https://doi.org/10.17221/112/2019-PPS

Influence of oxathiapiprolin on preinfectional and early infection stages of *Plasmopara halstedii*, downy mildew of the sunflower

Otmar Spring, Javier Gómez-Zeledón*

Institute of Botany, Faculty of Natural Science, University of Hohenheim, Stuttgart, Germany

**Corresponding author: O.Spring@uni-hohenheim.de*

Citation: Spring O., Gómez-Zeledón J. (2020): Influence of oxathiapiprolin on preinfectional and early infection stages of *Plasmopara halstedii*, downy mildew of the sunflower. Plant Protec. Sci., 56: 83–91.

Abstract: Oxathiapiprolin is a recently introduced fungicide with particular activity against hemibiotrophic and biotrophic oomycetes. For *Plasmopara halstedii*, the downy mildew of the sunflower, no detailed studies for the activity of the pure compound on the preinfectional and early infection stages in which the fungicide could most effectively interfere with the life cycle of the pathogen have been reported. The fungicide was shown to be active against all stages of the pathogen starting from the release of the zoospores to the development of the mycelia and the formation of the sporangia. Differences in the sensitivity of the different developmental stages are in accordance with the suggested mechanism of the fungicide activity which targets sterol-binding proteins. The experiments showed that, in preventive treatments against very sensitive stages of the pathogen (such as germination of spores), extremely low concentrations of less than 1 ng/mL can completely protect the plants. Coating the seeds with oxathiapiprolin successfully prevented the seedlings from soil-borne infections. This is of particular importance in sunflower cultivation, because wind-borne infections on plants are much rarer and less harmful than soil-borne infections, which usually become systemic and lead to complete yield loss. The curative effects of oxathiapiprolin were shown on the leaf disks as well as on the infected young plants. However, this seems to be less important in sunflower cultivation than, for instance, in viticulture, because spraying sunflowers in the field after the germination of the seeds is uncommon (except in the ornamental cultivation of cut sunflowers).

Keywords: Oomycetes; plant pathogens; sporangial development; zoospore sensitivity; fungicide

Oxathiapiprolin, a compound of the piperidinyl thiazole isoxazoline class (Pasteris et al. 2008) discovered and developed by DuPont, which has recently been shown to be a highly effective fungicide for controlling oomycetes. Its activity, which is directed against the oxysterol binding protein-related protein 1 (ORP1), particularly affects biotrophic pathogens depending on the host derived sterol supply, whereas necrotrophic oomycetes such as *Pythium* are less sensitive (Pasteris et al. 2016). Several studies have revealed a very high effectiveness of oxathiapiprolin against various *Phytophthora* species (Ji et al. 2014; Ji & Csinos 2015; Qu et al. 2016; Belisle et al. 2019), *Pseudoperonospora cubensis* (Cohen 2015; Cohen et al. 2018) and *Plasmopara viticola* Leonian 1922 (Pasteris et al. 2016). For *Plasmopara halstedii* (Farl.) Berl. & De Toni 1888, one of the most severe pathogens in sunflower cultivation, no such reports had been published at the time that this study had started. However, very recently Cohen et al. (2019) reported strong effects of Plenaris™, an oxathiapiprolin containing commercial fungicide, against sunflower downy mildew and they particularly found synergistic effects in combination with other fungicides. Sunflower downy mildew has a nearly worldwide distribution (Spring 2019) and the fast evolution of new aggressive pathotypes makes chemical disease control inevitable (Viranyi & Spring 2011; Spring et al. 2018). Moreover, resistance to mefenoxam (metalaxyl M), the most widely employed systemic fungicide used for seed coating in sunflower cultivation has developed over decades of application in many

https://doi.org/10.17221/112/2019-PPS

countries (Albourie et al. 1998; Gulya 2000; Spring et al. 2006). Hence, it was the goal of the current study to investigate the reaction of *P. halstedii* on the oxathiapiprolin treatment in the different stages of its life cycle. This included the preinfectional stages of the zoospore release from the sporangia, the encystment of the spores and the germ tube formation as well as the mycelium growth in the early infection stages. Moreover, the sporulation after the preventive and curative application of the fungicide was assessed. To compare the reaction range of the pathogen, the study was conducted using three genetically homogeneous single spore strains of different pathotypes and tolerances to metalaxyl.

MATERIAL AND METHODS

Fungicide. Oxathiapiprolin granulate obtained from Syngenta (Switzerland) was refrigerated at 8 °C until use. After grinding the particles to a fine powder, a stock solution of 100 µg/mL was prepared in pure dimethyl-sulfoxide (DMSO). Then a 1/10 dilution with water was possible to be used without affecting the solubility of the compound. Out of this 10 µg/mL stock, lower concentrations down to 1 pg/mL were prepared and kept at 8 °C until use.

