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Original Research Paper

Application of the detached-leaf technique to evaluate the pathogenicity of isolates of fungi *Ascochyta fabae* and *Botrytis fabae* to faba bean

Zastosowanie metody odciętych liści do oceny patogeniczności izolatów grzybów *Ascochyta fabae* i *Botrytis fabae* wobec bobiku

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The most important diseases of the faba bean include Ascochyta blight caused by the fungus *Ascochyta fabae* and chocolate spot caused by the fungus *Botrytis fabae*. The pathogenicity of isolates of *A. fabae* and *B. fabae* was investigated against six cultivars of faba bean. The technique of detached leaves was used. The leaves were inoculated with pycnidial and conidial spores of the pathogens tested. After incubation, the sizes of the necrotic spots produced by the pathogens were determined. Significant variation in the pathogenicity of isolates of both fungi was found. The varieties showed differential resistance to Ascochyta blight and chocolate spot. The variation in resistance of cultivars to chocolate spot was lower than resistance to Ascochyta blight. The possibility of using the ImageJ image analysis software to measure the surface of necrotic lesions was described.

Keywords: Ascochyta blight, chocolate spot, image analysis, resistance, *Vicia faba*

Do najważniejszych chorób bobiku należą askochytoza powodowana przez grzyb *Ascochyta fabae* oraz czekoladowa plamistość powodowana przez grzyb *Botrytis fabae*. Przebadano patogeniczność izolatów *A. fabae* i *B. fabae* wobec sześciu odmian bobiku. Zastosowano technikę odciętych liści. Liście inokulowano zarodnikami piknidialnymi i konidialnymi badanych patogenów. Po inkubacji określano rozmiary plam nekrotycznych wytwarzanych przez patogeny. Stwierdzono istotne zróżnicowanie patogeniczności izolatów obu grzybów. Odmiany wykazały zróżnicowaną odporność na askochytozę i czekoladową plamistość. Zróżnicowanie odporności odmian na czekoladową plamistość było mniejsze niż odporności na askochytozę. Opisano możliwość wykorzystania programu do analizy obrazu ImageJ do pomiaru powierzchni plam nekrotycznych.

Słowa kluczowe: analiza obrazu, askochytoza, czekoladowa plamistość, odporność, *Vicia faba*

Introduction

Faba (field, horse) bean (*Vicia faba* L. ssp. *minor*) is a grain legume crop (Fouad et al., 2013). It is cultivated for seeds with a high protein content (25-30%) and can be a raw material for producing protein animal feed (Grela and Czech, 2019). An additional advantage of faba bean cultivation is improving the soil structure by loosening it and enriching it with organic matter. Thanks to faba bean cultivation, the soil is enriched with nitrogen assimilated by rhizobia bacteria. This is particularly important in organic farming, where artificial nitrogen fertilizers are not used.

The area of faba bean cultivation in Poland was approximately 35,000 hectares in 2022 (Panek 2022). In the last ten years, it has increased almost four times. This area is lower than that of lupins, field peas, and soybeans. This is mainly due to the high soil requirements of faba bean and the high water needs during the period from the formation of buds to the formation of pods. Faba bean is also strongly affected by pests (aphids, pea leaf weevil, broadbean weevil) and pathogens causing fungal diseases. Two fungal diseases, Ascochyta blight, and chocolate spot, pose a significant threat to the growth of faba bean crops. Ascochyta blight is caused by the fungus *Ascochyta fabae* Speg (teleomorph *Didymella fabae* Jellis & Punith), while chocolate spot is caused by the fungus *Botrytis fabae* Sard. (Díaz-Ruiz et al., 2009; Kaur et al., 2014; Zakrzewska, 2004, 1988). Ascochyta blight is a common disease in faba bean crops, causing yield losses of up to 90% in susceptible cultivars when environmental conditions favor disease development (Hanounik and Robertson, 1989). The fungus infects all above-ground parts of plants, including seeds (Sillero et al., 2010).

The resistance of faba bean to Ascochyta blight (*A. fabae*) is a complex trait. It is controlled by single main genes and polygenically (Avila et al., 2004; Kohpina et al., 2000a; Ondřej and Huňady, 2007; Román et al., 2003; Sillero et al., 2010). Genes associated with resistance of both leaves and stems have also been identified, as well

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as groups of genes that determine resistance of leaves or stems only (Kharrat et al., 2006). Furthermore, the presence of resistance QTL effective against various isolates of *A. fabae* was found, but also QTL effective only against single isolates (Avila et al., 2004). This indicates the need for a broad spectrum of *A. fabae* isolates to test the resistance of the faba bean. Therefore, some researchers have suggested the existence of races of the pathogen, but this is not fully documented (Díaz-Ruiz et al., 2009; Tivoli et al., 2006). Rather, in the *A. fabae* population, pathogenicity groups are identified according to the severity of disease symptoms caused by isolates on a set of cultivars with different resistance (Blake et al., 2022). Sexual reproduction allows the emergence of new combinations of virulence, which is associated with the possibility that the pathogen responds to the selection exerted by the introduction of host resistance genes (Ozkilinc et al., 2015; Rubiales and Trapero-Casas, 2002).

