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REVIEW ARTICLE

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Phytopythium: origin, differences and meaning in modern plant pathology

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Abstract

The genus *Phytopythium* is a relatively new group of organisms distinguished from the *Pythium* genus. These are organisms closely related to *Pythium* and *Phytophthora*, with similar structure and biology. Despite many similarities, this genus is characterised by several morphological features that allow it to be easily identified. Until now, more than 20 species belong to this genus, most of which are saprophytic organisms. However, there is also a group of species particularly dangerous to plants. These species include, among others, *Pp. litorale*, *Pp. helicoides* and *Pp. vexans*. The purpose of this work was to collect information about these organisms and present them in a condensed form. The study contains basic information about the history of the type of separation, differences in morphology distinctive genus of *Phytopythium*, *Phytophthora* and *Pythium* and information about diseases and host plants. Information is also provided on the potential threat posed by these organisms to forest ecosystems.

Key words

Phytophthora, *Pythium*, *Phytopythium*, Oomycetes, host, disease symptoms

Introduction

Initially, *Phytopythium* was combined with closely related species belonging to the genus *Pythium*, which was first described in 1858 by the German botanist Nathanael Pringsheim (Pringsheim 1858). Species belonging to the genus *Pythium* have been classified as dangerous plant pathogens causing rotting of various parts of the plant (e.g. fruits, roots). Research related to the importance of this genus has developed very quickly, which makes it possible to get to know more and more species (Lévesque and de Cock 2004; Broders et al. 2009; Karaca et al. 2009; Senda et al. 2009; Bala et al. 2010; Uzuhashi et al. 2010). Lévesque and de Cock (2004) divided this type into 11 clades, based on molecular studies. The clades are well supported by morphological features. The *Pythium* genus is characterised by well-developed mycelium and a specific way of releasing spores (zoospores). Zoospores are released from sporangia in the form of undifferentiated protoplasm, followed by the process of differentiation of individual spores beyond sporangium (Marano et al. 2014). This method of releasing zoospores is similar for all species belonging to the genera *Pythium*, *Pythiogeton* and *Lagenidium*, which differ from each other by other morphological characteristics. In turn, species belonging to the genus *Pythium* differ in e.g. the shape of sporangia, which seems to be significant from

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the point of view of evolution. For this type, one can distinguish several different shapes of sporangia from filamentous to round with an expanding base (van der Plaats-Niterink 1981). In their studies, Uzuhashi et al. (2010) confirm that the *Pythium* type really consists of five different groups, each of which is characterised by a special and unique type of sporangium. On this basis, a new division was proposed: *Pythium*, *Ovatisporangium*, *Globisporangium*, *Elongisporangium* and *Pilasporangium*. Analysis of rDNA and mtDNA (COI) regions showed that clade within *Pythium* (*Pythium* clade K; Lévesque and de Cock 2004), which has previously been separated and included in the genus *Ovatisporangium* (clade 1 z; Uzuhashi et al. 2010), in fact, is more closely related to *Phytophthora* than to the type of *Pythium* (Bala et al. 2010; Uzuhashi et al. 2010; Robideau et al. 2011). Ultimately, however, the name *Ovatisporangium* became a synonym for *Phytopythium* (de Cock et al. 2015).

To sum up the genesis of the genus *Phytopythium*, it is a relatively young genus distinguished from the *Pythium* genus (Bala et al. 2010; Rai et al. 2020). This genus is strongly associated with the freshwater environment, where many of its representatives can be found (Nam and Choi 2019; Resekar et al. 2019). Some of *Phytopythium* species are plant pathogens developing in moist habitats, e.g. *Pp. helicoides*, which spread through water, develop in the soil and infect plant roots. These organisms are considered to be particularly important in agricultural production (Baten et al. 2014). To date, more than 20 species have been described, including *Pp. sindhum*, *Pp. helicoides* or *Pp. citrinum*.

