species of choice for the development of dedicated markers (if missing) and proceed with a

910 phytopathological validation of the involvement of the species in the disease, highlighting synergism
911 or antagonism in symptom causation. In parallel, concepts and tools developed in community ecology
912 can be transposed to plants infected by species complexes to understand the assembly, dynamics, and
913 management of within-individual host–species complex ecosystems (Rynkiewicz et al., 2015). Keeping
914 in mind that many species within disease complexes have identical or closely similar infection and host
915 exploitation feeding strategies, such tools may help to define 'functional groups' in which parasites are
916 grouped in terms of their infection characteristics (infection site, consumed resources) and how the
917 host immune system responds to their infection. This could allow predictions of one pathogen's likely
918 direct and indirect effects on another and help to determine if a fungal pathogen will successfully infect
919 a host and how that infection will affect the host condition (Rynkiewicz et al., 2015). In the same line
920 of thought, Elena et al. (2014) proposed that evolutionary game theory provides an adequate
921 theoretical framework to analyze mixed plant-virus infections and to predict the long-term evolution
922 of the mixed populations. It would be interesting to evaluate the same approaches to analyze fungal
923 infections due to species complexes.

924 Understanding the species at play necessitates experimentally investigating their interactions in
925 axenic cultures (growth characteristics, favored growth environment and nutrient requirements,
926 confrontations) and *in planta* following mixed interactions (**Fig. 1**). An exciting avenue would be
927 deciphering within-host interactions, mainly dissecting the molecular mechanisms underpinning
928 disease establishment during single and multiple plant-pathogen interactions. Indeed, the dynamics
929 of multiple pathogen infections within the complex (and its modulation by the immune system and
930 host resource) may modulate the outcome of the interactions between species composing this
931 complex. Exploiting what has been done in the *L. maculans*-*L. biglobosa* model, coupling these
932 experiments with extensive analysis of multi-partner gene expression using RNA-seq analyses is an
933 avenue of choice to understand the interactions functionally.

934 Practical strategies must then be set up to combat the diseases, with the knowledge that
935 disequilibrium in the species complexes due to human action may render the strategy obsolete (Tran
936 et al., 2014b). One favored route would be to breed varieties with resistance to different species of
937 the complex or combining both sources of resistance and architectural characteristics to condition the
938 environment in the plant canopy. Knowing that sources of resistance may be scarce, that breeding for
939 resistance in perennial plants is quite complex, and that only a few cases of resistance are identified
940 to combat at least one species of the complex (**Table 1**), such a prospect may be out of reach for most
941 systems. However, in one case at least, that of leaf blotch diseases of wheat, Justesen et al. (2021)
942 noted that if current varieties are mostly highly susceptible to *Z. tritici*, *P. nodorum*, and *P. tritici-*
943 *repentis*, some cultivars had “relatively good resistance” to both *Z. tritici* and *P. nodorum*. Only very
944 few cultivars had resistance to all three pathogens. Other approaches, such as crop associations,
945 should also be investigated. Indeed, numerous studies show that the structure and composition of
946 ecological niches can condition the potential for establishing and developing certain diseases.
947 Generating heterogeneous canopies could impact the composition and epidemic dynamics of disease
948 complexes. The development of resistance to fungicides has contributed significantly to changes in the
949 composition and prevalence of pathogenic species composing particular disease complexes (Shaw et
950 al., 2008; Leroux et al., 2012; Hay et al., 2019). As for varietal resistance, phytosanitary treatments are
951 rarely considered in studies dealing with disease complexes. In both cases, even if we sometimes have
952 “targeted pathogen” data, it is rare to find references dealing with the cross-effects of one or the other
953 of the control methods. As part of our understanding of how disease complexes work, it is essential to
954 integrate these two components (e.g., by monitoring the effectiveness of the fungicides used and
955 assessing their spectrum of action against the species composing a given disease complex), which will
956 affect the ability of pathogen species to establish and develop. Other cultural practices (e.g., the choice
957 of cultivar, the date of sowing, the sowing density, or the use or non-use of fertilizer) are also important
958 parameters of the agroecosystem that will impact the dynamics of both plant canopy and disease
959 complexes. For example, Barbetti et al. (2021) showed that changes in cultural practices impact the

960 Ascochyta blight complex composition and its impact on yield production: all the practices investigated
961 (sowing date, plant density, cultivar) influenced the prevalence and the severity of the different
962 pathogens within the complex. Last, it is essential to have a larger scale approach when tackling co-
963 occurring pathogens and disease complexes and to reason the connectivity between compartments
964 at the landscape scale (**Fig. 1**). The choice of agronomic practices applied to the crops, and also to the
965 surrounding landscape (Plantegenest et al., 2007) are critical in the constitution of the disease complex
966 infecting a crop; they drive the dynamics of inoculum reservoirs, the connectivity between hosts, and
967 hence the local disease pressure and kinetics. This implies that detailed and documented epidemic
968 survey data at the landscape scale are essential for correctly appreciating the set-up and dynamics of
969 disease complexes.