This level of specialization is not observed in *B. fabae* causing chocolate spot. The degree of resistance of faba bean to this disease is low and only a few sources of resistance have been identified (Gela et al., 2022; Sillero et al., 2010).

Testing the resistance of faba bean to Ascochyta blight and chocolate spot is laborious and requires the creation of appropriate provocative conditions for the development of these diseases (Zakrzewska, 2004, 1988). Acceleration of the selection process of resistant genotypes can be achieved using the technique of detached leaves (Herath et al., 2001; Kohpina et al., 2000b; Tivoli et al., 2006). In the case of Ascochyta blight, it should be considered that the reaction of leaves and stems to infection with *A. fabae* can vary (Avila et al., 2004). Therefore, Kohpina et al. (2000b) also propose using stem fragments to obtain more reliable results showing the resistance of faba bean genotypes to Ascochyta blight. The complexity of faba bean reaction to Ascochyta blight shows that the correlation between laboratory and field results is not always high. This is due to the above-mentioned different resistance of leaves and stems to *A. fabae* infection and the fact that the fungus also infects pods (Zakrzewska, 2004). The development of the disease under field conditions is also influenced by the length of the stem and the type of inflorescence (Jellis et al., 1985; Pritchard et al., 1989; Zakrzewska, 2004). In the case of the chocolate spot, there was satisfactory concordance between detached leaf tests and field tests (Zaki, 2010). This concordance was lowest for genotypes with moderate resistance to the disease (Bouhassan et al., 2004; Tivoli et al., 2006; Villegas-Fernández et al., 2011).

The research aimed to study the pathogenicity of *A. fabae* and *B. fabae* isolates and the resistance of faba bean cultivars to diseases caused by these pathogens using the detached-leaf technique. The possibility of using image analysis software for a precise and automated assessment of the severity of the above diseases on detached faba bean leaves was also tested.

Material and methods

The pathogenicity of *Ascochyta fabae* and *Botrytis fabae* isolates to faba bean cultivars was evaluated. Six cultivars of faba bean were used in the research: Bobas – traditional cultivar, high in tannin; Albus, Amulet, Kasztelan – traditional, low -tannin cultivars; Granit, Optimal – determinate (SK), high tannin cultivars. Faba bean seeds were obtained from breeding companies (Albus, Amulet, Granit, Kasztelan – Hodowla Roślin Strzelce; Bobas, Optimal – DANKO Hodowla Roślin).

The isolates of *A. fabae and B. fabae* were obtained from leaves showing symptoms of Ascochyta blight and chocolate spot and from seeds with disease symptoms (Table 1). Isolates are stored in the collection of the IHAR-PIB Department of Applied Biology.

To obtain spores, *A. fabae,* and *B. fabae* were cultured in a medium of PDA (potato glucose agar) (Carl Roth GmbH, Karlsruhe, Germany) with the addition of faba bean meal (Zakrzewska, 2004). The dishes were irradiated with UV light (black light, 360 nm) in a 12/12h cycle to stimulate sporulation (Fig. 1). The surface of the dishes on which the sporulation of fungi was observed was washed with distilled water. The resulting suspension was filtered through gauze to remove mycelium fragments. The suspension concentration of pycnidiospores of *A. fabae* was established at 100,000 spores per milliliter and the conidiospores of *B. fabae* at 500,000 spores per milliliter. Measurements of spore concentration were made using a Thoma hematology chamber. The same concentrations of spores were adjusted for all isolates tested from both species.

The detached leaf assay was used to assess the pathogenicity of isolates and resistance of cultivars (Walentyn-Góral and Góral, 2011; Góral and Walentyn-Góral, 2012). Sterilized faba bean leaves were placed in 12.5 × 12.5 cm square Petri dishes (Bionovo, Legnica, Poland). The medium was water agar (Carl Roth GmbH, Karlsruhe, Germany) supplemented with 100 mg of benzimidazole (Merck Life Science Sp. z oo, Poznań, Poland) per liter. Each leaflet was inoculated with a drop (50 µl) of either *A. fabae* or *B. fabae* spore suspension. The drop was placed in the middle of the leaflet in a place punctured with a needle. Four to five leaflets were inoculated on each leaf. Plates with leaves were incubated in a culture chamber (Sanyo Electric, Moriguchi, Japan) at 20°C. The duration of the day was 12 h. After the symptoms appeared, necrotic lesions' size was measured five times (7, 10, 14, 16, and 21 days post-inoculation).

