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ALTERNARIA BLACK SPOT OF CRUCIFERS: SYMPTOMS, IMPORTANCE OF DISEASE, AND PERSPECTIVES OF RESISTANCE BREEDING

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Summary

Alternaria black spot of cruciferous vegetables, incited by different species of *Alternaria,* remains an increasing threat to *Brassicaceae* crops throughout the world, including Poland. *Brassica* plants are attacked by conidia of *A. brassicae* (Berk.) Sacc., *A. brassicicola* (Schw.) Wiltsh., *A. raphani* Groves & Skolko, and *A. alternata* (Fr.) Kreissler. The pathogens have a wide spectrum of hosts, such as head cabbage, Chinese cabbage, cauliflower, broccoli, and other crucifers including cultivated and wild grown plants. *Alternaria* pathogens usually cause damping-off of seedlings, spotting of leaves of cabbages, blackleg of heads of cabbages, and spotting of cauliflower curds and broccoli florets. In oilseed rape, *A. brassicae* is the dominant invasive species, while in the cruciferous vegetables, both species, *A. brassicae,* and *A. brassicicola* are encountered. Infected seeds with spores on the seed coat or mycelium under the seed coat are the main means of distribution for these pathogens. The fungus can overwinter on susceptible weeds or crop debris and on seed plants, as well as on stecklings.

Methods for disease prevention and control are based on combining agricultural management practices with chemical control. Using disease-free seeds or seeds treated with fungicides can greatly reduce disease incidence. After appearance of the first symptoms of disease, stringent fungicide spray program is an effective way to reduce losses. Many authors seem to agree, that the most economically feasible method of disease control is the development of resistant *Brassicaceae* crops varieties, as transgenic approach proved unsuccessful. Due to our increasing understanding of pathogen-host plant interactions, identification of resistance sources, and assessment of the resistance trait inheritance mode, breeding programs of *Brassica* crops for *Alternaria* resistance can be enhanced. This is of particular importance since recent years experience dynamic development of ecological and integrated plant production with an emphasis on plant biotic stress resistance. Highly resistant genetic resources have not been reported in *Brassica* cultivated species, although some varieties differ in their resistance/susceptibility level.

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Strong cross-incompatibility, polygenic background of the resistance (additive and dominant gene interactions), as well as the differences in ploidy between the *Brassica* species of interest, render the transfer of *Alternaria* resistance from the wild species into the cultivated forms difficult. Additionally, it is often connected with employment of *in vitro* hybridization techniques, including somatic hybridization, embryo and ovary rescue, or protoplast fusion.

key words: *Alternaria* spp., cruciferous plants, resistance, testing methods

INTRODUCTION

Cruciferous plants (*Brassicaceae*) worldwide are severely affected by the *Alternaria* fungi. *A. brassicae* (Berk.) Sacc., *A. brassicicola* (Schw.) Wiltsh., *A. raphani* Groves and Skolko, and *A. alternata* (Fr.) Kreissler belong to species of major negative influence on *Brassicaceae* plants. Although both, *A. brassicicola* and *A. brassicae* occur on oleiferous and vegetable (oleraceous) Brassicas, the former is the dominant invasive species of the vegetable Brassicas, while the oleiferous crucifers are primary hosts for the latter fungus (Maude & Humpherson-Jones 1980, Humpherson-Jones 1989).

Both most common *Alternaria* pathogens usually cause black spot disease, manifested by damping-off of seedlings, spotting of leaves of cabbages, blackleg of heads of cabbages (head cabbage and Chinese), and spotting/browning of cauliflower curds and broccoli florets. Black spot is the most common disease in the crucifers plantations located in tropical and sub-tropical regions. Due to weather conditions conducive to infection (see below), however, the disease constitutes a serious problem in crucifers plant production in long-lasting high humidity or intensive rains regions, including Poland.

A. brassicicola and *A. brassicae* cause severe economic losses in several different ways (Humpherson-Jones & Maude 1982, Humpherson-Jones 1989). Seed infection causes reduced germination and seedling vigour, in addition to pre- and post-emergence damping-off, and affects the sale and use of infected/infested seed. Lesions on leaves, stems, and siliques reduce the photosynthetic area and accelerate senescence in the plant. These pathogens are responsible for major seed yield losses in the oleraceous Brassicas and this is the most important component of their economic impact. The unsightly cosmetic blemishing or rotting of the head or wrapper leaves in vegetable Brassicas as a consequence of pathogens' toxins and disease causes downgrading and crop losses in both fresh and stored produce. *A. brassicicola* often occurs in conjunction with *A. brassicae* and some other pathogens of the *Brassicaceae*. This confounds precise estimates of losses caused individually by this pathogen in the field.

In Europe, alone the seed losses due to both pathogens were estimated at up to 86% in *B. oleracea* in several years (Maude & Hampherson-Jones 1980, Humpherson-Jones 1989). Under Polish climate conditions, the disease takes particularly high toll on

the late and medium-late varieties of head-cabbage grown for sauerkraut processing, as well as for storage. Cabbage heads with characteristic symptoms of *Alternaria* black spot do not store well, and their processing value is very low. This translates onto significant economic impact of the disease: In 2010, Polish cabbages and other Brassicas production ranked 7th, while cauliflower and broccoli ranked 8th globally (1141200 tonnes and 252325 tonnes, respectively) with net worth of production of Int.$171 M and Int.$60,5 M, respectively (FAOSTAT: http://faostat.fao.org).

