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Review

Ascomycete Fungi (*Alternaria* spp.) Characterization as Major Feed Grains Pathogens

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Abstract: The emergence of new infectious plant diseases is driven by anthropogenic and environmental change, including trade, land use, and climate changes. The movement of infected plant material through trade in plant products, germplasm, grafts, and live plants has been recognized as the most significant contributing factor to the emergence of new plant diseases. *Alternaria* spp. are ubiquitous fungi. They are present in the human and animals' environment, being commonly found in environmental dust samples and air conditioning systems, while spore traps often show evidence of *Alternaria* dispersal. *Alternaria* spp. have even been shown to be associated with insects, having been isolated from the backs of cockroaches. Little work has been performed to investigate the saprotrophic lifestyle of *Alternaria* spp., which probably accounts for the majority of *Alternaria* species in nature. *Alternaria* spp. can persist on low nutrient media, suggesting that they can complete their lifecycle in poor nutrient environments. This review aims to present the lifestyle of ascomycete fungi such as *Alternaria* spp. and show their characterization as major feed grains pathogens in agricultural feed production.

Keywords: mycotoxins; wheat; *Alternaria*; fungi; feed; toxins.

1. Introduction

The appearance of new transmittable plant diseases is determined by anthropogenic and conservational change, including changes in trade, land use, and climate [1–4]. The growth of infected plant material through trade in plant products, germplasm, grafts, and live plants has been recognised as the greatest contributing factor to the emergence of new plant diseases [5–7]. A pathogen may be introduced without disease emergence initially until a second factor, such as the introduction of disease vectors, more appropriate hosts, or changes in the environment, leads to an increase in disease incidence, geographic range, or severity [8]. Fungi are responsible for many introduced plant diseases, with more fungal infections introduced to Europe and Africa over the 20th Century than bacteria and viruses combined [9]. Understanding the evolutionary history, evolutionary potential, and pathogenicity of fungal diseases will help manage and identify emerging pathogens [10].

Alternaria spp. are ubiquitous fungi [11]. They are present in the human and animals' environment, being commonly found in environmental dust samples and air conditioning systems, while spore traps often show evidence of *Alternaria* dispersal [12]. *Alternaria* spp. have even been shown to be associated with insects, having been isolated from the backs of cockroaches [13]. Little work has been performed to investigate the saprotrophic lifestyle of *Alternaria* spp., which probably accounts for the majority of *Alternaria* species in nature. *Alternaria* spp. can persist on low nutrient media, suggesting that they can complete their lifecycle in poor nutrient environments [14]. *Alternaria* is best known for its role as plant pathogens. The USDA Fungal Host Index contains over 4,000 plant-host associations in this genus, ranking it 10th in the total number of host associations of nearly 2000 fungal genera. The *Alternaria alternata* species group alone is recorded as causing disease on over 100 host plants. This includes economically essential crops including cereals, ornamentals, vegetables, and fruits, with losses incurred through direct crop damage, postharvest spoilage, or through contamination with mycotoxins [15].

Alternaria infections usually occur on the leaves and stems of the host plant [16]. Leaf spots are recognized by black necrotic lesions surrounded by chlorotic halos. Leaf necrosis may lead to reduced marketability for leafy crops such as *Brassica*. It may also result in the host abscising leaves, reducing photosynthetic potential and crop yields indirectly, as is the case in apple and pear [17]. *Alternaria* spp. also causes fruit spot. They are leading to reduced crop marketability, a significant problem in citrus fruits. *Alternaria* spp. also incurs economic losses postharvest [18,19]. In Red Delicious varieties of apple in South Africa, annual losses of 6-8% have been attributed to *Alternaria* dry core rot. Such postharvest diseases are often not thought to be attributed to a single *Alternaria* sp. but may be caused by a range of species. Infections of wheat grains by *Alternaria* spp. occur in the field and in storage, where low temperatures favor them. This reflects the saprotrophic/opportunistic necrotrophic lifestyle common through *Alternaria* species [20].

Postharvest spoilage may not just be a result of visual blemishes or reduced palatability but may also be caused by mycotoxin contamination [7]. Mycotoxins are non-host selective toxins produced by fungi, and more than 30 have been isolated from *Alternaria*. Toxins are produced by *Alternaria* infecting crushed and whole grains as well as fruits and vegetables [21]. These have been shown to pose a range of animal and human health risks [22]. *Alternaria* mycotoxins are frequently detected in fresh produce, including fruit products and juices and grains such as wheat and plant oils [23]. The species responsible for contamination are often reported to be *Alternaria infectoria* or *A. alternata* [24].

The *Alternaria* genus, and particularly the species *A. alternata*, are also of clinical signs often associated with human airway disorders, including allergy, asthma, and chronic rhinosinusitis [25]. As a result, *Alternaria* spp. are considered to have an enormous contribution to the 3 billion US dollars spent on relieving allergenic rhinitis each year in the USA. *Alternaria* spp. are also gaining recognition as human invasive pathogens. This usually occurs in immunocompromised patients, occurring as lung or sub-cutinal infections. Infection also occurs following surgery requiring antifungal treatments or further operations to remove the infection [26].

