The Incidence and Spreading of *Macrophomina phaseolina* (Tassi) Goidanovich on Sunflower in the Czech Republic

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Abstract

Veverka K., Palicová J., Křížková I. (2008): **The incidence and spreading of** *Macrophomina phaseolina* (Tassi) Goidanovich **on sunflower in the Czech Republic**. Plant Protect. Sci., **44**: 127–137.

The warm climate pathogen *Macrophomina phaseolina* (Tassi) Goid., which causes charcoal disease, has been reported in the Czech Republic since 1999. The aim of our work was to study the incidence of disease between 2000–2007 in the main sunflower growing regions and analyse the relationship between weather conditions and the occurrence of the pathogen. The first and highest incidence of disease was in the Žatec region. However, in the region south of Brno there was no disease in 2000, 2001 and 2003, and only individual low incidence in other years until 2007, when the disease was found in nine localities. The incidence of the disease is increasing year by year indicating a spread of the pathogen. The warm and dry periods throughout the end of July and August have promoted the disease. However in some years where weather conditions were favourable for the pathogen, in several cases no disease was found. It is assumed, that the pathogen was not yet present in these localities. Soil conditions are critical. Diseased plants are usually distributed in several dispersed groups in the stand, especially on higher and drier parts of the field. Disease is also often found on plants suffering from compressed roots. Diseased plants had poorly developed heads and seeds.

Keywords: M*acrophomina phaseolina*; sunflower; charcoal rot; climatic changes

Macrophomina phaseolina (Tassi) Goidanovich is regarded as a warm climate pathogen, infecting more than 500 hosts in a broad range of crops and weeds. Symptoms of charcoal rot (dry-weather wilt or summer wilt) appear in hot, dry weather (Blanco-López & Jiménez-Díaz 1983) or when unfavourable environmental conditions stress the plant, e.g. in irrigated soya fields when water is withheld after flowering (Sinclair & Backman 1989). It is one of the most important sunflower pathogens in southern Europe (Acimović 1988; Spain: Jimenéz-Diaz *et al.* 1983; Italy: Zazzerini 1980; Rumania: Jinga *et al.* 1992; Bulgaria: Alexandrov 1999; Alexandrov & Kotev 2001; Hungary: Békési *et al.* 1970; Italy: Manici *et al.* 1995). The pathogen was reported as far north as Germany only once in an extremely hot and dry year (Müller & Grill 1991).

Charcoal disease caused by *M. phaseolina* had not been reported in the Czech Republic on any plant before 1999, when it was found in the Žatec region on several sunflower stands. In 2000 and 2001 it was found on sunflowers in the Žatec region, but in other regions occurred on two fields only (Kudlíková *et al.* 2002; Šárová *et al.* 2003). High levels of this pathogen were reported in Slovakia in 2005 and 2006 (Bokor 2007).

Although *M. phaseolina* is an important pathogen in subtropical, tropical, semiarid and arid

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parts of the world, it is not easy to assess the factors that have promoted its spread north in the last few decades. Strains of the pathogen from northern Italy were adapted to lower temperatures than strains from the south of Italy (Manici *et al.* 1995). However, none of the Czech isolates from the Žatec region grew better at low temperatures than the isolates from Hungary or Egypt (Šárová *et al.* 2003).

The temperatures at Žatec in the summer months of 1999–2001 were higher then the local 30-year average, and often were on the level of the 30-year average of Pécs, Ljubljana, Zagreb or were close to that of Belgrade. The Seljaninov hydrothermic coefficient was often far below 1, indicating severe water stress of the plants (Šárová *et al.* 2003). In southern Europe, where sunflower is usually sown, develops and ripens a fortnight or one month earlier than at Žatec. Therefore we should compare the weather conditions in July at Žatec with those of Belgrade in June. This comparison has shown that weather conditions at Žatec could be even more suitable for *M. phaseolina* than in the south of Europe (Šárová *et al.* 2003).

The aim of our work was to monitor the incidence of charcoal disease in the main sunflower-growing regions of the Czech Republic between 2000 and 2007 and analyse the relationship between weather conditions and charcoal disease.

MATERIAL AND METHODS

Temperature requirements of our isolate No. 72 of *M. phaseolina* from the Žatec region, were studied *in vitro* on malt agar and compared with isolate No. 36 of *Botryotinia fuckeliana* (de Bary) Whetzel from strawberries.

The incidence of charcoal disease was studied in the three main sunflower growing regions of the Czech Republic (Figure 1): in the region around Žatec (1), the region west and east of Prague between Slaný and Čáslav (2) and the region of South Moravia between Brno and the Austrian border – meteorological station Lednice (3).

