11

Fungicide Resistance in Cucurbit Powdery Mildew Fungi

Aleš Lebeda[1], Margaret T. McGrath[2] and Božena Sedláková[1]
[1]*Palacký University in Olomouc, Faculty of Science, Department of Botany, Olomouc*
[2]*Cornell University, Department of Plant Pathology and Plant-Microbe Biology, Riverhead, NY*
[1]*Czech Republic*
[2]*USA*

1. Introduction

Powdery mildew is the major cause of losses in production of cucurbits worldwide (Cohen et al., 2004; Křístková et al., 2009; Lebeda et al., 2007b; McCreight, 2006) (Fig. 1a,b and 2a,b). This disease is caused by two obligate biotrophic ectoparasites: *Golovinomyces cichoracearum s.l.* (*Gc*) (syn. *Erysiphe cichoracearum s.l.*) and *Podosphaera xanthii* (*Px*) (syn. *Sphaerotheca fuliginea*) (Křístková et al., 2009; Lebeda, 1983) (Fig. 3a,b and 4a,b). Their distribution and relative occurrence varies throughout the world (Bardin et al., 2009; Křístková et al., 2009; Pérez-García et al., 2009). For example, both *Px* and *Gc* are important in Central Europe whereas *Px* occurs almost exclusively in the USA.

Both cucurbit powdery mildew (CPM) species (*Gc* and *Px*) have high evolutionary potential and according to the terminology of McDonald & Linde (McDonald & Linde, 2002) could be considered as "risky" pathogens (Lebeda et al., 2007a). Pathogen populations with a high evolutionary potential are more likely to overcome plant genetic resistance and/or develop fungicide resistance than pathogen populations with a low evolutionary potential (Kuck & Rusell, 2006). CPM species are highly variable in their pathogenicity and virulence which is illustrated by the existence of a large number of different pathotypes and races (Jahn et al., 2002; Lebeda & Sedláková, 2006; McCreight, 2006).

Breeding of cucurbit crops for powdery mildew resistance has a long and successful history, with many resources of race-specific resistance now known in muskmelon (*Cucumis melo*) (McCreight, 2006). There is also excellent resistance in cucumbers (*Cucumis sativus*) (Jahn et al., 2002). Resistance has been bred in some cultivars of squash and pumpkin (*Cucurbita pepo*) and in gourds (*Cucurbita* spp.) (Jahn et al., 2002; Lebeda & Křístková, 1994). Degree of suppression achieved with resistant cultivars is variable, partly due to pathogen adaptation. Additionally, there is tremendous variation within the different cucurbit crops, and incorporating resistance into all horticultural types is an enormous task. Thus utilising plant disease resistance is not an option for managing CPM for all farmers. Furthermore, resistant cultivars do not always provide adequate suppression to be utilized as the sole management practice.

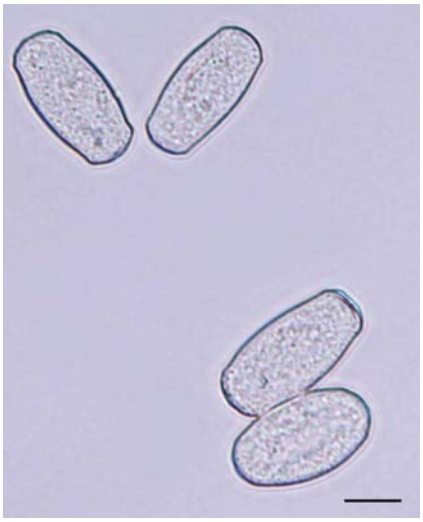
Application of fungicides continues to be the principal approach for managing powdery mildews around the world (Hollomon & Wheeler, 2002). This is due to the limitations of resistance and lack of other management practices. Systemic and translaminar fungicides are especially important for controlling CPM because they provide adequate protection of abaxial leaf surfaces, where conditions are more favorable for disease development than on adaxial surfaces (McGrath, 2001). These fungicides have specific single-site mode of action, thus they are active at one point of one metabolic pathway of the pathogen, and therefore are generally more at-risk for resistance development than other fungicides (McGrath, 2001). Several reports have been published about the appearance and increase of CPM populations (mainly *Px*) resistant to six groups of single-site inhibitors (benzimidazole, DMI, morpholine, hydroxypyrimidine, phosphorothiolate, QoI) (Hollomon & Wheeler, 2002; McGrath, 2001, 2006; Sedláková & Lebeda, 2008). On the other hand, contact fungicides have

1a 1b

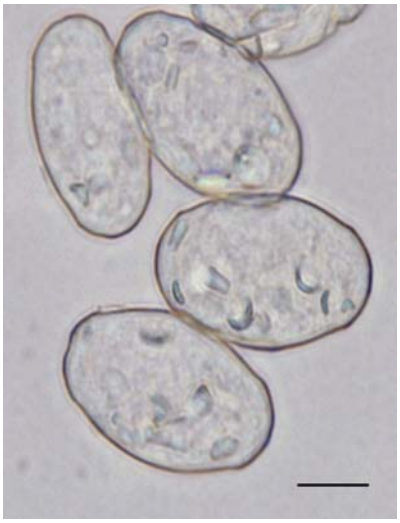
Fig. 1. a,b. Symptoms of cucurbit powdery mildew developing under field conditions (a – limited infection, b – serious infection)

2a 2b

Fig. 2. a,b. *Cucurbita pepo* leaf with powdery mildew (a – adaxial leaf surface, b - abaxial leaf surface)

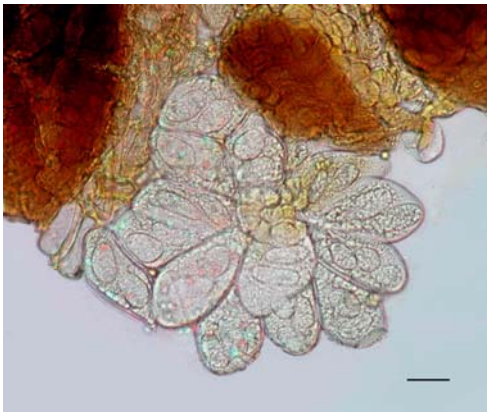


3a

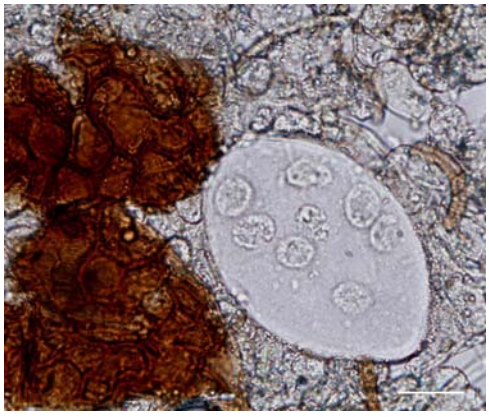


3b

Fig. 3. a,b. The cucurbit powdery mildew fungi. a - conidia of *Golovinomyces cichoracearum* lacking fibrosin bodies, b – conidia of *Podosphaera xanthii* with fibrosin bodies (scale bar: 10 μm)



4a



4b

Fig. 4. a,b. Asci with ascospores of two powdery mildew fungi. a – *Golovinomyces cichoracearum* (7-15 pedicelate asci per chasmothecium each with two ascospores), b – *Podosphaera xanthii* (one ascus without pedicelus per chasmothecium containing eight ascospores) (scale bar: 20 μm)

low potential for resistance development because they are multi-site inhibitors (Kuck & Russell, 2006; McGrath, 2001); however, they are less important for controlling CPM because of their inherent lower efficacy due to inability to protect abaxial surfaces. To date, CPM resistance has been detected to only two groups of multi-site inhibitors (quinoxaline and

miscellaneous) (McGrath, 2001). Current recommendation for managing CPM and fungicide resistance is an integrated disease management program for CPM that entails using fungicides with different modes of action, including multi-site inhibitors, as well as, resistant cultivars, together with information from epidemiological studies and disease monitoring (Sedláková & Lebeda, 2008).

2. Fungicide resistance in cucurbit powdery mildew fungi – historical overview and recent status

CPM pathogens have demonstrated ability to develop resistance, often quickly, to fungicides prone to resistance due to their single-site mode of action (McGrath, 2001). With all at-risk fungicides, repeated use typically in commercial production has resulted in selection of strains with lower sensitivity or resistance to the fungicide then were present before use. Some strains are fully resistant and thus not affected by the fungicide (aka practical or field resistance). The history in the USA of the development of fungicides for CPM and of resistance provides an illustration of the potential of the CPM pathogens. A similar history has occurred in other countries. The dominant CPM pathogen in the USA is *Px*.

MBC (methyl benzimidazole carbamate) fungicides aka benzimidazoles (FRAC Group 1) were the first chemical class of fungicides with a single-site mode of action used for CPM (McGrath, 2001). Resistance developed very quickly to benomyl, the first fungicide in this group. In the USA, benomyl-resistant strains were detected in 1967, the first year of field evaluations at USA university facilities. This was the first documented case of resistance in the USA. At that time, global experiences with fungicide resistance were limited and thus the potential impact on control and the need for management were not recognized. Benomyl formulated as Benlate was registered in 1972 for commercial use on cucurbit crops in the USA. The first case of control failure in the field occurred the next year. Another MBC fungicide, thiophanate-methyl, formulated as Topsin M, is still labeled for CPM and thus available for use in production fields. It is not recommended because resistant strains continue to be found widely and commonly despite limited use of thiophanate-methyl for other diseases. Resistance to this group of fungicides is qualitative, thus pathogen strains are sensitive or fully resistant. And cross resistance occurs among the fungicides, thus resistant strains are insensitive to all fungicides in the group.