Pathogen and plant material. Inoculation was made on the seedlings and leaf disks (Benary, Germany) of *Helianthus annuus* Linnaeus cv. Giganteus using an untreated (control) or a seed-coated material. The seed-coating was performed by R. Zeun (Syngenta Crop Protection AG, Switzerland) with oxathiapiprolin in concentrations of 30 g (recommended full dose) or 10 g a.i. per 100 kg of seeds which is equal to ca. 24 µg a.i./seed. The cultivation of the plants was performed in a heat-sterilised soil under constant conditions (18 °C, 80% rel. humidity, 14 h/d light) in a climate chamber. The leaf disk experiments were conducted with cut outs of 1 cm in diameter taken from the primary leaves of the two-week old plants.

Sporangia of three genetically homogeneous pathogen strains (single spore strains; Spring et al. 1998) of *P. halstedii* were used for the inoculation. The strains differed in pathotype and tolerance against Mefenoxam (metalaxyl M) (Rozynek & Spring 2001; Spring et al. 2006) as follows:

A – strain 1343-A25, pathotype 354, selected from the field isolate (voucher in Herbarium HOH 17099, collected in Germany, 2016); **B** – strain IN-B5z, pathotype 703, selected from the field isolate (HOH 16622, France, 2000); **C** – strain BL-A4z, pathotype 710, metalaxyl tolerant, selected from the field isolate (HOH 16619), France, 1999.

The strains were maintained on the untreated sunflower plants through biweekly rounds of infection. The sporangia were freshly collected from the sporulating plants after overnight induction in a wet chamber at 100% rel. humidity. The number of the sporangia in the inoculum was determined in a Fuchs-Rosenthal counting chamber and diluted accordingly.

Zoospore release from the sporangia. The sporangial suspensions from strains A–C were prepared in distilled water and the concentration was adjusted to 100 000 sporangia/mL as described previously (Gómez-Zeledón & Spring 2018). From this suspension, 90 µl were dispensed on each of the 3 wells of a Multitest 3-well glass slide (Menzel Gläser, Germany). At least 5 slides were prepared for each concentration of the oxathiapiprolin treatment. Oxathiapiprolin (10 µL) was added to each well from the 10-fold stock solutions and mixed by pipetting. Subsequently, the Multitest slides were incubated in a wet chamber (100% relative humidity) for 24 h in the dark at 18 °C. After incubation, photographic documentation was performed from each well using an inverted microscope (Axioplan; Zeiss, Germany) with a 20 × magnification. The pictures were evaluated using the software ImageJ (Version J1) (Schneider & Rasband 2012) and the ratio of the zoospore release was calculated from the number of empty to full sporangia. The ratio of the zoospore release in pure water was taken as the control. For each concentration of oxathiapiprolin, at least 3 independent experiments were performed with the different generations of the sporangia.

Encystment and formation of germ tubes. The sporangial suspensions that were prepared as described above were incubated for 2.5 h in the dark at 18 °C. After confirming that the zoospore release had taken place, 80 µL of the suspension was dispensed on each of the wells of a Multitest 3-well slide. To induce encystment and germ tube formation, 10 µL of $CaCl_2$ (200 mM) was added to each well to achieve a final concentration of 20 mM. This step was important to initiate the synchronised encystment of the zoospores (Gómez-Zeledón & Spring 2018). On each of the wells, 10 µL of oxathiapiprolin (10-fold stocks) was given and mixed by pipetting to obtain the desired concentration. Water was used as the control. Pictures of each well were taken 18 h after contact with the oxathiapiprolin and $CaCl_2$.

https://doi.org/10.17221/112/2019-PPS

The formation of germ tubes was evaluated using the software ImageJ1. The germination ratio was determined by dividing the number of spores bearing a germ tube (the size of the germ tube did not play a role in this evaluation) by the number of encysted spores without a germ tube. For each concentration of oxathiapiprolin, at least 3 experiments were performed with the different generations of the sporangia.

Soilborne infection experiment with the oxathiapiprolin-coated seeds. The sunflower seeds, which had been coated with 30 g of oxathiapiprolin/100 kg seeds (recommended full dose) or 10 g/100 kg, were planted in the soil and cultivated for 3–4 days in the climate chamber. After emergence, each seedling was drenched with 20 000 sporangia of strain IN-B5z. The uncoated seeds were used as a control. For each sample, 12 seedlings were treated and all the experiments were performed independently four times. Two weeks after inoculation, the sporulation was induced by transferring the plants overnight to 100% rel. humidity.

To avoid the possibility of overlooking vanishing or latent infections (the pathogen invades the host, but does not sporulate; Spring 2001), three seedlings of each sample were randomly selected and studied microscopically by means of freehand cross sections through the main root, hypocotyl and epicotyl. The cuttings were investigated using a microscope (Axioplan, Zeiss, Germany) coupled to a digital camera (Leica DCM 2900, Leica, Germany). For better recognition of the hyphae and haustoria, the cross sections were analysed using fluorescence microscopy after aniline blue staining (Filter II, 02/G365, excitation 365 nm) (Zeiss, Germany).