Diseased plants were collected in producing fields in the second half of August and later. We concentrated on parts of the field with pronounced signs of disease – premature drying of plants. Plants were regarded as infected only when microsclerotia were observed in the inner tissues of the stem base. Incidence was assessed as: sporadic – plants with microsclerotia were found very

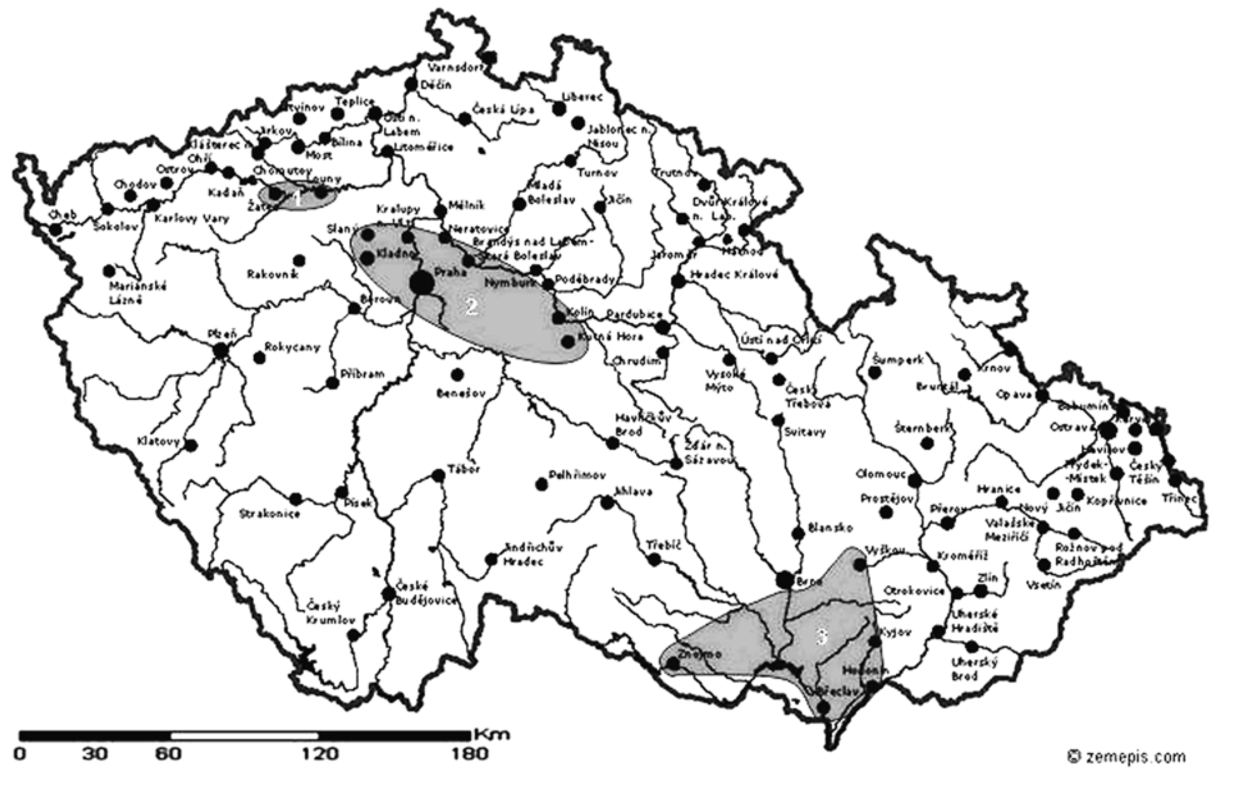

Figure 1. Main sunflower growing regions in the Czech Republic: (1) region Žatec; (2) region west and east of Prague between Slaný and Čáslav; (3) region of South Moravia between Brno and the Austrian border

rarely, medium – infection was found in 10–75% of plants, and high – more than 75% of plants had microsclerotia in the base of the stem, at least in the part of the field.

For analyses of the weather conditions, climate diagrams were used according to Walter and Lieth (1967). The diagrams display monthly temperature averages and a monthly sum of precipitation over a year. Each tic mark along the horizontal axis indicates a month, and 20 mm of monthly precipitation is equivalent to a 10°C change in average temperature. Where the precipitation curve undercuts the temperature curve, the area in between is hatched, indicating a dry season. For the ratio 20 mm:10°C none of the Czech regions had any part of the year dry (Anonymous 2008). We therefore used a modified climate diagrams that is applied in agriculture and forestry with a rate of 30 mm:10°C.

RESULTS AND DISCUSSION

Temperature requirements of *M. phaseolina*

Growth of *M. phaseolina* and *B. fuckeliana* were compared at temperatures from 10–40°C. Figure 2 shows that the growth of warm climate *M. phaseolina* is negligible at lower temperatures and starts its growth at 15°C, in comparison with *B. fuckeliana* which begins to grow at 5°C. For *M. phaseolina,* the optimum temperature is 30°C in comparison with *B. fuckeliana* at 20°C. Growth of *B. fuckeliana* was strongly inhibited by temperatures above 30°C, whereas *M. phaseolina* was inhibited by temperatures of 40°C or above. These data are approximations and differ between isolates (Šárová *et al.* 2003). The data are presented to show substantially different temperature requirements for the local and widespread *B. fuckeliana* compared with the new expansive warm climate fungus *M. phaseolina.*

Symptoms

We did not observe all of the symptoms that may appear on infected plants as described in the literature. Symptoms on the plants were manifested much later than in southern Europe. No plants died during emergence or until after flowering. The first symptoms appeared in the middle of August. Most often the microsclerotia were in the stem base and up to 20 cm high. Infected plants were as high as healthy ones, but often had thinner stems and smaller heads. Only in one case did plants have very abundant microsclerotia from the stem base up to 75–100 cm and the plants were, on average, 40 cm shorter (2004, Hrušovany locality near Žatec).