The next chemical class developed for CPM was the DMI (demethylation inhibitor) fungicides (FRAC Group 3) (McGrath, 2001). The first active ingredient in this group was triadimefon. It was registered for CPM in the USA in April 1984. The first reported control failure documented through university fungicide efficacy experiments occurred just two years later. Control failure became widespread during the early 1990s. Resistance to DMIs is quantitative, thus pathogen strains exhibit a range in sensitivity. While cross resistance exists among fungicides in this group, there are inherent differences in activity. The next DMI fungicide developed, myclobutanil, when used at a high concentration was effective in university experiments against pathogen strains fully resistant to triadimefon and where this fungicide was ineffective. Myclobutanil was granted registration in the USA in 2000. For two years prior to 2000 an emergency exemption from registration (FIFRA Section 18) for myclobutanil was granted in some states because neither benomyl nor triadimefon, the only mobile fungicides registered for this use at the time, were adequately effective due to resistance. The degree of DMI insensitivity in the CPM pathogen population continued to

shift during the 1990s. As a result, myclobutanil applied at its lowest label rate no longer controlled CPM as well as at the highest rate. USA federal (Section 3) registration was granted for another new DMI, triflumizole, in 2002. Subsequently, sensitivity to the DMI fungicides has remained fairly stable through 2009. Myclobutanil and triflumizole have provided effective control of CPM in most, but not all, field efficacy evaluations conducted over those years. None of the DMI fungicides developed recently, which are difenoconazole, tebuconazole, and metconazole, have exhibited greater inherent activity than the DMIs currently registered, unlike the situation with myclobutanil being substantially more active than triadimefon.

QoI (quinone outside inhibitor) fungicides (FRAC Group 11) were the next chemical class developed for CPM (McGrath, 2001). Azoxystrobin was registered in the USA in spring 1999. It could be used in some states in 1998 where an emergency exemption was granted. Additional QoIs were registered in fall 1999 (trifloxystrobin) and 2002 (pyraclostrobin). Resistance to QoIs was first detected in the USA in 2002 (McGrath & Shishkoff, 2003). Control failures were reported from several states throughout the USA; resistant strains were confirmed to be present in Georgia, North Carolina, Virginia, and New York. Impact on control was dramatic, with failure occurring where QoIs were highly effective the previous year, reflecting the qualitative nature of resistance to this group of fungicides. Resistant strains of CPM have been common in the USA based on bioassays conducted recently in several states. QoI fungicides are no longer recommended for CPM because resistant strains are common, they are fully resistant due to the qualitative nature of the resistance, and there is cross resistance among QoI fungicides. Resistance to QoI and also to MBC fungicides has been detected at the start of CPM development where tested. There continues to be selective pressure to maintain QoI resistance in the CPM pathogen population in the USA because the only fungicide available with a new active ingredient, boscalid, also contains a QoI fungicide.

Carboximide (FRAC Group 7) was the fourth chemical class of mobile fungicides at risk for resistance development available for managing CPM in the USA. The first product, which was registered in 2003, contained boscalid plus pyraclostrobin. Pathogen strains have exhibited a range in sensitivity to boscalid. Strains fully resistant to this fungicide were first detected in 2008 (McGrath, *unpublished*). These strains were able to tolerate label rates (500 ppm) in a leaf-disc bioassay (McGrath & Fox, 2010). Control failure in a fungicide evaluation in 2009 was associated with their presence (Wyenandt, *personal communication*). It is feasible but not known yet whether the new carboximides in development are sufficiently different chemically from boscalid that their efficacy will not be compromised due to cross resistance with boscalid.

Quinoline (FRAC Group 13) is the chemical class most recently to become available for use in the USA. Quinoxyfen was registered for use on melon in 2007 and on pumpkin and winter squash in 2009. It has been highly effective in university fungicide evaluations (e.g. McGrath & Fox, 2009).

There are a few additional fungicides in development with high inherent activity based on baseline sensitivity of *Px*.

Experience with resistance in CPM has revealed challenges in predicting resistance specifically and in managing resistance (McGrath, 2001). While it is well established that whether or not a fungicide has a risk of developing resistance can be predicted based on if it has single-site mode of action, it is not as straight forward to predict details such as how quickly resistance will develop, the type of resistance (qualitative or quantitative), degree of cross resistance, and how long a fungicide will continue to provide control of CPM after

resistance is detected. For example, when the first QoI fungicide was commercialized, it was predicted that relative risk for this group was low (compared to the benzimidazoles), it would take several years for resistance to develop, and it would be quantitative. Additionally, resistance was expected to develop first in *Px*. However, the risk proved to be high, qualitative resistance developed and quickly, with control failure occurring during the fourth year of commercial use for CPM, and resistance was detected prior to this in *Didymella bryoniae*. In Europe resistance was detected in just one year of use (Hollomon & Wheeler, 2002). In contrast, resistance has developed slowly in the DMI fungicides. Fungicides developed since the first one (triadimefon), which became ineffective due to resistance, have continued to provide some control of CPM. Another fungicide commercialized after the DMI fungicides (boscalid) appears to be becoming ineffective as the result of *Px* developing a very high level of resistance. Inherent activity of the newest DMI fungicides has not proven higher than older products as expected. Pathogen strains resistant to a fungicide have often exhibited resistance to unrelated fungicides (correlated resistance).

Managing resistance has been challenging partly due to lack of tools (McGrath, 2001). The general recommendation for a fungicide program to manage resistance is to alternate among at least two fungicides at risk for resistance development and to mix these with a contact fungicide that has low resistance risk. In the USA, there has rarely been a period when more than one at-risk fungicide was available for commercial use to which the CPM pathogen had not already exhibited development of insensitivity. Host plant resistance is the only other management practice for CPM to use in an integrated management program. While there is good potential utility of using resistant cultivars in an integrated program for managing fungicide resistance, it has limitations due to resistance not being incorporated into all horticultural types and pathogen ability to also evolve to overcome genetic resistance as well as fungicides.

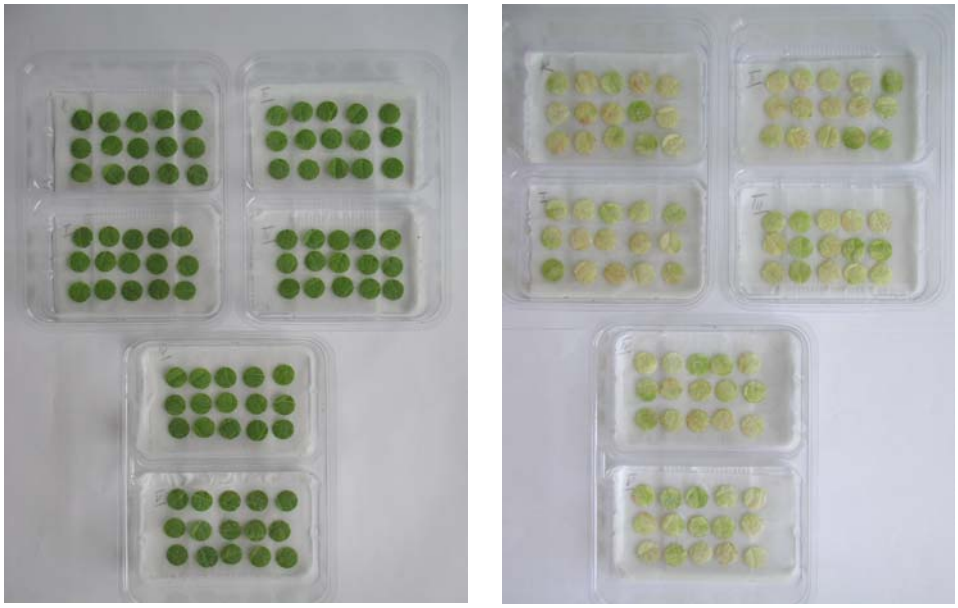
3. Methodology of fungicide resistance research

3.1 Laboratory approach

3.1.1 Leaf-disc bioassays for determining fungicide sensitivity of CPM isolates

A modified leaf-disc bioassay was developed for fungicide resistance screening in Czech CPM populations (Sedláková & Lebeda, 2004a,b, 2006, 2008) (Fig. 5a,b). All screened fungicides were tested at five concentrations (one recommended by the producer; two others below and above this). Treatment with distilled water served as the control. There were five leaf discs (15 mm in diameter) in three replicates for every concentration of each fungicide. Discs were placed into plastic boxes (190 × 140 × 65 mm) containing the fungicide solutions and soaked for 30 minutes. The discs were removed from the fungicide suspension and placed with the adaxial surfaces up on wet filter paper in plastic boxes with the septum (190 × 140 × 65 mm) lined with five layers of moistened cellulose cotton-wool and one layer of filter paper. There were five leaf discs of each of two different fungicide concentrations in each box and three boxes for each isolate. Boxes were open for approximately 1 h (at laboratory temperature in a sterile room) to allow the discs to dry. The discs were inoculated 24 h later by tapping spores off primary leaves of cucumber 'Stela F_1' which were covered with 3- to 4-day-old sporulating mycelium of the isolate of CPM to be tested. Incubation proceeded under the same conditions as for the maintenance of isolates (see 4.1.2).