The importance of *Phytopythium* in agriculture and horticulture

Although a large part of the species belonging to the genus *Phytopythium* is considered saprophytic (water and soil) organisms, it is also possible to distinguish among the group of dangerous plant pathogens such as *Pp. litorale* (pumpkin fruit rot), *Pp. helicoides* (root rot and the stems of many plants) and *Pp. vexans* (root rot of many plants including kiwi) (Javadi and Sharifnabi 2016; Afandi et al. 2018; Prencipe et al. 2020). In 2011, Yang et al. (2013) described disease symptoms on begonia (*Begonia* × *semperflorens-cultorum* cv. Vodka Dark Red) plants. Infected leaves, blackened rotting roots and stems and, as a consequence, the dieback of entire plants were observed even on the surface of nearly 80% of the crop. From infected tissues, *Pp. helicoides* was isolated. In the USA, Radmer et al. (2017) conducted research on the cause of soya bean death. From the 12 fields on which soya bean was grown, samples were taken for molecular identification. The study showed the presence of various species from the genera *Phytophthora*, *Pythium* and *Phytopythium*. For each of the groups, pathogenicity tests on soya beans were also carried out. *Pp. litorale* turned out to be a pathogenic species in relation to soya bean. Radmer et al. (2017) also showed that increasing the temperature at which infected seeds were incubated from 15°C to 25°C significantly increased the damage caused by *Pp. litorale*. Also in China, research related to the activity of pathogens from the genus *Phytopythium* was carried out. In 2010–2012, the dieback of the kiwi plant (*Actinidia chinensis*) in orchards was observed. Initial symptoms include necrosis on the leaf edges and leaf curling, which led to the weakening of the entire plant (Wang et al. 2015). In infected plants, dark necrotic spots on the roots and the root collar were also observed. Wang et al. (2015) from necrotic spots isolated *Pp. helicoides*. Similar symptoms (root rot, root collar and dark discoloration) as well as fruit rot were also observed on kiwifruit in Turkey (Polat et al. 2017). The dying region included up to 20% of the entire area on which the breeding was carried out. From infected fruit, *Pp. vexans* was isolated.

Comparison of *Phytopythium* with *Phytophthora* and *Pythium*

During further detailed molecular studies on the division of the *Pythium* genus, particular attention was paid to the K clade, which based on phylogenetic analysis showed a very high affinity between *Pythium* and *Phytophthora* (Lévesque and de Cock 2004). Briard et al. (1995) and Cooke et al. (2000), based on the ribosomal large subunit and internal transcribed spacer (ITS), showed that *P. vexans* is significantly different from other species belonging to both *Pythium* and *Phytophthora*. In other studies, Villa et al. (2006) proved that species belonging to clade K were in fact closer to the type of *Phytophthora*. The specific nature of the K clade has also been confirmed by Bedard et al. (2006) through analysis of the organisation of the 5S gene family. In species in clade K, the 5S rRNA genes were predominantly linked to the rDNA repeat mostly in tandem arrays in the same orientation as the rRNA genes. As a result of the differences discussed above in the phylogenetic tree proposed by Villa et al. (2006), by the analysis that was based on sequences of ITS region rDNA and the cytochrome oxidase II and β-tubulin genes, the K clade so far belonging to the *Pythium* genus has been separated as a different group.

Species of the genus *Phytopythium* also significantly differ in morphological features. One of the typical features for this type is sporangia of oval to spherical shape with a distinct papilla (except for the *Pp. vexans*), common internal proliferation similar to that of *Phytophthora* and the type of development and release of zoospores typical of the *Pythium* genus. However, species of the genus *Phytophthora* never produce internal proliferation for papillate sporangia. This unusual combination (internal proliferation and papillation) for sporangia is common in species of the genus *Phytopythium* and for some *Pythium* species such as: *P. marsupium*, *P. middletonii*, *P. multisporum* (belonging to clade E); *P. nagaii* (G clade), *P. grandisporangium* (clade C); *P. anandrum*, *P. dimorphum*, *P. helicandrum*, *P. prolatum*, *P. undulatum* (clade H) (De Jesus et al. 2018). Another different feature is the development of sporangia itself. In *Phytopythium*, young sporangia are not equipped with papilla, which grow only when sporangia mature, and do not consist of hyaline 'apical thickening' as in *Phytophthora* (Blackwell 1949). In contrast to *Phytophthora*, species of the genus *Phytopythium* can produce shorter or larger discharge tubes. At the same time, in some species, the papilla is not the place from which mature zoospores are released. Another difference in the construction of the papilla itself is the fact that when its prolonged form is observed, it is also possible to branch it out (de Cock et al. 2015). The last two features are rare but may be an important diagnostic feature. At the end of the papilla molded tip, there are vesicles. Protoplasm released by sporangia goes to the follicle, where it is differentiated into single zoospores (Baten et al. 2014). According to Marano et al. (2014), *Pp. kandeliae* releases zoospores mainly as species of the *Pythium* genus. However, there are also situations when zoospores partially form already inside the sporangium, and partly in the vesicle, which is the intermediate phase between *Phytophthora* and *Pythium*.

Another characteristic feature of the *Phytopythium* genus is the shape of antheridia. In most species, they are elongated, cylindrical and sometimes there are narrowings on them (in the genus *Phytophthora* antheridia, they usually take on the spherical form). The fertilisation tube unlike the other types is in the side position and not the apical position. Despite this, from time to time it is possible to observe club-shaped antheridia with a vertebral joint. For example, for *Pp. vexans*, there are antheridia widely connected with oogonium. A group of species of the genus *Pythium*, which similarly to *Phytopythium* may simultaneously produce sporangia with papilla and internal proliferation, has been mentioned before. However, in most cases, these species do not develop elongated cylindrical antheridium. The only one species of the *Pythium* genus that produces this type of antheridium is *P. helicandrum*. However, this species can be distinguished from *Phytopythium* based on ornamented oogonia and much larger oogonium that exceeds the ranges typical of the genus *Phytopythium*. There are several species that produce antheridia similar to *Phytopythium* such as *P. marsipium* or *P. grandisporangium*, but the first of these species has oogonia utriform instead of ovoid, while the other is a marine species with very large sporangia with stenosis at the base (de Cock et al. 2015).