Table 1
Tabela 1

List of isolates of *Ascochyta fabae* and *Botrytis fabae*
Lista izolatów *Ascochyta fabae* i *Botrytis fabae*

Species Gatunek	Isolate Izolat	Source Źródło	Cultivar Odmiana	Isolation year Rok izolacji
A. fabae	AF 5-1	seeds / nasiona	Titus	*
A. fabae	AF 5-2	seeds / nasiona	Titus	*
A. fabae	AF 6-1	seeds / nasiona	Amulet	2009
A. fabae	AF 6-2	seeds / nasiona	Amulet	2009
A. fabae	AF 7-1	leaves / liście	Kasztelan	2012
A. fabae	AF 7-3	leaves / liście	Granit	2012
A. fabae	AF 8-1	leaves / liście	Bobas	2013
A. fabae	AF 8-2	leaves / liście	Amulet	2013
A. fabae	AF 15-1	seeds / nasiona	Albus	2017
A. fabae	AF 15-4	seeds / nasiona	Granit	2017
B. fabae	BF 1	leaves / liście	Optimal	2017
B. fabae	BF 2	leaves / liście	Optimal	2017
B. fabae	BF 3	leaves / liście	Granit	2017
B. fabae	BF 4	leaves / liście	Granit	2017

* – seed stored in refrigerator, collected in 2004
* – nasiona przechowywane w zamrażarce, zebrane w roku 2004

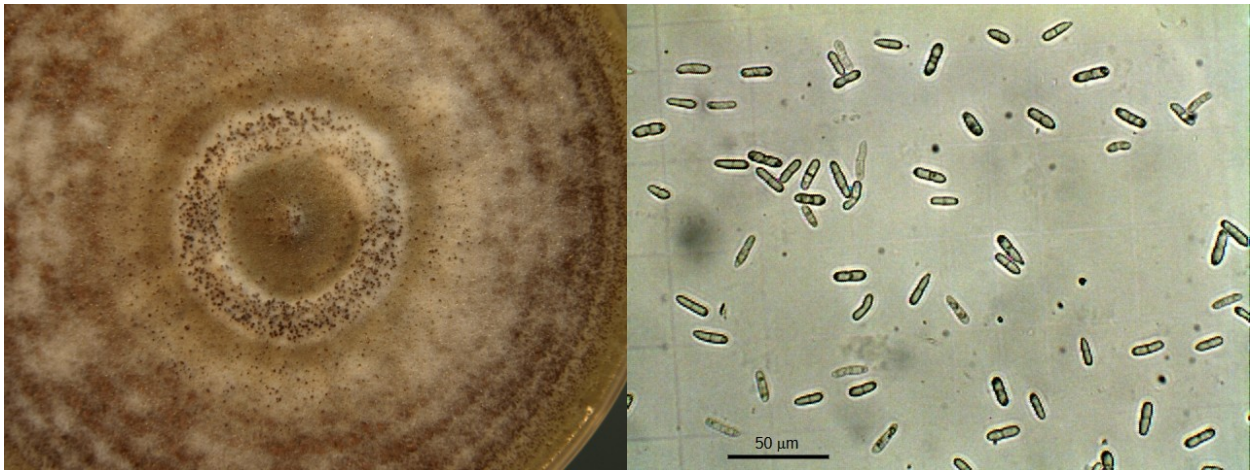

Fig. 1. Isolate of *Ascochyta fabae* growing on a PDA medium with faba bean seed meal (left); pycnidispores of *A. fabae* (right)
Rys. 1. Izolat *Ascochyta fabae* rosnący na pożywce PDA z mączką z nasion bobiku (z lewej); piknidiospory *A. fabae* (z prawej)

The average area of the lesion was calculated for each leaf. Photographs of the infected leaves were taken at 21 dpi. For each pathogen, two infection experiments were performed in two replications.

Statistical analysis was performed using the XLSTAT Life Science package (Version 2021.3.1.1177, Lumivero, Denver, CO, USA). An analysis of variance was performed on the mean area of necrotic lesions produced by *A. fabae* and *B. fabae* (XLSTAT: ANOVA procedure). The factors were: isolate, cultivar, and date of measurement. Fisher's NIR multiple comparison test was used to compare the means for cultivars and isolates.

In selected images, the area of the lesions was measured using ImageJ (https://imagej.net) (National Institutes of Health, Bethesda, MD, United States and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, Madison, WI, USA) (Scheider et al., 2012). This work aimed to verify the possibility of using image analysis to assess the size of necrotic lesions on faba bean leaves.

After opening the leaf image (TIFF, GIF, JPEG, BMP, and others), initial image adjustments were made (e.g. *Image* > *Adjust* > *Brightness/ Contrast*, *Image* > *Adjust* > *Color balance*). The measurement scale of the image was then determined to obtain the actual area of the measured necrotic lesion (*Analyze* > *Set Scale*). A necrotic lesion was marked and its area was measured using the *Image* > *Adjust* > *Color Threshold* com-

mand. The highlighted area was outlined using the *Wand Tool* command (icon in the ImageJ icon bar). Options of *Wand Tool* can be modified using the command *Edit* > *Options* > *Wand Tool*. The area of the selected area was calculated using the *Analyze* > *Analyze Particles* command. The process can be automated by recording a macro including the above commands (*Plugins* > *Macros* > *Record*).