Infected plants with microsclerotia, ripen and dry sooner, and seeds are poorly developed. However, premature ripening and spots on the stem base are not specific, and only plants with microsclerotia in the stems were regarded as being infected by *M. phaseolina.* The difference between healthy and infected plants is most noticeable when the stands begin to yellow – diseased plants begin drying. Infection of the plants can be identified also in the stubble, but it is not possible to select potentially infected areas of the fields from the distance by the presence of early drying.

The main problem with assessment of the proportion of infected plants is the presence of latent infections. After stem inoculation in mid July,

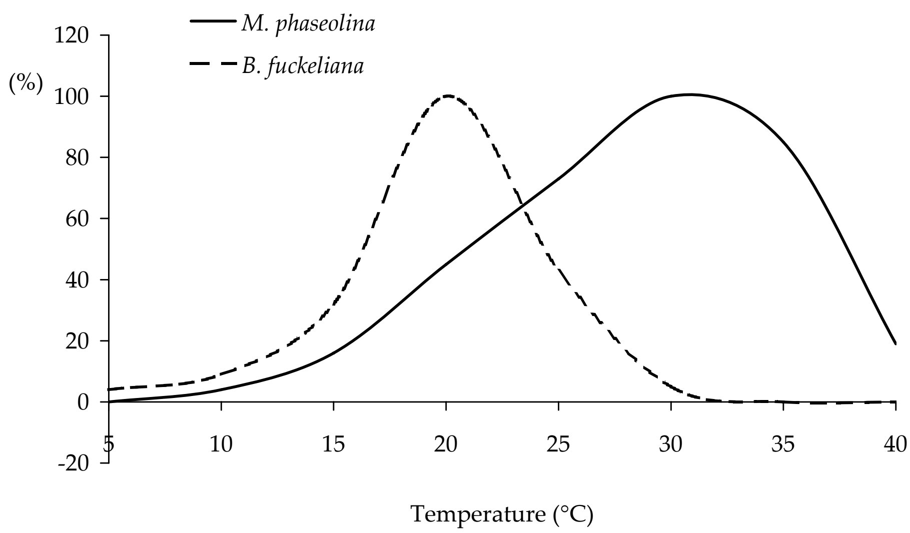

Figure 2. Growth of *M. phaseolina* and *B. fuckeliana* at different temperatures

large necroses developed within three weeks, but microsclerotia were not found sooner than at the beginning of October (Palicová & Veverka 2004 – unpublished results). This shows that pathogenesis proceeds very slowly. For that we can conclude that microsclerotia found after mid August, at the beginning of ripening, are the result of infections in the spring. Water stress at the beginning of senescence is the reason the fungus ceases its development and forms resting organs – microsclerotia. The microsclerotia were more abundant in the years with a long dry autumn, when stands with natural infections were left to dry without chemical dessication, and we were able to collect samples until the middle of October.

Incidence

The disease is promoted by water stress and for that it occurred more frequently on the plants with compressed roots (pipe-like roots) (Veverka *et al.* 2006) and in the driest parts of the fields. When the incidence was very low, diseased plants were found individually, but at higher levels, were usually seen in dispersed groups.

For this reason, the incidence was not expressed as an average for the field, but as the highest incidence found in the field. Since only plants with developed microsclerotia were regarded as infected, and because of the uneven distribution in the field, it was not possible to express the disease incidence precisely.

