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Pathogen Spotlight

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REVIEW

Peeling the Onion: Towards a Better Understanding of *Botrytis* Diseases of Onion

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ABSTRACT

Onion is cultivated worldwide for its bulbs, but production is threatened by pathogens and pests. Three distinct diseases of onion are caused by species that belong to the fungal genus *Botrytis.* Leaf blight is a well-known foliar disease caused by *B. squamosa* that can cause serious yield losses. Neck rot is a postharvest disease that manifests in bulbs after storage and is associated with three species: *B. aclada*, *B. allii*, and *B. byssoidea*. The symptomless infection of onion plants in the field makes it difficult to predict the incidence of neck rot in storage, although progress on the detection of latent infection has been made. In onion cultivation for seed production, blighting of the inflorescence is caused by all four onion-specific *Botrytis* species plus the broad host range pathogen *B. cinerea*. Flower blight can reduce seed yield and contaminate seed. In this review, the long history of *Botrytis* diseases of onion is discussed, as well as recent and future approaches to acquire a better understanding of the biology and ecology of *Botrytis* spp. pathogenic on onion. New fundamental insights in the genetic, biochemical, and physiological aspects of *Botrytis*–onion interactions are essential to improve the breeding of *Botrytis*-resistant onion cultivars.

Keywords: *Allium cepa*, *Botrytis*, disease control and pest management, flower blight, fungal pathogens, host–parasite interactions, leaf blight, neck rot, onion, pathogen detection, postharvest pathology and mycotoxins

BACKGROUND

Onion (*Allium cepa*) is one of the most important vegetable crops worldwide. It is grown for its edible bulbous organs, which have high nutritional value and give a specific pungent flavor to the diets of many people worldwide. For bulb production, onion is cultivated as an annual crop that can be sown from seed or planted from pregrown sets that enable early harvest. For seed production, however, onion is cultivated as a biennial crop because inflorescences are formed in the second year. After harvest, bulbs can be consumed directly or processed, but some varieties are also well suited for storage, which makes onion available year round. In 2018, nearly 100 million tonnes of onion was produced worldwide, on a total production area of more than 5 million ha (FAOSTAT 2020).

As for many other vegetable crops, the production of onion bulbs is threatened by numerous pathogens and pests from a wide variety of taxa (Schwartz and Mohan 2008). Notorious threats are the insect pest *Thrips tabaci*; the fungal disease Fusarium basal rot, caused predominantly by *Fusarium oxysporum* f. sp. *cepae*; and the Iris yellow spot virus. All are capable of causing serious diseases that can reduce yield (Cramer 2000; Gent et al. 2006; Gill et al. 2015). Remarkably, there is one fungal genus, *Botrytis*, that contains as many as five species that cause multiple distinct diseases on all organs of the onion plant: bulbs, leaves, and inflorescences.

The Ascomycete genus *Botrytis* consists of approximately 35 species that are known as necrotrophic plant pathogens (Garfinkel et al. 2019; Hyde et al. 2014). The most well known and extensively studied model species is *B. cinerea*, which is able to infect >1,400 plant species including onion (Elad et al. 2016). Phylogenetic analysis of *Botrytis* has divided the genus into two clades. *B. cinerea* and *B. pseudocinerea*, both pathogens with a broad host range, belong to clade 1, whereas the species in clade 2 are mostly host specific to one or a few taxonomically related plant species (Hyde et al. 2014; Staats et al. 2005). Four *Botrytis* species in clade 2 are exclusively pathogenic on onion and known as causal agents of diseases that form a serious threat in onion cultivation, and another four *Botrytis* species are pathogenic on related *Allium* hosts.

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This review focuses on diseases that are caused by *Botrytis* spp. on onion (Table 1). The most well-known disease is leaf blight, a foliar disease caused by *B. squamosa*, which is characterized by necrotic spots on onion leaves that eventually result in blighting. Another important disease of onion is neck rot, a troublesome infection that manifests in bulb tissue. Three different *Botrytis* species are known as causal agents of neck rot: *B. allii*, *B. aclada*, and *B. byssoidea*. Furthermore, there is a blight disease of the inflorescence, known herein as flower blight, to which all the aforementioned onion-infecting species are associated, including *B. cinerea*. Besides the pathology of the diseases, recent and future approaches to elucidate infection biology and host specificity of *Botrytis* spp. pathogenic on onion are discussed, as well as perspectives to guide breeding for onion cultivars resistant to *Botrytis* spp.

BOTRYTIS LEAF BLIGHT

Pathology. Botrytis leaf blight is a foliar disease of onion caused by *B. squamosa.* This fungus was first described in 1925 and was reported to cause a disease of onion named small sclerotial neck rot (Walker 1925a). Several decades later, *B. squamosa* was isolated from diseased leaves, and the species was recognized as the causal agent of onion leaf blight (Hickman and Ashworth 1943; Page 1953). When artificially inoculated on mature onion leaves, *B. cinerea* is also able to cause symptoms. However, the superficial leaf flecks caused by *B. cinerea* never grow into the intercellular spaces of the leaf and do not cause blighting (Hancock and Lorbeer 1963). Occasionally *B. cinerea* is detected on plants showing symptoms of leaf blight, but it is usually accompanied by *B. squamosa* and is thus not considered the causal agent of the disease (Hickman and Ashworth 1943; Misawa and Takeuchi 2015). Leaf blight is a major disease in almost all onion production areas worldwide, including Europe, Asia, Australia, and North and South America (Carisse et al. 2011). Although information on yield losses caused by leaf blight is limited, in untreated plots losses of ≤30% have been reported (De Visser 1996; Shoemaker and Lorbeer 1977b).