Results and Discussion

All tested isolates of *A. fabae* and *B. fabae* showed pathogenicity towards the faba bean cultivars. Symptoms in the form of necroses appeared about seven days after inoculation. Then necroses increased, in some cases covering the entire surface of a single leaflet (Fig. 2). The area of necrosis produced by *B. fabae* was on average three

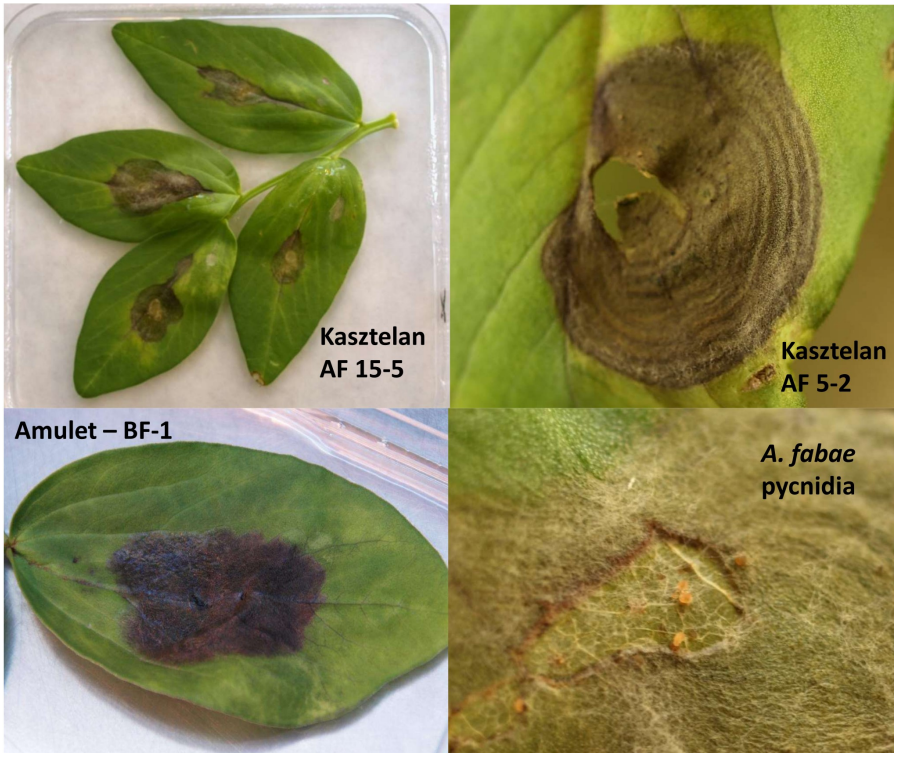

Fig. 2. A. Necrotic lesions on faba bean leaves (cultivar Kasztelan) caused by *A. fabae* isolate AF15-5 after point inoculation with pycnidiospores; B. Necrotic lesion on faba bean cultivar Kasztelan with formatting pycnidia of *A. fabae*, isolate AF5-2; C. Necrotic lesion on faba bean leaves (cultivar Amulet) caused by *B. fabae* after point inoculation with conidiospores; D. Pycnidia of *A. fabae* on a faba bean leaves releasing pycnidiospores.
Rys. 2. A. Plamy nekrotyczne na liściu bobiku (odmiana Kasztelan) powstałe na skutek inokulacji punktowej zarodnikami grzyba *A. fabae*, izolat AF 15-5; B. Plama nekrotyczna na liściu bobiku odmiany Kasztelan z tworzącymi się piknidiami grzyba *A. fabae*, izolat AF 5-2; C. Plama nekrotyczna na liściu bobiku (odmiana Amulet) powstała na skutek inokulacji punktowej zarodnikami grzyba *B. fabae*, izolat BF-1; D. Piknidia grzyba *A. fabae* na liściu bobiku uwalniające piknidiospory.

times larger than that caused by *A. fabae*. In necrotic lesions produced on leaves inoculated with *A. fabae,* pycnidia, releasing pycnidiospores were formed (Fig. 2D).

The pathogenicity of *A. fabae* isolates varied (Table 2, Fig. 3). Two groups of isolates were distinguished according to the size of the necrotic lesions. The first included four highly pathogenic isolates: 15-4, 15-1, 7-3, and 8-2. The highest pathogenicity was shown by isolates 15-4 and 15-1 obtained in 2015 from infected faba bean seeds from a field experiment in Radzików, Central Poland. Isolates 7-3 and 8-2 were obtained from infected leaves collected in 2012 and 2013. The second group included six isolates with significantly lower pathogenicity. The differences between them were not statistically significant. A. fabae isolates show a wide variation in aggressiveness toward field beans (Blake et al., 2022). This is because *A. fabae* is a heterothallic species and the presence of two mating types MAT-1 and MAT-2 is required for the formation of pseudothecia and ascospores (Kaiser et al., 2007).