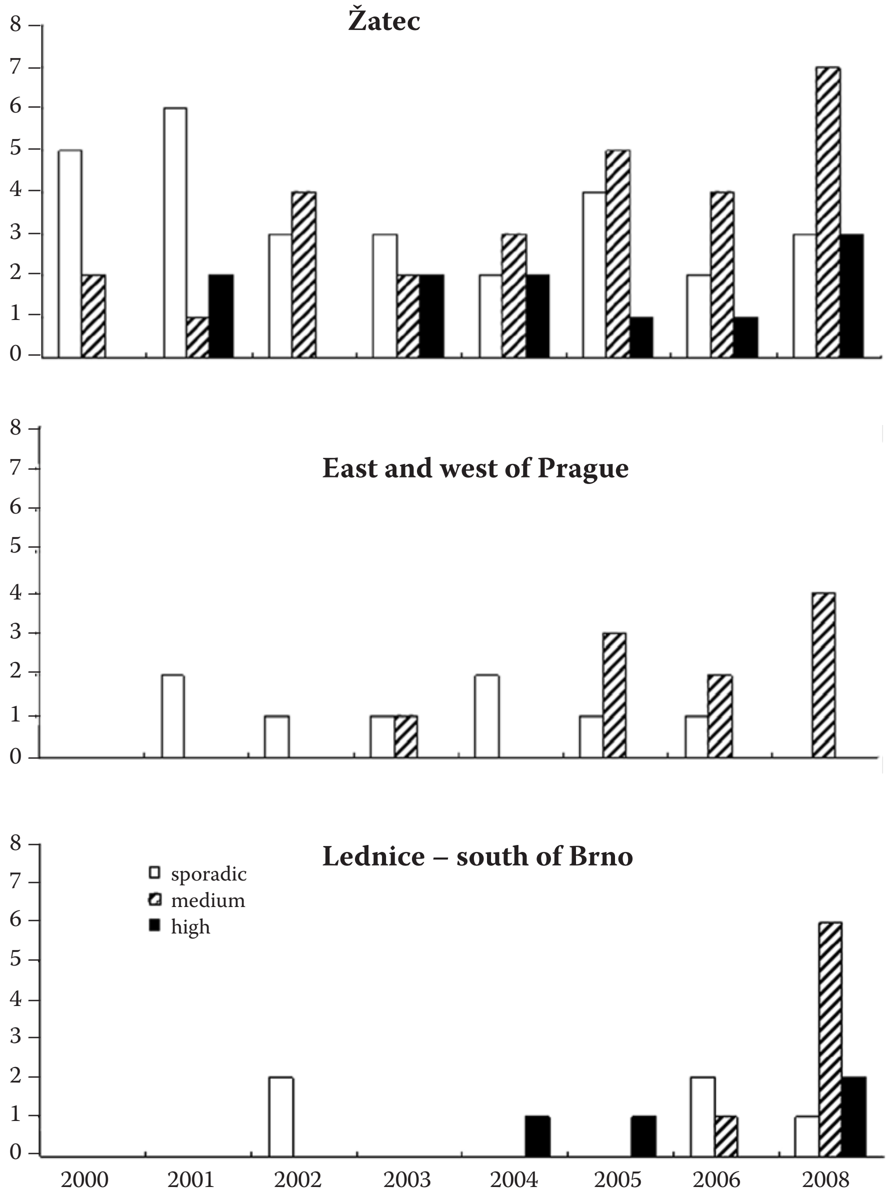

Figure 3. Number of localities of charcoal disease in the main sunflower growing regions of the Czech Republic

The data in Figure 3 shows, that there is an increasing incidence of charcoal disease in all of the three regions. First it appeared in region 1, Žatec, in 1999 (Šárová *et al.* 2003) and the incidence varied in the following years. In region 2, east and west of Prague, the incidence was much lower.

In region 3, south of Brno meteorological station Lednice, no disease was observed in 2000 and 2001. In 2002 only 2 stands with sporadic infections were recorded and in 2004 and 2005 only one field with a high incidence was found. This high incidence in 2004 and 2005 was on two fields 200 m apart near Podivín. Disease was not found in any other field in this region in both these years. Surprisingly, a high incidence was recorded in this region in 2007.

List of the localities with the incidence of *M. phaseolina*

Region 1: Žatec – Bítozeves, Citoliby, Drahomyšl, Hrušovany, Lažany, Lipenec, Postoloprty, Seménkovice, Staňkovice, Žatec.

Table 1. Monthly mean temperatures during the vegetative period in the main sunflower-growing regions of the Czech Republic. Temperatures higher than corresponding 30-year average are in bold face type

Locality	Period	Temperature (°C)					
	1971–2000	8.5	13.4	16.7	18.0	17.4	13.5
	2000	**11.1**	**15.5**	**18.3**	17.1	**19.3**	**14.0**
	2001	**8.8**	**16.3**	16.6	**20.2**	**20.7**	**13.0**
	2002	**9.6**	**16.9**	**19.6**	**20.6**	**21.2**	**14.8**
Žatec	2003	**9.3**	**16.6**	**21.6**	**20.2**	**22.2**	**15.1**
	2004	**9.8**	12.4	16.6	**18.4**	**19.0**	**13.6**
	2005	**10.2**	**14.4**	**17.5**	**19.0**	16.8	**14.4**
	2006	**8.9**	**13.7**	**18.2**	**22.8**	16.7	**16.2**
	2007	**12.8**	**15.8**	**19.6**	**19.5**	**18.9**	13.3
	1971–2000	7.9	13.3	16.2	18.1	18.1	13.7
	2000	**11.6**	**15.8**	**17.8**	15.9	**19.1**	14.0
	2001	7.8	**15.0**	14.7	**18.2**	**18.7**	12.1
	2002	**8.5**	**15.4**	**17.6**	**18.6**	**19.5**	13.2
Prague	2003	**8.5**	**15.8**	**20.4**	**19.5**	**21.4**	**14.7**
	2004	**10.1**	12.3	**16.3**	**18.3**	**19.6**	**14.4**
	2005	**10.5**	**14.4**	**17.3**	**19.2**	17.3	**15.7**
	2006	**9.8**	**14.2**	**18.1**	**22.9**	16.6	**17.4**
	2007	**12.2**	**16.0**	**19.3**	**19.5**	**19.0**	13.0
	1971–2000	9.4	14.4	17.4	19.1	18.2	14.3
	2000	**14.1**	**17.6**	**20.0**	18.2	**20.8**	**14.4**
	2001	9.4	**17.0**	**16.8**	**20.7**	**20.5**	13.1
	2002	**9.9**	**17.5**	**19.5**	**21.4**	**20.1**	14.1
Lednice	2003	**9.7**	**17.6**	**21.4**	**20.7**	**22.9**	**15.0**
	2004	**11.5**	13.7	**17.6**	**19.7**	**20.3**	**14.8**
	2005	**11.2**	**15.7**	**18.4**	**20.2**	**18.4**	**16.0**
	2006	**11.6**	**15.0**	**19.0**	**23.5**	17.3	**17.0**
	2007	**12.6**	**17.1**	**20.9**	**21.3**	20.8	13.3