2. *Alternaria* spp. Characterization

2.1. Description of the Genus

The genus *Alternaria* was first described in 1817, with *Alternaria tenuis* as the type isolate. Keissler [27] found ambiguities in descriptions of *A. tenuis* and synonymized both *A. tenuis* and *Torula alternata* to *A. alternata* [28]. No sexual stage was evident in the genus, and as such, it was classified in the Phylum Fungi Imperfecti with other asexual fungi. Since the genus' conception, over 1000 *Alternaria* species have been described [29]. Many of these species' names are invalid as they have since been classified into other genera or because they lack type specimens. The continued revision of the genus reflects its diverse nature, possessing considerable variation in spore structure and being identified in many different ecological niches [30].

2.2. Morphological Descriptions

Most classification of the *Alternaria* spp. has been based on morphology [31]. This understanding was brought by published 355 essays and papers on *Alternaria* morphology, which was subsequently summarised in an identification guide for the *Alternaria* genus, re-describing 275 morphological species [32]. The *Alternaria* genus is characterized by large, multicellular, melanized conidia, which can possess longitudinal and transverse septae. Spores are typically broadest at the base and taper towards the end [28]. The tapering at the end of spores is commonly referred to as a "beak". Spores are often produced on conidiophores in chains that may branch or lead to secondary conidiophores that produce other spores [15]. It is mainly the individual spore characters and the sporulation patterns that are used to differentiate morphological species within the genus. Identification of *Alternaria* taxa has long been considered problematic. Over 1000 species have been described, and 275 names are in current use [33].

Frequent revision of groups in the genus has resulted in the species boundaries being unclear. Taxonomic keys based on morphology have been attempted but have not contained appropriate characters to identify taxa at a commonly considered species level [33]. Overlapping spore characters and natural variation in response to culturing conditions made these keys hard to follow. This was particularly true for many small-spored *Alternaria* spp. (including *A. alternata*), which display considerable morphological diversity, are present ubiquitously in the environment, and exhibit adaptation to various lifestyles, from economically essential plant pathogens to human allergens [34]. Therefore, broader groups of spore morphologies were developed to categorize these species. This "lumping" of morphologically described species did much to simplify the identification of *Alternaria* spp. Whether these morphological groups represent multiple distinct species or represent, a smaller number of highly variable species is still unresolved [35].

2.3. Toxin Characterisations

Concurrent to significant revisions of taxa based on *Alternaria* morphology, mycotoxins were being identified and characterized in *Alternaria* species [28]. Toxins that were associated with plant disease on major grains were of particular interest. Morphologically similar *A. alternata* species were found to produce toxins that conferred "host-specific" pathogenicity on fruits, vegetables, and citruses [15]. Later it was shown that these toxins had a broader host range than initially thought, leading to them being referred to as host-selective toxins (HSTs). The conflict between *Alternaria* morphological species descriptions and results from introduced molecular techniques has resulted in ambiguity over which morphological descriptions constitute species [35]. Multiple morphological species descriptions are available for HST producing *Alternaria*. Still, all these taxa possess identical DNA sequences for the internal transcribed spacer region (ITS) and have been considered a single species, *A. alternata*. As a result of differences of opinion in naming the HST-producers, some *Alternaria* pathotypes have both morphological species descriptions and pathotype designations. This has led to confusion when calling the agents of disease; for example, *Alternaria mali* was described as the causal agent of infection of apple trees, and the description of this species was based on spore morphology [24]. Separate from the morphological characterization of *A. mali* is its pathotype designation. Individuals that can produce apple HSTs are termed *A. alternata* apple pathotypes and were first identified in Japan. In general, current literature describes HST growing individuals as pathotypes of their host. Scientific literature and disease regulation often use the two names interchangeably, despite the name representing two different species concepts, and there is little evidence that morphological species even cause the same disease [36].

2.4. Approaches Based on Deoxyribonucleic Acid

The development of molecular approaches has advanced our understanding of evolutionary relationships in *Alternaria* genus [37]. Many morphological described species have been confirmed as distinct evolutionary lineages, including *Alternaria brassicicola*, *Alternaria infectoria*, *Alternaria porri*, and

Alternaria radicina. However, in many cases, multiple morphological species are associated with a single phylogenetic lineage. These lineages generally reflect taxa that have previously been morphological species groups and have recently been described using the taxonomic level section, and subsequently in Woudenberg et al. [38]. The *Alternaria* section *Alternaria* relates to what was previously considered the "*Alternaria alternata* species group". This group's taxonomic status is still unresolved, as molecular approaches have shown limited resolution between morphological species [38]. Individuals within this group are generally considered to represent a single species *A. alternata*. This group's accurate classification is required due to its diversity of roles as an environmental saprophyte, human allergen/pathogen, and plant pathogen [39].

3. Conclusions

Host ranges of individual pathotypes within *A. alternata* are not yet understood; for example, pathotypes of *A. alternata* thought to be specific to lettuce, tomato, and strawberry have each been shown to be capable of causing leaf lesions on European pear (*Pyrus communis*). Furthermore, there is evidence that some European *Malus* and *Pyrus* cultivars may be less resistant to *Alternaria* diseases than cultivars grown inside the disease's natural host range. The European and Mediterranean Plant Protection Organisation (EPPO) lists *Alternaria gaisen* as a documented pest. It lists *A. mali* as an A1 quarantine pest, meaning that it is not present and is recommended for regulation throughout the EPPO region. Keeping in mind those mentioned above, it is essential to focus on more sophisticated methodologies in identifying *Alternaria* spp., especially in feed samples such as the most often consumed wheat and corn.

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