Leaf disk experiments. The leaf disk inoculation with the sporangia of strain IN-B5z (10 000/leaf disk) was performed as previously described (Rozynek & Spring 2001). Leaf disks from the primary leaves of the two-week old plants were inoculated with 10 000 sporangia/disk of strain IN-B5z. The disks were incubated by floating upside down on water. Three and five days after inoculation, oxathiapiprolin was added in different concentrations to the water, from where it could be taken up through translaminar transport. Seven days after inoculation, the sporulation was recorded using a stereo microscope.

Experiments to test the longevity of the protection from the seed-coating were carried out in the same way with the leaf disks from the plants (seed-coated with the full and 1/3 dose of oxathiapiprolin) after the primary leaves had reached a size of ca. 3 cm (approximately three weeks after the seedling emergence). No oxathiapiprolin was added to the water and the sporulation was recorded 7 days after the inoculation.

Post-infectional treatment of the seedlings with oxathiapiprolin. The two-day old seedlings were inoculated with 20 000 sporangia of strain IN-B5z /mL through the whole-seedling-inoculation and then planted in the soil. The application of oxathiapiprolin (5 mL/12 seedlings) was performed 2 days later by spraying the above ground parts (cotyledons and hypocotyl). The plants were subsequently covered by plastic foil for 4 hours. Two weeks later, the sporulation was induced and the rate of infection was recorded.

Statistics. At least three independent experiments were performed for each experiment and strain or concentration. The statistical analyses (ANOVA/Tukey's HSD) were conducted using InfoStat (version 2019) (Di Rienzo et al. 2019).

RESULTS

Inhibition of zoospore release from sporangia. The release of zoospores from the sporangia is the first step that the pathogen required for infection. The natural release rate in the pure water ranged between 48 to 58% in the three tested strains of *P. halstedii* (Table 1). The effective dose of oxathiapiprolin for 75% inhibition of the sporangium dischargement compared to the water control was 50 ng/mL with the metalaxyl tolerant strain BL-A4z. Strain IN-B5z

Table 1. The effect of oxathiapiprolin on the zoospore release of three strains of *Plasmopara halstedii*

Strain	Oxa (ng/mL)	Inhibition ratio (%)
1343-A25	0	0^{b}
	50	91.6^{a}
	100	92.7^{a}
	500	94.3^{a}
BL-A4z	0	0^{d}
	50	75.2^{c}
	100	$84.3^{a,b}$
	500	$83.3^{a,b}$
IN-B5z	0	0^{b}
	50	57.7^{a}
	100	69.0^{a}
	500	71.9^{a}

Oxa – oxathiapiprolin; the different letters indicate a significant difference ($P < 0.05$)

https://doi.org/10.17221/112/2019-PPS

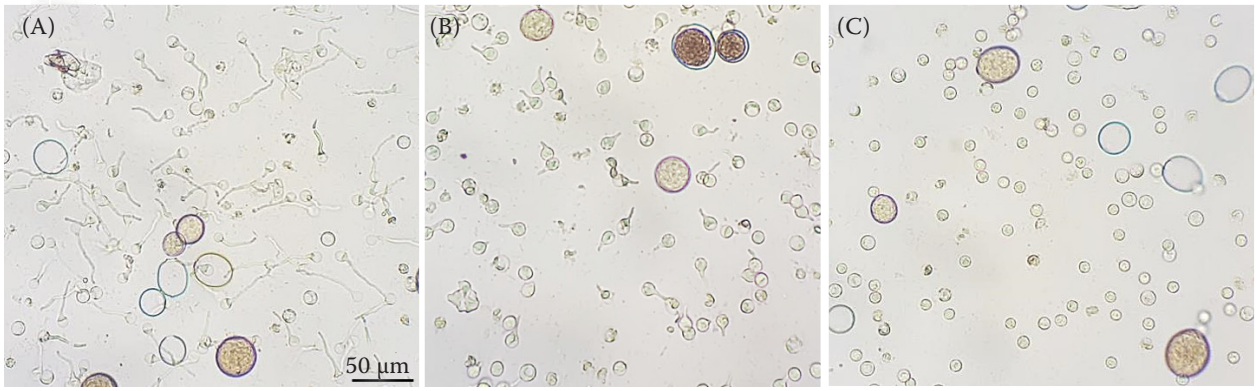

Figure 1. The oxathiapiprolin effect on the germ tube development of strain 1343-A25 18 h after application (A) water control, (B) 5 pg a.i./mL, (C) 50 pg a.i./mL

was slightly less sensitive and showed 58% inhibition at this concentration, whereas, in the highly aggressive strain 1343-A25, the release was reduced by more than 90% and even 5 ng/mL were sufficient for nearly 88% inhibition (data not shown).