Faba bean cultivars used for the investigation differed statistically significantly in terms of resistance to Ascochyta blight caused by four isolates with the highest pathogenicity (Tab. 3, Fig. 4). The Bobas cultivar showed the highest resistance. However, it did not differ significantly from the Optimal (SK) cultivar.

Albus was the most susceptible cultivar. Kasztelan, Granit (SK), and Amulet cultivars were less susceptible and did not differ significantly. The determinate cultivars of faba bean Granit and

Table 2
Tabela 2

Analysis of variance of area of necrotic lesions produced by *A. fabae* and *B. fabae* isolates on six cultivars of faba bean
Analiza wariancji powierzchni plam nekrotycznych wytwarzanych przez izolaty *A. fabae* i *B. fabae* na sześciu odmianach bobiku

Source of variation Źródło zmienności	*Ascochyta fabae* (10 izolates / 10 izolatów)		*Botrytis fabae* (4 izolates / 4 izolaty)	
	F	Pr > F	F	Pr > F
Cultivar / Odmiana	10,037	0,000	3,570	0,005
Isolate / Izolat	19,557	0,000	55,716	0,000
Date / Termin	62,114	0,000	153,423	0,000
Cultivar × isolate Odmiana × Izolat	1,971	0,001	3,723	0,000
Cultivar × experiment Odmiana × date	2,137	0,064	0,857	0,576
Izolate × experiment Izolat × date	6,801	0,000	10,649	0,000

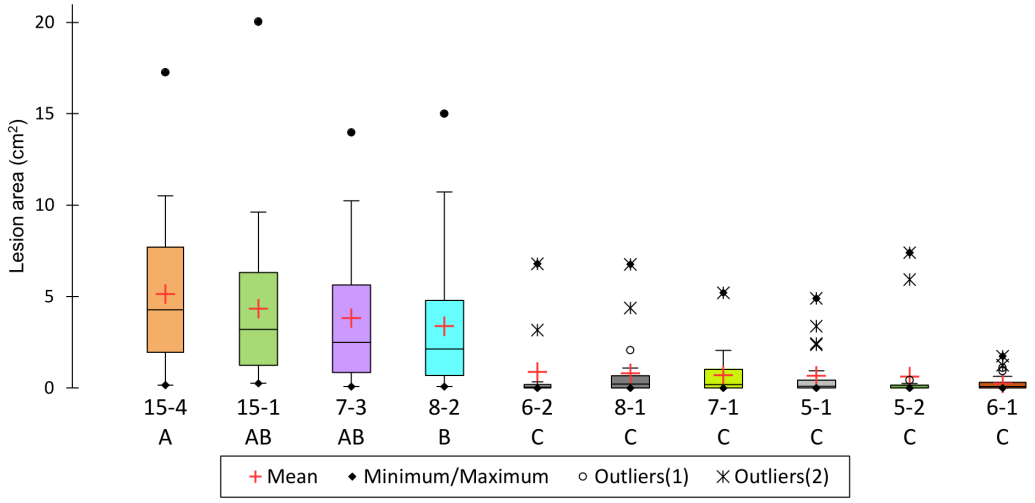

Fig. 3. Pathogenicity of isolates of *A. fabae* towards six faba bean cultivars evaluated using detached leaves technique. Isolates marked with the same letter are not significantly different at α = 0.05 (analysis of variance, Fisher's LSD test)
Rys. 3. Zróżnicowanie agresywności izolatów *A. fabae* wobec sześciu odmian bobiku określone z wykorzystaniem techniki odciętych liści. Izolaty oznaczone tą samą literą nie różnią się istotnie statystycznie dla α = 0,05 (analiza wariancji, test NIR Fishera)

Table 3
Tabela 3

Analysis of variance of area of necrotic lesions produced by *A. fabae* and *B. fabae* isolates on six cultivars of faba bean-
Analiza wariancji powierzchni plam nekrotycznych wytwarzanych przez izolaty *A. fabae* i *B. fabae* na sześciu odmianach bobiku

Source of variation Źródło zmienności	*Ascochyta fabae* (4 izolates / 4 izolatów)		*Botrytis fabae* (2 isolates / 2 izolaty)	
	F	Pr > F	F	Pr > F
Cultivar / Odmiana	6,963	0,000	2,634	0,036
Isolate / Izolat	1,223	0,309	0,183	0,670
Date / Termin	58,043	0,000	110,024	0,000
Cultivar × Isolate Odmiana × Izolat	1,177	0,314	6,116	0,000
Cultivar × Date Odmiana × Termin	1,817	0,123	0,711	0,710
Izolate × Date Izolat × Termin	0,089	0,966	0,492	0,615