A laboratory procedure for assessing sensitivity to fungicides was independently developed in NY (McGrath et al., 1996). *Cucurbita pepo* seedlings at the cotyledon stage were sprayed to coverage with fungicide solutions using atomizer bottles connected to an air compressor



5a 5b

Fig. 5. a,b. A modified leaf-disc method for fungicide resistance screening in cucurbit powdery mildew fungi, tested fungicide Topsin M 70 WP (active ingredient: thiophanate-methyl) (a – before inoculation, b – after 14 inoculation days, resistant isolate, profuse sporulation on leaf discs at every fungicide concentration tested)

operated at 30 psi. The fungicide concentrations used were identified through preliminary testing. Treated plants dried over night in a fume hood, then leaf discs were cut and placed with adaxial surface up on water agar in segmented Petri dishes. Discs were cut with a #9 cork borer. Up to six discs treated with the same fungicide concentration were placed in each section. Non-treated discs were placed in one of the four sections. Each disc was inoculated in its center by transferring spores of the isolate to be tested from a leaf culture. Clumps of spores were transferred using a disposable pipette whose tip had been melted in a Bunsen burner flame to form a fine, sealed tip suitable for selecting small groups of spores and for sterilizing in alcohol. Assay plates were incubated for about 10 days under constant light in the laboratory. This bioassay has been used with experimental fungicides to obtain baseline sensitivity data as well as with registered fungicides to investigate shifts in pathogen sensitivity.

3.1.2 Evaluations of the fungicide bioassays

Evaluations for the Czech bioassay were conducted 6-14 days after inoculation by using a 0-4 scale (0 = no sporulation, 1 = sporulating mycelium covering ≤ 25% of leaf disc surface, 2 = > 25% - ≤ 50%, 3 = > 50% - ≤ 75%, 4 = > 75%) (Lebeda, 1984, 1986). The total degree of infection for each isolate was expressed as a percentage of the maximum scores according to Towsend & Heuberger (1943): $P = \Sigma (n \cdot v) \cdot 100/x \cdot N$, where P = the total degree of infection, n = number of discs in every category of infection, v = the category of infection, x

= the maximum level of sporulation, N = the total number of evaluated discs. Three types of reactions were assigned: sensitive (degree of infection, DI = 0-10%); tolerant (DI = 10.1-34.9%); and resistant (DI ≥35%) (Fig. 6).

Fig. 6. Three types of assigned reactions: a – sensitive (degree of infection, DI = 0-10%), b – tolerant (DI = 10.1-34.9%), c – resistant (DI ≥ 35%)

The leaf-disc bioassay conducted in NY was assessed 9-14 days after inoculation (Fig. 7). Percent of each disc with visible powdery mildew was estimated. Ability of an isolate to produce conidia when growing on fungicide-treated leaf tissue, and thereby multiple, was considered an important measure of tolerance/resistance. An isolate was rated sensitive to the fungicide concentration on the disc if no conidia had formed, tolerant if there was growth on fewer than half of the discs, and resistant if there was growth on most of the discs. Where there was no pathogen growth evident with the unaided eye, the disc was examined with a dissecting microscope to ensure inoculum was present. For tolerant and resistant isolates, average percent CPM growth on the fungicide-treated discs was compared to the non-treated discs to determine whether growth was suppressed by the fungicide.

3.2 Field approach

An in-field seedling fungicide sensitivity bioassay was developed in NY to assess fungicide sensitivity of CPM pathogen populations in commercial production fields (Fig. 8 and 9). Seedlings were sprayed with various fungicides and concentrations, placed for at least 4 hours in fields where powdery mildew was developing, then kept in a greenhouse until symptoms developed about 10 days later (Fig 9). Severity was visually estimated on each leaf. Severity on treated seedlings was compared to non-treated ones to estimate frequency of the pathogen population able to tolerate each fungicide concentration tested.

Additionally, efficacy of individual fungicides at risk for resistance development was determined under field conditions in NY with naturally-occurring pathogen strains to assess whether resistance was affecting control. Each fungicide tested was applied weekly with a tractor-sprayer to fungicide plots in a replicated experiment (e.g. McGrath & Fox, 2009). Powdery mildew severity was also assessed weekly by rating severity on both surfaces of leaves.

These approaches are being utilized in some other states.

Fig. 7. Leaf-disc bioassay conducted in segmented Petri dish with sensitive reaction in section of dish on left (no growth of tested CPM isolate), tolerant reaction in upper section (limited growth on some discs), and resistant reaction on right (isolate growth on treated discs similar to growth on non-treated discs in lower plate section)

Fig. 8. Fungicide-treated *Cucurbita pepo* seedlings left with non-treated seedlings for at least 4 hours in a squash crop affected by powdery mildew for an in-field fungicide sensitivity bioassay

Fig. 9. Leaves from in-field bioassay seedlings eleven days after exposure to a cucurbit powdery mildew pathogen population (treatments clockwise from severely-affected non-treated control leaf at left: 50 ppm thiophanate-methyl, 80 ppm myclobutanil, 100 ppm boscalid, 1 ppm quinoxyfen, 80 ppm triflumizole, and 50 ppm trifloxystrobin)

4. Case studies of fungicide resistance in cucurbit powdery mildew fungi

4.1 Czech Republic (Central Europe)

4.1.1 Origin and characterization of cucurbit powdery mildew isolates

Occurrences of powdery mildews (*Golovinomyces cichoracearum* (*Gc*), *Podosphaera xanthii* (*Px*)) on cucurbits was monitored in the Czech Republic (CR) during 2001 to 2007. Each year, at least 100 locations were visited mostly in the main production areas, but also in areas with non-optimal climatic conditions for growing cucurbits (e.g. hilly areas) (Lebeda & Sedláková, 2004a, Lebeda et al., 2007a, 2009a). The timing of visits was focused on the main harvest period (August & first half of September). Severity of symptoms on host plants was evaluated on a 0–4 scale (Lebeda & Křístková, 1994). Whole leaves were collected. Discrete colonies of CPM on the leaves were selected for isolation. Before isolation, spores were microscopically examined in a 3% KOH solution (Lebeda, 1983) for species determination. Isolates were not obtained where a mixture of powdery mildew species were found.

A total of 196 isolates (130 *Gc,* 66 *Px*) originating from the Czech Republic and collected in the years 2001-2007 were screened for tolerance and/or resistance to the two frequently used fungicides (fenarimol and dinocap) and a fungicide ineffective due to resistance (benomyl). A total of 88 (52 *Gc*, 36 *Px)* CPM isolates from 2005-2007 were also tested for tolerance and/or resistance to thiophanate-methyl and 35 (21 *Gc*, 14 *Px*) CPM isolates from 2007 for tolerance and/or resistance to azoxystrobin. 179 CPM isolates originated from infected leaves of *Cucurbita pepo* and *C. maxima*, 13 were from *Cucumis sativus*, three from *C. melo* and one from *Cucurbita moschata* (Sedláková & Lebeda, 2010). All tested isolates were first screened for pathogenic variation (pathotypes, races) by a leaf-disc method (Bertrand et al., 1992; Lebeda, 1986). These isolates were characterized by using a differential set of six cucurbitaceous taxa (Bertrand et al., 1992) and found previously to belong to the various

pathotype groups (Lebeda & Sedláková, 2004a,b, unpublished data). Races were identified by using 11 differential genotypes of *Cucumis melo* (Bardin et al., 1999). Most isolates expressed medium or high pathogenicity (Lebeda et al., 2007a, Lebeda & Sedláková, 2006, Sedláková & Lebeda, 2010).

4.1.2 Pathogen isolation, multiplication and maintenance of isolates

The infected leaf samples collected from production fields were placed on wet filter paper in plastic containers (110 × 85 × 45 mm) in a mobile ice-box for transportation. Individual colonies from these leaves were used to establish CPM cultures. Conidia from pure cultures were transferred by tapping spores on to primary leaves of susceptible cucumber (*Cucumis sativus*) 'Stela F_1'. Isolates were cultured in plastic boxes (190 × 140 × 130 mm) in a growth chamber at 24°C/18°C day/night and 12 h photoperiod (Lebeda et al., 2010) (Fig. 10a,b).

10a

10b

Fig. 10. a, b. Maintenance of cucurbit powdery mildew (CPM) isolates (a – CPM isolates in plastic boxes, b – sporulation of CPM on cotyledons of *Cucumis sativus,* susceptible cv. 'Stela F_1'

Fig. 11. Maintenance of a cucurbit powdery mildew (CPM) isolate on cotyledon in 60-mm Petri dish containing 1.5% water agar

4.1.3 Plant material

Highly susceptible cucumber 'Stela F_1' was used for leaf discs. Plants were grown in mixed substrate (ratio of volume 2 : 1) containing mould and Florcom SB (horticultural substrate based on peat; produced by BB Com, s.r.o., Letohrad, Czech Republic) and under optimal growth conditions (25°C/15°C day/night, with daily watering and weekly fertilization by Kristalon Start (NU3 B.V., Vlaardingen, the Netherlands), 10 ml/10 l of H_2O) in the glasshouse and without any pesticide treatment (Fig. 12). Discs were cut with a cork borer from the leaves of 6- to 8-week-old plants (3- to 6-true-leaf stage) (Lebeda, 1986).