970 **5- Concluding remarks**

971 In agricultural settings and in the wild, coinfections of plants are the rule. Several review
972 papers recently addressed the issues linked to these coinfections with ecological concepts turning the
973 plant and its environment into a whole ecosystem for a cohort of pathogens (Lamichhane and Venturi,
974 2015; Rynkiewicz et al., 2015; Tollenaere et al., 2016). Except in the case of species complexes due to
975 viruses and, more rarely, bacteria (Tollenaere et al., 2016), such review papers tend to focus on multi-
976 infection by different genotypes of the same species or on different species sequentially or
977 concomitantly infecting a host, without a focus on fungi and without explicitly focusing on species
978 complexes as defined here. Here, we expanded part of the review by Lamichhane and Venturi (2015)
979 to refine the species complex concept in fungal pathogens and define commonalities between them.
980 Needless to say, only some models listed in **Table 1** really are species complexes, or that information
981 may need to be included for some of them to decide whether they are actual complexes or if their
982 perimeter is correctly defined. Notwithstanding this, our review allows us to stress a series of
983 commonalities for fungal species complexes governing the concept, from assemblage rules to
984 functioning: (i) the disease name encompasses the species complex. In other words, a species complex

985 is the causative agent of the disease, (ii) the involvement of more than one species may have been
986 unsuspected for a long time due to taxonomic complexity, identity of symptoms, lack of precise
987 identification tools, etc., (iii) most if not all species complexes are characterized by one dominant
988 species associated more or less tightly to a few or many other species, often closely related
989 taxonomically and with similar feeding strategies but (iv) the dominant species and the cohort of
990 associated species sometimes vary in different geographic locations while the disease name is
991 unchanged. (v) Species complexes are highly dynamic at all temporal scales, with documented
992 historical replacement of one prior dominant species by another, and, for some annual crops, reports
993 of variation in composition within a growing season; (vi) differences in epidemiology between
994 different members of the species complex may categorize pioneer species paving the route for the
995 following ones (including opportunistic pathogens) or to niche preferences and colonization of
996 different tissues by different members of the species complex. (vii) Anthropization of agricultural
997 settings (use of host genetic resistance against one species of the complex, differences in sensitivity to
998 fungicides between species of the complex), as well as the extension of cropping area (e.g., as it was
999 the case for banana expansion and the propagation of Sigatoka disease), strongly impact the
1000 equilibrium within species complexes. (viii) In most cases, disease control is focused on control of the
1001 dominant species (which may not always be the most aggressive), and more information is needed on
1002 how the species interact to increase or lower disease severity. (ix) As a consequence, studies
1003 investigating the biological and molecular bases of the interactions between species, either *in vitro* or
1004 *in planta*, and the plant responses to individuals vs. complexes are generally lacking and need to be
1005 more robust.

1006

1007 **Author contributions**

1008 CLM, ALB, and TR performed literature review, data acquisition and the first drafting of the manuscript.

1009 MA contributed to data acquisition. DA contributed to the drafting and writing of the manuscript. CLM,

1010 ALB and TR coordinated the overall study and the manuscript drafting. All authors read and approved
1011 the final version of the manuscript.