Most species belonging to this genus produce large, smooth oogonium, thick-walled oospores and one or two paragynous antheridium cells (Bala et al. 2010) that are laterally connected to the oogonium.

High optimum and maximum temperature of growth is also characteristic of this type. The optimum temperature for most classified species is 30°C, while the maximum temperatures range from 35°C to 40°C (Lévesque and de Cock 2004).

The importance of *Phytopythium* in orchards and forestry

There are many reports about the harmful effects of pathogens from the genus *Phytopythium* not only on agricultural plants but also on trees and shrubs (Jung et al. 2020). An example of this may be the study by Boari et al. (2018), who analyzed the causes of soft rot of manioc roots (*Manihot esculenta*). This disease in Brazil is considered the greatest threat to the cultivation of this shrub (Poltronieri et al. 1996; Silva et al. 2019). At that time, a single organism responsible for the occurrence of the symptoms of this disease has not been diagnosed, but rather it is considered to be related to the action of many soil organisms (Hillocks and Wydra 2002). Boari et al. (2018) consider organisms from the genus *Phytopythium* as one of the perpetrators of this disease. Another example of the impact of organisms from the *Phytopythium* genus is reports on the deaths of young pistachio trees in California (USA). Fichtner et al. (2016), when writing about this phenomenon, emphasise root necrosis and the rapid process of dying (from June to July). As the species responsible for this damage *Pp. helicoides* was identified. This pathogen can cause similar symptoms also at the roots of the aforementioned begonia (Yang et al. 2013) or rhizomes of lotus (Yin et al. 2016). Root diseases caused by soil pathogens are also a major problem in Iran. Javadi and Sharifnabi (2016) pay attention to damage to almond crops (*Prunus amygdalus* L.). Their research showed that the causal agent of root rot and crown dieback in almond crops in Isfahan is *Pp. litorale*. Pathogens of the genus *Phytopythium* also cause damage to avocado (*Persea americana* Mill.) crops. Reports on this topic are presented by Rodriguez-Padron et al. (2018). In the studies conducted on avocado plantations in the Canary Islands, where tree dieback was observed, species of the genera *Phytophthora* and *Phytopythium* (*Pp. vexans*) were isolated. An attempt was also made to compare the pathogenicity of the obtained isolated organisms with the main pathogen for avocado crop damage such as *Ph. cinnamomi*. The results of the analyses obtained were very divergent. On six tested *Pp. vexans* isolates, three were found to be non-pathogenic and one was moderately aggressive, while the other two were highly aggressive, however, under specific conditions (Rodriguez-Padron et al. 2018). One isolate was as damaging as *Ph. cinnamomi* in hydroponic conditions but did not cause significant root necrosis in potted plants, and the opposite results were obtained with other isolates. In addition, the presence of, inter alia, *Pp. vexans* also caused necrosis on the root collar, which was not observed in other analyzed species. Two new species for knowledge are also described in the mangrove forest in the Philippines (Bennett et al. 2017a). The newly described species are *Pp. leanoi* and *Pp. dogmae*. Bennett et al. (2017a) in their research potentially describe the threats resulting from the presence of these species in mangrove forests (leaf discoloration). Other researchers also found species belonging to this genus in similar conditions (Leanio 2001; Thines 2014; Bennett et al. 2017b).

Summary

Information on the importance of species of the genus *Phytopythium* and prevalence are not as well known as other types belonging to the Oomycota (*Phytophthora* or *Pythium*). Nevertheless, these organisms are often equally aggressive towards host plants as their close relatives (Rodriguez-Padron et al. 2018). Particularly noteworthy is the fact that these organisms develop and are more aggressive at temperatures between 25°C and 35°C. This is particularly important because of the climate change observed in recent years. This process allows *Phytopythium* organisms to shift their natural ranges of occurrence and to search for new host plants. A good example of this may be the reports from Poland about the occurrence of *Pp. citrinum* in weakened oak stands (Jankowiak et al. 2015). The importance of these pathogens in the process of dieback of oaks is not known while in other parts of Poland this species was successfully isolated from rhizosphere soil, in dying oak and alder stands, where it accompanied more dangerous pathogens such as *Ph. plurivora* (in oak stands) and *Ph. alni* (in stands alder) (unpublished data). The challenge for the coming years is to learn more about the importance of *Phytopythium* organisms not only in both agricultural and horticultural crops but also in stands where they can threaten the sustainability of ecosystems as well as closely related organisms of the genus *Phytophthora*.

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