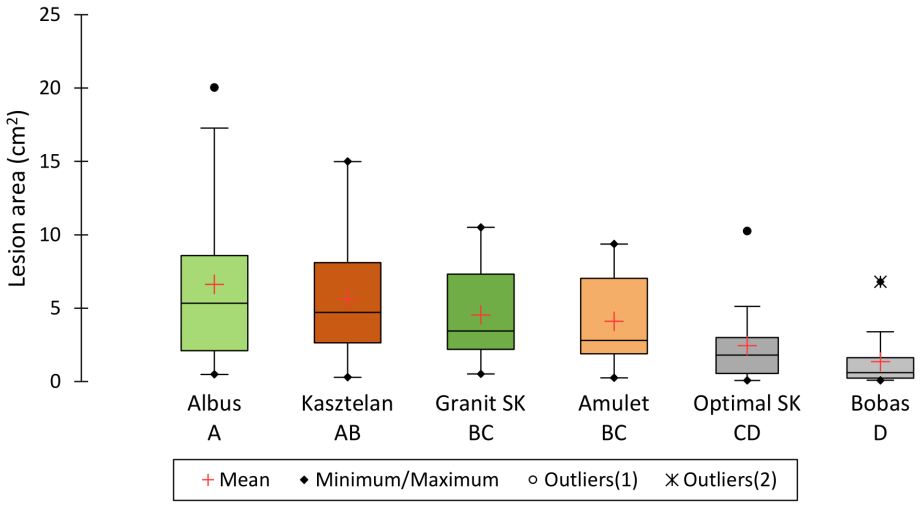

Fig. 4. Resistance of fabae bean cultivars to 4 isolates of *A. fabae* evaluated using detached leaves technique. Cultivars marked with the same letter are not significantly different at α = 0.05 (analysis of variance, Fisher's LSD test)
Rys. 4. Odporność odmian bobiku na porażenie 4 izolatami *A. fabae* określona z wykorzystaniem techniki odciętych liści. Odmiany oznaczone tą samą literą nie różnią się istotnie statystycznie dla α = 0,05 (analiza wariancji, test NIR Fishera)

Optimal showed medium or low susceptibility to infection of detached leaves with Ascochyta blight. Meanwhile, under natural infection conditions in the field, they were most severely affected by Ascochyta blight (Boros et al. 2014; T. Góral, unpublished). Similar results were obtained by Zakrzewska (2004) comparing the resistance of traditional forms and determinate forms of faba bean after inoculation with *A. fabae* in several years of field experiments. The results may suggest that there is no direct influence of the gene determining the terminal inflorescence (*Vf_TFL1*) on the resistance of faba bean to Ascochyta blight (Avila et al., 2007). The increased susceptibility of determinate cultivars to Ascochyta blight in field conditions is somewhat due to the much shorter stem length of these cultivars (about 30-40 cm according to COBORU data) (Jellis et al., 1985; Pritchard et al., 1989; Zakrzewska, 2004).

The isolates of *B. fabae* also varied (Table 1, Fig. 5). Two groups of isolates were distinguished according to the size of the necrotic lesions. The first included two isolates with high pathogenicity: BF 3, and BF 1. The second group had two isolates with significantly lower pathogenicity: BF 2 and BF 4. The differences between the isolates in both groups were not statistically significant.

The faba bean cultivars differed statistically significantly in terms of resistance to chocolate spot caused by the two isolates with the highest pathogenicity (Table 2, Fig. 6). The cultivars Optimal (SK) and Kasztelan showed the highest re-

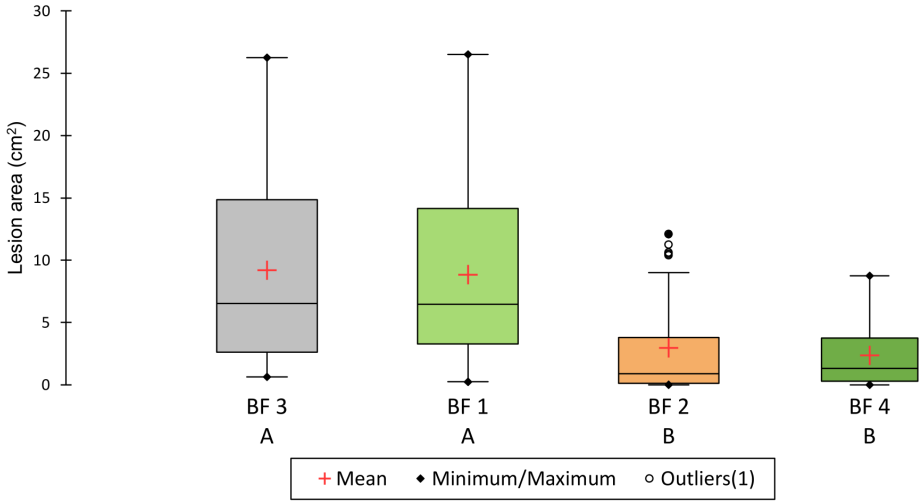

Fig. 5. Pathogenicity of isolates of *B. fabae* towards six faba bean cultivars evaluated using detached leaves technique. Isolates marked with the same letter are not significantly different at α = 0.05 (analysis of variance, Fisher's LSD test)
Rys. 5. Zróżnicowanie agresywności izolatów *B. fabae* wobec sześciu odmian bobiku określone z wykorzystaniem techniki odciętych liści. Izolaty oznaczone tą samą literą nie różnią się istotnie statystycznie dla α = 0,05 (analiza wariancji, test NIR Fishera)