Fig. 12. Highly susceptible cucumber cv. ' Stela F_1' used for preparation of leaf discs

4.1.4 Fungicides and leaf-disc bioassay

Efficacy of four widely used fungicides was tested: fenarimol, formulated as Rubigan 12 EC, producer: Margarita International, Camercio e Servicios, Ltd., Funchual, Portugal; dinocap, formulated as Karathane LC, producer: Dow AgroSciences, Mozzanica, Italy; thiophanate-methyl, formulated as Topsin M 70 WP, producer: Nippon Soda Co. Ltd., Tokyo, Japan; azoxystrobin, formulated as Ortiva, producer: Syngenta Limited, Guildford, Great Britain). They are registered in the CR for field application to control CPM (Kužma, 2005; Minář, 2006, 2007). Fungicide benomyl, formulated as Fundazol 50 WP, producer: Chinosin Pharmaceutical Works Ltd. Budapest, Hungary, was included to serve as a resistant fungicide control. Fundazol is no longer effective and its registration has been cancelled in the CR (Kužma, 2004). These five fungicides are from different chemical groups and have specific features (FRAC 2010; Hollomon & Wheeler, 2002; McGrath, 2001, Table 1).

All mentioned fungicides were tested using a modified leaf-disc bioassay (Sedláková & Lebeda, 2004a,b, 2006, 2008) with five concentrations (one recommended by the producer;

plus two others below and above this) (Table 2, see section 3.1.1). Evaluations of the fungicide bioassay were conducted 6-14 days after inoculation by using a 0-4 scale (Lebeda, 1984, 1986, see section 3.1.2)

Target site and code	Group name	Chemical group	Common name	Source preparation used	Type of resistance	FRAC Code
G1: C14-demethylase in sterol biosynthesis	**DMI**-fungicides (**De**-**M**ethylation **I**nhibitors) (SBI: Class I)	Pyrimidines	Fenarimol	Rubigan 12 EC	Quantitative	3
C5: uncouplers of oxidative phosphorylation		Dinitrophenyl crotonates	Dinocap	Karathane LC	Quantitative	29
B1: β-tubuline assembly in mitosis	**MBC**-fungicides (**M**ethyl **B**enzimidazole **C**arbamates)	Benzimidazoles	Benomyl	Fundazol 50 WP	Qualitative	1
		Thiophanates	Thiophanate-methyl	Topsin M 70 WP		
C3: complex III: cytohrome bc1 (ubiquinol oxidase) at Qo site (*cyt b gene*)	**QoI**-fungicides (**Q**uinone **o**utsite **I**nhibitors)	Methoxy-acrylates	Azoxystrobin	Ortiva	Qualitative	11

*According to: FRAC Code List©2010, Hollomon & Wheeler (2002), Lebeda & Sedláková (2004b), McGrath (2001), Sedláková & Lebeda (2008), Tomlin (2003)

Table 1. Features of the fungicides* used for resistance screening

Fungicide	Concentration of fungicide (µg a.i./ml)/concentration of formulated product* (%)				
	1	2	3**	4	5
Fenarimol	9.6/0.008	18/0.015	36/0.03	72/0.06	144/0.12
Dinocap	28/0.008	52.5/0.015	105/0.03	210/0.06	420/0.12
Benomyl	62.5/0.0125	125/0.025	250/0.05	500/0.1	1000/0.2
Thiophanate-methyl	131.25/0.018	262.5/0.037	525/0.075	1050/0.15	2100/0.3
Azoxystrobin	125/0.05	250/0.1	500/0.2	1000/0.4	2000/0.8

*Formulated products used were Rubigan 12 EC for fenarimol, Karathane LC for dinocap, Fundazol 50 WP for benomyl, Topsin M 70 WP for thiophanate-methyl, and Ortiva for azoxystrobin.
**the concentration recommended by the producer

Table 2. Concentrations of fungicides tested

4.1.5 Results and discussion

Significant differences were observed in fungicide sensitivity of CPM isolates within and between the years 2001-2005 and 2006-2007. Resistant and/or tolerant isolates of both CPM species were detected in different locations (Lebeda et al., 2008, 2009b, 2010; Sedláková & Lebeda, 2004a,b, 2006, 2008, 2010; Sedláková et al., 2009) (Tables 3, 4, 5 and 6).

Fenarimol (Rubigan 12 EC) exhibited decreasing activity in bioassays in 2002 (only for *Gc*) and in 2005 (for both CPM species) (Table 3). Despite the increasing frequency of fenarimol-

tolerant strains, efficacy of fenarimol has remained sufficient to achieve control of CPM under field conditions. Detection of change in sensitive to fenarimol in Czech CPM populations reveals the potential for selection of fenarimol-insensitive strains. Fenarimol belongs to the DMI pyrimidine group of fungicides which have a specific, single-site mode of action that is active against only one point in one metabolic pathway in a pathogen, therefore it is recognized as being at risk of resistance development in CPM population (McGrath, 2001). The genetic structure of the pathogen population is determined mainly by windborne conidia from cucurbit crops in countries surrounding the CR as the main source of initial inoculum for annual reestablishment of the disease (Lebeda et al., 2010; McGrath, 2001; Sedláková & Lebeda, 2008). Overwintering as chasmothecia is considered to be rare (Křístková et al., 2003, 2009). Resistance to DMI fungicides is commonly referred to as "quantitative resistance" because it results from modification of several interacting genes and thus loss of effectiveness due to resistance can be regained by using higher rates or more frequent applications (McGrath, 2001). These facts combined with the use of other effective fungicides might be the explanation for the apparent disappearance of locally-developed resistance to fenarimol. The occurrence of fenarimol-resistant *Px* strains has been reported from Greece, Spain, Israel, Japan, Australia and the Netherlands (López-Ruiz et al., 2010; McGrath, 2001). Resistance to fenarimol is described also in some other pathogens, e.g. *Blumeria graminis* f.sp. *hordei* (Buchenauer et al., 1984) and *Uncinula necator* (Ypema et al., 1997). Cross-resistance of fenarimol-resistant *Px* strains to some other DMI fungicides was documented in the Netherlands and Israel (Scheppers, 1983; 1985), the United Kingdom (Kendall, 1986) and Spain (López-Ruiz et al., 2010).

Efficacy of fungicide (active ingredient), conc. µg a.i./ml^{-1} , ppm)						Total no. of isolates/frequency (%)		
Fenarimol						Σ	*Gc*	*Px*
C	9.6	18	36*	72	144			
+	-	-	-	-	-	153/78	97/63	56/37
+	(-)	-	-	-	-	17/9	16/94	1/6
+	+	-	-	-	-	3/1.5	3/100	-/-
+	(-)	(-)	-	-	-	8/4	4/50	4/50
+	(-)	(-)	(-)	-	-	6/3	5/83	1/17
+	+	(-)	-	-	-	3/1.5	2/67	1/33
+	+	(-)	(-)	-	-	3/1.5	3/100	-/-
+	+	+	(-)	-	-	1/0.5	-/-	1/100
+	+	+	(-)	(-)	-	2/1	-/-	2/100

Gc = Golovinomyces cichoracearum, Px = Podosphaera xanthii;
C = control (untreated by fungicide, characterized by profuse sporulation), *concentration recomended by the producer.
Reaction of CPM: - = sensitive reaction (no sporulation), (-) = tolerant reaction (limited sporulation), + = resistant reaction (profuse sporulation)
**acording to Lebeda et al. (2010), Sedláková & Lebeda (2008, 2010)

Table 3. Response of CPM populations in the years 2001-2007 to different concentrations of Rubigan 12 EC (active ingredient: fenarimol)**

Dinocap (Karathane LC) showed a similar decreasing activity as fenarimol during the years 2001-2005 (Table 4). This phenomenon could be interpreted as the persistence of strains with increasing levels of insensitivity, which were incorporated into the CPM populations by mutation, migration, and/or gene flow (McDonald & Linde, 2002). Elimination of these strains in the same manner as occurred for fenarimol (Ypema et al., 1997) could be a possible explanation for increased efficacy of dinocap in 2006 and 2007. This fungicide appears to still be highly effective for control of Czech CPM populations. Dinocap belongs to the multi-site activity contact fungicides with far lower risk of developing resistance than fungicides with single-site activity (Gisi, 2002; Kuck & Russell, 2006; McGrath, 2001). There are few reports of dinocap resistance worldwide and only for *Px*. It has been reported only from southern Spain, Japan and Taiwan (McGrath, 2001).

Efficacy of fungicide (active ingredient), conc. μg a.i./ml^{-1} , ppm)						Total no. of isolates/frequency (%)		
Dinocap						Σ	*Gc*	*Px*
C	28	52.5	105*	210	420			
+	-	-	-	-	-	150/76.5	99/66	51/34
+	(-)	-	-	-	-	17/9	9/53	8/47
+	(-)	(-)	-	-	-	16/8	13/81	3/19
+	(-)	(-)	(-)	-	-	10/5	8/80	2/20
+	+	-	-	-	-	2/1	1/50	1/50
+	+	(-)	-	-	-	1/0.5	-/-	1/100

Gc = Golovinomyces cichoracearum, Px = Podosphaera xanthii;
C = control (untreated by fungicide, characterized by profuse sporulation), *concentration recomended by the producer.
Reaction of CPM: - = sensitive reaction (no sporulation), (-) = tolerant reaction (limited sporulation), + = resistant reaction (profuse sporulation)
**according to Lebeda et al. (2010), Sedláková & Lebeda (2008, 2010)

Table 4. Response of CPM populations in the years 2001-2007 to different concentrations of Karathane LC (active ingredient: dinocap)**

Benomyl (Fundazol 50 WP) and **thiophanate-methyl** (Topsin M 70 WP) were both ineffective during our seven-year study (Table 5). The screened CPM populations were highly resistant to benomyl as well as to thiophanate-methyl. Both benomyl and thiophanate-methyl belong to benzimidazole fungicides to which there is a great risk of resistance developing (McGrath, 2001). Benzimidazole resistance is commonly referred as "qualitative resistance" because it results from modification of a single major gene and is seen as a complete loss of disease control that cannot be regained by using higher rates or more frequent fungicide applications (McGrath, 2001). The type of resistance combined with the fact that cross resistance occurs between these fungicides explains why benomyl and thiophanate-methyl have been ineffective in the CR. Benomyl registration was cancelled in the CR in 2004 (Kužma, 2004). Despite this, benomyl-resistant strains were common in Czech CPM populations during the years 2005-2007, thus documenting their ability to persist in pathogen populations in CR. Benzimidazole resistance is the most frequently mentioned form of resistance reported from the USA, Australia, the Netherlands and Japan (Brown, 2002; McGrath, 2001, 2006; McGrath & Shishkoff, 2001). Most reports pertain to

benomyl. Thiophanate-methyl has been mentioned in reports of resistance only from the USA (Matheron & Porchas, 2007; McGrath, 2001, 2005,). Occurrence of benzimidazole resistance has been reported in other pathogens, including *Didymella bryoniae* (Keinath et al., 1998 and *Uncinula necator* (Ypema et al., 1997).

