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Chapter

Downy Mildew of Basil: A New Destructive Disease Worldwide

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Abstract

Oomycete pseudofungus (*Peronospora belbahrii*) is a causal of devastating basil downy mildew disease because once infected basil plants are no longer marketable. The host range is limited to basil and hyssop. Coleus was previously considered as host as well, but pathogen causing downy mildew on coleus has been shown genetically different and specified as *P. belbahrii sensu lato*. Therefore, *P. belbahrii* is described as a complex species, likely defined by plant host. The *P. belbahrii* is air-borne and seed-borne pathogen and it does not need a vector for dispersal. The disease was firstly reported from Africa where it is assumed to have originated on sweet basil and 70 years later it was reported from Europe. Currently, basil downy mildew is of pandemic occurrence and the pathogen is present in almost all areas around the world where basil is cultivated. Since the pathogen is transmitted by the seed, there is a high risk of the pathogen spread by the seed trade.

Keywords: *Agastache*, downy mildew, *Lamiaceae*, *Ocimum*, *Peronospora*

1. Introduction

Downy mildew caused by *Peronospora belbahrii* Thines is one of the most destructive diseases of sweet basil (*Ocimum basilicum*) of the family *Lamiaceae* Lindl. (alternatively *Labiaceae* Dulac) which except field farming is also grown as a specialty crop in greenhouses. Downy mildew of basil was first reported in 1932 from Uganda, Africa as *Peronospora* spp. and again in 1937 as *P. lamii* from where it is assumed to have originated on sweet basil [1, 2].

First report from Europe was in 2001 from Switzerland where it was observed in greenhouses [3]. After that, the disease was detected in Italy in 2003 on sweet basil in several greenhouses located in Liguria region (Northern Italy). In 2004, it was found in France on some basil crops near Saint Tropez (Southern France) [4]. In the same year, it was found in Belgium, but there are no data about the first detection.

After those first European reports, the pathogen was rapidly spread through Europe. In summer 2009, it was detected in United Kingdom in *Agastache* (hyssop) plants (*Agastache mexicana* and *Agastache* sp.) at Wisley gardens (Surrey) and on the summer of 2010 in protected basil plants in south-east England [5, 6]. In 2010, a significant incidence of downy mildew was reported in Hungary at two plant stands at Budapest-Soroksár and Tordasal though a similar disease had been observed in 2003 in a greenhouse at Albertirsa [7]. It was reported from Czech Republic in 2012 as well as from Cyprus [8]. In 2014, it was found again in United Kingdom but in several plants of coleus (*Solenostemon scutellarioides* cv. 'Chocolate Mint') but in 2016 has been shown that the pathogen causing coleus downy mildew is *P. belbahrii*

sensu lato [9]. In 2016, it has been reported from Spain on basil collected from the island of Tenerife (Islas Canarias) and afterwards was also noted that has been causing severe symptoms and economic losses in Almería, Andalucía [10].

In the United States, downy mildew of basil is considered as relatively new disease but the pathogen has been detected in October 2007 in South Florida [11]. Since its first detection in the United States, it has been observed on basil in at least 42 states [11, 12]. Interesting is founding in 2008 on basil plants produced in various nurseries in Sebastopol, Sonoma County because trace-back investigation revealed that the seeds had originated from Italy. This disease was also reported in Argentina in February 2008 [13] and Canada in 2011 [14]. In 2011, it was reported in Hawaii for the first time and in Mexico in 2015 [15].

First report in Asia was in Iran, where a severe outbreak of downy mildew was observed in sweet basil fields in 2006 [16]. A year later, in April 2007, it has been found in Japan on coleus plants cultivated in a greenhouse in Chiba Prefecture (Honshu) [17]. In the spring of 2009, it has been found in Taiwan in the field of Nantu and Yunlin [18]. In Israel, it was firstly found in December 2011 near Bet She'an, and in 2012 the disease has been spread throughout the country to all basil-growing areas [19]. Recently, it has been found in China in July 2014 on basil on the island of Hainan in Sanya City and in 2016 in the Shunyi and Daxing districts of Beijing which is concerned as first report in mainland China [20, 21]. Last Asian report is from Korea, where it has been first observed in November 2015 on sweet basil plants growing in plastic greenhouses in Gwangmyeong [22].