Effect of oxathiapiprolin on the germ tube formation. After the sporangia release, the zoospores pass through an active phase of mobility and orientation before starting the encystment and formation of a germ tube which tries to penetrate the host surface. The duration of this process in nature is highly variable and can last for hours. As this is not very suitable for the quantitative analysis of the fungicide effects, we artificially initiated and synchronised the encystment by adding $CaCl_2$ (Gómez-Zeledón & Spring 2018) after the sporangia had been allowed to set the zoospores free in pure water for 2.5 hours.

The addition of oxathiapiprolin to the zoospores inhibited the formation of the germ tubes at extremely low concentrations, but did not inhibit the encystment process (Figure 1C). It was possible to add both substances, $CaCl_2$ and oxathiapiprolin, at the same time and to study the number of germinated spores in relation to the number of encysted spores. The ED_{50} (50% effective dose) was as low as 1.44 pg/mL of oxathiapiprolin for this sensitive phase. A concentration of 5 pg/mL was sufficient to reduce germ tube formation (only short germ tubes developed; Figure 1B) and 50 pg/mL completely suppressed the germination in all three strains (Figure 2). Bl-A4z (tolerant to metalaxyl) was the most sensitive strain tested.

Effect of the seed-coating on the soilborne infection. Soil drenching with sporangia of strain IN-B5z resulted in an average infection rate of 60% (SD 18) on the seedlings grown from the untreated seeds. In contrast, the plants raised from the oxathiapiprolin-coated seeds showed no sporulation or any external symptoms of infection, no matter if they had been treated with the full (30g/100kg seeds) or 1/3 dose of the fungicide. Moreover, the leaf disks taken from the primary leaves (of about 3 cm in size) from the 3-week old plants raised from the oxathiapiprolin-coated seeds could not be infected with the sporangia of strain IN-B5z in the leaf disk experiments (data not shown).

The microscopic investigation of the cross sections through the main root, hypocotyl and epicotyl of the plants from all three samples revealed that only the cuts from the untreated control plants showed infection structures of the pathogen in the inner tissues (Figure 3A–C). The hyphae and haustoria were present in the intercellular space of the parenchyma in the cortex and in the central cylinder (black arrows).

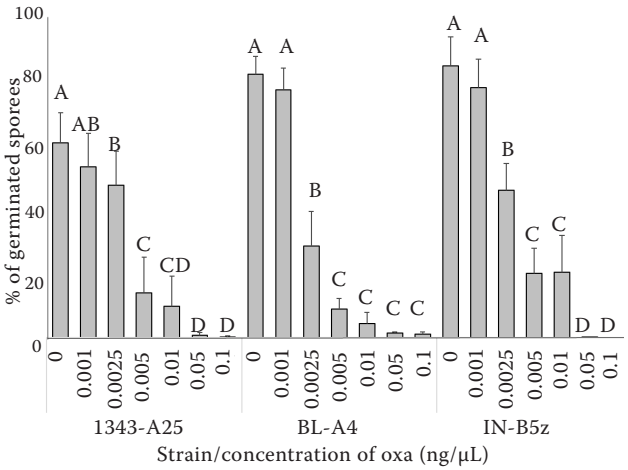

Figure 2. The effect of oxathiapiprolin on the spore germination (germ tube formation) in three strains of *Plasmopara halstedii* 18 h after the fungicide treatment

The bars indicate the SD; the different letters indicate a significant difference ($P < 0.05$); oxa – oxathiapiprolin