Efficacy of fungicide (active ingredient), conc. µg a.i./ml^{-1} , ppm)						Total no. of isolates/frequency (%)		
Benomyl						Σ	*Gc*	*Px*
C	62.2	125	250*	500	1000			
+	(-)	-	-	-	-	1/0.5	1/100	-/-
+	(-)	(-)	-	-	-	4/2	3/75	1/25
+	(-)	(-)	(-)	(-)	-	1/0.5	-/-	1/100
+	(-)	(-)	(-)	(-)	(-)	3/1.5	2/67	1/33
+	+	(-)	(-)	(-)	(-)	2/1	2/100	-/-
+	+	+	(-)	-	-	1/0.5	1/100	-/-
+	+	+	(-)	(-)	(-)	3/1.5	2/67	1/33
+	+	+	+	(-)	-	2/1	1/50	1/50
+	+	+	+	(-)	(-)	7/3.5	4/57	3/43
+	+	+	+	+	(-)	11/6	9/82	2/18
+	+	+	+	+	+	161/82	105/65	56/35
Thiophanate-methyl								
C	131.25	262.5	525*	1050	2100			
+	(-)	(-)	(-)	(-)	(-)	1/1	1/100	-/-
+	+	+	(-)	(-)	-	2/2	1/50	1/50
+	+	+	+	-	-	2/2	-/-	2/100
+	+	+	+	(-)	-	6/7	1/17	5/83
+	+	+	+	(-)	(-)	7/8	6/86	1/14
+	+	+	+	+	(-)	9/10	7/78	2/22
+	+	+	+	+	+	61/70	36/59	25/41

Gc = Golovinomyces cichoracearum, Px = Podosphaera xanthii;
C = control (untreated by fungicide, characterized by profuse sporulation), *concentration recomended by the producer.
Reaction of CPM: - = sensitive reaction (no sporulation), (-) = tolerant reaction (limited sporulation), + = resistant reaction (profuse sporulation)
**according to Lebeda et al. (2010), Sedláková & Lebeda (2008, 2010)

Table 5. Response of CPM populations in the years 2001-2007 to different concentrations of Fundazol 50 WP (active ingredient : benomyl) and in 2005-2007 to Topsin M 70 WP (active ingredient: thiophanate-methyl)**

Azoxystrobin (Ortiva) exhibited decreasing efficacy in 2007 (Table 6). Even though 40% of CPM isolates were highly sensitive, most of the screened CPM isolates expressed a high level of tolerance or resistance to this fungicide. The screened Czech CPM population showed a high potential for developing resistance to azoxystrobin. Before this time, occurrence of azoxystrobin-resistant strains had not been reported from the CR, therefore results of our one-year study could be considered a base for future experiments. Azoxystrobin belongs to the strobilurin QoI fungicides which have a single-site mode of

action that binds to the subunit protein of cytochrome bc_1 complex of the electron transport chain located in the inner-mitochondrial membrane, thereby inhibiting fungal respiration (Sauter et al., 1995). It is generally known now that these site-specific fungicides have a high risk of resistance development in a pathogen population (McGrath, 2001). The type of resistance to strobilurins is qualitative (same as for benomyl) which means that individuals of the CPM pathogen are either highly sensitive to strobilurins or highly resistant (Ishii et al., 2001; McGrath & Shishkoff, 2003). This fact does not correspond to the situation in Czech CPM population in relation to azoxystrobin in the year 2007. This could reflect the structure of the CPM population in Central Europe, where *Gc* is propably the most important CPM pathogen on field cucurbits (Lebeda, 1983; Křístková et al., 2003, 2009) and CPMs are highly variable in their pathogenicity and virulence (Jahn et al., 2002; Lebeda & Sedláková, 2004 a,b, 2006; Sedláková & Lebeda, 2008, 2010). In many parts of eastern Asia and the northern Mediterranean area, strobilurin resistance in CPM has developed, sometimes within the first season of use (Hollomon & Wheeler, 2002). Resistance to azoxystrobin in *Px* was recorded from Spain (Fernández-Ortuño et al., 2006), Japan (Ishii et al., 2001) and the USA (McGrath & Shishkoff, 2003; McGrath, 2005, 2006). Reduced efficacy of azoxystrobin was also claimed in cucurbit downy mildew (*Pseudoperonospora cubensis* (Berk. & M. A. Curtis) Rostovzev.) in Japan (Ishii et al., 2001) and the USA. Cross-resistance among QoI fungicides has been documented with *E. graminis* f. sp. *tritici* and *Plasmopara viticola* (Heaney et al., 2000). For most of the plant pathogens in which QoI resistance has been described, resistance was conferred by a point mutation in cytochrome b (*cyt b*) gene leading to an amino acid change from glycine to alanine at position 143 (G143A) (Gisi et al., 2002). Based on recent published data, it is evident that field resistance to QoI fungicides in *Px* is not supported by typical mutations in the mitochondrial cytochrome *b* gene (Fernández-Ortuño et al., 2008).

Efficacy of fungicide (active ingredient), conc. µg a.i./ml^{-1} , ppm)						Total no. of isolates/frequency (%)		
Azoxystrobin						Σ	*Gc*	*Px*
C	125	250	500*	1000	2000			
+	-	-	-	-	-	14/40	11/79	3/21
+	(-)	-	-	-	-	6/17	1/17	5/83
+	(-)	(-)	-	-	-	1/3	-/-	1/100
+	(-)	(-)	(-)	(-)	(-)	1/3	-/-	1/100
+	+	-	-	-	-	1/3	1/100	-/-
+	+	+	(-)	(-)	-	1/3	1/100	-/-
+	+	+	+	+	+	11/31	7/64	4/36

Gc = Golovinomyces cichoracearum, Px = Podosphaera xanthii;

C = control (untreated by fungicide, characterized by profuse sporulation), *concentration recomended by the producer.

Reaction of CPM: - = sensitive reaction (no sporulation), (-) = tolerant reaction (limited sporulation), + = resistant reaction (profuse sporulation)

**according to Lebeda et al. (2010), Sedláková & Lebeda (2008, 2010)

Table 6. Response of CPM populations in the year 2007 to different concentrations of Ortiva (active ingredient: azoxystrobin)**

4.2 New York (northeastern USA)

4.2.1 Investigation of fungicide sensitivity in cucurbit powdery mildew populations

The in-field seedling fungicide sensitivity bioassay (see section 3.2) has been used to assess fungicide sensitivity of CPM pathogen populations most growing seasons on Long Island, NY. Spring-planted zucchini and summer squash crops (*Cucurbita pepo*) were used for the first bioassay conducted in a year because this is where powdery mildew starts to develop each season. Additional bioassays were conducted in main season crops and research plantings of jack-o-lantern pumpkin (*C. pepo*) to examine the impact of fungicide use on fungicide sensitivity.

Seedlings of pumpkin cv. 'Sorcerer' were started in a growth chamber, then transplanted to pots and grown in a greenhouse until the 1st to 4th true leaf stage. Their growing point and unexpanded leaves were removed just before treatment. Seedlings were treated with various doses of fungicides using a back-pack CO_2-pressurized sprayer equipped with a single nozzle boom operated at 40 psi. Treated seedlings were left overnight to dry. Then they were placed in fields amongst cucurbit plants with powdery mildew symptoms. Each group of seedlings had 1 treated seedling for each fungicide dose tested plus two non-treated seedlings. They were left for about 4 hours during the middle of the day to be exposed to the wind-dispersed spores of the CPM pathogen in the fields. Afterwards the seedling were kept in a greenhouse until symptoms of powdery mildew were visible, which took at least 10 days. Then severity (percent tissue with symptoms) on upper leaf surfaces was visually estimated for each true leaf. Frequency of pathogen strains in a field able to tolerate each fungicide dose was estimated by calculating the ratio of severity on fungicide-treated plants relative to non-treated plants.

4.2.2 Determination of fungicide sensitivity for cucurbit powdery mildew isolates

Isolates of *Px* were obtained from field-grown cucurbit plants for determining fungicide sensitivity of individual members of CPM populations with the leaf-disc bioassay developed in NY (see section 3.1.1). Leaves with discrete colonies of CPM on the abaxial surface were collected from commercial and research fields. In the laboratory leaves were cut to remove pieces with discrete colonies, which were placed on wet filter paper in Petri dishes, with abaxial surface facing upward, to incubate for at least one day to obtain ample conidia for transferring to cotyledons in culture plates (Fig. 11). Conidia were moved with the sealed pipette transfer tool. Cultures were incubated for 9-21 days under constant light in the laboratory before use in a bioassay or transfer to a new leaf. Optimum period was 11 days. Cultures could be held for a longer period before transferring had to be done, due to declining culture condition, when leaves used were in peak condition and conidia were transferred to one location rather than multiple locations to obtain ample quantity of conidia for bioassays. Pumpkin cv. 'Sorcerer' was grown to the cotyledon stage in 48-cell trays filled with Pro-mix in a growth chamber at 29°C/26°C day/night with 18-hr day and daily watering. Isolates were tested in successive bioassays with three fungicide concentrations each (see section 3.1.1).