Until 2017, the disease was considered exotic to Australia, when it has been reported from South-east Queensland. Within 6 months, the disease was present along the eastern seaboard from north Queensland to Victoria, South Australia and the Northern Territory. In the scientific literature, Australia has been listed as a host country as early as 2015; however, no records of detections could be traced [23].

Finally, the first report of *P. belbahrii sensu lato* detection on coleus (*Plectranthus* spp.) in Brazil was reported in 2019 [24].

The first official report for Croatia was done in 2015 by Croatian Agency for Agriculture and Food based on symptoms and morphological characteristic not confirmed by molecular diagnostic [25]. In October 2015, as part of regular reporting reviews conducted by the Advisory Service, infected plants were found in greenhouses in the Varaždin County. The disease was spread soon after that founding on areas of four more counties (Krapina-Zagorje, Međimurje, Split-Dalmatian and Zagreb). Interestingly, in Dubrovnik-Neretva County, the disease was found on pot-plants imported from Italy. Up to date in Croatia, downy mildew is recorded only in production of basil in greenhouses. This chapter authors are currently investigating the occurrence on basil in the greenhouse 'Green friends' of eco-grower of culinary herbs and spices, situated in Rakovica in the Zagreb County. We confirmed determination of the *P. belbahrii* by molecular diagnostic (PCR sequence comparison of the ITS rDNA sequences and Cox2 region) (unpublished).

2. Disease symptoms

The first symptoms can be spotted on lower leaves, where infection starts and progresses upwards. The most noticeable symptom is yellowing (slightly chlorotic) of the leaves with the veins remaining green. The initial yellowing can be misinterpreted as a nutritional deficiency and so disease can go unrecognised. With time on upper surface of leaves, large chlorotic lesions with soft margins are developing. Chlorosis often involved the entire leaf surface. Since the pathogen is a biotroph, it causes dying of cells from which it absorbed nutrients and therefore necrotisation

Figure 1.
Brown growth of Peronospora belbahrii *on abaxial side of basil leaf.*

occur after chlorosis and the central portion of a chlorotic lesion become necrotic. This can lead to slight curvature of leaves. Necrotic spots are variable in size and of irregular shape as they are limited by the main veins. In some cases, entire area of the leaf surface is affected. In humid conditions, necrotic regions can become dark brown to black in colour. On abaxial leaf surfaces, both in chlorotic and necrotic regions, a typical greyish to brown, furry or downy moulds could be observed giving the leaves a dirty appearance (**Figure 1**). Parasitisation results in shrinkage of leaf and premature leaf fall.

Disease can go asymptomatic under cool and dry conditions [26] and sometimes plants not showing symptoms at harvest can develop symptoms during transport [27]. In report from Taiwan, it was noted that the pathogen caused chlorosis and leaf shrinkage on basil in the field, but did not cause any symptom on coleus, Pai-tsai Chinese cabbage (*Brassica rapa*), leaf lettuce (*Lactuca sativa*) and melon (*Cucumis melo*) [18].

3. Hosts range

Sweet basil is the natural host of *P. belbahrii* and the majority of *P. belbahrii* findings have been on sweet basil. In 2009, Thines et al. concluded that coleus is also the natural host of this pathogen [28]. They also investigated the downy mildew of sage, but were unable to confirm that it is caused by *P. belbahrii* so did not considered sage as natural host. Coleus has been confirmed as host of *P. belbahrii* in Japan, United States, United Kingdom and Germany [29]. Species concept has been refined recently and pathogen causal of coleus downy mildew was specified as *P. belbahrii sensu lato* [9]. Moreover, an unidentified species of *Peronospora* sp. infects coleus in Israel [30]. Interestingly, Israeli isolates of *P. belbahrii* from sweet basil do not infect coleus although infects other *Lamiaceae* species: rosemary (*Rosmarinus officinalis*) Nepeta (*Nepeta curviflora*), Clinopodium (*Micromeria fruticosa*) and two species of sage (*Salvia pinnata* and *S. fruticosa*) [30]. Further, the conidia from mentioned species failed to infect sweet basil and therefore the role of these species in the epidemiology of basil downy mildew in Israel is unknown [30]. The *Peronospora* sp. on coleus was reported in 2005 Louisiana, New York

and Florida in U.S. [31, 32]. In 2015, *Peronospora* sp. on coleus was reported in Tennessee, and the morphological and molecular characteristics were consistent with Thines description of P. *belbahrii sensu lato* [9, 28]. So, Rivera et al. concluded that *P. belbahrii* can be described as complex of species likely defined by plant host [9]. Recently, coleus downy mildew causal pathogen is confirmed as *P. belbahrii sensu lato* host based on pathogenicity test in Brazil, and this is the first such report for the South America [24].