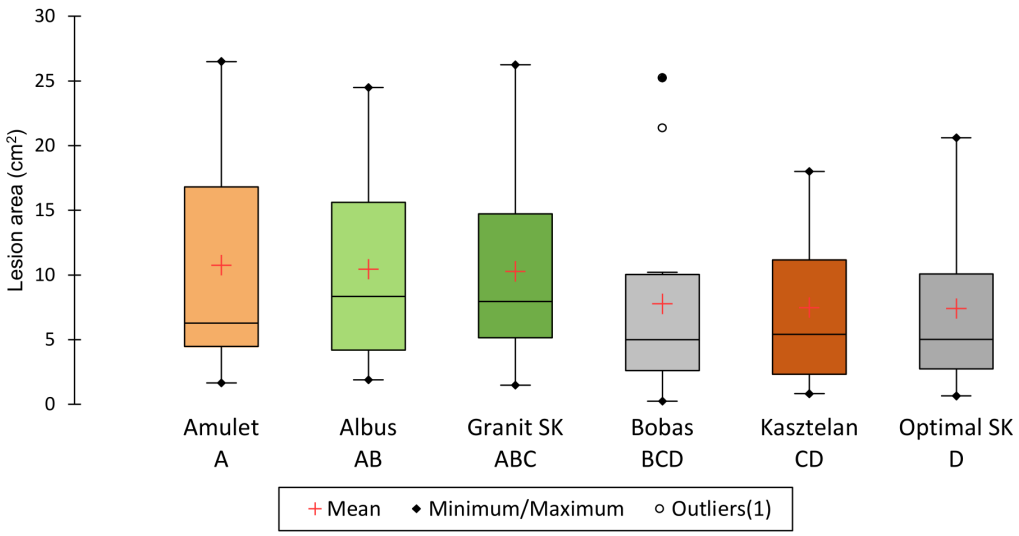

Fig. 6. Resistance of fabae bean cultivars to 2 isolates of *B. fabae* evaluated using detached leaves technique. Cultivars marked with the same letter are not significantly different at α = 0.05 (analysis of variance, Fisher's LSD test)
Rys. 6. Odporność odmian bobiku na porażenie 2 izolatami *B. fabae* określona z wykorzystaniem techniki odciętych liści. Odmiany oznaczone tą samą literą nie różnią się istotnie statystycznie dla α = 0,05 (analiza wariancji, test NIR Fishera)

sistance, the cultivars Amulet and Albus the lowest. However, the variation in cultivar resistance to chocolate spot was lower than that observed for Ascochyta blight (Table 2). This was also confirmed by several years of observations of the occurrence of chocolate spot in field conditions in Radzików (Boros et al. 2014; T. Góral, unpublished). In years with high severity of chocolate spot, most cultivars were affected similarly. Only determinate cultivars stood out, in which the severity of the disease (assessed as the degree of leaf infection on the entire plant) was greater. The results of published studies also show slight variation in the resistance of faba bean genotypes to chocolate spot (Sillero et al., 2010). The problem in resistance breeding is the lack of good sources of resistance to this disease. Most of the identified resistant genotypes come from the Andean regions of South America and are not adapted to European conditions (Bond et al., 1994; Maalouf et al., 2016).

Figure 7 shows the successive stages of measuring the area of necrotic lesions described in the Material and Methods chapter. The defined measurement process is only an example of using the ImageJ program. It can be modified with extensive software options, adapting them to your needs (Abd-El-Haliem, 2012; Mutka et al., 2015). Stewart et al. (2016) developed a macro for the ImageJ program to assess the severity of Septoria blight (caused by *Zymoseptoria tritici*) in wheat leaves. For each leaf, the macro enabled measurement of the total leaf area, total necrosis area, number of pycnidia, average size of pycnidia, and the pycnidia gray value. The latter feature indicates the intensity of melanin production by isolates of *Z. tritici,* a compound that plays an essential role in the pathogen's virulence (Lendenmann et al., 2014). Elliott et al. (2022) compared image analysis methods using ImageJ and machine learning to assess cassava *Xanthomonas* infection. The authors found that both ways accurately distinguished and quantified the different types of lesions caused by *Xanthomonas* on cassava leaves. The ImageJ method was more helpful in analyzing smaller datasets because it required the user to create a mask for each image.

Obtaining precise measurements requires unifying the photos taken of leaves with disease symptoms. The methodology is described, for example, in the work of Bartosiak (2020) on the assessment of leaf infection of wheat seedlings inoculated with the *Parastagonospora nodorum* fungus. Bartosiak (2020) also proposes automating disease symptom assessment using Phyton language applications in his work. It is an application specially developed to evaluate leaf damage and makes the work faster and easier than the rather complicated ImageJ program. Similarly, Alheeti et al. (2021) indicated that the use of ImageJ to assess leaf infection by two fungal pathogens is more laborious than the Leaf Doctor application (free application for iPhone) (Pethybridge and Nelson, 2015). The development of plug-ins for ImageJ may be a solution to this problem. An example is an HTPheno plugin developed at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) (http://htpheno.ipk-gatersleben.de/) (Hartmann et al., 2011). This plug-in can be used for automated image analysis in high-throughput plant phenotyping.