4.2.3 Fungicides

The fungicides tested were:

thiophanate-methyl at 50 ppm (µg/ml) (formulated as Topsin M 70 WP®; FRAC Group 1; producer: Nippon Soda Co. Ltd., Tokyo, Japan);

trifloxystrobin at 50 ppm (Flint®; FRAC Group 11; producer: Bayer CropScience, Research Triangle Park, NC);
myclobutanil at 20, 40, 80, 100, 120 and 150 ppm (Nova 40W®; FRAC Group 3 fungicide; producer: Dow AgroSciences LLC, Indianapolis, IN);
triflumizole at 80, 100, 120 and 150 ppm (Procure 50WS®; FRAC Group 3; producer: Crompton Manufacturing Co., Inc., Middlebury, CT);
boscalid at 125, 150, 175, 200 and 500 ppm (Endura®; FRAC Group 7; producer: BASF Corporation, Research Triangle Park, NC); and
quinoxyfen at 1, 5, and 10 ppm (Quintec®; FRAC Group 13; producer: Dow AgroSciences LLC, Indianapolis, IN).
Endura was used rather than the fungicide with boscalid registered for this use, Pristine, because it also contains pyraclostrobin. All other fungicides used are registered and labeled for managing CPM in the USA. There are several FRAC Group 3 and 11 fungicides registered in the USA and labeled for CPM. Myclobutanil and trifloxystrobin were used as representatives for these groups, respectively. Since MBC and QoI resistance is qualitative only one concentration is needed for its detection. A range of concentrations was used for the other fungicides because resistance is quantitative. All concentrations listed were not included in each assay. The concentrations tested were usually selected based on previous results with the goal of having discriminatory concentrations that some isolates would be resistant to.

	Proportion of population fungicide tolerant [average (range)]				
Year	Thiophanate-methyl 50 ppm	Trifloxystrobin 50 ppm	Myclobutanil 80 ppm	Boscalid 175 ppm	Quinoxyfen 10 ppm
2006	74 (50-89)	30 (5-48)	62 (38-86)	ND	ND
2007	ND	70 (0-100)	71 (13-100)	14 (1-22)	0 (0-0)
2008	72 (18-100)	66 (21-100)	28 (7-46)	12 (6-21)	1 (0-2)
2009	59 (20-100)	80 (28-100)	21 (7-29)	20 (4-48)	2 (0-5)

ND = not determined.

Table 7. Fungicide sensitivity of *Podosphaera xanthii* populations in NY based on results from seedling bioassays conducted in spring crops early in disease development

	Proportion of population fungicide tolerant (average for all fields assayed)							
	Myclobutanil		Triflumizole		Boscalid		Quinoxyfen	
Date	100 ppm	120 ppm	100 ppm	120 ppm	125 ppm	175 ppm	1 ppm	5 ppm
8/10/07	20	7	55	31	21	16	38	1
8/23/07	38	13	15	6	45	14	18	2
9/7/07	4	0	0	0	23	11	4	3
10/2/07	3	0	0	0	20	13	ND	2

ND = not determined.

Table 8. Fungicide sensitivity of *Podosphaera xanthii* populations in NY based on results from seedling bioassays conducted in pumpkin crops during the 2007 season

	Proportion of population fungicide tolerant (average for all fields assayed)							
	Trifloxy-	Myclobutanil		Triflumizole	Boscalid		Quinoxyfen	
Date	strobin	80 ppm	120 ppm	80 ppm	100 ppm	175 ppm	1 ppm	5 ppm
8/6/08	9	2	1	2	15	14	7	2
8/12/08	42	6	4	7	44	24	25	4
8/21/08	ND	9	5	8	20	15	6	9
9/9/08	42	7	ND	7	22	15	4	4
9/30/08	16	14	ND	ND	7	ND	4	2

ND = not determined.

Table 9. Fungicide sensitivity of *Podosphaera xanthii* populations in NY based on results from seedling bioassays conducted in pumpkin crops during the 2008 season

	Proportion of population fungicide tolerant (average for all fields assayed)							
	Trifloxy-	Myclobutanil		Triflumizole	Boscalid		Quinoxyfen	
Date	strobin	80 ppm	120 ppm	80 ppm	50 ppm	175 ppm	1 ppm	10 ppm
9/3/09	77.4	4.1	0.4	4.6	17.3	10.9	6.3	0.5

ND = not determined.

Table 10. Fungicide sensitivity of *Podosphaera xanthii* populations in NY based on results from seedling bioassays conducted in pumpkin crops during the 2009 season

4.2.4 Results and discussion

Both bioassays proved to be useful tools for investigating fungicide resistance in CPM. The in-field seedling fungicide sensitivity bioassay conducted in spring-planted crops provided information quickly (11 days) about the CPM population that could be used to guide fungicide recommendations for main season crops. The leaf-disc bioassay provided precise information about the sensitivity of individuals in the population, but required a lot of labor and time to obtain. Both procedures were used to examine impact of fungicide use on pathogen sensitivity to fungicides.

A range in response from very sensitive to resistant was detected to the five fungicide chemical groups tested with the seedling bioassay conducted in spring crops in NY in 2006-2009 (Table 7). On average, greater than 50% of the CPM population was resistant to MBC (FRAC Group 1) fungicides. Resistance to QoI (FRAC Group 11) fungicides was also common most years. There evidently was a lot of variation among fields where the bioassay was conducted. During each production season there was little evidence of change in the pathogen population (Tables 8-10).

5. Conclusions and future prospects

1. Currently there are two fungi predominantly responsible for causing cucurbit powdery mildew (CPM). They are distributed worldwide and considered economically important on almost all commonly grown cucurbits (Křístková et al., 2009). *Podosphaera xanthii* is considered more common than *Golovinomyces cichoracearum* (McGrath & Thomas, 1996).

2. Protection of cucurbits against CPM is primarily accomplished with fungicides; resistant cultivars are not available in all horticultural types and host plant resistance is often used in combination with fungicides for CPM to achieve a high degree of control and to manage selection of pathogen races able to overcome genetic resistance (McGrath, 2001, 2006).
3. Fungicide resistance is known in both powdery mildew species (McGrath, 2001; Sedláková & Lebeda, 2008).
4. Based on published literature, there is very limited information about fungicide resistance/susceptibility of *Golovinomyces cichoracearum* in comparison with *Podosphaera xanthii* (McGrath, 2001; Sedláková & Lebeda, 2008). The dominant pathogen is considered to be *P. xanthii*. However, *G. cichoracearum* is widespread around the world in temperate regions, and probably is the most important CPM pathogen on field-grown cucurbits in Central Europe (Lebeda, 1983; Křístková et al., 2003; 2009).
5. During the last several decades, many new fungicides effective against CPM (e.g. Kuck & Russell, 2006; Tomlin, 2003) have been introduced to the market, providing superior control over the contact fungicides relied upon earlier (López-Ruiz et al., 2010; McGrath, 2006; McGrath & Shishkoff, 2003).
6. These new fungicides are mostly single-site inhibitors in a metabolic pathway of the pathogens and thus have a high risk of resistance developing to them (McGrath, 2001, 2005, 2006; McGrath & Shishkoff, 2001; Sedláková & Lebeda, 2008).
7. There have been reports of failure to control CPM with fungicides; some cases have been shown to be associated with resistance in this group of pathogens (McGrath, 2001, 2006; Sedláková & Lebeda, 2008).
8. Distribution and dynamics of fungicide resistance in CPM fungi in large growing areas or continents are not known. The goal of recent research has been to obtain comprehensive data about fungicide resistance pertaining to the geographic distribution of resistant pathogen strains, their spatial and temporal variability, and changes in the fungicide resistance status of the pathogen in Central Europe (Czech Republic). Temporal aspects of fungicide resistance and impact of fungicide use on pathogen sensitivity is being investigated in the USA (NY).
9. Various procedures, described in this chapter, have been developed and utilized to investigate fungicide resistance in CPM fungi in the laboratory and field.
10. Nevertheless, additional detailed studies on CPM fungi are needed focused on the mechanisms of fungicide resistance, its genetic background, epidemiology, and spatial and temporal changes to gain a better understanding of this phenomenon. More international cooperation and coordination are required for significant progress in this field, and for more efficient plant protection of cucurbits against both powdery mildew species.

6. Acknowledgements

The authors thank Dr. Michaela Sedlářová (Department of Botany, Faculty of Science, Palacký University, Olomouc, Czech Republic) for assistance and practical advice during preparation of microphotos used in this chapter, and Mr. George M. Fox (formerly of Cornell University) and Dr. Monica Miazzi (Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, Via Amendola 165/a, 70127 Bari, Italy) for assistance with research conducted in NY. Research described was supported by grants

NAZV QH 71229, MSM 6198959215, and PrF_ 2010_ 001; Friends of Long Island Horticulture Grant Program; Hatch funds; and by funds from agricultural chemical industries. Some isolates of cucurbit powdery mildew used in this research are maintained in the Czech National Collection of Microorganisms (http://www.vurv.cz/ collections/ vurv.exe/) at Palacký University in Olomouc, Department of Botany (http://botany.upol.cz).