The symptoms and histopathology of *B. squamosa* have been reviewed in the past (Lacy and Lorbeer 2008b; Lorbeer 1992; Lorbeer et al. 2007) and will be summarized here. When a conidium of *B. squamosa* lands on the surface of an onion leaf, it can germinate and form a germ tube. At the tip of the germ tube an appressorium develops that is able to penetrate the leaf cuticle. The appressorium is often formed on top of anticlinal walls of epidermal cells and enables the fungus to enter the leaf tissue by growing through the middle lamella that separates the anticlinal walls. Alternatively, germ tubes grow into the leaf tissue directly by entering via stomata. Upon leaf entry, hyphae grow mostly intercellularly and enter the cavity of the hollow onion leaf. The collapse of epidermal and mesophyll cells results in the first characteristic symptoms of leaf blight, which can be observed as small necrotic spots on the onion leaves (Fig. 1A). The subsequent spreading of hyphae results in expansion of the lesion. The lesions are often surrounded by a chlorotic halo, and sometimes a characteristic longitudinal slit develops within the lesion. In a later stage of the infection leaves start to blight, leading to early leaf senescence and consequently reduced plant growth. On necrotic plant tissue the fungus produces new conidia that are dispersed by wind to other leaves and initiate a new infection cycle, providing a source of secondary inoculum.

Besides conidia, *B. squamosa* produces sclerotia on colonized plant parts, especially under conditions that are not conducive to conidiation. Sclerotia can last on decomposing plant material for

TABLE 1
Overview of the different *Botrytis* species pathogenic on onion, with names of diseases on corresponding plant parts

Species	Leaves	Bulbs	Inflorescence
B. squamosa	Leaf blight	Small sclerotial neck rot	Flower blight
B. aclada	na[a]	Neck rot	Flower blight
B. allii	na	Neck rot	Flower blight
B. byssoidea	na	Mycelial neck rot	Flower blight
B. cinerea	Superficial leaf flecks	Brown stain	Flower blight

[a] na, not applicable.

FIGURE 1
Symptoms of **A,** leaf blight on a mature onion leaf caused by *Botrytis squamosa* showing necrotic leaf spots; **B,** neck rot on a sliced onion bulb; and **C,** flower blight on the umbel of an onion flower.

several months and function as overwintering structures (Ellerbrock and Lorbeer 1977c). When the new growing season starts, sclerotia are able to form apothecia, which play a role in the sexual reproduction of the fungus (Bergquist and Lorbeer 1972). Alternatively, sclerotia can produce new conidia as a primary source of inoculum (Ellerbrock and Lorbeer 1977a). Other sources of primary inoculum include conidia grown on colonized leaves of sprouted bulbs in cull piles, onion plants in seed production fields, and volunteer onion plants that grow around bulb production fields and remain from bulbs from the previous year (Ellerbrock and Lorbeer 1977a; Lorbeer 1992; Lorbeer et al. 2007).

The severity of a leaf blight epidemic is strongly influenced by environmental factors. For development of lesions a temperature of about 20°C is optimal, and a leaf wetness period of at least 6 to 8 h is needed for initiation of infection (Alderman and Lacy 1983; Alderman et al. 1985; Sutton et al. 1984). Longer leaf wetness periods of ≤48 h increase the number of lesions (Shoemaker and Lorbeer 1977a). Conidiation on colonized plant parts is promoted by moderate temperatures of 14 to 20°C and also requires moist conditions in the form of leaf wetness or high relative humidity (Lorbeer et al. 2007; Sutton et al. 1983, 1978).

Control. Because of the fast progression of the disease, onion growers heavily rely on preventive fungicides applied at 7- to 10-day intervals for ≤14 sprays per growing season (Carisse et al. 2011; Carisse and Tremblay 2007). Forecasting models for leaf blight help growers time their fungicide sprays based on weather conditions and the amount of airborne conidia above the onion canopy, measured by spore traps (Carisse et al. 2012, 2008; Lacy and Pontius 1983; Sutton et al. 1986; Vincelli and Lorbeer 1989). Forecasting models allow fungicides to be used more efficiently, reducing the total number of sprays per growing season (Carisse et al. 2005; De Visser 1996). Increasing awareness of the negative effects of fungicide use on the environment, as well as the risk of resistance development in the target fungus, has resulted in restrictions on the number of admitted fungicides. Some strains of *B. squamosa* have become insensitive to active ingredients that have been used for control of leaf blight, such as iprodione. Because of cross-resistance, these strains have also become insensitive to vinclozolin, which, like iprodione, belongs to the dicarboximide fungicides (Carisse et al. 2011; Carisse and Tremblay 2007; Presly and Maude 1982; Tremblay et al. 2003). Currently, fungicides against leaf blight are based on active ingredients from different classes, such as mancozeb (dithiocarbamate), prothioconazole (triazole), and fluoxastrobin (strobilurin), and using these products in tandem reduces the probability of resistant *B. squamosa* populations. However, relying on fungicide treatments alone is not a durable strategy to combat leaf blight. Some studies have focused on biocontrol of *B. squamosa* by suppressing its sporulation via antagonists, but this research has not led to commercially available products against leaf blight (Köhl et al. 1992, 2003).