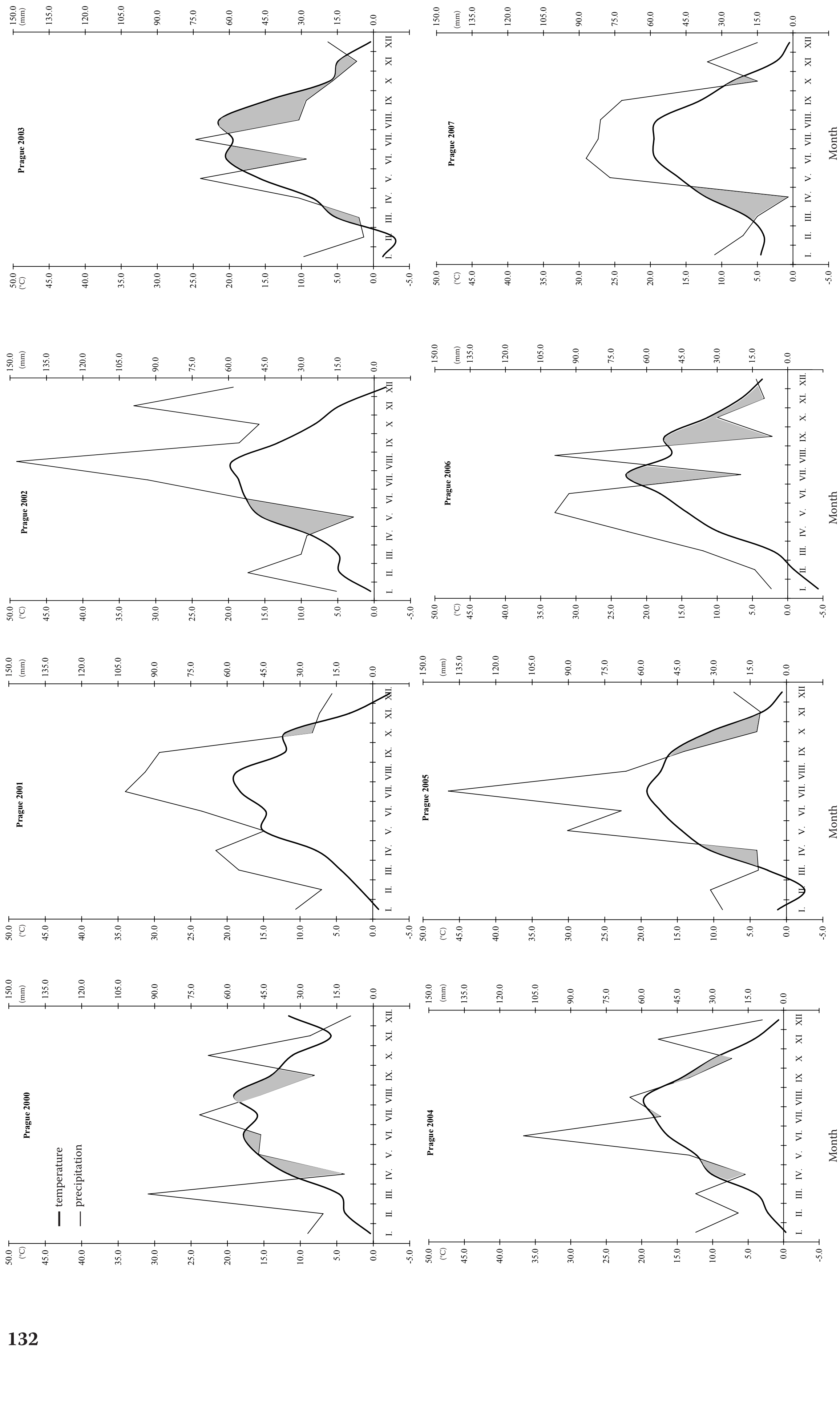

Figure 4. Walter-Lieth climate diagrams of the sunflower growing region Prague

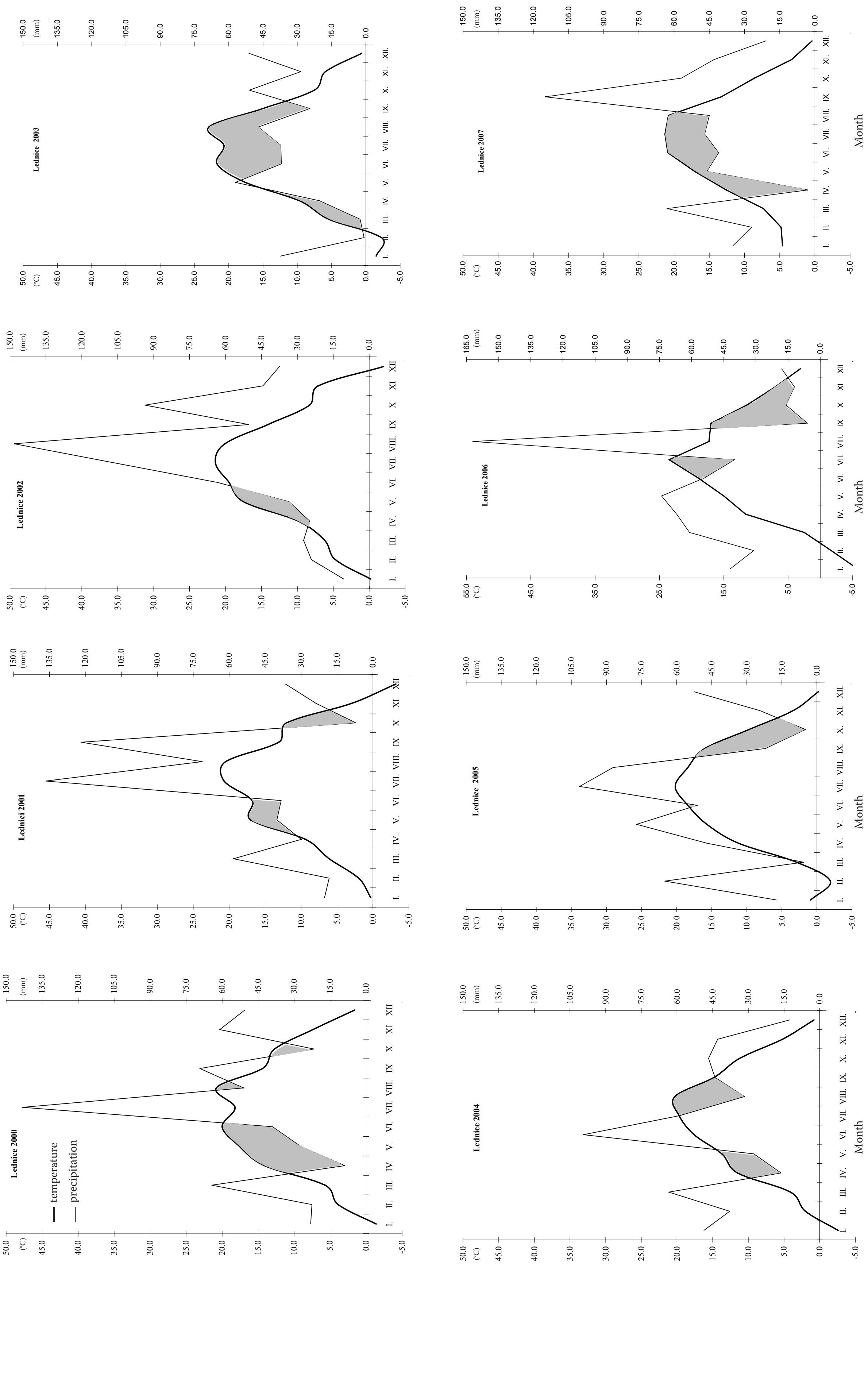