In 2009, the *Agastache* species (*Lamiaceae*) was also named as the new *P. belbahrii* host by Henricot et al. [6]. The host range is today broadened and as alternative hosts are considered culinary and ornamental varieties related to basil and coleus from *Lamiaceae* family and here are mint (*Mentha* spp.) and sage (*Salvia* spp.). All cultivars of sweet basil are hosts and as highly susceptible ones are cv. Genovese Nufar, Italian Large Leaf, Queenette, Superbo, Poppy Joe's and Milita [27]. Some of the exotic, spice and ornamental basils cultivars such as red types (*O. basilicum purpurescens* cv. Red Rubin, Red leaf), lemon basil (*O. citridiorum* cv. Lemon std., Mrs. Burn's Lemon, Lemona & Lime) and lime basil (*O. americanum* cv. Blue Spice, Spice & Blue Spice F1) have been found less susceptible or even resistant to downy mildew [9, 28, 33]. This chapter's authors detected downy mildew on spice cultivars of basil, and *P. belbahrii* was confirmed as causal pathogen by molecular analysis (unpublished).

4. Description of pathogen

The causal pathogen of basil's downy mildew is pseudofungus *Peronospora belbahrii* Thines and has been formally introduced under name *P. belbahrii* by Thines et al. in 2009 as dedication to Lassaard Belbahrii who first suggested that the pathogen on basil might be a distinct undescribed species and distinguished it from a different closely related species that parasitizes sage (*Salvia officinalis*) [28]. It is assumed that *P. belbahrii* is of African origin, as its host basil is native to this continent [28]. As oomycete it is classified in *Chromysta*, *Oomycota*, *Oomycetes*, *Peronosporales* and *Peronosporaceae*. The pathogen was molecularly determined in 2005 by Belbahri et al. [3] and showed through ITS sequencing that it is a newly occurring species on basil that differs from *P. lamii*, the only previously reported downy mildew on sweet basil and also differs from *Peronospora* species that is affecting lamiaceous hosts worldwide [1, 2]. Perhaps, previous findings of *Peronospora* sp. on sweet basil and coleus may be *P. belbahrii* but have been misidentified as *P. lamii* before sequence identification was carried out and before it was first described as a new species *P. belbahrii*. Confusion between species is likely to occur without sequence data; therefore, samples must be submitted to a competent testing laboratory for identification. Using morphological comparison and molecular phylogenetic reconstructions, Thines et al. also confirmed that *P. belbahrii* is not identical to *P. swingleii* on *Salvia reflexa* [28]. *P. belbahrii* on basil and coleus seems to be closely related yet; it has been shown that they are morphologically and genetically different [28]. Limited potential to infect basil has been reported for the isolates from coleus, as it was described earlier [30]. The significance of differences between causal pathogen of downy mildew on basil and coleus needs to be investigated further; but for now, the pathogen on coleus is determined as *P. belbahrii sensu lato.*

The growths on the underside of the symptomatic leaves in a form of a brown downy mould are asexual organs, sporangia bearing sporangiophores which emerge from leaf stomata. Microscopic observations will show that they are consistent with the characteristics of a genus *Peronospora*. The first descriptions of sporangia and sporangiophores on basil and coleus that were confirmed by molecular determination were provided by Thines et al. in 2009 [28]. The sporangia of genus *Peronospora*

are spore-like structures and they act as conidia and germinate into a germ-tube when they are near a leaf stoma. Therefore, the use of synonym conidia, or simply spore, has become commonplace for sporangia.

Conidia are dark brown to olive in colour and pedicel is absent. They are rounded and egg-shaped with a length 24–29–30.8–33–36 μm on basil and 26–29–31.3–33–37 μm on coleus. They width are 20–23–24–26–29 μm on basil and 20–23–24.5–26–29 μm on coleus. Ratio of length and width is 1.1–1.2–1.29–1.4–1.5 on basil and 1.1–1.2–1.28–1.4–1.5 on coleus [28].