To reduce the reliance on fungicides, scientists and breeders are searching for sources of natural resistance against Botrytis leaf blight. In general, all commercial onion varieties are susceptible to leaf blight (Bergquist and Lorbeer 1971; Tremblay et al. 2003), but variation has been observed in levels of susceptibility between cultivars (Araújo et al. 2018). Strong resistance to *B. squamosa* has been reported in a wild relative of onion, *A. roylei*. Resistance was determined to be conferred by a single gene (named *Bs1*), which behaved in a partially dominant manner and resulted in high levels of resistance in progeny plants of *A. cepa* × *A. roylei* (de Vries et al. 1992; van der Meer and de Vries 1990). A quantitative trait locus for resistance to *B. squamosa* from *A. roylei* was identified on chromosome 6 via the use of single nucleotide polymorphism markers in an interspecific three-way cross population [*A. cepa* × F1(*A. roylei* × *A. fistulosum*)] segregating for resistance (Scholten et al. 2016). The large size of the quantitative trait locus region indicated that, apart from the *Bs1* gene, minor genes from *A. roylei* or *A. fistulosum* may have affected resistance levels in plants of this population. Backcrossing of the *Bs1* resistance gene into *A. cepa* resulted in lines that resemble cultivated onion with a quantitative level of resistance against leaf blight (Hyde et al. 2015). A second source of resistance was found in another relative of onion, the Japanese bunching or Welsh onion (*A. fistulosum*) (Bergquist and Lorbeer 1971; Currah and Maude 1984).

Onion related *Allium* species may be used in breeding programs to breed for resistance (Kik 2002), but crossing *Allium* species that range in genome sizes may result in low fertility in progeny plants (Labani and Elkington 1987; van Raamsdonk et al. 2003). An example of a successful introgression of disease resistance is the introgression of downy mildew resistance from *A. roylei* into onion. After many years of backcrossing and subsequent selfing, breeding has led to the development and release of downy mildew–resistant onion varieties (Scholten et al. 2007). For optimal exploitation of related species in breeding programs, it is important to gain a better understanding of the molecular mechanisms underlying leaf blight resistance, for which a lot of research is still needed.

BOTRYTIS NECK ROT

Pathology. Botrytis neck rot is a serious disease of onion bulbs that causes postharvest losses. As the name suggests, it results in rotting of the neck area of the onion bulb, where tissue softens and turns brown as it decays (Fig. 1B). Often, sclerotia develop between the rotting scales of the bulb. Additionally, mycelium and gray conidia can be observed on the outer surface of the neck area (Lacy and Lorbeer 2008a; Lorbeer et al. 2007). Although infection takes place in the field, symptoms of neck rot are typically observed after bulbs are taken from storage (Maude and Presly 1977b). Neck rot is reported in all major onion production areas worldwide, and yield losses can reach 50%, although they vary widely between years (Chilvers and du Toit 2006; Chilvers et al. 2004; Hwang et al. 2016; Khan et al. 2013; Maude and Presly 1977a, b).

Multiple *Botrytis* species are known to be associated with neck rot. *B. allii* and *B. aclada* are considered to be the main causal agents (Chilvers and du Toit 2006). Also, *B. byssoidea* can cause neck rot, but because the disease usually displays more pronounced mycelial growth and less sclerotia and conidia formation, the disease caused by *B. byssoidea* is sometimes called mycelial neck rot (Lacy and Lorbeer 2008a; Lorbeer et al. 2007; Owen et al. 1950). *B. squamosa* has also been associated with a neck rot disease of onion, as can be seen from the original name "small sclerotial neck rot" (Walker 1925a), which is sporadically observed in onion plants and is more regarded as a late-stage symptom of severe leaf blight infections (Lorbeer et al. 2007). Occasionally, *B. cinerea* is isolated from rotting onion necks, often together with *B. allii* or *B. aclada* (Rod 1984) and is therefore not considered a causal agent of the disease (Yohalem et al. 2003). Furthermore, *B. cinerea* is able to cause brown stain, a rarely observed superficial discoloration of the outer dry scales of bulbs that does not cause problems in bulb production (Clark and Lorbeer 1973a, b).

B. aclada and *B. allii* occupy the same ecological niche, and the morphological differences between them are subtle. Therefore, *B. aclada* and *B. allii* were not distinguished for a long time, and their names were used synonymously for one species causing onion neck rot (Hennebert 1973). Based on chromosome number and size of conidia, two subgroups could be distinguished, one that has 16 chromosomes, like other *Botrytis* species, and one that has 32 chromosomes, with conidia approximately double in volume (Shirane et al. 1989). Molecular fingerprinting studies confirmed the existence of two subgroups and showed that both differ from *B. byssoidea* (Nielsen et al. 2001). Sequencing of DNA fragments demonstrated that the subgroup containing 32 chromosomes was the result of a hybridization event with one ancestor from the subgroup containing 16 chromosomes and the other ancestor being

B. byssoidea (Nielsen and Yohalem 2001). The subgroup with 16 chromosomes was named *B. aclada*, and the subgroup with 32 chromosomes was named *B. allii* (Yohalem et al. 2003). Because the names *B. aclada* and *B. allii* were used synonymously until 2003, literature until 2003 could refer to either of them. Sequence analysis of phylogenetically informative gene sequences of two *B. allii* isolates revealed that these isolates arose from independent hybridization events (Staats et al. 2005).