https://doi.org/10.17221/112/2019-PPS

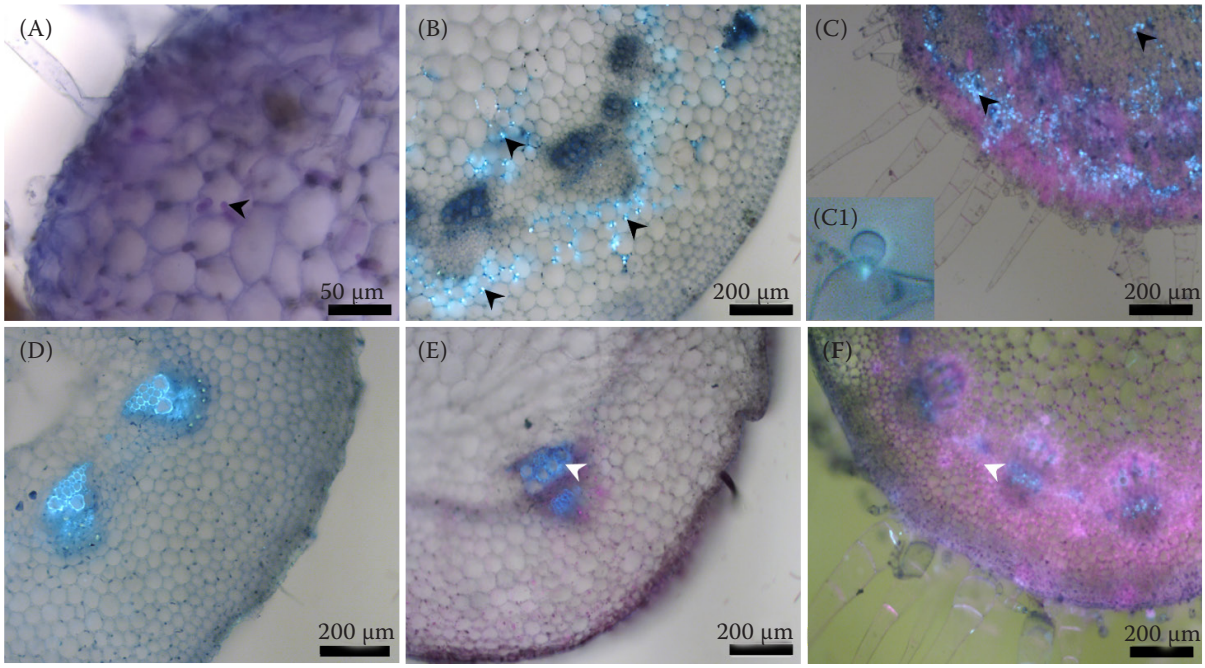

Figure 3. (A) The light (B–F) and fluorescence microscopy pictures of the cross cuttings of: (A) the main root; (B) the hypocotyl; (C) the epicotyl of the non-treated seedlings (control) from the seed-coating experiment two weeks after the inoculation; (D–F) the cuttings of the same parts from a plant treated with the 1/3 dose of oxathiapiprolin in the seed coating; (C1) A detail of the haustorium

The hyphae and haustoria (black arrows) only occurred in the untreated plants. The violet and light blue fluorescence in vessels (white arrows) are due to auto fluorescence

In the plants from the coated seeds of both oxathiapiprolin concentrations, no structures of the pathogen were found (Figure 3 D–F), thus, indicating that the infection had been inhibited before or at a very early stage of penetration.

Curative effects of oxathiapiprolin on the leaf disks. When the leaf disks from the primary leaves of the 2-week old plants were inoculated with sporangia of strain IN-B5z and oxathiapiprolin was added to the water, 3 or 5 days postinoculation, the rate and severity of the infection was reduced considerably.

Application of 12–48 ng oxathiapiprolin on the third day after the inoculation reduced the sporulation by about 65–81% in comparison to the control (Table 2). The application of oxathiapiprolin on the fifth day after the inoculation showed a reduced inhibition of only 25–40%. However, this appears to be due to the much faster development of the infection in the leaf disk test when compared to the whole seedlings. Hence, in some cases, sporulation already appeared on the 5th–6th day after the inoculation. The sporangia, which developed after the oxathiapiprolin treatment partly showed a morphological deformation (Figure 4).

Curative effects of oxathiapiprolin on the whole seedlings. The curative effects of oxathiapiprolin were also investigated on the whole seedlings. The concentrations of 0.012–0.048 µg/mL oxathiapiprolin, which were effective in leaf disk experiments, were insufficient to control the pathogen

Table 2. The infection ratio (sporulating leaf disks) and infection reduction (average) after the oxathiapiprolin treatment three or five days after inoculation with 10 000 sporangia of strain IN-B5z

Oxa (µg/mL)	Application	Infection ratio (%)	Infection reduction (%)
0.012	control	85	–
	3 dpi	30	65
	5 dpi	65	24
0.024	control	80	–
	3 dpi	25	69
	5 dpi	60	25
0.048	control	80	–
	3 dpi	15	81
	5 dpi	50	38

Oxa – oxathiapiprolin

https://doi.org/10.17221/112/2019-PPS

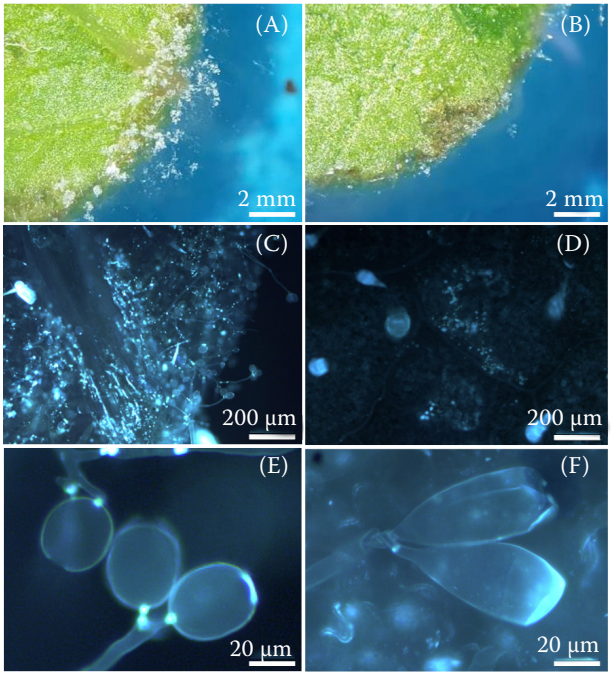

Figure 4. Microscopy of leaf disks. The dense sporulation of controls (A–C) was significantly reduced in samples where oxa (0.012 µg/mL) had been applied three days post inoculation (D–F). Although sporulation occurred on treated disks, deformed sporangia were observed