Alternaria prevention and control methods include combining the proper agro-technique with chemical protection. An essential disease prevention method is production of healthy seeds, obtained from plantations with heavy fungicide protection. In the 2-year lasting crucifers seed production periods, good effects of protection against *Alternaria* infections during the 1st year of growth were expedited by fungicides containing iprodione as an active ingredient (Maude *et al.* 1984, Survilienė *et al*. 2010). In Poland, the only product containing it is Seed Protector T 75DS WS (Zaprawa Nasienna). Since both pathogens survive on crop debris, seeds, and in association with weed hosts (Humpherson-Jones & Maude 1982, Humpherson-Jones 1989), crop debris management (for example through crop rotation and deep tillage) and use of clean seed and proper weed control should alleviate the disease. After appearance of disease symptoms, one may achieve limitation of the infection by repeated spray with fungicides containing strobilurines as active ingredients (Amistar 250 SC, Signum 33 WG, Zato 50 WG) and fungicides based on iprodione (Rovral FLO 255 SC) (Maude *et al.* 1984, Survilienė *et al*. 2010). This method, however, carries an economic disadvantage and may prove ineffective under pathogen infection-conducive weather conditions, particularly among the seed crops. An alternative protection method to be employed is use of antagonistic fungi; deployment of *Aureobasidium pullulans* and *Epicoccum nigrum* on the crucifers leaves reduced the infection level under controlled conditions (Pace & Campbell 1974). Field studies concerning the biological control efficacy are yet to be carried out.

Pathogen profile and infection progress

The current classification of *Alternaria* fungi is as follows: Kingdom Fungi, Phylum Ascomycota; Subdivision Pezizomycotina; Class Dothideomycetes; Order Pleosporales; Family *Pleosporaceae*; Subfamily mi-tosporic Pleosporaceae; Genus *Al-ternaria* (http://www.uniprot.org/taxonomy/5598). There are 299 species listed in the genus (Kirk *et al.* 2008); most *Alternaria* species are saprophytes that are commonly found in soil or on decaying plant tissues (Bart & Thomma 2003). *Alternaria* fungi proliferation is vegetative in character, and takes place by means of conidial spores, airborne and found in the soil and water, as well as indoors and on objects. Sexual recombination (teleomorphy) occurs very rarely.

Alternaria spp. may be grown on artificial agar media, with PDA (potato-dextrose agar) and V8 (V8 juive - agar) being the most popular in use.

As observed in the *in vitro* cultures, the pathogens develop fast-growing thick colonies which are usually green-black, or white-gray, with brown to black reverse. Dark septa divide the branched or unbranched conidiophores, carrying the conidial chains. Growing hyphae develop light-brown or dark-tawny, club-shaped spores - single or forming long chains with longitudinal and transverse septa (Fig. 1A).

Primary source of pathogens are the infected seeds or non-decomposed plant debris in the top soil layers with over-wintering hyphae or spores. Another important source of the pathogen are the Brassica weeds, which promote infestations as pathogen host plants (Humpherson-Jones 1989). During the vegetation period, the rain- and wind-transported fungal conidial spores are also an important source of infection. A majority of conidia are released during harvest and cleaning the crops from the infected leaves; such released spores are then spread approximately within 1,800 m (Humpherson-Jones & Maude 1982). Under Polish climate, highest *Alternaria* spore concentrations are detected in the air mainly in June and July (Nowakowska *et al*. 2011).

Alternaria black spot symptoms appear on all host plant parts and at every developmental stage. *A. brassicicola* and *A. brassicae* cause the damping off of the crucifers seedlings. Elongated brownings develop on the sub-cotyledonous part of the stem and on the cotyledons, often leading to narrowing and breaking of the stems, and thus, to seedlings' decease. Most often infected are the lower, older leaves of head cabbage (Fig. 1B,C), Chinese cabbage, cauliflower, broccoli, and of other crucifers. Infected cauliflower curds or broccoli florets develop slight dents with brownish spots covered with black bloom of spores. In these plants, infection usually remains on the surface and does not reach deep in the curd or the floret; however, symptomatic cauliflower or broccoli florets lose their commercial value. In case of radish, turnip, or rutabaga, the disease affects the root thickenings as well; the disease symptoms manifest themselves as brown rots only during their storage.

Three ways of *Alternaria* infection have been reported: Through epidermis penetration, through stomata, and through insects- or agrotechnique-derived host plants wounding. Regardless of their means of entry, *A. brassicicola* and *A. brassicae* exhibit distinct differences in the host plant tissue penetration. *A. brassicae* invades host plants solely through their stomata, while for *A. brassicicola*, direct plant tissue penetration prevails over stomatal infections. Hyphae of both pathogens develop well on the epidermis, directly beneath the leaf waxes, and exhibit low cell penetration ratio. Upon successful pathogen attack, dark-brown spots of different sizes (0.5 cm to several cm in diameter) appear on the leaves; the spots of characteristic concentric circumferences sometimes have a yellow chlorotic halo (Fig. 1B,C). Host plants' reaction to the perceived infection is manifested as almost immediate browning of cell walls, in particular in the parastomatal cells. Under favorable conditions, lesions become covered with brown-black downy-like bloom of sporulating hyphae. *A. bras-*

sicicola-derived spots are darker and less regular in shape compared with those of *A. brassicae* origin. As the disease progresses, the spots enlarge, and the infected plant tissue perishes and crumbles, giving rise to dents and hollows (Fig. 1C).