Botrytis neck rot typically remains symptomless during the growing season of onion plants. First symptoms are generally observed after bulbs have been harvested and stored. The asymptomatic infection makes it difficult to predict yield losses and to develop control strategies that can be applied in the field to mitigate the damage. To unravel the infection biology of neck rot and to elucidate how the fungus reaches the neck area without causing symptoms, several hypotheses about the infection strategy have been proposed.

One of the infection strategies proposed is a symptomless infection of the leaves followed by endophytic growth toward the bulb. A study by Tichelaar (1967) suggests that conidia of *B. allii* are able to germinate and penetrate the surface of onion leaves without causing a plant response. The hyphae grow in the epidermis, and when the leaves senesce the fungus colonizes the underlying mesophyll tissue without causing symptoms (Tichelaar 1967). In this way, the fungus grows through the leaves toward the bulb, resulting in latent infection of the neck area. By contrast, other studies have reported that inoculation of onion leaves with spores of *B. allii* triggers leaf flecks, and sometimes expanding lesions are observed (Presly 1985b; Stewart and Mansfield 1984). Because such symptoms are not observed in the field, artificial inoculations may not be representative for studying the infection biology of neck rot in the field.

Another infection strategy that may contribute to the incidence of neck rot is infection through seed. *B. allii* was detected in commercial onion seed batches, with ≤71% of seed samples being infected (du Toit et al. 2004; Maude and Presly 1977b; Stewart and Franicevic 1994). The fungus was detected externally on the seed coat and internally, and it was found to survive for ≤3 years (du Toit et al. 2004; Maude and Presly 1977b). *B. allii* was reported to spread from the infected seed to the seedling, first being detected in the cotyledon, later in the true leaves, and eventually in the neck of the mature onion (Maude and Presly 1977b; Stewart and Franicevic 1994). Although growth of the fungus throughout living plant tissue was always symptomless, conidiophores were observed after colonized leaf tissue senesced and turned necrotic, indicating that seed transmission is not the only way the disease spreads (Maude and Presly 1977b; Tichelaar 1967). Although several studies reported a high correlation between the percentage of infected seed and the incidence of neck rot in storage (Maude 1983; Maude and Presly 1977a; Stewart and Franicevic 1994), in a wet growing season the incidence of bulbs with neck rot was greater than the occurrence of infection in seeds (Maude and Presly 1977a). During storage, no further spread of the fungus was observed from infected to healthy bulbs, suggesting that infection occurs only in the field (Maude and Presly 1977a).

In addition to infection through seed and latent infection of leaves, it is hypothesized that bulb infection occurs just before harvest at the moment the foliage is cut, leaving a wound above the neck area providing a perfect point of entry for germinating conidia of neck rot fungi (Maude et al. 1984).

Control. To reduce the incidence of neck rot in storage, several control strategies have been developed that aim to reduce infection during onion cultivation. Application of preventive fungicides to control neck rot is a common practice in onion cultivation (Kritzman 1983; Presly 1984), but it has led to *B. allii* populations that are resistant to active compounds such as benomyl and carbendazim (Gladders et al. 1994; Kritzman 1983; Viljanen-Rolinson et al. 2007). Today, fungicide sprays with the active ingredients fluopyram, tebuconazole, boscalid, and pyraclostrobin are commonly applied in onion cultivation and are simultaneously effective against both leaf blight and neck rot. Fungicides are also applied on seeds to reduce the level of seedborne inoculum. Treatment of seeds has been reported to drastically reduce the incidence of neck rot, but its effectiveness can be countervailed by spread of the disease in the field under favorable weather conditions (Maude and Presly 1977a). To prevent seed becoming a source of neck rot, seed batches are tested for the presence of *Botrytis* spp., and contaminated batches are disinfected. Also, after harvest bulbs can be treated with fungicides to reduce fungal growth inside the bulbs and decrease the incidence of neck rot in storage (Ali and El Shabrawy 1979; Grinstein et al. 1992).

As an alternative to fungicides, several studies have focused on biological control of neck rot. Rod (1984) explored the potential of antagonistic fungi as biocontrol agents to inhibit the growth of *B. allii* and identified several candidates. Different strains of *Trichoderma viride* were found to have an inhibitory effect on growth of *Botrytis* spp. (Morris and Lane 1990; Roulston and Lane 1988). Köhl et al. (1997) reported that *Ulocladium atrum* could be used as a potential biocontrol agent against neck rot. Sporulation of *B. aclada* on necrotic leaf tissue could be suppressed by competition for tissue colonization by the antagonistic fungus, but growth into living leaf tissue could not be stopped (Köhl et al. 1995, 1999; Yohalem et al. 2004).