Sporangiophores are colourless (hyaline) with a long, straight trunk and monopodially with a length 270–300–400–520–680 μm on basil and 330–380–466–570–650 μm on coleus [28]. Numbers of ramifications were 3–4–4.9–5–7 per sporophore on basil and 4–5–5.2–6–7 μm on coleus. Ultimate branchlets were in pairs, curved, longer one in length 13–18–20.6–26–31 μm on basil and 12–13–18–22–31 μm on coleus while the shorter one in length 3.8–7.7–9.80–10–15 μm on basil and 5.1–7.7–10.7–13–17 μm on coleus. Ratio of longer to shorter branches is 1.3–1.8–2.25–2.7–4 on basil and 1.1–1.6–1.71–1.9–2.5 on coleus. Ultimate branches end dichotomically and tips (sterigmata) are acute to subacute on both, basil and coleus. Tips are bearing single sporangia.

The shortest sporangiophores were reported in Iran and were 130–290 μm (avg. 194 μm) long and branched two to five times [16]. The longest sporangiophores were recorded in Hungary, and they were in length of 416–784 μm (avg. 572 μm) and monopodially branched five to seven times [7].

There are two oospore detections published up to date, both from Israel, found in leaves of susceptible sweet basil cultivar 'Peri'. In 2013, Cohen et al. identified and described oospores as thick-walled, brown in colour, measuring of 46.2 ± 2.8 μm in diameter [34]. Oospores never occurred on the infected leaf surface, but inside the mesophyll [30]. In 2016, in walk-in tunnel experiments that simulated commercial production conditions, oospores were observed attached to the leaf surface, to older parts of the infection area, and also found to water washes of the leaf surface by Elad et al. [35]. Discovery of oospores suggests the potential for sexual reproduction, but little is known on *P. belbahrii* oospore formation or is it homothallic or heterothallic. Currently, only one mating type has been found [22], although it is already presumed that it is heterothallic [26, 36, 37]. The pathogenicity of oospores is investigated, but without positive infections [30, 38], and their role in the basil downy mildew epidemiology is not known.

5. Disease cycle and dispersal

The *P. belbahrii* thrives in warm, humid conditions and produce conidia that can infect in temperatures as low as 15°C (59°F) [26]. For example, downy mildew is present in Israeli basil-cropping regions where in the cooler season temperatures may reach minimum of 5–10°C at night and a maximum of 10–25°C during the day [35]. This corroborates with our observations. Pathogen can tolerate cold weather (10–15°C) but, like its host basil, cannot survive freezing winter temperatures at continental climate. Conidia cannot survive harsh winters and as pathogen is biotroph it needs living host. Therefore, in climates with harsh winters and with just one mating type of the *P. belbahrii* it can survive only on living plants in greenhouse production operations that produce basil year round. In mild winters and in warm, temperate regions where the host, basil will not freeze, the second overwintering inoculum are mycelium and conidia in infected plant buds, plant stems, leaf tissue and shoots. Congruently, the most devastating damage is often seen in warm and humid conditions, late summer and in greenhouses.

Most of *Peronospora* species can reside in soil as soil-borne oospores that are formed in leaf tissue and may overwinter in leaf litter or may be released into the soil as leaves decay and considered as soil-borne inoculum. Any movement of soil particles with soil-borne oospores inoculum can spread it from infected plants to non-infected ones. Although *Peronospora* species are biotrophs, they can survive without host as oil-borne oospores because they are in dormancy and can be viable for few years depending on species. Until now, there are no reports about *P. belbahrii* soil-borne oospores even in cases when oospores were detected inside the mesophyll of the leaves [30, 35]. Large-scale experiments were conducted to elucidate the pathogenicity of oospores to basil plants. Soil was infested with oospores (10 oospores/5 g of soil/well) and three to four basil seeds were planted in each well. Plants were grown until the four-leaf stage, but none of the 2000 plants that developed showed symptoms of downy mildew or sporulation of *P. belbahrii* [30]. Also, the experiments conducted in the Israeli walk-in tunnels lead to a conclusion that oospores are minimally affected by high temperature, and therefore the high temperature presumably did not affect pathogen survival [35].