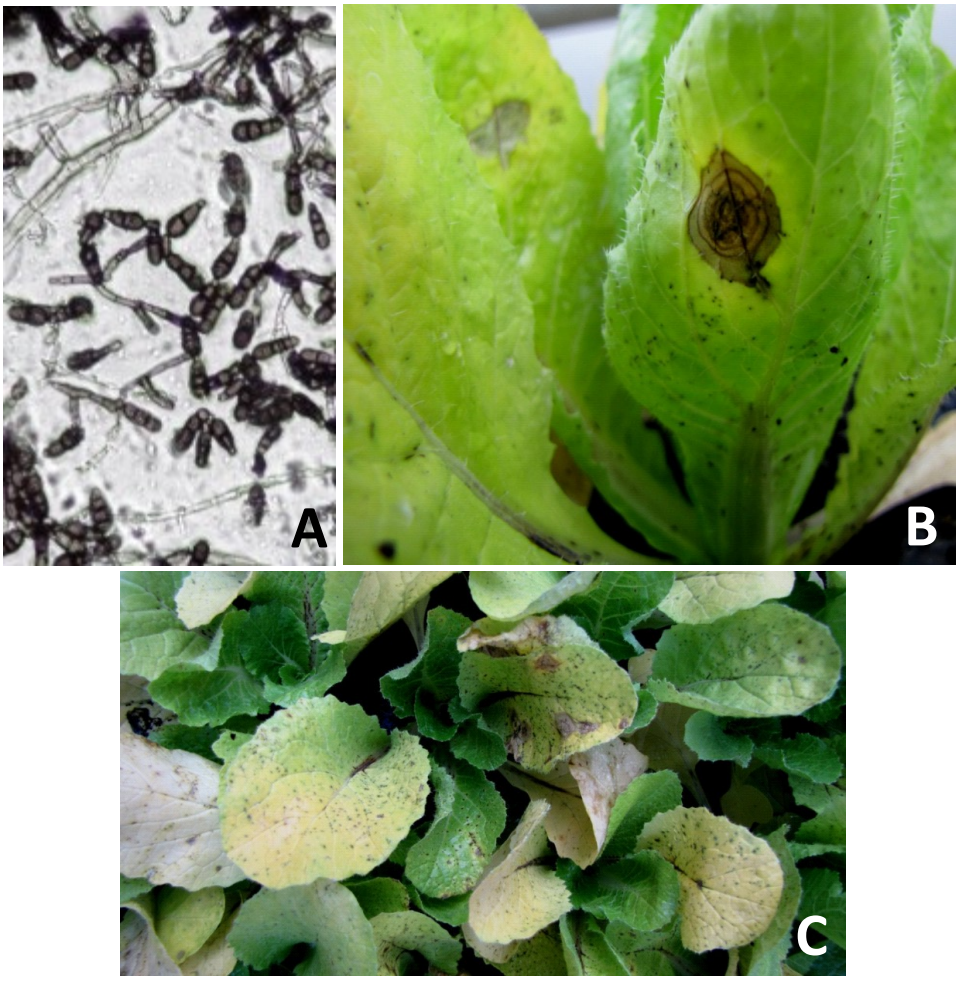

Fig. 1. *Alternaria brassicicola* and cabbage damping off symptoms. (A) Pathogen mycelium as visible under light microscope [40x]. (B) Characteristic concentric lesions on the abaxial leaf side. (C) Due to heavy infection, seedlings display black spot symptoms, followed by damping off.

Development of the pathogen's infection structures and of the disease symptoms on the oleraceous Brassicas depends primarily on the incubation temperature (Bassey & Gabrielson 1983), and relative air humidity. While spores of both *Alternaria* species germinate in a rather broad temperature range, germination effectiveness is correlated with the temperature (Degenhardt *et al*. 1982). Optimal hyphae growth temperature for *A. brassicae* is

18-24ºC, and for *A. brassicicola* 20-30ºC. According to the *in vitro* studies, sporulation of *A. brassicae* is temperature-dependant: At 8-24ºC, fully-developed spores are detectable at 24 or 14 h, respectively. Temperature spectrum of *A. brassicicola* sporulation is broader (8-30ºC), with fully-developed spores detectable after 43 or 14 h, respectively. High air humidity (95-100% RH) lasting at least 9-18 h is a crucial requirement displayed by both pathogen species during plant infection (Humperson-Jones & Phelps 1989). Reports of massive infestations under air temperatures of 20-27ºC and constant plant moisture of at least 5 h, or RH exceeding 95% lasting at least 12-20 h are commonplace (reviewed in Bart & Thomma 2003).

During plant infection, both *Alternaria* fungi produce and exude phytotoxins that belong to HST clade (host-specific toxins or host-selective toxins; Parada *et al*. 2008, Wight *et al.* 2009). These compounds play a major role in the pathogenesis by determining the host plant spectrum, as well as the isolates' virulence and pathogenicity levels (Nishimura & Kohmoto 1983). To date, two HSTs have been characterized in detail: AB toxin of *A. brassicicola* (Otani *et al*. 1998) and ABR of *A. brassicae* (Parada *et al*. 2008). Both phytotoxins are proteins with a suggested role in evoking disease symptoms on the infected cruciferous plants.

In addition to the above, *A. brassicae* produces a number of phytotoxins (destruxin B and derivatives, such as homodestruxin B, desmethyldestruxin B, and destruxin B_2) responsible for typical black spot symptoms, such as necrotic and chlorotic lesions. Destruxin B was shown to be a major one responsible for inducing necrotic lesions on plant leaves and eliciting the phytoalexins brassilexin and sinalbin A (Sodelade *et al*. 2012). Majority of researchers classify destruxin B as an HST (Sodelade *et al*. 2012), while others have demonstrated its unspecific character and questioned its role in the initial host plant colonization (Buchwaldt & Green 1992, Parada *et al*. 2008). While some isolates of *A. brassicae* produce destruxin B as their sole toxin, others are capable of production of their derivatives (homodestruxin B, desmethyldestruxin B, and destruxin B_2; Parada *et al*. 2008).

Depudecin, an eleven-carbon linear polyketide and histone deacetylase (HDAC) inhibitor made by *A. brassicicola*, proved a minor virulence factor. Depudecin-minus mutants have a small (10%) but highly significant ($p<0.01$) reduction in lesion size on cabbage, but not on *Arabidopsis*, including the *pad3* mutants, a susceptible control for *Alternaria* inoculation (Zhou *et al.* 1999, Wight *et al.* 2009). Likely reasons for only a minor role of this polyketide in *A. brassicicola* pathogenicity on cabbage and *Arabidopsis* are: Depudecin concentrations or penetration insufficient to effectively inhibit host plant's HDAC; HDAC inhibition playing major role in pathogenicity in grasses but not in other plants (cabbage, *Arabidopsis*); redundancy of HDAC inhibitors in *A. brassicicola*, masking the depudecin loss (Wight *et al.* 2009).