Breeding for onion varieties resistant to neck rot is difficult because of the different causal agents and the complexity of performing biologically relevant disease assays. There are differences in susceptibility between onion varieties, and the few studies that focused on resistance breeding against neck rot concluded that susceptibility is a quantitative trait that shows continuous variation and is at least in part heritable (Lin et al. 1995; Vik and Aastveit 1984).

Cultural control methods also are applied to limit the chances of neck rot infection in bulb storage. To prevent infection through the wound that arises after the leaves are cut, bulbs are lifted from the soil and left on the field to dry for several days, a process called curing. During that process, the top of the onion dries, making it more difficult for fungi to grow into the neck of the bulb (Maude et al. 1984). To speed up that process and to further inhibit fungal growth, bulbs are often cured with heated air (Gunkel et al. 1971; Harrow and Harris 1969; Maude et al. 1984; Walker 1925b).

Despite the development of control strategies, neck rot remains difficult to control and is thus a major problem in storage of onions. The latent nature of the infection makes it difficult to predict the incidence of neck rot in storage. However, diagnostic methods have been developed that detect the presence of neck rot fungi in seeds, plants, or bulbs with the aim of predicting the incidence of neck rot in storage. The first method that was developed is based on the outgrowth of fungi on selective media in which only *Botrytis* species are capable to grow (Kritzman and Netzer 1978; Lorbeer and Tichelaar 1970). Subsequently, the different neck rot species can be distinguished based on their macroscopic and microscopic morphological characteristics. An accurate guideline for fungal isolation and characterization of species is described in Chilvers and du Toit (2006). To predict disease incidence by using diagnostic tools, a more efficient and direct method than traditional fungal isolation is preferred. Nielsen et al. (2002) developed a diagnostic tool to detect and distinguish *B. aclada*, *B. allii*, and *B. byssoidea*. This method, based on PCR followed by restriction fragment length polymorphism, enables detection of *B. aclada* in symptomless onion leaves (Nielsen et al. 2002). An adaptation of the PCR protocol that includes magnetic capture hybridization increased the speed and sensitivity of detection (Walcott et al. 2004). A real-time PCR (qRT-PCR) method to quantify the amount of Botrytis neck rot fungi in onion seed was developed by Chilvers et al. (2007). This assay uses specific primer pairs that are optimized to anneal only to *B. aclada*, *B. allii*, and *B. byssoidea* without targeting other *Botrytis* species or other fungi commonly detected on onion seed. In addition to this qRT-PCR method, which was based on SYBR Green chemistry, another qRT-PCR was developed for determining the quantity of *B. aclada* in bulb tissue based on TaqMan probe-based chemistry (Coolong et al. 2008). Khan et al. (2013) developed a high-resolution melting analysis assay that allowed fast and simple discrimination between neck rot species. Alternatively to DNA-based detection, an enzyme-linked immunosorbent assay was developed to detect *B. allii* by using polyclonal antisera (Linfield et al. 1995). Also, volatiles produced by infected bulbs can be used in metabolite profiling to detect neck rot in bulbs (Li et al. 2011). More specifically, Prithiviraj et al. (2004) distinguished bulbs infected with *B. allii* from uninfected bulbs, and also from bulbs infected with other fungi and bacteria. Bulbs infected with *B. allii*, bulbs infected with *Burkholderia cepacia*, and mock-treated bulbs could be distinguished based on their volatile profile (Li et al. 2011). Although several methods for the detection and identification of neck rot fungi have been developed, for detecting either latent leaf infection or contaminated seed, sampling is the most problematic factor. Optimization of sample size, distribution of samples, and timing are difficult to assess in advance, although they are essential for an accurate prediction of the incidence of neck rot in storage. The latent nature of neck rot infection remains a significant problem in developing strategies to mitigate neck rot.

BOTRYTIS FLOWER BLIGHT

Pathology. In onion cultivation, *Botrytis* spp. present a serious threat not only in bulb production but also in seed production. *B. cinerea*, *B. squamosa*, *B. allii*, *B. aclada*, and to a lesser extent *B. byssoidea* are known to cause blighting and girdling of the scapes (seed stalks) and blighting of the umbel and flowers (Chilvers and du Toit 2006; du Toit et al. 2004; Ellerbrock and Lorbeer 1977b; Lorbeer 1992; Lutyńska 1968; Schwartz and Mohan 2008b, c; Ramsey and Lorbeer 1986b). Furthermore, there is one report describing the identification of *Botryotinia porri* (causal agent of Botrytis rot of garlic and leek) from blighted onion flowers (du Toit et al. 2002). *B. allii* was isolated from both blighted flowers and asymptomatic flowers, suggesting the possibility of a latent infection in flower parts, as described for neck rot (du Toit et al. 2004). Here, the term *flower blight* is used for all blighting and girdling of florets, umbels, scapes, and immature seed capsules of onion caused by *Botrytis* spp.