Figure 5. Walter-Lieth climate diagrams of the sunflower growing region Lednice

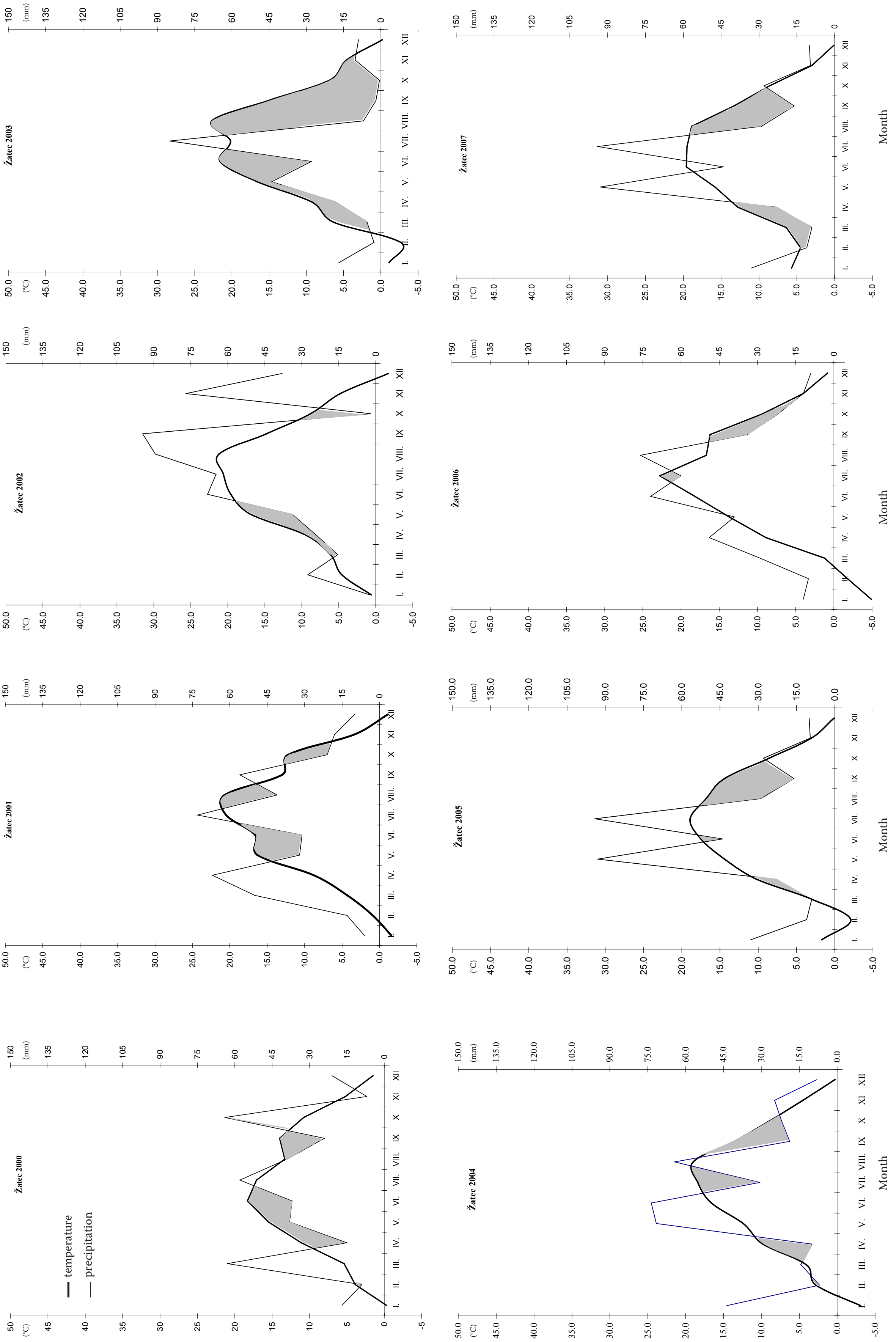

Figure 6. Walter-Lieth climate diagrams of the sunflower growing region Žatec