Alternaria spp. pathogens are also capable of production of other unspecific toxins. In all *Alternaria* - infected organs, alternariol and tenuazonic acid

have been detected. Alternariol-induced cytotoxicity is mediated by activation of the mitochondrial pathway of apoptosis. High concentrations of tenuazonic acid inhibit protein synthesis and, thus, negatively affect seed germination (Tylkowska *et al*. 2003, Bart & Thomma 2003). Moreover, *A. brassicae*- and *A. brassicicola*-produced cytokines cause green discolorations within the diseased spots (Tylkowska *et al*. 2004). *A. brassicae*-derived abscisic acid causes premature leaf aging and defoliation, dropping flowers, or premature breaking of the siliques (Tewari 1991a).

Disease impact

The highest toll the *Alternaria*s collect is on the seed plantations of the oleraceous Brassicas, including the cabbages. Infected silique tissue perishes and withers, as a result of which the siliques shrink, break open, and the seeds drop (Maude & Humpherson-Jones 1980), which generates significant economic losses. Upon strong infections of young siliques, the seeds do not develop, or remain underdeveloped, and exhibit decreased vigor and germinability (Chirco & Harman 1979). Infected seeds may display the hyphae present on their surface (surface infection), but the hyphae is able to grow through the seed cover (internal infection). Spores localized both internally and externally may survive several years, although the internal infection seems to be more durable (Maude & Humpherson-Jones 1980). Seedlings developing from infected seeds show typical symptoms of damping off (small black spots on the bottom leaf surface or dark stripes on the hypocotyls).

Sources of *Alternaria* spp. resistance

Brassica crops endangered with *Alternaria* black spot necessitate complex projects on production of varieties with high levels of genetic resistance. An accomplishment of new varieties of these crops exhibiting resistance against the disease is regarded as potentially most economically feasible solution to limiting the yield losses. Such a task remains crucial from the standpoint of the vegetable producers, as well as of the consumers. It allows for a decrease in pesticide use, which is of particular importance in the integrated and ecological vegetable production. Unfortunately, such resistance breeding of the crops is currently hindered due to bottlenecks experienced in transfer of resistance from the wild species into commercial lines.

Until now, no high-level resistance sources against *A. brassicicola* or *A. brassicae* have been identified among the cultivated species of the *Brassica* genus; however, individual varieties among the cabbages may differ in the exhibited levels of susceptibility to black spot (Otani *et al.* 2001). The highest level of *Alternaria* resistance from among the *Brassica* crops is displayed by the Ethiopian mustard (*B. carinata*). Among the wild cruciferous plants closely related to the *Brassica* genus, the highest *A. brassicae* resistance levels were confirmed for white mustard (*Sinapis alba*; Kolte 1985, Brun *et al.* 1987, Ripley *et al.* 1992, Sharma & Singh 1992, Hansen & Earle 1995, 1997); however, the highest overall *Alternaria* spp. resistance has been identified in the crucifers species more distant from the *Brassica*, such as camelina (*Came-*

lina sativa; false flax), shepherd's-purse (*Capsella bursa-pastoris*), rucola (*Eruca sativa*), and ball mustard (*Neslia paniculata*) (Conn & Tewari 1986, Conn *et al*. 1988, Tewari 1991b). Resistance against *Alternaria* black spot has also been reported among other wild members of the *Brassicacae* family (Sharma *et al.* 2002, Tewari & Conn 1993; reviewed and referenced in Warwick 2011): *Alliaria petiolata*; *Barbarea vulgaris*; *Brassica elongate, B. desnottessi, B. fruticulosa*, *B. maurorum*, *B. nigra*, *B. souliei*, *B. spinescens*; *Camelina sativa*; *Capsella bursa-pastoris*; *Coincya* spp.; *Diplotaxis catholica*, *D. berthautii*, *D. creacea*, *D. erucoides*, *D. tenuifolia*; *Erucastrum gallicum*; *Eruca vesicaria* subsp. *sativa*; *Hemicrambe fruticulosa*, *H. matronalis*; *Neslia paniculata*; *Rhaphanus sativus*; *S. alba*, and *S. arvensis*. The completely-immune plants remained symptom-free both, under natural field infection, as well as under controlled artificial inoculation (Sharma *et al*. 2002). Comparatively, broccoli and cauliflower varieties exhibited only moderate *Alternaria* resistance, while the cabbages turned out susceptible.

Resistance background

Depending on the plant material studied, *A. brassica*/*A. brassicicola* resistance was said to be controlled by one or several nuclear genes of partially-dominant interaction (Zhang *et al*. 1997) or is conditioned by additive inheritance (Krishnia *et al*. 2000). On the biochemical level, resistance against *Alternaria* pathogens seems to be connected with high activities of phenolases (polyphenol oxidase, peroxidase, catalase), high levels of leaf sugars (Singh *et al*. 1992), and thicker epicuticular wax layer forming a hydrophobic coating to reduce the adherence of water-borne inoculum, as well as limiting spore germination rate (Meena *et al*. 2010). Presence of intensive leaf wax deposition seems correlated with the resistance exhibited by other *Brassicacae* plants (Meena *et al*. 2010).

Wild crucifers are found to elicit phytoalexins upon challenge inoculation (Conn *et al*. 1988). Among the *Alternaria*-resistant species, camelina stands out for its immunity against *A. brassicicola* infection, originating in the plant's ability to synthesize camalexin, a compound with antibiotic properties, and thus to hamper pathogen's development. Indeed, it has been demonstrated that camalexin deficient *Arabidopsis* mutant, *pad-3* is more susceptible to *A. brassicicola* than wild-type plants (Zhou *et al*. 1999). Additional evidence that camalexin plays a major role in resistance came from the observation that different *Arabidopsis* ecotypes with varying levels of camalexin show correlative differential resistance (Kagan & Hammerschmidt 2002). Finally, the *esa1* mutation affects resistance against *A. brassicicola* through a severe reduction in both camalexin production, as well jasmonate-depen-dent gene induction, although the *Esa1* gene has yet to be cloned (Tierens *et al*. 2002).