7. References

Bardin, M.; Carlier, J. & Nicot, C.P. (1999). Genetic differentiation in the French population of *Erysiphe cichoracearum*, a causal agent of powdery mildew of cucurbits. *Plant Pathol.*, 48, 531-540, ISSN 0032-0862

Bertrand, F. ; Pitrat, M. ; Glandard, A. & Lemaire, M.J. (1992). Diversité et variabilité des champignons responsables de l' oïdium des cucurbitacées. *Phytoma-La Défense des Végétaux,* 438, 46-49, ISSN 1164-6993

Brown, M.K.J. (2002). Comparative genetics of avirulence and fungicide resistance in the powdery mildew fungi. In: *The Powdery Mildews. A Comprehensive Treatise,* R.R. Bélanger; R.W. Bushnell; J.A. Dik & W.L.T. Carver (Eds.), 56-65, APS Press, ISBN 0-89054-291-0, St. Paul, MN, USA

Buchenauer, H.; Budde, K.; Hellwald, H.K.; Taube, E. & Kirchner, R. (1984). Decreased sensitivity of barley powdery mildew isolates to triazole and related fungicides, *Proceedings of the British Crop Protection Conference of Pests and Diseases*. pp. 483-488, ISBN 1901396576 9781901396577, Brighton Hilton Metropole Hotel, Brighton, UK, November 13-16, 2000, Farnham, Surrey: BCPC, UK

Cohen, R.; Burger, Y. & Katzir, N. (2004). Monitoring physiological races of *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*), the causal agent of powdery mildew in cucurbits: factors affecting race identification and the importance for research and commerce. *Phytoparasitica,* 32, 174-183, ISSN 0334-2123 (print version)

Fernández-Ortuño, D.; Pérez-García, A.; López-Ruiz; F., Romero, D.; de Vincente, A. & Toréz, J.A. (2006). Occurrence and distribution of resistance to QoI fungicides in populations of *Podosphaera fusca* in south central Spain. *Europ. J. Plant Pathol*., 115, 215-222, ISSN 0929-1873

Fernández-Ortuño; D., Toréz, J.A.; de Vincente, A. & Pérez-García, A. (2008). Field resistance to QoI fungicides in *Podosphaera fusca* is not supported by typical mutations in the mitochondrial cytochrome *b* gene. *Pest Manag. Sci.,* 64, 694-702, ISSN 1526-498X

FRAC Code List© 2010. Fungicides sorted by mode of action (including FRAC Code numbering). pp. 10. http://www.frac.info/frac/publication/anhang/FRAC Code List 2010.pdf

Gisi, U. (2002). Chemical control of downy mildews. In: *Advances in Downy Mildew Research,* N.T.P. Spencer-Phillips, U. Gisi & A. Lebeda (Eds.), 119-159, Kluwer Academic Publishers, ISBN 1402006179 9781402006173, Dordrecht, the Netherlands

Heaney, S.P.; Hall, A.A.; Davies, S.A. & Olaya, G. (2000). Resistance to fungicides in the QoI-STAR cross-resistance group: current perspectives. *Proceedings of BCPC Conference-Pests and Diseases*, pp. 755-762, ISBN 1901396576 9781901396577, Brighton Hilton Metropole Hotel, Brighton, UK, November 13-16, 2000, Farnham, Surrey: BCPC, UK

Hollomon, W.D. & Wheeler, E.I. (2002). Controlling powdery mildews with chemistry. In: *The Powdery Mildews. A Comprehensive Treatise.,* R.R. Bélanger; R.W. Bushnell; J.A. Dik & W.L.T. Carver (Eds.), 249-255, APS Press, ISBN 0-89054-291-0, St. Paul, MN, USA

Ishii, H.; Fraaije, B.A.; Sugiyama, T.; Noguchi, K.; Nishimura, K.; Takeda, T.; Amano, T. & Hollomon, D.W. (2001). Occurrence and molecular characterization of strobilurin resistance in cucumber powdery mildew and downy mildew. *Phytopathology,* 91 (12), 1166-1171, ISSN 0031-949X

Jahn, M.; Munger, M.H. & McCreight, D.J. (2002). Breeding cucurbit crops for powdery mildew resistance. In: *The Powdery Mildews. A Comprehensive Treatise,* R.R. Bélanger; R.W. Bushnell; J.A. Dik & W.L.T. Carver (Eds.), 239-248, APS Press, ISBN 0-89054-291-0, St. Paul, MN, USA

Keinath, A.P. (1998). Resistance to benomyl and thiophanate-methyl in *Didymella bryoniae* from South Carolina and New York. *Plant Dis.,* 82 (5), 479-484, ISSN 0191-2917

Kendal, S.J. (1986). Cross-resistance of triadimenol-resistant fungal isolates to other sterol C-14 demethylation inhibitor fungicides. *Proceedings of BCPC Conference - Pests and Diseases,* pp. 539-546, Brighton Hilton Metropole Hotel, Brighton, UK, November 17-20, 1986, Thornton Heath: BCPC, UK

Křístková, E.; Lebeda, A. & Sedláková, B. (2009). Species spectra, distribution and host range of cucurbit powdery mildews in the Czech Republic, and in some other European and Middle Eastern countries. *Phytoparasitica,* 37, 337-350, ISSN 0334-2123 (print version)

Křístková, E.; Lebeda, A.; Sedláková, B.; Duchoslav, M. & Dančák, M. (2003). Distribution of powdery mildew species on Cucurbitaceaous vegetables in the Czech Republic. Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture. *Hortic. and Veget. Growing* 22 (3), 31-41, ISSN 0236-4212

Kuck, K.-H. & Russell, E.P. (2006). FRAC: Combined resistance risk assessment. *Aspects of Appl. Biol*., 78, 3-10, ISSN 0265-1491

Kužma, Š. [Ed.] (2004). *List of the Registered Plant Protection Products 2004.* The State Phytosanitary Administration in Agrospoj, Prague, ISBN 8023942891 9788023942897, Czech Republic (in Czech, with English summary)

Kužma, Š. [Ed.] (2005). *Bulletin of The State Phytosanitary Administration. List of the Registered Plant Protection Products 2005.* Special issue, vol. 2, March, 2005, The State Phytosanitary Administration, Prague, Czech Republic. (in Czech, with English summary)

Lebeda, A. (1983). The genera and species spectrum of cucumber powdery mildew in Czechoslovakia. *Phytopathol. Zeitschrift,* 108, 71-79, ISSN 0031-9481

Lebeda, A. (1984). Screening of wild *Cucumis* species for resistance to cucumber powdery mildew (*Erysiphe cichoracearum* and *Sphaerotheca fuliginea*). *Sci. Hortic.,* 24, 241-249, ISSN 0304-4238

Lebeda, A. (1986). Padlí okurkové. *Erysiphe cichoracearum*, *Sphaerotheca fuliginea* (Cucumber powdery mildew. *Erysiphe cichoracearum*, *Sphaerotheca fuliginea*). In: *Methods of Testing Vegetable Crops for Resistance to Plant Pathogens*, A. Lebeda (Ed.), 87-91, VHJ Sempra, Research Institute of Vegetable Crops, Olomouc

Lebeda, A., Křístková, E. (1994). Field resistance of *Cucurbita* species to powdery mildew (*Erysiphe cichoracearum*). *J Plant Dis. Prot.*, 101, 598-603, ISSN 1861-38371861-3829

Lebeda, A. & Sedláková, B. (2004a). Disease impact and pathogenicity variation in Czech populations of cucurbit powdery mildews. In: *Progress in Cucurbit Genetics and Breeding Research. Proceedings of Cucurbitaceae 2004,* A. Lebeda & H.S. Paris (Eds.), pp. 281-287, ISBN 80-86636-04-6, 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding, Palacký University in Olomouc, July 12-17, 2004, Palacký University, Olomouc, Czech Republic

Lebeda, A. & Sedláková, B. (2004b). Species spectrum, pathogenic variability and fungicide resistance in cucurbit powdery mildew. *Rostlinolékař,* 15 (6), 15-19, ISSN 1211-3565 (in Czech, with English summary)

Lebeda, A. & Sedláková, B. (2006). Identification and survey of cucurbit powdery mildew races in Czech populations. In: *Proceedings of Cucurbitaceae 2006*, G.J. Holmes (Ed.), pp. 444-452, Asheville, NC, USA, September, 17-21, 2006, Universal Press, Raleigh, NC, USA

Lebeda, A. & Sedláková, B. (2008). Fungicide resistance in populations of cucurbit powdery mildew. *J. Plant Pathol.*, 90, (2, Suplement) S2.142, ISSN 1125-4653

Lebeda, A. & Sedláková, B. (2010). Screening for resistance to cucurbit powdery mildew (*Golovinomyces cichoracearum, Podosphaera xanthii*). In: *Mass Screening Technigues for Selecting Crops Resistant to Diseases*, chapter 19, 295-307, IAEA, ISBN 978-92-0-105110-3, Vienna, Austria

Lebeda, A.; Sedláková, B. & Křístková, E. (2007a). Temporal changes in pathogenicity structure of cucurbit powdery mildews populations. *ISHS 2007, Acta Hort.*, 731, 381-388, ISBN 978-90-66050-79-2, ISSN 0567-7572

Lebeda, A.; Sedláková, B.; Křístková, E. & Vysoudil, M. (2009a). Long-lasting changes in the species spectrum of cucurbit powdery mildew in the Czech Republic – influence of climate changes or random effect? *Plant Protect. Sci.*, 45 (Special Issue), S41-S47, ISSN 1212-2580