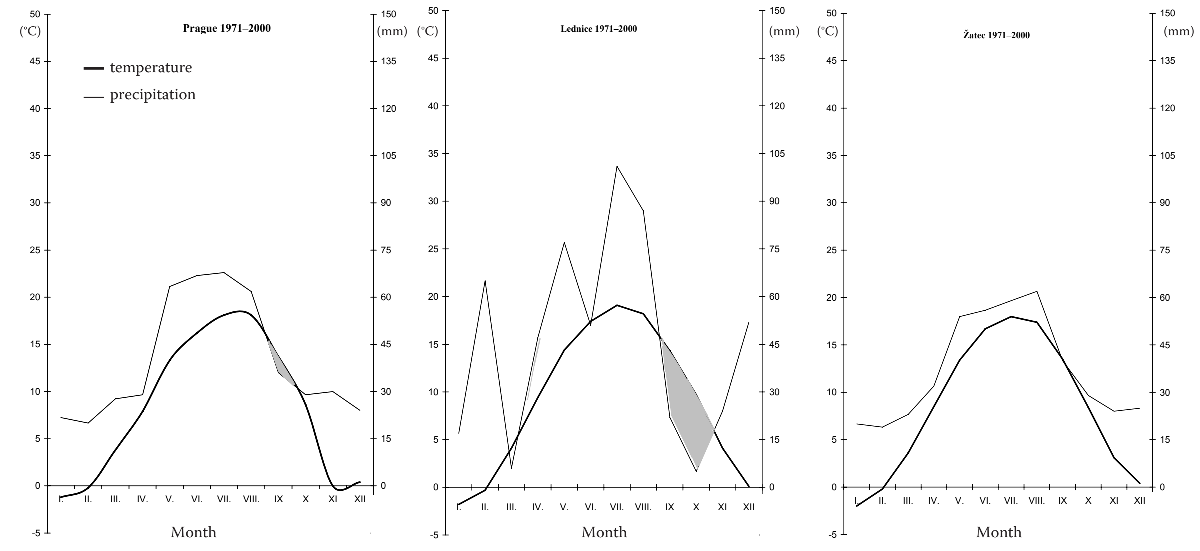

Figure 7. Walter-Lieth climate diagrams of the three sunflower growing regions (average 1971–2000)

Region 2: Prague – Škvorec, Přišimasy, Vitice, Středokluky, Slaný.