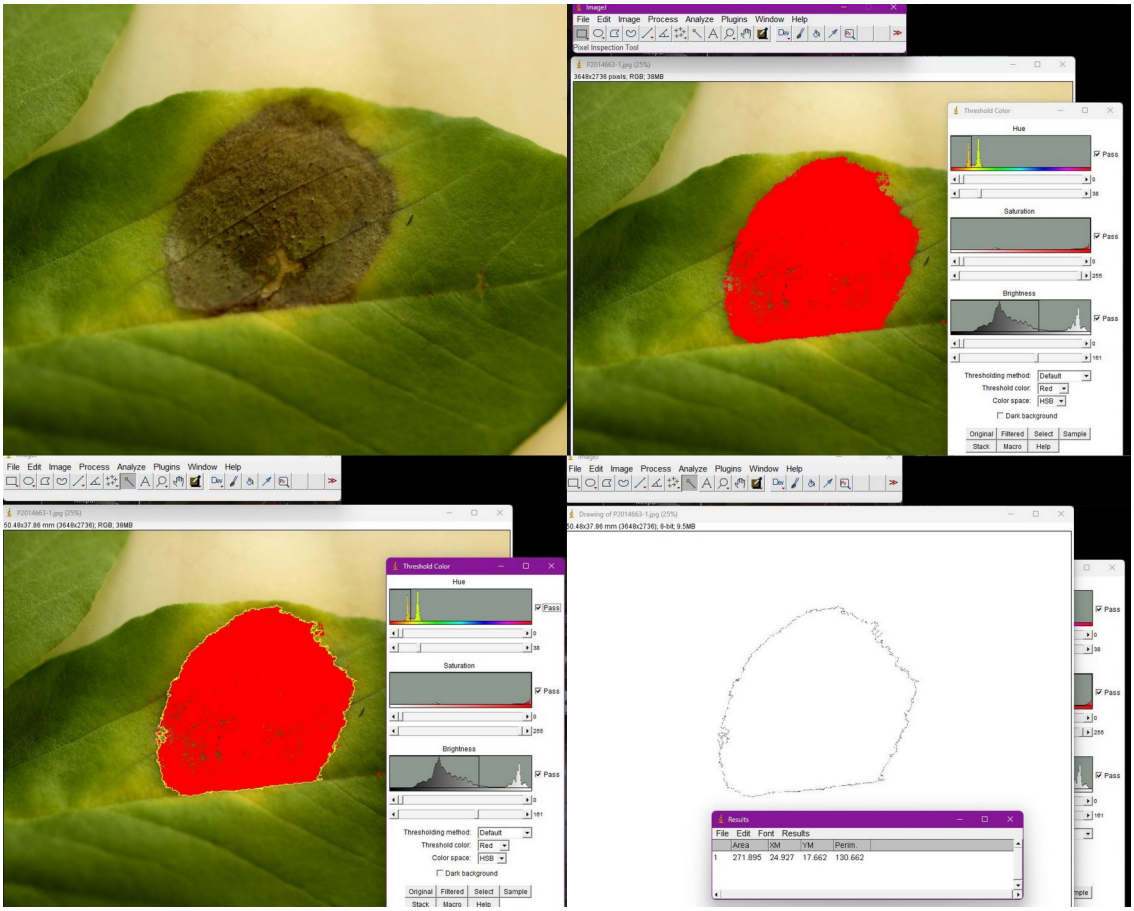

Fig. 7. Necrotic area measurement using ImageJ software. A. Necrotic lesion on faba bean leaf caused by *A. fabae*; B. Highlighting necrotic lesion; C. Outline of the highlighted area; D. Measuring the area of the lesion.
Rys. 7. Pomiar powierzchni plamy nekrotycznej przy użyciu programu ImageJ. A. Nekroza na liściu bobiku spowodowana przez grzyb *A. fabae*; B. Wyróżnianie obszaru plamy; C. Obrys wyróżnionego obszaru; D. Pomiar powierzchni plamy

Summary of results

1) The variation in the pathogenicity of *A. fabae* and *B. fabae* isolates towards the studied faba bean cultivars was statistically significant.
2) Faba bean cultivars showed statistically significant differences in resistance to Ascochyta blight (*A. fabae*) and chocolate spot (*B. fabae*).
3) The results of the analysis of variance showed that the variation in the resistance of the cultivars to the chocolate spot was lower than that of the resistance to Ascochyta blight.
4) The image analyses show the usefulness of the ImageJ software for assessing leaf infection by faba bean pathogens.

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