in the whole seedling test. Significant effects of ca. 30% inhibition were observed at 0.5 µg/mL when the treatment was performed two days post-inoculation. The use of 50µg/mL inhibited sporulation by nearly 80% (Table 3). When the fungicide treatment was performed later (three dpi and five dpi), the effects were gradually reduced (data not shown).

Table 3. The inhibition of the sporulation (the means compared to the controls) in the seedlings which had been inoculated two days before spraying with oxathiapiprolin (oxa) in the concentrations of 0.5–50 µg/mL

Oxa (µg/mL)	Infection reduction (%)
0.5	31[b]
5	33[b]
50	77[a]

Oxa – oxathiapiprolin; the different letters indicate a significant difference with $P < 0.05$

DISCUSSION

Fungicide effects on the preinfectional stages. Before penetrating the host and forming the haustoria for the biotrophic phase of the life cycle, *P. halstedii* has to pass through several host-independent stages (such as the zoospore discharge, attachment to the host surface, encystment of the spore and subsequent formation of a germ tube), which differ in sensitivity to fungicides (Viranyi & Oros 1991). The zoospore release occurs after the enzymatic lysis of a papilla at the frontal part of the sporangium when reorganisation of the cytoplasm for the cytogenesis of the spores has been accomplished. This latter process apparently does not require high metabolic activities or may already be prepared in the late stage of the sporangium formation. This could explain the relatively low sensitivity (ED_{50} ca. 5–50 ng/mL) that we have found against oxathiapiprolin in comparison to the subsequent developmental stages of the pathogen. Obviously, sterol-binding proteins, the target of oxathiapiprolin (Weber-Boyat et al. 2013), do not play a key role during the zoospore release.

The concentrations of oxathiapiprolin necessary for the nearly complete inhibition of the zoospore release in strain A–C are clearly above the range of 1 g/mL reported as ED_{100} by Cohen et al. (2019) for three different strains from Russia, Spain and Switzerland. It is not very likely that the pathotype or origin of the isolates is responsible for this difference. Instead, we assume that the different methodologies in the test could be the reason. Moreover, Cohen et al. (2019) used Plenaris™ 20 SC (a commercial fungicide containing 20% oxathiapiprolin) for their experiments and it appears that formulation of the ingredients could possibly influence the uptake or efficacy of the oxathiapiprolin.

When compared to other oomycetes, the zoospore release of *P. halstedii* was relatively sensitive to oxathiapiprolin. Miao et al. (2015) reported ED_{50} values of 130 ng/mL for *P. cubensis* and up to 4 µg/mL for *P. capsici*, whose release of spores required more than 10 µg/mL for 90% inhibition. It should be noted, however, that the data in the literature vary considerably in this aspect. Cohen (2015) mentioned, that 10 ng/mL inhibited 99% of the zoospore release in *P. cubensis*.

For the inhibition of the germ tube formation, Cohen et al. (2019) reported an ED_{100} of 100 pg/mL with Plenaris, which is slightly higher than our results, but in a similar range. Thus, the spore germination in *P. halstedii* is more sensitive to oxathiapiprolin than in any of the other oomycetes investigated so far. The ED_{50} values of *Phytophthora nicotianae* (Bittner & Mila 2016; Qu et al. 2016)

https://doi.org/10.17221/112/2019-PPS

with 2–16 ng/mL and *P. capsici* (Miao et al. 2015) with 6 ng/mL are ca. 1000-times higher.

Effect of the seed-coating on the soilborne infection. Foliar fungicide sprays against pathogens are rarely applied on the sunflower, in contrast to other crops such as grapevines, tobacco or cucumbers that are endangered by downy mildew. Therefore, our experiment aimed at unravelling the preventive effects of the seed-coating with oxathiapiprolin against a soilborne infection. This type of infection is naturally initiated by overwintering oospores or soil contamination with fresh sporangia and usually leads to a typical systemic infection known to affect the sunflower (Spring 2001).

The results of the experiments with the oxathiapiprolin-coated seeds showed that the fungicide from the external seed hull is readily uptaken and systemically distributed in the plant, thus providing complete protection even at 1/3 of the recommended dose. In addition, the failure to infect the leaf discs of the seedlings raised from the coated seeds showed that this protection reached the host tissues that had never been in direct contact with the applied compound. Cohen et al. (2019) also reported the high effectiveness of the seed coating with Plenaris^{TM} (at 30 µg a.i./seed) as a preventive measure against a soil borne infection, which is comparable to our full dose seed coating (calculated 24 µg a.i./seed for a thousand grain weight of 80 g). They found synergistic effects with Bion (Acibenzolar-S-Methyl) and Apron (mefenoxam; metalaxyl M) that allowed the amount of oxathiapiprolin to be reduced by 50, 66 or even 83%, but the activity of these lower doses with oxathiapiprolin alone was not shown.