Infection of scapes starts at any point along the seed stalk, with the formation of lesions that consist of shriveling necrotic tissue. Expanding lesions can lead to girdling of the entire seed stalk and eventually to senescence of the umbel (Fig. 1C). Scapes and umbels that show severe symptoms of blight may fall over, resulting in reduction of seed yield (Ellerbrock and Lorbeer 1977b; Schwartz and Mohan 2008b; Netzer and Dishon 1966). Often conidia are formed on the surface of the lesions in a patchy distribution or in a pattern of concentric rings (du Toit et al. 2004; Ramsey and Lorbeer 1986b). Infection may also occur on individual florets. Open florets are more susceptible than unopen florets or immature seeds (Ramsey and Lorbeer 1986c). Floral infection may lead to infected seeds or, depending on the moment of infection, drastically reduced seed set as a result of the shriveling of immature seeds (Blodgett 1946; Ellerbrock and Lorbeer 1977b; Schwartz and Mohan 2008c). Seed transmission may be especially important for *B. aclada* and *B. allii*, for which correlations exist between the presence of the pathogen in the seeds and the incidence of neck rot in storage bulbs (Lorbeer 1992).

Flower blight in seed production fields can cause ≤80% reduction in seed yield, especially in years with high precipitation (Ellerbrock and Lorbeer 1977b; Netzer and Dishon 1966; Ramsey and Lorbeer 1986a, b). The influence of environmental conditions on flower blight has been determined via artificial inoculations. Optimal conditions to promote floret blighting are similar for the three species *B. squamosa*, *B. allii*, and *B. cinerea*, with an optimum temperature between 21 and 24°C. Periods of free moisture of up to 48 h are necessary for infection and blighting of all inflorescence parts (Ramsey and Lorbeer 1986a). Inoculations with *B. cinerea*, *B. squamosa*, and *B. allii* resulted in blighting of flowers and seed production losses of 98, 93, and 47% respectively, as compared with noninoculated controls. Inoculations with *B. byssoidea* did not lead to blighting (Ramsey and Lorbeer 1986c) or to significantly lower seed production (Ellerbrock and Lorbeer 1977b). *B. squamosa* blighted a higher percentage of florets than *B. cinerea* and *B. allii* independent of inoculum concentration, suggesting that *B. squamosa* is the most aggressive species (Ramsey and Lorbeer 1986c), although the relative significance of the different *Botrytis* spp. to flower blight is difficult to assess and may differ between regions and growing seasons.

Control. In seed production fields flower blight is commonly controlled with fungicide treatments. Sprays with chlorothalonil, benomyl, and mancozeb resulted in increased seed yield of 142, 93, and 60% respectively, as compared with untreated fields (Ellerbrock and Lorbeer 1977b). In a study in Israel, treatments of seed production fields with iprodione or vinclozolin resulted in yields, with a 45% increase in total seed weight per plant (Kritzman 1983). Also, sprays with boron increased seed yield and quality (El-Magd et al. 1989). Fungicide treatments are still used to reduce seed yield losses and to minimize the chance of contaminated seed. Development of biological control agents and breeding for resistant onion varieties against flower blight have not yet been studied.

PERSPECTIVE

Research on *Botrytis* species in onion has a long history, and most of the work described was performed several decades ago. It is remarkable how little progress has been made in the past two decades in increasing our knowledge of *Botrytis*–onion interactions, which can be exploited to rationally develop novel concepts for controlling these diseases in onion. New approaches are needed to acquire a better fundamental understanding of the biology and ecology of these fungi and of the genetic, biochemical, and physiological aspects of their interactions with onion tissues, either leaf, bulb, or inflorescence, which is essential to improve the breeding of *Botrytis*-resistant onion cultivars.

What makes *Botrytis* spp. pathogenic on *Allium* unique? As many as eight *Botrytis* species are pathogenic exclusively on plants in the genus *Allium*. In addition to *B. squamosa*, *B. aclada*, *B. allii*, and *B. byssoidea*, which exclusively infect *A. cepa*, four other species are host specific to other *Allium* species. *B. porri* is a pathogen of leek and garlic (*A. porrum*, *A. sativum*), *B. sphaerosperma* is pathogenic on three-cornered leek (*A. triquetrum*), *B. globosa* causes disease on wild garlic (*A. ursinum*), and *B. sinoalli* is a pathogen of Japanese bunching or Welsh onion (*A. fistulosum*) (Chilvers and du Toit 2006; Elad et al. 2016; Zhang et al. 2010). The *Allium*-pathogenic *Botrytis* species are not each other's closest relatives but are dispersed throughout the *Botrytis* phylogeny (Valero-Jiménez et al. 2020). This suggests that their common ancestor was able to infect *Allium* and that either this ability was lost multiple times during evolution or the ability to infect *Allium* has been acquired multiple times independently. To elucidate why so many *Botrytis* species are specialized on the same plant genus, genomic information about these species could provide insights to unravel mechanisms underlying host specificity. For example, in *F. oxysporum*, isolates with different host specificities (formae specialis) contain unique lineage-specific chromosomes that are necessary for infecting a particular host (Armitage et al. 2018; Ma et al. 2010; van Dam et al. 2017). Also, in *Verticillium* *dahliae*, lineage-specific regions, embedded within core chromosomes, have been shown to be important for pathogenicity and host specificity (Chen et al. 2018; de Jonge et al. 2013; Faino et al. 2016). An attempt was made to resolve the host specificity of *Botrytis* species infecting *Allium* by using comparative genomics. Genomes of six *Allium*-infecting *Botrytis* species were sequenced, assembled, and annotated (Table 2) and compared with those of sister species nonpathogenic on *Allium* to unravel the unique features of *Botrytis* species pathogenic on *Allium* (Valero-Jiménez et al., *unpublished data*). Genomes of *Botrytis* species appeared to be highly syntenic and similar, but indications for host specificity determinants were not identified from the genomic features (Valero-Jiménez et al., *unpublished data*). This study contributed to a deeper understanding of the molecular evolution of *Botrytis* species by obtaining high-quality genomes and increased the number of sequenced *Botrytis* species to 16 (Valero-Jiménez et al. 2019; van Kan et al. 2017).