The life cycle of *P. belbahrii* is initiated as abundantly produced air-borne conidia which can readily be spread by moist wind [37]. The conidia can be carried by rain drops, by wind, and can be splashed by rain to wet leaves near the ground. It does not need a vector for dispersal. Survivability of conidia, contrary to oospores, are strongly affected by temperature and duration of exposure so, a longer exposure period and higher temperature weakened the infection capacity of the conidia. Wetted-dried conidia lost their activity after 55 h at 25°C, 20 h at 30°C and 9 h at 40°C [30]. Therefore, conidia are short lived and viable just for few days so, they will endanger only susceptible host within the conidia dispersal area. McGrath conducted an experiment with field-grown basil at the Long Island Horticultural Research and Extension Center (LIHREC) in Riverhead, NY and considered the primary source of initial inoculum in this area to be long-distance wind dispersed conidia from affected plants [39] although the distance is not specified. The possibility to use frozen conidia as inoculum was also tested and those collected from infected leaves frozen for 3 months at −20°C or 2 years at −80°C retain high germination capability [30]. In other trial, frozen conidia germinated at 25% in contrast to nearly 90% germination rate of freshly harvested conidia [40]. Their germination was favoured between 5 and 15°C on water agar *in vitro*. Inoculation of basil plants with frozen or fresh conidia (3×10^4 mL^{-1}) resulted in high disease severity 14 days post inoculation [40].

Sporulation occurs in moisture saturated atmosphere at an appropriate temperature and often during the night, in the dark and in chlorotic lesions 5–15 days old [41]. In controlled greenhouse experiments, sporulation occurs 6–7 days post inoculations [37]. The sporulation starts when pathogen biomass in the leaf mesophyll reached a certain threshold and complete within 8–12 h from onset of darkness in optimal conditions (saturated atmosphere at 18°C). During the first 6 h, hyaline sporophores are formed and as they emerge from stomata gradually become dichotomously. In the subsequent 5 h, dark spores are produced on the tips of the sporophore branchlets (sterigmata) [41]. The light strongly inhibits spore formation, but not sporophore development and emergence through leaf stomata. Yet, sporophores formed under the light are abnormal and unable to form spores. Cohen et al. in 2013 discovered that lightning during the second half of the night inhibits spore formation, and narrow band led illumination showed that red light (λmax 625 nm) was most inhibitory to spore formation comparing to blue light (λmax 440 nm) while in other oomycetes is quite the opposite [41]. They speculate that probably *P. belbahrii* has a different photoreceptor sensitive to red light. The sporulation is greater when the portion of carbohydrates in the leaf is higher [41].

The carbohydrates accumulating during the day are hydrolysed to hexoses during the first half of the night which pathogen uses for formation of conidia during the rest of the night [41]. Therefore, the greater the accumulation of carbohydrates in infected leaves during the daytime contributes to the greater sporulation in the following dark, wet period of the night. This all suggests that the sporulation terminates with necrosis of leaf which obstructs assimilation as plant cells die because of pathogen absorbed all nutrients from it.

Conidia germinate in 3–5 days into one or two germ tubes and infect plant tissues via a germ-tube which penetrates through leaf stomata [28, 36] and it takes 3 h [35]. Germ tubes rarely form an appressoria-like structures prior infection. Developing hypha grows into intercellular spaces within the leaf mesophyll, proliferate and eventually invaginate the host cell plant cells through special globuse structures called haustoria (a hallmark oomycete structure) for nutrient acquisition [37]. Further branching and spreading of this initial hypha lead to forming of a cushion of intercellular mycelia just below the stomata. From this cushion, sporangiophores arise and emerge through stomata on sterigmata bearing sporangia. Conidia are produced simultaneously and are carried by wind and rain to new infection sites of the same or different plant. Leaf wetness of at least 6 h is required for conidial infection [42, 43]. Under favourable conditions, sporulation progresses in the polycyclic disease cycle leading to an epidemic of downy mildew disease.

P. belbahrii is also a seed-borne pathogen. Detection of *P. belbahrii* in several commercially produced basil seed batches confirmed that the pathogen is seed-borne [3, 28, 44]. It is considered that infected seed act as primary inoculum source in basil production, and is so far considered to be the most important way of this pathogen spreading as it can explain the rapid global spread of *P. belbahrii*. Great example for the spreading of *P. belbahrii* with seed transport and seed-marketing to long distances is that the biotype that was detected for the first time in US in 2007 was genetically identical to the one reported in Switzerland in 2001 [27]. Also, the disease occurrence in US Sonoma County in 2008 was connected with the origin of the used seed that was introduced from Italy. Investigation conducted by Farahani-Kofoet and Römer detected *P. belbahrii* on 80–90% of randomly selected commercial seed stocks [45] and assumed that *P. belbahrii* can be spread by transport and marketing of seed stocks.