1012

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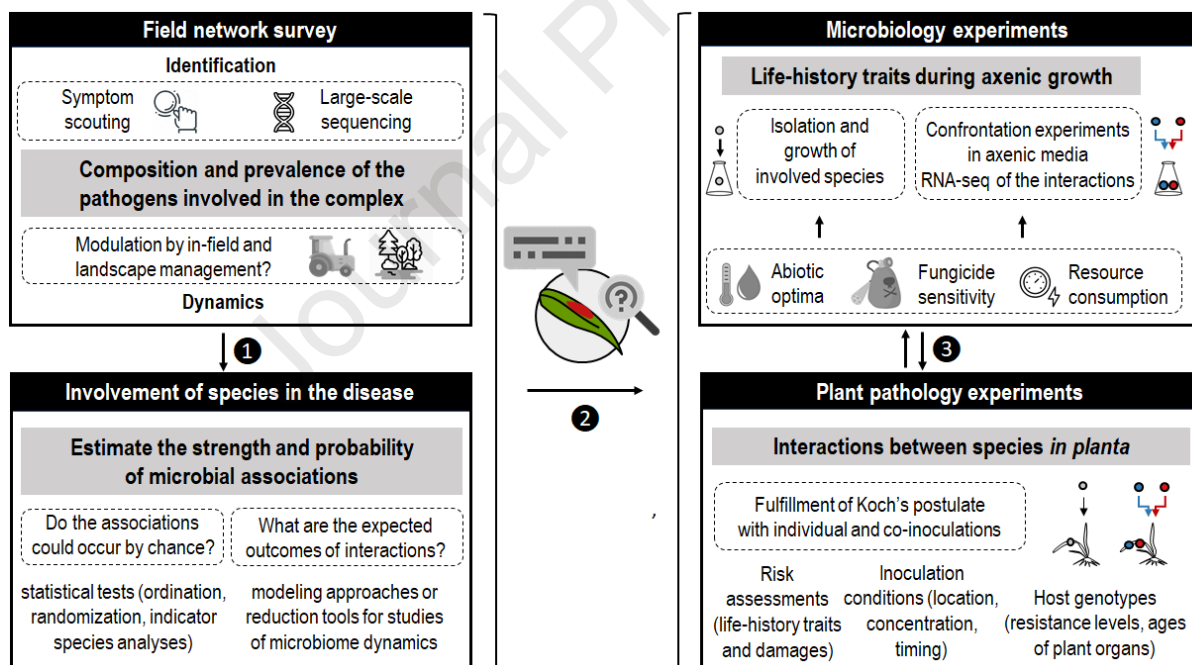
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1459 **Figure 1: Experimental steps to study how disease complexes operate.** Methods allowing
 1460 detection and characterization of plant disease complexes in the field are a prerequisite to
 1461 effectively characterize the composition and prevalence of pathogen species potentially
 1462 involved in the complex. (1) The identification of causal agents from a pool of potential
 1463 pathogens through statistical and modeling approaches enables (2) narrowing the pool of
 1464 pathogens as candidates or revealing previously unrecognized causal agents (3) microbiology
 1465 and pathogenicity tests to couple symptom recognition in the field with re-isolation of the
 1466 species associated with it (microbiology experiments conducted from field samples of diseased
 1467 and asymptomatic plant tissues) and fulfillment of the Koch's postulates (characterization of
 1468 the interactions between species *in planta*).



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1474 Table 1. An overview of currently known fungal pathogen associations that may be considered as disease complexes (for more complete information - list of
1475 species involved, taxonomic complexity, trophic mode, epidemiology- and associated references, see Suppl. Table 1).

Disease name and host plant	Number of described species involved/ Dominant species	Discrimination by symptoms ₁	Differences between species of the complex in distribution, prevalence, and sensitivity to control methods					In planta studies of interactions ₂
			Documented historic shift between species	Regional/temporal differences in complex composition	Host specificity	Sensitivity to fungicides	Plant resistance genes	
Stem canker (blackleg) of crucifers	Four/ <i>Leptosphaeria maculans</i>	Partly	Yes	Yes	Narrow host range	Yes	<i>Rlm</i> genes against <i>L. maculans</i>	Field and controlled conditions
Alternaria leaf spot of Brassicae	14/ <i>Alternaria brassicae</i> or <i>A. brassicicola</i>	Partly	No	Yes, highly variable	Narrow to broad host range	Unknown	No	Field observations
Alternaria blight of potatoes	Eight/ <i>Alternaria alternata</i> or <i>A. solani</i>	Partly	No	Yes, highly variable	Narrow to broad host range	Individual variation	No	Field observations
Ascochyta blight of pea	Four/ <i>Peyronelleae pinodes</i> ; <i>Phoma medicagenis</i>	Partly	No	Unknown	Narrow host range	Unknown	No	Field and controlled conditions
Wheat eyespot	Two/ None	No	No	Unknown	Narrow host range	Yes	Yes	No
Leaf blotch diseases of wheat	Three/ <i>Zymoseptoria tritici</i>	Difficult	Yes	Yes	Narrow host range	Probable	Resistance genes to <i>Z. tritici</i> ; susceptibility genes to <i>P. nodorum</i> and <i>P. tritici-repentis</i>	Field and controlled conditions
Fusarium head blight of wheat	16/ <i>Fusarium graminearum</i>	Difficult	Yes	Yes	Broad host range	Yes	No	Field and controlled conditions
Maize ear rot	22/ <i>Fusarium graminearum</i> ; <i>F. verticillioides</i>	No	Yes	Yes	Broad host range	Unknown	No	Field observations
Fusarium stalk rot of maize	15/ <i>Fusarium verticillioides</i> ;	No	No	Yes	Broad host range	No	Unknown	Field observations