Resistance testing

A direct method of determination of *Alternaria* resistance are the phytopathological tests: Field-, greenhouse-, or phytotron-based. Field observations can be carried out upon natural pathogen infection, or after controlled artificial inoculation with fungal spore

suspension. Advantages of the greenhouse or phytotron tests are: Speediness, reproducibility, and a possibility of control of the conditions. Phytopathological tests require *Alternaria* spp. conidia, collected directly from the infected plant tissue or maintained on the artificial media. On the commonly used PDA artificial media, fungal growth and effective spontaneous sporulation take place at 25±2ºC, in darkness. A choice of other methods exist towards *Alternaria* spp. growth and maintenance. *A. brassicicola* hyphae has been successfully cultured on artificial media V8A (V8 juice - agar) at 25ºC, resulting in spontaneous sporulation under 12 h photoperiod (Otani *et al*. 1998).

Controlled-conditions phyto-tests are carried out on whole plants (*in vivo*) or on detached leaves (*in vitro*; Sharma *et al*. 2002). Plants are routinely tested at 3-6 weeks seedlings stage, but cotyledon phyto-tests have been published as well (Doullah *et al*. 2006). The detached leaf method is one of the most often employed ways to assess the *Alternaria* spp. resistance levels displayed by the tested plants under controlled conditions. Differences exist, however, regarding the inoculation method and assay conditions. As described in several studies, inoculum of 5×10^4 spores×ml^{-1} was placed at the upper (adaxial) leaf side (Doullah *et al*. 2006), while other authors described spraying the lower (abaxial) leaf side with inoculum of 3×10^5 spores×ml^{-1} (Parada *et al*. 2008). Yet others inoculated only the 4th and 5th leaves (seedling 45 days old; Sharma *et al*. 2002). Wet swabs have been used to remove the leaf wax layers on both nerve sides on the adaxial leaf surface, due to which the aqueous spore suspension gets uniformly distributed on the leaf surface, without the need of adding agar or adjuvants (Sharma *et al*. 2002). Better adhesion of the water-suspended spores to wax-covered leaf surface of cabbages is granted by addition of agar (Ho *et al*. 2007) or Tween (Doullah *et al*. 2006). Thin needle has been employed to make small surface cuts, onto which a droplet of inoculum (4×10^3 spores×ml^{-1}) was placed.

Disease symptoms have been scored in 24 h increments for 3 dpi. Resistance scoring of individual plants included three parameters: Percentile of infected leaf surface (0-60 pts), lesion size (0-30 pts), and incubation duration (0-10 pts). Plants exhibiting maximum susceptibility scored 100 points. Individual plants have been grouped into the resistance classes, according to their points scoring: 0-fully resistant; 1-15 pts – moderately resistant; 16-25 pts – susceptible; above 25 pts – highly susceptible. As discussed above, optimal phyto-test conditions are temperatures of about 20°C, relative humidity of at least 90% lasting for 6 h and more, and inoculum load of 6×10^4 spores×ml^{-1} (Sharma *et al*. 2002, Doullah *et al*. 2006).

Bottlenecks in resistance breeding

Since resistance against *Alternaria* black spot is generally governed by polygenes, breeding for resistance could involve pyramiding of minor genes to provide additive/polygene resistance. Rapid advances in techniques of tissue culture, protoplast fusion, embryo rescue, and genetic engineering have made possible the transfer of disease resistance traits

across the otherwise impassable self-incompatibility barriers. Transgenic plants with disease resistance which over-express different antifungal compounds like pathogenesis-related (PR) proteins (chitinase, glucanase, osmotin, etc.) and ribosome inhibiting proteins (RIPs) such as thionins, defensins, and phytoalexins (Zhou *et al*. 2002) to inhibit growth of the pathogen, seem less efficacious.

To introduce camelina-derived *A. brassicicola* resistance into commercial varieties, somatic hybrids between *C. sativa* and *B. carinata* have been procured; however, the researchers failed to multiply the resulting hybrids (Narasimhulu *et al.* 1994). Similar strategy of protoplast fusion between *C. sativa* and *B. oleracea* with subsequent hybrid regeneration also proved unsuccessful (Hansen 1998). Several research groups attempted, but not succeeded, to introduce the *E. sativa*-deriving black spot resistance into various species of cultivated crucifers (Fahleson *et al*. 1988, Sikdar *et al*. 1990, Sigareva & Earle 1997). The first somatic hybrids to be obtained as a result of protoplast fusion were those of *B. napus* (rapeseed) and *S. alba* (Primard *et al*. 1988). None of the hybrids procured that way showed *A. brassicae* resistance comparable to that exhibited by *S. alba*. Chevre *et al*. (1991) used these species towards interspecies crosses through somatic hybridization and bidirectional crosses. Having employed the embryo rescue technique, the researchers succeeded in regeneration of *B. napus* plants carrying 38 chromosomes typical for that species, and displaying *A. brassicae* resistance at levels close to this of *S. alba*, *B. oleracea* var. *botrytis*, or *B. carinata* (Ryschka *et al*. 1996). Seeds of developed intertribal somatic hybrids between *B. napus* and *C. sativa* (by means of protoplast electrofusion) exhibited phenotype intermediate compared with the parental species. They also exhibited higher level of linolenic and eicosanoic acids, but the hybrid plants await determination of their *Alternaria* resistance (Jiang *et al.* 2009).

In general, it has been postulated, that introduction of *Alternaria* resistance genes into commercial cultivars of crucifers is dependent on cumulation of horizontal resistance genes (Sharma *et al*. 2002). Hence, it is imperative to identify various sources of horizontal resistance among the *Brassica* plants (see above), and subsequently to combine them towards increase in durable *Alternaria* protection. Strong cross-incompatibility, polygenic background of the resistance (additive and dominant gene interactions), as well as the differences in ploidy (differing number of chromosomes) between respective *Brassicaceae* species render the transfer of *Alternaria* resistance from the wild species into the cultivated forms difficult. Additionally, it is often connected with employment of advanced *in vitro* hybridization techniques, including somatic hybridization, embryo and ovary rescue, or protoplast fusion.