On contaminated seeds, *P. belbahrii* has been found in form of conidia and oospore [38, 45]. Until now, *P. belbahrii* was not reported inside the basil seed or embryo. Based on their observations, Farahani-Kofoet and Römer concluded that *P. belbahrii* is able to survive for several years on seeds [45]. Generally, oospores of *Peronospora* species can also be formed on seeds and infect the emerging seedling. Their oospores germinate in a way similar to that described for conidia and the infection process is similar. Investigation of *P. belbahrii* oospore infection of basil seeds was conducted, but plants developed from seeds planted in soil infested with oospores were symptomless and sporulation characteristic for *P. belbahrii* did not occur [30].

It has not yet been clarified whether the pathogen infects the seed deeply and systematically or is just a contaminant. In some European investigations, systemic infections in seeds and in different plant parts (leaves, stems) even in a symptomless plant have been detected [44, 45]. Novel investigation of seed transmission conducted in Israel showed that *P. belbahrii* is seed-borne but not seed-transmitted, as seeds produced by infected plants in the field can be externally contaminated with conidia that were embedded in the surface, but not entirely [46]. Further, plants grown in growth chambers until 5–6 leaf stage from contaminated seeds did not show any symptom of downy mildew and did not carry latent infection. Also, systemic infections were rarely seen in the field. They confirmed systemic spread

of mycelium in the basil plants which corroborated with previous finding [45]. Systemically infected plants remained stunt and produced no seeds. Therefore, the Israeli investigators postulated that seed infections and seed transmission may occur in Europe, as it was reported [44, 45], and other locations with wetter summers, especially under prolonged wetness periods at the flowering and seed production.

Both investigations, European and Israeli, confirmed that contaminated seeds can be harvested from symptomless, latently infected plants and also, that contaminated seeds can give symptomless, latently infected plants [44–46].

Peronospora belbahrii can also be spread through vegetative materials like contaminated plant cuttings, transplants and fresh leaves. Novel Israeli investigation showed that *P. belbahrii* is spread systematically in basil plants [46]. Mycelium has been found to grow acropetally to the stem apex and basipetally to the cotyledons and hypocotyl and laterally to the axillar buds but, mycelium has never reached the roots. Especially in young basil plants, this pathogen systemically runs through tissue and causes plant stunt and fail to produce seeds.

6. Management of basil downy mildew

The control methods of the downy mildew pathogen today involve fungicides, seed treatment and breeding for resistance. In the greenhouses, they can be augmented with physical measures: nocturnal illumination, ventilation and daytime solar heating. The last one is also suitable for net-houses [47].

Current control measures rely mainly on fungicide application. In conventionally produced basil, it can be controlled in a preventive program with conventional foliar fungicides. The efficient once are based on mefenoxam, azoxystrobin, cyazofamid, mandipropamid, fluopicolide and fenamidone [11, 12, 26, 34, 38]. There are also phosphorous acid fungicides which are in most cases labelled and allowed in greenhouses. The best control of 98% was achieved with preventive fungicide application, before symptoms occurred, on a weekly schedule [38].

The *P. belbahrii* developed mefenoxam-resistance within 1 year of use in Israel and was reported in 2013 [34]. It was also detected in Italy were mefenoxam (metalaxyl-M) plus copper has been the most widely used and effective product against *P. belbahrii*, since its registration on basil in Italy in 2004 [48]. As the systemic fungicides are prone to the resistance development, ingredients with different modes of action are needed [32]. The novel fungicides with extremely high efficacy against oomycete including *P. belbahrii* are oxathiapiprolin [30] and valifenalate [49]. Oxathiapiprolin acts at multiple stages of the pathogen's asexual life cycle at extremely low concentrations and due to translaminar and acropetally systemic movement, it protects treated leaves and new leaves as they emerge and grow. In *P. belbahrii*, it inhibits sporangia germination and curatively, it stops mycelial growth within the host plant before visible lesions occur and inhibits further lesion expansion, offering protection at 1 and 2 days post-infection [50]. It was found to be effective against mefenoxam-resistant biotypes as well [30]. But, as it is a single-site inhibitor and its target is the oxysterol binding protein, the resistance to oxathiapiprolin assume to be medium to high and resistance management is required [51]. The soil application of mixture of oxathiapiprolin and benthiavalicarb or their single application against *P. belbahrii* was tested. Application to the root of 1 mg active ingredient per plant in the field experiment provided durable protection of up to 4 weeks against *P. belbahrii* [52]. The mixture performed better than single applications of those two compounds suggesting a synergistic interaction between them. The valifenalate is also a single-site inhibitor and acts as the inhibitor of cellulose

synthesis in the Oomycete plant pathogens [49]. The resistance to valifenalate is assumed to be low to medium risk [51].