Region 3: South Moravia – Kobeřice, Lednice, Mikulov, Moravský Žižkov, Nížkovice, Podivín, Rakvice, Uherský Brod, Velké Bílovice, Židlochovice.

Effect of weather conditions on disease occurrence

The effect of weather on fungal infection of plants is different depending on whether the fungi infect aerial parts of the plants or roots. It is possible to use mathematical models to define the short term conditions needed for infection by e.g. *Phytophthora infestans,* or for the development of insect larvae (Kocmánková *et al.* 2008). However, for root infecting fungi, no such data are available that include the complexity of soil conditions and the potentially long infectious period. There are no detailed data available on temperature requirements for charcoal rot development. It is known from field experience that the disease appears when the plants are stressed by hot dry weather.

Weather records over the vegetative seasons 2000–2007 shows that the monthly mean temperatures were mostly higher than the 30-year average 1961–1990, and this has to be considered as one of the predisposing factors that promote charcoal disease (Table 1). The most important factor seems to be the August temperatures. The average month and year temperatures were continuously increasing in the last decades. In July and August it was in Žatec:

Decades 1971–1980: July 17.1°C, August 17.2°C, year average 7.9°C.

Decades 1981–1990: July 18.2°C, August 18.1°C, year average 8.4°C.

Decades 1991–2000: July 18.9°C, August 19.1°C, year average 9.2°C.

Analysis of the climate diagrams

The definitive precondition for *M. phaseolina* infection and disease development is water stress. The applied climate diagrams used here were based on a rate of 30 mm:10°C, which was lower than the original Walter-Lieth diagrams. The thirty years average for 1971–2000 showed very mild dry periods in August and September in the regions of Prague and Lednice, but no dry period in Žatec. However, dry periods occurred in Žatec in most of the years 2000–2007.

Since the microsclerotia appeared after mid August, we assumed that the critical factor was water stress at the end of July and in August. Comparison of the incidence of charcoal disease (Figure 2) with corresponding climate diagrams for the same years shows that this was true in some cases, but not all (Figures 4–7).

Žatec – good coincidence except for 2002 – dry period since March till June and September–October, but no dry period in July and August. No coincidence in 2007.

Prague – good coincidence only in 2003 and 2006.

Lednice – no dry period in July and August 2000–2002, sporadic disease in 2002 in two localities only

2003 very remarkable dry period – no disease incidence

2004 dry period – high disease incidence in one locality

2005 no dry period – high disease incidence in one locality

2006 dry period in July – low disease incidence

2007 very intensive dry period from April till August – high incidence.

Unfortunately, the reference climate station can not represent individual fields across the whole region. Local precipitation or drought may be very different from the data of climatic stations.

CONCLUSION

The incidence of diseases, pests and weeds, and their effect, is usually related to climatic changes that result in conditions being more or less appropriate for individual agents. It is necessary to keep in mind the whole chain of events. In the case of charcoal disease this is as follows:

– Sunflower is a new crop in the Czech Republic, and was planted on a very limited scale 15 years ago. The area sown has increased step by step. Some stands are in fields where sunflowers have not previously been sown. Also the incidence of other fungal diseases is much lower than on other crops.

– The pathogen is also seedborne. All seeds are of foreign origin, which facilitates the introduction of new pathogens. Seeds are normally treated with a selective fungicide, effective only against *Plasmopara halstedii* (Farlow) Berlese et de Toni. The critical role of seed-born inoculum of fungus is indirectly supported by the early and elevated occurrence of the disease in the isolated Žatec region, but only later in South Moravia, which is in direct contact with Hungary and the Balkan regions where charcoal rot is common.

– The first conclusion is that infection is via an inoculum in the soil or on the seed, but it is not known if the pathogen survives in the soil under normal conditions in the Czech Republic. Seeds are not tested for the presence of the pathogen. We can also conclude that the absence of charcoal disease in any of the regions was not due to weather conditions, but due to the absence of the pathogen.

– Soil conditions are critical. Diseased plants are usually distributed in several dispersed groups in the stand, especially in the higher and drier parts of the field. Disease is also often seen on plants suffering from compressed roots.

– Diseased plants had poorly developed heads and seeds. The high incidence of charcoal rot, especially in coincidence with compressed roots would have a negative effect on yield.

Very high incidence of charcoal disease on sunflower in Slovakia (Bokor 2007) and the increasing incidence in the Czech Republic shows the potential risk of losses caused by this disease. Critical questions for further research are whether the pathogen survives in the soil or on other hosts, especially weeds, and whether the seed has to be treated against *M. phaseolina.*

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