Curative effects of oxathiapiprolin. Due to the different testing systems, it is difficult to compare the curative effects of oxathiapiprolin on the sunflower downy mildew with the effects found in other systems. The reported ED_{50} values of 1–10 ng/mL for the inhibition of mycelial growth in the *Phytophthora* species (Bittner & Mila 2016; Qu et al. 2016; Miao et al. 2015) is in the same range as the results recorded here for the sporulation of *P. halstedii* on the infected sunflower leaf disks. However, it should be noted that the mycelia of the hemibiotrophic *Phytophthora* can be cultivated and tested on agar plates. For the growth of the mycelia in the leaves of *P. cubensis*, ED_{50} values of ca. 0.3 ng/mL were reported (Miao et al. 2015). Cohen (2015) found a 50% inhibition of symptoms with 0.1 ng/mL oxathiapiprolin, when the application was performed one day after inoculation with the same pathogen, but required a ten times higher concentration for the same effect when the application was made two days after inoculation, and three days p.i. required 100 ng/mL. This shows a similar reduction in the curative effects as in our experiments with *P. halstedii* (Table 2) when the infection process had progressed before the fungicide treatment.

Curative effects of oxathiapiprolin on the whole seedlings. Oxathiapiprolin, although only in µg/mL concentrations, was able to inhibit the sporulation of *P. halstedii* in the sunflower seedlings which had been inoculated up to two days before the fungicide application (Table 3). In the literature, we found no reports from other plant/pathogen systems which tested the curative oxathiapiprolin effects on the pre-infected seedlings in a comparable manner as we did with sunflower. Cohen (2015) showed that a 3 µg/mL oxathiapiprolin treatment of naturally infected cucumber leaves could strongly inhibit the sporulation. It should be considered, however, that similar to what was found out in the leaf disk experiment, the effectiveness of the fungicide treatment is highly dependent on the time of the application and on the progress of the pathogen development at the point of treatment.

CONCLUSION

The observations presented here on the effects of oxathiapiprolin on the preinfectional developmental stages of *P. halstedii* and the early infection stages of the sunflower show that the pathogen generally reacts in a manner that is similar to other previously tested oomycetes of the genera *Phytophthora*, *Peronospora*, *Pseudoperonospora* and *Plasmopara*, which are all dependent on their hosts in their phytosterol metabolism. The fungicide was shown to be active against all the stages of the sunflower downy mildew pathogen starting from the release of the zoospores to the development of the mycelia and the formation of the sporangia. Only the motility of the zoospores seems to be unaffected (Cohen et al. 2019). The differences in the sensitivity of the different developmental stages are in accordance with the suggested mechanism of the fungicide activity which is targeted against sterol-binding proteins. Thus, the release of the zoospores was much less sensitive than

https://doi.org/10.17221/112/2019-PPS

the phase of the mycelium formation in which sterol metabolism is required for the membrane synthesis. The experiments showed that in the preventive treatments against the very sensitive stages of the pathogen (such as the germination of the spores) extremely low concentrations of less than 1 ng/mL can completely protect the plants. Coating the seeds with oxathiapiprolin successfully prevented the seedlings from soil-borne infections and lasted for several weeks. This is of particular importance in the sunflower cultivation, because wind-borne infections on plants are much rarer and less harmful than soil-borne infections which usually become systemic and can lead to complete yield loss. The curative effects of oxathiapiprolin could be shown on the leaf disks as well as on the infected young plants. However, this seems to be less important in the sunflower cultivation than, for instance, in viticulture, because spraying the sunflower in the field after the germination of the seeds is uncommon (except in the ornamental cultivation of cut sunflowers or in the production of high-quality seeds for propagation) and the curative applications should also generally be avoided to decrease the likelihood of resistance development to this new mode of action.

REFERENCES

Albourie J.-M., Tourvieille J., Tourvieille de Labrouhe D. (1998): Resistance to metalaxyl in isolates of the sunflower pathogen *Plasmopara halstedii*. European Journal of Plant Pathology, 104: 235–242.

Belisle R.J., Hao W., McKee B., Arpaia M.L., Manosalva P., Adaskaveg J.E. (2019): New oomycota fungicides with activity against *Phytophthora cinnamomi* and their potential use for managing avocado root rot in California. Plant Disease, 103: 2024–2032.

Bittner R.J., Mila A.L. (2016): Effects of oxathiapiprolin on *Phytophthora nicotianae*, the causal agent of black shank of tobacco. Crop Protection, 81: 57–64.