In organic farming, conventional fungicides are not allowed, so neem oil, potassium bicarbonate and hydrogen dioxide can be used for protection only, but they do not give satisfied protection [38]. Organic fungicides are contacts and do not go into plant tissue where is the pathogen and they are not able to translocate to abaxial side of leaves where sporulation occur. Therefore, their performance is not commercially acceptable and as they provide limited to no control, including when applied twice weekly on a preventive schedule to a moderately resistant variety [12, 38, 39]. As alternative, there are some bio-products based on *Bacillus amyloliquefaciens*, *Streptomyces lydicus* and the extract of *Reynoutria sachalinensis* [38]. Organic production should be in protected conditions and better transplanting then seeding as pathogen-free seed is not available. In greenhouse, it is important to prevent favourable conditions for disease development.

Certain cultural practices which create less optimal conditions for the pathogen can be helpful in reducing the amount of infection. Such practices include providing good soil drainage and good air circulation among plants. Increasing plant spacing in the field or greenhouse prevents the creation of high-humidity conditions on plant surfaces and can inhibit infection as *P. belbahrii* requires humidity for sporulation as well as free leaf moisture for infection. The humidity should be keeping below 85% and this is crucial. In the greenhouse, the use of plastic mulch and drip irrigation is recommended instead of bare ground and overhead irrigation. Effective measure for reducing ambient relative humidity and avoids vapour deposition of leaves surface is ventilation [47]. In some experiments, combining daytime solar heating with nocturnal illumination without fungicide applications showed to be an effective control in organic farming [30, 47]. High temperature is detrimental to the *P. belbahrii* and exposure of infected plant of 35–45°C for 6–9 h suppressed survival of conidia and mycelia [47]. Subsequently, solar heating has been used to cure plants. In Israel, solar energy was captured by closing greenhouse windows or covering the house with a transparent IR polyethylene sheet during sunny hours of the days: best is to use three consecutive daily exposures of 3–4 h starting at 8 am [47]. Solar heating should be conducted cautiously to avoid plant heat damage [47]. Ensuring light during the night, especially red light should prevent sporulation. The protective effect of nocturnal illumination was determined in laboratory and greenhouse trials; but in Israel, field trials (net-houses) also demonstrated that light can be successfully used to supress downy mildew in field-grown basil [41]. The inhibitory effect of incandescent or CW fluorescent light of 3.5 or 6 μmoles.m^2.s^{-1} on sporulation was 100% on lower leaf surface even when only the upper leaf surface was exposed to light [41].

The rapid global spread of the downy mildew may be related to transmission of *P. belbahrii* by infected seeds and/or trade of basil cuttings and plants with latent infection [3, 38, 45, 46]. Infested seeds are a great risk for spreading the pathogen by transport and seed-marketing to long distances. Implementation of seed-certification schemes to exclude seed batches infested with *P. belbahrii* from marketing would be of great value for both seed-producing companies and growers [45]. Therefore, improving seed production; developing and implementing seed testing, certification protocols, and standards for the basil seed industry and strict following of import restriction may have halted *P. belbahrii* [38, 45]. To limit the spread of the pathogen by seed shipments, it is crucial for breeders and growers to draw on an early, fast and specific detecting test [45].

Seed should be tested on the presence of *P. belbahrii* and for that purpose real-time PCR have been designed [3]. Belbahri et al. have designed a specific primer pair (Bas-F/Bas-R) based on sequences within the unique genomic ribosomal DNA