	<i>Macrophomina phaseolina</i>								
Sheath diseases of rice	Three/ <i>Rhizoctonia solani</i>	No	No	Unknown	Broad host range	Unknown	No	Field and controlled conditions	
Bakanae disease of rice	Seven/ <i>Fusarium fujikuroi</i>	Partly	No	Likely	Broad host range	Unknown	Yes, but not deployed	No	
Banana Sigatoka disease	Three/ <i>Pseudocercospora fijiensis</i>	No	Yes	Yes	Narrow host range	Unknown	Yes	No	
Purple leaf blotch of leek and onion	Two/ <i>Alternaria. porri</i>	No	Yes	Yes	Narrow to broad host range	Yes	Yes, but not deployed	Field observations	
Root, crown, and vascular symptoms of winter squash	Six/ Unknown	No	No	Unknown	Broad host range	Unknown	Unknown	Field observations	
Cassava root rot disease	Eight/ <i>Fusarium solani</i> ; <i>F. oxysporum</i>	Difficult	No	Yes	Broad host range	Unknown	Unknown	No	
Stem rot of <i>Aglaonema modestum</i>	Two/ Unknown	No	No	Unknown	Unknown	Unknown	Unknown	Field and controlled conditions	
Kiwifruit decay	Eight/ Unknown	No	No	Yes, highly variable	Polyphagous	Unknown	Unknown	No	
Grey mold	Two/ <i>Botrytis cinerea</i>	No	No	Yes, highly variable	Broad host range	Yes	No	No	
Coffee berry disease	Nine/ <i>Colletotrichum kawahae</i>	No	No	Yes	Unknown	Unknown	Probable	No	
Cranberry fruit rot	12/ Three or four dominant species	No	No	Yes	Unknown	Yes	No	No	
Mango malformation disease	Six/ <i>Fusarium mangiferae</i>	No	No	Yes	Unknown	Unknown	Unknown	No	
Brown apical necrosis of walnut	Five/ <i>Fusarium semitectum</i> ; <i>F. graminearum</i>	No	No	Yes	Broad host range	Unknown	Unknown	No	
Sooty blotch / fly-speck of apple	More than 100/ Unknown	No	No	Yes, highly variable	Broad host range	Yes	No	No	

Apple replant disease	Nine/ None	No	No	Yes, highly variable	Polyphagous	Unknown	Unknown	Field and controlled conditions
Ink disease of chestnut	Seven/ <i>Phytophthora cinnamomi</i>	Partly	No	Yes	Broad host range	Unknown	Probable	Field observations
Stem canker of eucalyptus	Two/ <i>Teratosphaeria gauchensis</i> ; <i>T. zuluensis</i>	No	No	Yes	Narrow host range	Unknown	Yes	No
Oak downy mildew	Three/ <i>Erysiphe alphitoides</i>	No	No	Yes	Narrow host range	Unknown	Unknown	Field observations
Oak and tanoak mortality	Three/ <i>Phytophthora ramorum</i>	No	Yes	Yes	Broad host range	Unknown	Unknown	Field and controlled conditions
Black foot of grape	30/ None	No	No	Yes	Polyphagous	Unknown	No	Field and controlled conditions
Petri disease of grape	31/ <i>Phaeoconiella chlamydospora</i> ; <i>Phaeoacremonium</i> spp.	No	No	Yes	Polyphagous	Unknown	No	Field and controlled conditions
Esca disease of grape	24/ <i>Phaeoconiella chlamydospora</i> ; <i>Phaeoacremonium aleophilum</i> ; <i>Fomitiporia mediterranea</i>	No	No	Yes	Polyphagous	Unknown	No	Field and controlled conditions
Botryosphaeria dieback of grape	26/ None	No	No	Yes	Polyphagous	Unknown	No	No

1476

1477 ¹ Discrimination by symptoms between individual species of the complex: clearcut (yes), impossible (no), difficult or partly (possible for some species of the
 1478 complex only; possible on certain organs or at different ages of one organ of the host; dependent on the host plant).

1479 ² For artificial inoculations, only cases where co-inoculation experiments have been done are mentioned.

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Slipping through the cracks: Challenges and prospects for investigating fungal plant disease complexes

Highlights

- 1- Disease caused by complexes of fungal phytopathogens are common but often unsuspected
- 2- Symptoms are often insufficient to discriminate between members of a complex
- 3- Complexes often encompass one dominant species and a variable cohort of accompanying species
- 4- Disease complex composition and dynamics are driven by intrinsic and extrinsic factors
- 5- Interactions between members of the complex govern severity and are understudied

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Le May Christophe reports article publishing charges was provided by Agro Institute Rennes-Angers Department of Ecology. Le May Christophe reports a relationship with Agro Institute Rennes-Angers Department of Ecology that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.