Cohen Y. (2015): The novel oomycide oxathiapiprolin inhibits all stages in the asexual life cycle of *Pseudoperonospora cubensis* – causal agent of cucurbit downy mildew. PLoS ONE 10(10): e0140015.doi: 10.1371/journal.pone.0140015

Cohen Y., Rubin A.E., Galperin M. (2018): Oxathiapiprolin-based fungicides provide enhanced control of tomato late blight induced by mefenoxam-insensitive *Phytophthora infestans*. PLoS ONE 13(9): e0204523. doi: 10.1371/journal.pone.0204523

Cohen Y., Rubin A.E., Galperin M. (2019): Novel synergistic fungicidal mixtures of oxathiapiprolin protect sunflower seeds from downy mildew caused by *Plasmopara halstedii*. PLoS ONE 14(9): e0222827. doi: 10.1371/journal.pone.0222827

Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. (2019): InfoStat versión 2019. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. Available at: http://www.infostat.com.ar

Gómez-Zeledón J., Spring O. (2018): Up-regulated RxLR effector genes of *Plasmopara viticola* in synchronized host-free stages and infected leaves of hosts with different susceptibility. Journal of Phytopathology, 166: 709–721.

Gulya T.J. (2000): Metalaxyl resistance in sunflower downy mildew and control through genetics and alternative fungicides. In: Proceedings of the 15th International Sunflower Conference, Jun 12–16, 2000, Toulouse, France: i79–i84.

Ji P., Csinos A.S., Hickman L.L., Hargett U. (2014): Efficacy and application methods of oxathiapiprolin for management of black shank on tobacco. Plant Disease, 98: 1551–1554.

Ji P., Csinos A.S. (2015): Effect of oxathiapiprolin on asexual life stages of *Phytophthora capsici* and disease development on vegetables. Annals of Applied Biology, 166: 229–235.

Miao J., Dong X., Lin D., Wang Q., Liu P., Chen F., Du, Y., Liu, X. (2015): Activity of the novel fungicide oxathiapiprolin against plant-pathogenic oomycetes. Pest Management Science, 72: 1572–1577.

Pasteris R.J., Hanagan M.A., Shapiro R. (2008): World Patent Appl.WO2,008,013,925. Date?

Pasteris R.J., Hanagan M.A., Bisaha J.J., Finkelstein B.L., Hoffman L.E., Gregory V., Andreassi J.L., Sweigard J.A., Klyashchitsky B.A., Henry Y.T., Berger R.A. (2016): Discovery of oxathiapiprolin, a new oomycete fungicide that targets an oxysterol binding protein. Biorganic & Medicinal Chemistry, 24: 354–361.

Qu T., Shao Y., Csinos A.S., Ji P. (2016): Sensitivity of *Phytophthora nicotianae* from tobacco to fluopicolide, mandipropamid, and oxathiapiprolin. Plant Disease, 100: 2119–2125.

Rozynek B., Spring O. (2001): Leaf disc inoculation, a fast and precise test for the screening of metalaxyl tolerance in sunflower downy mildew. Phytopathology, 149: 309–312.

Schneider C.A., Rasband W.S., Eliceiri K.W. (2012): NIH Image to ImageJ: 25 years of image analysis. Nature Methods, 9: 671–675.

Spring O., Rozynek B., Zipper R. (1998): Single spore infections with sunflower downy mildew. Journal of Phytopathology, 146: 577–579.

Spring O. (2001): Nonsystemic infections of sunflower with *Plasmopara halstedii* and their putative role in the dis-

https://doi.org/10.17221/112/2019-PPS

tribution of the pathogen. Journal of Plant Diseases and Protection, 108: 329–336.

Spring O., Zipper R., Heller-Dohmen M. (2006): First report of metalaxyl resistant isolates of *Plasmopara halstedii* on cultivated sunflower in Germany. Journal of Plant Diseases and Protection, 113: 224.

Spring O., Gomez-Zeledon J., Hadziabdic D., Trigiano R.N., Thines M., Lebeda A (2018): Biological characteristics and assessment of virulence diversity in pathosystems of economically important biotrophic oomycetes. Critical Reviews in Plant Sciences, 37: 439–495.

Spring O. (2019): Spreading and global pathogenic diversity of sunflower downy mildew – Review. Plant Protection Science, 55: 149–158.

Virany P., Oros G. (1991): Developmental stage response to fungicides of *Plasmopara halstedii* (sunflower downy mildew). Mycological Research, 95: 199–205.

Virányi F., Spring O. (2011): Advances in sunflower downy mildew research. European Journal of Plant Pathology, 129: 207–220.

Weber-Boyvat M., Zhong W.B., Yan D.G., Olkkonen V.M. (2013): Oxysterol-binding proteins: Functions in cell regulation beyond lipid metabolism. Biochemical Pharmacology, 86: 89–95.

Received: October 23, 2019
Accepted: January 13, 2020
Published online: March 9, 2020