(ITS1) and the primer pair generates a single fragment of approximately 134 base pairs [3]. The PCR method proved to be very sensitive for direct detection of *P. belbahrii* on seeds and plant samples [45]. The PCR detection limit of *P. belbahrii* in artificially infested seeds corresponded to the DNA amount of a single spore per seed (3.4 pg of *P. belbahrii* genomic DNA extracted from a pure spore suspension at a density of 103 spores ml^{-1} using 1 μl as a template) [45]. Further, with this PCR protocol, *P. belbahrii* can be detected with high sensitivity in leaves and stems as well and not only at seeds, even if symptoms are not evident. Finding that latent systemic infection can result in the contamination of basil seed and vice versa supported the necessity to implement PCR-based detection in a seed-certification scheme [45]. Pathogen-free seed is most important for greenhouse crops plantings not expected to be exposed to wind dispersed spores [53]. It should be emphasised that the presence of pathogen DNA in seeds does not implicate spontaneous disease outbreaks because the PCR test cannot assess the viability of spores as specific fragments can also be generated from DNA material of dead spores. Moreover, the disease inception and development depends on host-pathogen interaction and existing environmental conditions. Yet, PCR test allows the testing of high numbers of samples within a short time and rapidly gives accurate information on *P. belbahrii* presence. Considering all, it is recommendable to be admitted in seed-certification schemes for routine testing of seed materials in order to inhibit marketing of infested seeds. As the PCR test can be used for detection in different plant parts, it can also be used for evaluation of procedures to control the downy mildew pathogen.

Seed treatments and at-seedling fungicides may have the potential for the good start of basil production [38]. There are no fungicides labelled for use on seed, but the at-seedling fungicides are available although not labelled for use on basil seed. Mefenoxam can be applied at seedling into the soil in field growing basil [38]. In novel trails, the root treatment of mixture of oxathiapiprolin and benthiavalicarb given to the young seedlings, growing in the multi-cell trays in the nursery, may be effective basil downy mildew measure [52]. The only organic fungicide labelled for ground application is based on the extract of *Reynoutria sachalinensis* [38].

Novel, non-chemical basil seed treatment is steam-air treatment and USA seed companies start to implement it [53]. Steam-air treatment of basil seeds against seed-borne fungi was tested in 1997 in Australia [54]. Steam-air treatment at 54–58°C for 30 min was successful in two-cylinder configuration in the steam-air machine. They noticed that this configuration against sixth-cylinders configuration provides extra steam velocity that prevents basil seed clumping which happened because when wet basil seed easily stick together as they have very thick gelatinous coat. Therefore, basil seeds are not amenable to hot-water treatment as the seed clumping makes the seed challenging to handle [53, 54].

The cultivation of resistant sweet basil cultivars also can be efficient control strategy. Highly resistant cultivars will be especially welcome in organic farming. Earlier, some cultivars of red types, lemon and lime basil have been found less susceptible [9, 30, 31, 33, 39]. New resistant basil varieties started to be marketed in USA in 2018 [53]. The first commercially available resistant variety is Eleonora. The Rutgers University basil breeding program released Devotion, Obsession, Passion, and Thunderstruck. They are marketed by VDF Specialty Seeds. Organically produced seed is available and marketed like Prosperais (Johnny's Selected Seeds), Emma and Everleaf (aka Basil Pesto Party and M4828Z) [53].

That accurate monitoring can be of great importance in field growing as well as in protected conditions as instrumentation for optimization of plant protection measures was shown by USA monitoring programme. It started in 2009 by McGrath and augmented knowledge of basil downy mildew [38]. It is an online

spreadsheet program set-up in Google Docs accessible by anyone. Until 2019, each year a spreadsheet page was set up for anyone to log and view occurrence reports [53]. In 2019, a new website was launched with a mapping program that mapped reports by county plus information about basil downy mildew. The growers need to be educated to accurately based on first symptoms recognise the diseases on time. In greenhouses, monitoring should be on daily basis as downy mildew can develop very quickly [38, 41].

7. Conclusion

'*One touch of nature makes the whole world kin*' Shakespeare wrote and this is so valid for the pandemic downy mildew agent like *P. belbahrii* from sweet basil. The only way to deal with this pathogen is knowledge. To fill the existing knowledge gaps research into various aspects of the pathogen will be needed. The dual identification according to the morphology and ITS sequence analysis is recognised and implemented. It would be of the most value to investigate *P. belbahrii* sexual reproduction and to identify mating types and mechanisms of their compatibility if it is heterothallic. Further, valuable will be to obtain knowledge of the *P. belbahrii* natural distribution range because its present distribution is due to human activity and trades. More research into aspects of *P. belbahrii* physiology, asymptomatic infections and oospore role in epidemiology. The seed transmission still needs to be elucidated and the question of whether the pathogen penetrates into the seed should be answered. Because it has been already spread worldwide, the *P. belbahrii* is not on the quarantine lists; although, it will be beneficial to follow the quarantine guideline, in the context of global trade with seeds.

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