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REFRENCES

Bart P.H., Thomma J. 2003. *Alternaria* spp.: from general saprophyte to specific parasite. Molecular Plant Pathology 4(4): 225-236. [DOI: 10.1046/j.1364-3703.2003.00173.x]

Bassey E.O., Gabrielson R.L. 1983. The effects of humidity, seed infection level, temperature and nutrient stress on cabbage seedling disease caused by *Alternaria brassicicola.* Seed Science and Technology 11: 403-410.

Brun H., Plessis J., Renard M. 1987. Resistance of some crucifers to *Alternaria brassicae* (Berk.) Sacc. Proc. Seventh Int. Rapeseed Congr. Poznan, Poland: 1222-1227.

Buchwaldt L., Green H. 1992. Phytotoxicity of destruxin B and its possible role in the pathogenesis of *Alternaria brassicae.* Plant Pathology 41: 55-63. [DOI: 10.1111/j.1365-3059.1992.tb02316.x]

Chevre A.M., Eber F., Brun H., Plessis J., Primard C., Renard M. 1991. Cytogenetic studies of *Brassica napus – Sinapsis alba* hybrids from ovary culture and protoplast fusion. Attempts to introduce *Alternaria* resistance into rapeseed. Proceedings of International Rapeseed Conference 8: 346-351.

Chirco E.M., Harman G.E. 1979. The effects of *Alternaria brassicicola* infection on Brassica seed vigor and viability. Journal of Seed Technology 3: 12-22.

Conn K.L., Tewari J.P. 1986. Hypersensitive reaction induced by *Alternaria brassicae* in *Eruca sativa*, an oil-yielding crucifer. Canadian Journal of Plant Pathology 8: 348.

Conn K.L., Tewari J.P., Dahiya J.S. 1988. Resistance to *Alternaria brassicae* and phytoalexins-elicitation in rapeseed and other crucifers. Plant Science 56: 21-25. [DOI: 10.1016/0168-9452(88)90180-X]

Degenhardt K.J., Petrie G.A., Morrall R.A.A. 1982. Effects of temperature on spore germination and infection of rapeseed by *Alternaria brassicae, A. brassicicola,* and *A. raphani.* Canadian Journal of Plant Pathology 4: 115-118. [DOI: 10.1080/07060668209501311]

Doullah M.A.U., Meah M.B., Okazaki K. 2006**.** Development of an effective screening method for partial resistance to *Alternaria brassicicola* (dark leaf spot) in *Brassica rapa*. European Journal of Plant Pathology 116: 33-43. [DOI: 10.1007/s10658-006-9035-2]

Fahleson J., Rahlen L., Glimelius K. 1988. Analysis of plants regenerated from protoplast fusions between *Brassica napus* and *Eruca sativa*. Theoretical and Applied Genetics 76(4): 507-512. [DOI: 10.1007/BF00260900]

Hansen L.H. 1998. Intertribal somatic hybridization between rapid cycling *Brassica oleracea* (L.) and *Camelina sativa* (L) Cranz. Euphytica 104: 173-179. [DOI: 10.1023/A:1018604919488]

Hansen L.H., Earle E.D. 1995. Transfer of resistance to *Xanthomonas campestris* pv. *campestris* (L.) by protoplast fusion. Theoretical and Applied Genetics 91: 1293-1300. [DOI: 10.1007/BF00220944]

Hansen L.H., Earle E.D. 1997. Somatic hybrids between *Brassica oleracea* (L.) and *Sinapis alba* (L.) with resistance to *Alternaria brassicae* (Berk.) Sacc. Theoretical and Applied Genetics 94: 1078-1085. [DOI: 10.1007/s001220050518]

Ho W.C., Wu T.Y., Su H.J., Ko W.H. 2007. Effect of oriental medicinal plant extracts on spore germination of *Alternaria brassicicola* and nature of inhibitory substances from

speedweed. Plant Disease 91: 1621-1624. [DOI: 10.1094/PDIS-91-12-1621]

Humpherson-Jones F.M. 1989. Survival of *Alternaria brassicae* and *Alternaria brassicicola* on crop debris of oilseed rape and cabbage. Annals of Applied Biology 115: 45-50. [DOI: 10.1111/j.1744-7348.1989.tb06810.x]

Humpherson-Jones F.M., Maude R.B. 1982. Studies on the epidemiology of *Alternaria brassicicola* in *Brassica oleracea* seed production crops. Annals of Applied Biology 100: 61-71. [DOI: 10.1111/j.1744-7348.1982.tb07192.x]

Humpherson-Jones F.M., Phelps K. 1989. Climatic factors influencing spore production in *Alternaria brassicae* and *Alternaria brassicicola.* Annals of Applied Biology 114: 449-458. [DOI: 10.1111/j.1744-7348.1989.tb03360.x]

Jiang J.J., Zhao X.X., Tian W., Li T.B., Wang Y.P. 2009. Intertribal somatic hybrids between *Brassica napus* and *Camelina sativa* with high linolenic acid contents. Plant Cell**,** Tissue and Organ Culture 99: 91-95. [DOI: 10.1007/s11240-009-9579-x]

Kagan I.A., Hammerschmidt R. 2002. *Arabidopsis* ecotype variability in camalexin production and reaction to infection by *Alternaria brassicicola*. Journal of Chemical Ecology 28: 2121-2140.

Kirk P.M., Cannon P.F., Minter D.W., Stalpers J.A. 2008. Dictionary of the Fungi. 10th ed. Wallingford: CABI. p. 22. ISBN 0-85199-826-7.

Kolte S.J. 1985. Diseases of annual edible oilseed crops. Vol. II, Rapeseed-mustard and sesame diseases. CRC Press Inc. Boca Raton, Florida, pp. 135.

Krishnia S.K., Saharan G.S., Singh D. 2000. Genetic variation for multiple disease resistance in the families of interspecific cross of *Brassica juncea x B. carinata.* Cruciferae Newsletter 22: 51-53.