Differences in the biology of onion-infecting *Botrytis* species. Four *Botrytis* species are known that have specialized on infecting onions. However, there are large differences between them in terms of biology and infection strategy, especially between the leaf blight pathogen *B. squamosa* and the group of neck rot fungi comprising *B. aclada*, *B. allii*, and *B. byssoidea*. *B. squamosa* behaves like a true necrotroph that kills onion leaf cells, which eventually leads to blighting, whereas the neck rot fungi proliferate latently and eventually manifest in bulb tissue. Specialization into different niches within the same host plant can be illustrated by the different sporulation patterns that have adjusted to their infection strategy.

Under laboratory conditions, spore formation of *B. squamosa* is difficult and requires conditions that mimic favorable field conditions. Optimal conditions for induction of *B. squamosa* conidia formation in vitro are temperatures between 15 and 23°C and a moist environment (Alderman and Lacy 1984; Bergquist et al. 1972). In addition, nutrient-rich substrates are needed for sporulation, and best results are obtained when *B. squamosa* is grown in onion leaves (Presly 1985a). Furthermore, light is needed for induction of sporulation, specifically light in the near ultraviolet (UV-A) spectrum and a photoperiod between 12 and 16 h (Bergquist et al. 1972). By contrast, *B. allii* produces conidia freely in the dark on a synthetic medium (Presly 1985a), reflecting the natural habitat of *B. allii* within the watery scales of onion bulbs. Furthermore, the conidia of *B. squamosa* have a volume ≤30 times larger than the conidia of *B. aclada*, *B. allii*, and *B. byssoidea* (Chilvers and du Toit 2006). Altogether, the different sporulation needs and conidial dimensions illustrate the specialization into different niches within the same host plant, resulting in a different infection biology.

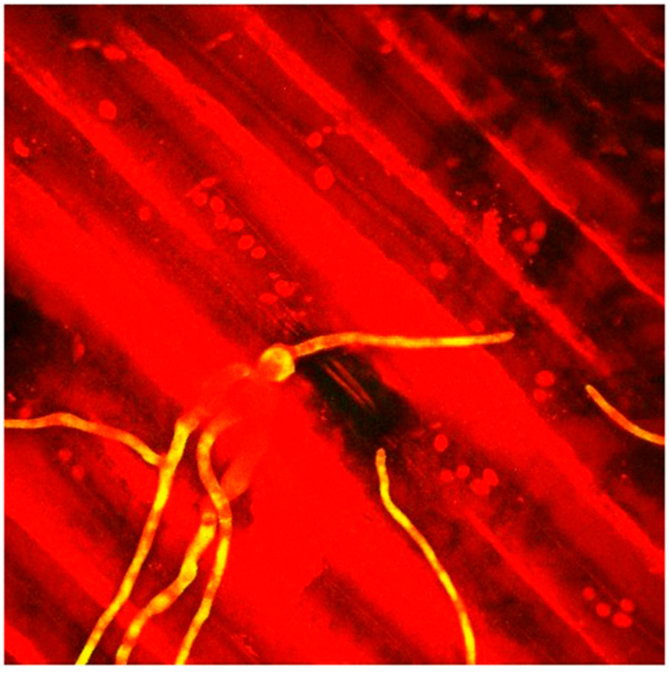
Understanding the switch from latent to visible infection. Despite the progress made in detection of neck rot fungi, prediction of yield losses and mitigation of damage remain difficult because the initial infection is latent. Information on how neck rot fungi infect and grow asymptomatically to reach the neck of the bulb is

TABLE 2
Genome assembly and gene prediction information of *Botrytis* spp. pathogenic on *Allium*

Species	Contigs	Assembly size	Largest contig	Predicted genes	Secretome size	Predicted effectors	Predicted cell wall degrading enzymes
B. aclada	16	48.31 Mb	4,155 Kb	11,870	867	137	120
B. byssoidea	59	42.98 Mb	2,599 Kb	12,212	898	152	119
B. globosa	27	45.68 Mb	4,093 Kb	12,073	864	142	116
B. porri	31	46.78 Mb	4,253 Kb	12,088	888	151	122
B. sinoallii	47	61.28 Mb	6,466 Kb	12,281	885	138	117
B. squamosa	29	54.60 Mb	4,659 Kb	11,963	897	132	132

FIGURE 2

Botrytis aclada–green fluorescent protein hyphae inoculated on the surface of an onion leaf.



lacking. *B. aclada*, *B. allii*, and *B. byssoidea* are not the only *Botrytis* species that display asymptomatic behavior. *B. deweyae* is an endophyte on daylily that can switch to necrotrophy (Grant-Downton et al. 2014). Also, *B. cinerea* has been reported to grow latently in primula (Barnes and Shaw 2003), lettuce (Sowley et al. 2010), grape (Keller et al. 2003), strawberry (Bristow et al. 1986), and roses (Elad 1988). The switch from endophytic, asymptomatic growth to visible damage and sporulation often occurs when colonized plant tissue becomes stressed, matures, or senesces (Shaw et al. 2016). In the case of neck rot, this switch might be induced when bulbs go into storage, leading to an altered bulb physiology and metabolism (Chope et al. 2012).