Lebeda, A.; Sedláková, B.; Pejchar, M. & Jeřábková, H. (2009b). Variation for fungicide resistance among cucurbit powdery mildew populations in the Czech Republic. *Proceedings of abstracts of The 4th International Cucurbitaceae Symposium*, p. 117, Changsha, Hunan, China, September 20-24, 2009, Hunan Agricultural University and ISHS, Changsha, Hunan, China

Lebeda A.; Sedláková B.; Pejchar M. & Jerábková, H. (2010). Variation for fungicide resistance among cucurbit powdery mildew populations in the Czech Republic. Acta Hort., 871, 465-475, ISSN 0567-7572

Lebeda, A.; Widrlechner, P.M.; Staub, J.; Ezura, H.; Zalapa, J. & Křístková, E. (2007b). Cucurbits (Cucurbitaceae; *Cucumis* spp., *Cucurbita* spp., *Citrullus* spp.). In: *Genetic Resources, Chromosome Engineering, and Crop Improvement*, Volume 3 – *Vegetable Crops*, R.J. Singh (Ed.), chapter 8, 271-376, CRC Press, ISBN 0-8493-9646-8 978-0-8493-9646-5, Boca Raton, FL, USA

López-Ruiz, F.J.; Pérez-García, A.; Fernández-Ortuño, D.; Romero, D.; García, E.; de Vincente, A.; Brown, J.KM. & Toréz, J.A. (2010). Sensitivities to DMI fungicides in populations of *Podosphaera fusca* in south central Spain. *Pest Manag. Sci.,* 66, 801-808, ISSN 1526-498X

Matheron, M.E. & Porchas, M. (2007). Comparative performance and preservation of chemical management tools for powdery mildew on muskmelon. *ISHS, Acta Hort.*, 731, 357-361, ISBN 978-90-66050-79-2, ISSN 0567-7572

McCreight, D.J. (2006). Melon-powdery mildew interactions reveal variation in melon cultigens and *Podosphaera xanthii* races 1 and 2. *J. Amer. Soc. Hortic. Sci*., 131, 59-65, ISSN 0003-1062

McDonald, B.A. & Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Ann. Rev. Phytopathol*., 40, 349-379, ISSN 0066-4286

McGrath, M.T. (2001). Fungicide resistance in cucurbit powdery mildew: experiences and challenges. *Plant Dis*., 85, 236-245, ISSN 0191-2917

McGrath, M.T. (2005). Evaluation of fungicide programs for managing pathogen resistance and powdery mildew of pumkin. *Fungicide and Nematicide Tests,* 60, V049, ISSN 0148-9038

McGrath, M.T. (2006). Occurrence of fungicide resistance in *Podosphaera xanthii* and impact on controlling cucurbit powdery mildew in New York. In: *Proceedings of Cucurbitaceae 2006*, G.J. Holmes (Ed.), pp. 473-482, Asheville, NC, USA, September 17-21, 2006, Universal Press, Raleigh, NC, USA

McGrath, M.T., & Fox, G.M. (2009). Efficacy of fungicides for managing cucurbit powdery mildew and treatment impact on pathogen sensitivity to fungicides, 2008. *Plant Disease Management Reports* 3, V125, ISSN 0148-9038

McGrath, M.T., & Fox, G.M. (2010). Efficacy of fungicides for managing cucurbit powdery mildew and treatment impact on pathogen sensitivity to fungicides, 2009. *Plant Disease Management Reports* 4, V147, ISSN 0148-9038

McGrath, M.T. & Shishkoff, N. (2001). Resistance to triadimefon and benomyl: dynamics and impact on managing cucurbit powdery mildew. *Plant Dis*., 85 (2), 147-154, ISSN 0191-2917

McGrath, M.T. & Shishkoff, N. (2003). First report of the cucurbit powdery mildew fungus (*Podosphaera xanthii*) resistant to strobilurin fungicides in the United States. *Plant Dis*., 87 (8), 1007, ISSN 0191-2917

McGrath, M.T., Staniszewska, H., Shishkoff, N. & Casella, G. (1996). Fungicide sensitivity of *Sphaerotheca fuliginea* populations in the United States. *Plant Dis.,* 80, 697-703, ISSN 0191-2917

McGrath, M.T. & Thomas, E.C. (1996). Powdery mildew. In: *Diseases compendium series,* T.A. Zitter, D.L. Hopkins & C.E. Thomas (Eds.), 28-30, APS Press, ISBN 0890542074 9780890542071, St. Paul, MN, USA

Minář, P. [Ed.] (2006). *Bulletin of The State Phytosanitary Administration. List of the Registered Plant Protection Products 2006.* Special issue, vol. 3, January, 2006, The State Phytosanitary Administration, Prague, Czech Republic. (in Czech, with English summary)

Minář, P. [Ed.] (2007). *Bulletin of The State Phytosanitary Administration. List of the Registered Plant Protection Products and Auxiliary Means 2007*. Special issue, vol. 4, May, 2007, The State Phytosanitary Administration, Prague, Czech Republic. (in Czech, with English summary)

Pérez-García, A.; Romero, D.; Fernández-Ortuño, D.; López-Ruiz, F.; De Vincente, A. & Toréz, J.A. (2009). The powdery mildew fungus *Podosphaera fusca* (synonym *Podosphaera xanthii*), a constant threat to cucurbits. *Mol. Plant Pathol.,* 10 (2), 153-160, ISSN 1464-6722

Sauter, H.; Ammermann, E.; Benoit, R.; Brand, S.; Gold, R.E.; Grammenos, W.; Köhle, H.; Lorenz, G.; Müller, B.; Röhl, F.; Schirmer, U.; Speakman, J.B.; Wenderoth, B. &

Wingert, H. (1995). Mitochondrial respiration as a target for antifungals: Lessons from research on strobilurins. In: *Antifungal Agents: Discovery and Mode of Action*, G.K. Dixon; L.G. Copping & D.W. Hollomon (Eds.), 173-161, BIOS Scientific Publisher, ISBN 1859961452 9781859961452, Oxford, UK

Schepers, M.A.T.H. (1983). Decreased sensitivity of *Sphaerotheca fuliginea* to fungicides which inhibit ergosterol biosynthesis. *Neth. J. Plant Pathol.*, 89, 185-187, ISSN 0028-2944

Schepers M.A.T.H. (1985). Changes during a three-year period in the sensitivity to ergosterol biosynthesis inhibitors of *Sphaerotheca fuliginea* in the Netherlands. *Neth. J. Plant Pathol.*, 91, 105-118, ISSN 0028-2944

Sedláková, B. & Lebeda, A. (2004a). Resistance to fungicides in cucurbit powdery mildew populations in the Czech Republic. *Acta fytotech. et zootech.*, 7, 269-271, ISSN 1335-258X

Sedláková, B. & Lebeda, A. (2004b). Variation in sensitivity to fungicides in Czech populations of cucurbit powdery mildews. In: *Progress in Cucurbit Genetics and Breeding Research. Proceedings of Cucurbitaceae 2004*, A. Lebeda & H.S. Paris (Eds.), pp. 289-294, ISBN 80-86636-04-6, 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding, Palacký University in Olomouc, July 12-17, 2004, Palacký University, Olomouc, Czech Republic

Sedláková, B. & Lebeda, A. (2006). Survey of fungicide resistance in cucurbit powdery mildews populations in the Czech Republic. In: *Proceedings of XVII. Czech and Slovak Plant Protection Conference*, G. Herda; J. Mazáková & M. Zouhar (Eds.), pp. 346-350, ISBN 80-213-1564-4, Prague, September 12-14, 2006, Czech University of Agriculture, Prague, Czech Republic

Sedláková, B. & Lebeda, A. (2008). Fungicide resistance in Czech populations of cucurbit powdery mildews. *Phytoparasitica*, 36 (3), 272-289, ISSN 0334-2123

Sedláková, B. and Lebeda, A. 2010. Temporal population dynamics of cucurbit powdery mildews (Golovinomyces cichoracearum and Podosphaera xanthii) in the Czech Republic. In: Proceedings of Cucurbitaceae 2010, J.A. Thies, S. Kousik & A. Levi (Eds.), pp. 244-247, ISBN 978-0-9830932-0-6, Cucurbitaceae 2010, Charleston, SC, USA, November 14-18, 2010, American Society for Horticultural Science, Alexandria, VA, USA

Sedláková, B.; Lebeda, A.; Pejchar, M. & Jeřábková, H. (2009). Occurrence of the strains with resistance to fungicides in Czech cucurbit powdery mildew populations. In: *XVIII. Czech and Slovak Plant Protection Conference*, *Book of abstracts*, Šafránková, I. & Šefrová, H. (Eds.), p. 19, ISBN 978-80-7375-316-0, Brno, September 2-4, 2009, Mendel University of Agriculture and Forestry, Brno, Czech Republic

Tomlin, S.D.C. [Ed.] (2003). *The Pesticide Manual: A World Compendium*. 13th ed., Alton, BCPC, ISBN 1-901396-13-4, Hampshire, UK.

Towsend, R.G. & Heuberger, W. (1943). Methods for estimating losses caused by diseases in fungicide experiments. *The Plant Dis. Reporter*, 27 (17), 340-343, ISSN 0032-0811

Ypema, L.H.; Ypema, M. & Gubler, D.W. (1997). Sensitivity of *Uncinula necator* to benomyl, triadimefon, myclobutanil and fenarimol in California. *Plant Dis.*, 81, 293-297, ISSN 0191-2917