Maude R.B., Humpherson-Jones F.M. 1980. Studies on the seed-borne phases of dark leaf spot (*Alternaria brassicicola*) and gray leaf spot (*Alternaria brassicae*) of brassicas. Annals of Applied Biology 95: 311-319. [DOI: 10.1111/j.1744-7348.1980.tb04752.x]

Maude R.B., Humpherson-Jones F.M., Shuring C.G. 1984. Treatments to control Phoma and Alternaria infections of brassica seeds. Plant Pathology 33: 525-535. [DOI: 10.1111/j.1365-3059.1984.tb02877.x]

Meena P.D., Awasthi R.P., Chattopadhyay C., Kolte S.J., Kumar A. 2010. Alternaria blight: a chronic disease in rapeseed-mustard. Journal of Oilseed Brassica 1(1): 1-11.

Narasimhulu S.B., Kirti P.B., Bhat S.R., Prakash S., Chopra V.L. 1994. Intergeneric protoplast fusion between *Brassica carinata* and *Camelina sativa*. Plant Cell Reports 13: 657-660. [DOI: 10.1007/BF00232940]

Nishimura S., Kohmoto K. 1983. Host-specific toxins and chemical structures from Alternaria species. Annual Review of Phytopathology 21: 87-116. [DOI: 10.1146/annurev.py.21.090183.000511]

Nowakowska M., Niezgoda A., Kozik E.U. 2011. [Alternaria disease of cabbage plants. Part I: Pathogen characteristic, symptoms and importance.] Nowości Warzywnicze/ Veget. Crops News 52: 33-40 [in Polish with English summary].

Otani H., Kohnobe A., Kodama M., Kohmoto K. 1998. Production of a host-specific toxin by germinating spores of *Alternaria brassicicola.* Physiological and Molecular Plant Pathology 52: 285-295. [DOI: 10.1006/pmpp.1998.0147]

Otani H., Kohnobe A., Narita M., Shiomi H., Kodama M., Kohmoto K. 2001. A new type of host-selective toxin, a

protein from *Alternaria brassicicola*. In: N.T. Keen, S. Mayama, J. E. Leach and S. Tsuyumu (Eds.). Delivery and perception of pathogen signals in plants. APS Press, St. Paul, MN, pp. 68-76.

Pace M.A., Campbell R. 1974. The effect of saprophytes on infection of leaves of *Brassica* spp. by *Alternaria brassicicola*. Transaction of the British Mycological Society 63: 193-196. [DOI: 10.1006/pmpp. 1998.0147]

Parada R.Y., Sakuna E., Mori N., Oka K., Egusa M., Kodoma M., Otani H. 2008. *Alternaria brassicae* produces a host-specific protein toxin from germinating spores on host leaves. Phytopathology 98: 458-463. [DOI: 10.1094/PHYTO-98-4-0458]

Primard C., Vedel F., Mathieu C., Pelletier G., Chevre A. M. 1988. Interspecific somatic hybridization between *Brassica napus* and *Brassica hirta* (*Sinapis alba L.*) Theoretical and Applied Genetics 75: 546-552. [DOI: 10.1007/BF00289119]

Ripley V., Thorpe M., Iler S., Mizier K., Beversdorf W.D. 1992. Isozyme analysis as a tool for introgression of *Sinapis alba* germ plasm into *Brassica napus*. Theoretical and Applied Genetics 84: 403-410. [DOI: 10.1007/BF00229500]

Ryschka U., Schumann G., Klocke E., Scholze P., Neumann M. 1996. Somatic hybridization in *Brassicaceae*. Acta Hortic 407: 201-208. http://www.actahort.org/books/407/407_24.htm

Sharma T.R., Singh B.M. 1992. Transfer of resistance to *Alternaria brassicae* in *Brassica juncea* through interspecific hybridization among *Brassica*. Journal of Genetics and Breeding 46: 373-378.

Sharma G., Kumar V.D., Haque A., Bhat S.R., Prakash S., Chopra V.L. 2002. *Brassica* coenospecies: a rich reservoir for genetic resistance to leaf spot caused by *Alternaria brassicae*. Euphytica, 125, 411-417. [DOI: 10.1023/A:1016050631673]

Sigareva M.A., Earle E.D. 1997. Direct transfer of a cold-tolerant Ogura male sterile cytoplasm into cabbage (*Brassica oleracea* ssp. *capitata*) via protoplast fusion. Theoretical and Applied Genetics 94: 213-220. [DOI: 10.1007/s001220050402]

Sikdar S.R., Chatterjee G., Das S., Sen S.K. 1990. "Erussuca" the inergeneric fertile somatic hybrid developed through protoplast fusion between *Eruca sativa* Lam. and *Brassica jencea* (L.) Czern. Theoretical and Applied Genetics 79: 561-567. [DOI: 10.1007/BF00226168]

Singh B.P., Singh S.P., Mohammad A., Sinha P.P. 1992. Effect of nitrogen, phosphorus and potash on the development of Alternatria leaf spot of cabbage. Indian Phytopathology 45(2): 245.

Sodelade M., Pedras C., Khallaf I. 2012. Molecular interactions of the phytotoxins destruxin B and sirodesmin PL with crucifers and cereals. Metabolism and elicitation of plant defenses. Phytochemistry 77: 129-139. [DOI: 10.1016/j. phytochem.2012.02.010]

Survilienė E., Valiuškaitė A., Duchovskienė L., Kavaliauskaitė D. 2010. Influence of fungicide treatment on grey mould of cabbage. Vegetable Crops Research Bulletin 73: 133-142. [DOI: 10.2478/ v10032-010-0025-8]

Tewari J.P. 1991a. Structural and biochemical bases of the black spot diseases of crucifers. Adv. Struc. Biol. 1: 25-34.

Tewari J.P. 1991b. Resistance to *Alternaria brassicae* in crucifers. Bulletin SROP 14 (6): 154-161.