For a better understanding of the latent infection and endophytic growth of neck rot, *B. aclada* was recently transformed with a green fluorescent protein label that can be used in fluorescence microscopy (Fig. 2) (M. B. F. Steentjes, *unpublished data*). Visualizing the fungal entry into the host tissue and tracing its latent growth toward the bulb will help in elucidating the infection biology of neck rot and might contribute to more accurate predictions of yield losses and development of control strategies.

Molecular aspects of *Botrytis*–onion interactions. Necrotrophic fungi such as *Botrytis* spp. kill cells of their host plants in order to obtain nutrients and colonize dead plant tissue. To do so, they secrete effector proteins and secondary metabolites that actively induce host cell death (Veloso and van Kan 2018). Effector proteins of several necrotrophic pathogens, such as of *B. cinerea*, *Sclerotinia sclerotiorum*, and *Parastagonospora nodorum*, have been studied (Heard et al. 2015; Mousavi-Derazmahalleh et al. 2019; Syme et al. 2018). In the case of necrotrophic fungi, susceptibility of host plants is the result of recognition of one or more necrotrophic effector proteins by receptors of the host (following a so-called inverse gene-for-gene model) (Shi et al. 2015). Studying the molecular aspects of *Botrytis*–onion interactions might provide tools that can be used in breeding programs to breed for partially resistant onion cultivars. If effector proteins of *Botrytis* spp. are identified that specifically trigger programmed cell death in onion, such proteins can be used for selecting onion genotypes lacking the receptor that recognizes the effector protein. In the case of the *P. nodorum*–wheat interaction, stacking insensitivities to effector proteins led to reduced susceptibility to the pathogen secreting the effector proteins (Friesen and Faris 2010; Shi et al. 2016).

All *Botrytis* species, including *B. squamosa*, have a repertoire of about 125 predicted effector genes. Culture filtrates of *B. squamosa* induce an onion-specific programmed cell death upon leaf infiltration (M. B. F. Steentjes, *unpublished data*), suggesting that some of the secreted effectors probably possess cell death–inducing activity. Furthermore, *B. squamosa* possesses a large array of secreted enzymes that degrade plant cell wall carbohydrates into consumable saccharides. Neither the effector repertoire nor the secreted enzymes revealed obvious key determinants that distinguish the host specificity of *B. squamosa* from that of its sister species *B. elliptica* and *B. deweyae*, which are host specific on lily and daylily, respectively (Valero-Jiménez et al. 2020). It is expected that the search for and characterization of secreted virulence factors in *B. squamosa* will be greatly facilitated by the ability to transform *B. squamosa* by using the CRISPR-Cas9 protocol recently developed for *B. cinerea* (M. B. F. Steentjes, *unpublished data*; Leisen et al. 2020).

Another fundamental concept of plant–pathogen interactions regards the study of antimicrobial compounds that inhibit fungal growth. Plants possess preventive defense mechanisms against invaders, such as antimicrobial compounds. However, plant pathogens have evolved mechanisms that confer tolerance to such compounds, which allows them to be pathogenic. In the cereal pathogen *Gaeumannomyces graminis*, which infects oat and wheat, detoxification of antimicrobial compounds was observed that determines pathogen host range. Isolates of *G. graminis* that infect oat produce an enzyme called avenacinase that detoxifies the antimicrobial secondary metabolite avenacin, whereas isolates that infect wheat are unable to detoxify avenacin and thus cannot infect oat. Both isolates are able to infect wheat, which does not produce avenacin. Avenacinase knockout mutants of the oat-infecting isolate became nonpathogenic on oat, and introduction of avenacinase into wheat-infecting isolates rendered pathogenicity on oat (Bowyer et al. 1995). Likewise, tomato produces the antifungal compound α-tomatine, which can be enzymatically degraded by the broad host range pathogen *B. cinerea* via a secreted tomatinase. However, one *B. cinerea* isolate collected from grapevine could not degrade α-tomatine and consequently was not aggressive on tomato (Quidde et al. 1998). By analogy, it seems logical to propose that pathogens of onion leaves, bulbs, and flowers must be tolerant to antifungal compounds in these tissues. In onion different antimicrobial compounds have been identified, such as saponins called ceposides and allicin, an antimicrobial compound found in garlic and other *Allium* species (Lanzotti et al. 2012; Marchese et al. 2016; Vu et al. 2012). Unraveling mechanisms that *Botrytis* spp. use to detoxify these compounds will enhance our understanding of the infection biology of *Botrytis* spp. pathogenic on onion. New molecular, cellular, and biochemical insights in the *Botrytis*–onion interaction might reveal leads for resistance breeding in onion as a durable solution against leaf blight, neck rot, and flower blight in onion cultivation.

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