Tewari J.P., Conn K.L. 1993. Reactions of some wild crucifers to *Alternaria brassicae*. Bulletin-OILS-SROP 16: 53-58.

Tierens K.F.M.J., Thomma B.P.H.J., Bari R.P., Garmier M., Eggermont K., Brouwer M., et al. 2002. *Esa1*, an *Arabidopsis* mutant with enhanced susceptibility to a range of necrotrophic fungal pathogens, shows a distorted induction of defense responses by reactive oxygen generating compounds. Plant Journal 29: 131-140. [DOI: 10.1046/j.1365-313x.2002.01199.x]

Tylkowska K., Grabarkiewicz-Szczęsna J., Iwanowska H. 2003. Production of toxins by *Alternaria alternata* and *A. radicina* and their effects on germination of carrot seeds. Seed Science and Technology 31 (2): 309-316.

Tylkowska K., Korbas M., Kurzawińska H., Rataj-Guranowska M. 2004. Objawy chorób powodowanych przez grzyby rodzaju Alternaria. s. 3-11. W: „Kompendium symptomów chorób roślin oraz morfologii ich sprawców" (M. Rataj-Guranowska, red.). Bogucki Wydawnictwo Naukowe, Poznań [in Polish].

Warwick S.I. 2011. Brassicaceae in agriculture. In: Schmidt R., Bancroft I. (Eds.), Genetics and genomics of the Brassicaceae. Plant genetics and genomics: Crop models 9. Springer Science+Business Media, LLC. pp: 33-65. [DOI: 10.1007/978-1-4419-7118-0_2]

Wight W.D, Kim K.-H., Lawrence C.B., Walton J.D. 2009. Biosynthesis and role in virulence of the histone deacetylase inhibitor depudecin from *Alternaria brassicicola*. MPMI 22(10): 1258-1267. [DOI: 10.1094/MPMI-22-10-1258]

Zhang F.L., Xu J.B., Yan H., Li M.Y. 1997. A study on inheritance of resistance to black leaf spot in seedlings of Chinese cabbage. Acta Agriculture Borealis Sinica 12: 115-119.

Zhou N., Tootle T.L., Glazebrook J. 1999. Arabidopsis *PAD3*, a gene required for camalexin biosynthesis, encodes a putative cytochrome P450 monooxygenase. Plant Cell 11: 2419-2428. [DOI: 10.2307/3870965] (print) [DOI: 10.1105/tpc.11.12.2419] (online)

ALTERNARIOZA ROŚLIN KAPUSTOWATYCH: OBJAWY, SZKODLIWOŚĆ I PERSPEKTYWY HODOWLI ODMIAN ODPORNYCH

Streszczenie

Alternarioza kapustowatych (syn. czerń krzyżowych, czarna plamistość roślin krzyżowych) powoduje duże straty gospodarcze w wielu krajach, w tym również w Polsce. Sprawcami choroby są różne gatunki grzybów z rodzaju *Alternaria,* najczęściej: *A. brassicae* (Berk.) Sacc., *A. brassicicola* (Schw.) Wiltsh., *A. raphani* Groves i Skolko oraz *A. alternata* (Fr.) Kreissler. Roślinami żywicielskim są kapusty i inne rośliny uprawne oraz dziko rosnące z rodziny krzyżowych. Szkodliwość czerni krzyżowych w uprawie kapusty głowiastej i pekińskiej polega na obniżeniu wysokości i jakości plonu. Grzyby z rodzaju *Alternaria* wywołują również zgorzel siewek, zgorzel podstawy główek kapusty, brązowienie róż kalafiora oraz brokułu. Najczęściej porażane organy wegetatywne roślin kapustowatych są infekowane przez *A. brassicicola* i *A. brassicae,* natomiast w uprawie nasiennej roślin oleistych w obrębie rodzaju *Brassica* dominującym sprawcą alternariozy jest *A. brassicae*. Pierwotnym źródłem choroby są głównie zakażone nasiona, ale także zimotrwałe rośliny z rodziny krzyżowych, resztki porażonych roślin, a na plantacjach nasiennych również materiał wysadkowy.

Metody zapobiegania i zwalczania alternariozy na plantacjach polegają na łączeniu zabiegów agrotechnicznych z ochroną chemiczną. Podstawową metodą zapobiegania chorobie jest produkcja zdrowych nasion, które otrzymuje się, stosując systematyczne opryskiwanie plantacji nasiennych fungicydami. W pierwszym roku uprawy zwalczanie ogranicza się do przedsiewnego zaprawiania nasion, a w czasie wegetacji, w okresach wzmożonego zagrożenia chorobą, znaczne obniżenie porażenia uzyskuje się dzięki regularnym opryskom środkami grzybobójczymi. Według wielu autorów, najbardziej ekonomicznym rozwiązaniem byłoby uzyskanie odpornych odmian warzyw kapustowatych. Pozwoliłoby to na zmniejszenie zużycia pestycydów, co ma szczególne znaczenie w uprawach integrowanych i ekologicznych. Pomimo identyfikacji źródeł odporności wśród roślin z rodziny krzyżowych, przeniesienie tej cechy do uprawnych gatunków kapustowatych jak dotąd nie powiodło się.

Silne bariery niezgodności krzyżowej, poligeniczne uwarunkowanie odporności (addytywne i dominujące współdziałanie genów) oraz różnice w ploidalności pomiędzy poszczególnymi gatunkami rodziny krzyżowych sprawiają, że przeniesienie genów odporności z dzikich gatunków do form uprawnych jest bardzo trudne i wymaga wykorzystania technik hybrydyzacji *in vitro* (w tym: somatyczna hybrydyzacja, „embryo/ovary rescue", fuzja protoplastów). Jednak dzięki coraz lepszemu poznaniu wzajemnych relacji patogen-roślina żywicielska, identyfikacji nowych źródeł odporności oraz określeniu mechanizmu dziedziczenia tej cechy, możliwy będzie postęp w hodowli roślin kapustowatych odpornych na alternariozę.