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First report of cyprodinil resistance in Monilinia laxa in Swiss apricots

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Short Report

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Abstract

Brown rot is an important disease of apricot in Switzerland. It is caused by the fungal species *Monilinia laxa*, *M. fructigena*, *M. polystroma*, and *M. fructicola*, with *M. laxa* and *M. fructicola* also causing blossom blight and twig blight. Management of *Monilinia* spp. in commercial apricot orchards relies largely on fungicide applications during flowering and fruit maturation. One fungicide registered for the treatment of *Monilinia* spp. is the anilinopyrimidine cyprodinil. In July 2024, diseased apricots were collected in a plot in Grône, Switzerland. Cyprodinil had been used in the plot, where only limited control was observed. A total of 30 single-spore isolates were obtained in this study, 15 from the apricots in Switzerland and 15 comparison isolates from untreated plum and cherry fruit in Germany. Isolates were identified as *M. laxa*, *M. fructigena*, or *M. polystroma* based on morphological characteristics and sequencing of the rDNA internal transcribed spacer region. Cyprodinil sensitivity was determined *in vitro* using a microtiter assay. Almost all *M. laxa* isolates from the Swiss apricots showed strongly reduced sensitivity to cyprodinil, with EC_{50} values > 3 mg/L. A few *M. fructigena* isolates also showed reduced sensitivity. Detached fruit assays on apricots showed that recommended rates of cyprodinil for apricot controlled sensitive *M. laxa* isolates, whereas adapted isolates were only slightly inhibited. To our knowledge, this is the first report documenting cyprodinil resistance in *M. laxa* from apricot in Switzerland and demonstrating reduced efficacy against resistant isolates in a detached-fruit assay.

Introduction

Brown rot, caused by species of the ascomycete genus *Monilinia*, is an economically important disease of stone fruits and results in substantial yield and postharvest losses worldwide. The major causal agents include *Monilinia laxa*, *M. fructigena*, *M. fructicola*, and *M. polystroma*, although their geographic distribution and relative importance vary by region and host species. In apricot (*Prunus armeniaca* L.), *M. laxa* is particularly important and, together with other *Monilinia* spp., can cause blossom and twig blight as well as brown rot of fruit. Integrated disease management practices are applied to reduce disease incidence. However, management of *Monilinia* diseases in commercial apricot orchards remains largely dependent on fungicide application during flowering and fruit maturation. Several classes of site-specific fungicides are currently authorized for disease management in apricot in Switzerland, including cyprodinil. Cyprodinil is registered in Switzerland as a single, active ingredient for the control of blossom and twig blight in apricot and, in combination with the phenylpyrrole fludioxonil, for the control of blossom and twig blight and brown rot of fruit (BLV 2026).

Fungicide resistance or reduced sensitivity to several classes of site-specific fungicides has been reported in different *Monilinia* species worldwide, including demethylation inhibitors (DMIs), methyl benzimidazole carbamates (MBCs), and succinate dehydrogenase inhibitors (SDHIs) (Chen et al. 2013; Egüen et al. 2016; Martini et al. 2016; Şenyurt et al. 2025; Veloso et al. 2026). Resistance risk depends on the fungicide mode of action, use pattern, and pathogen biology. Resistance risk may also differ among *Monilinia* species because of species-specific biological and genetic characteristics, emphasizing the

importance of considering species identity when interpreting fungicide sensitivity data (Miessner and Stammler 2010; Pagano et al. 2025).

The site-specific fungicide cyprodinil belongs to the anilinopyrimidines (FRAC Group 9), which interfere with methionine biosynthesis and are also associated with inhibition of the secretion of hydrolytic enzymes (FRAC 2026). Anilinopyrimidines are classified by the Fungicide Resistance Action Committee (FRAC) as having a medium risk of resistance development. Reduced sensitivity to cyprodinil has previously been detected in *M. laxa* populations from stone fruit in Hungary, Italy, and Türkiye (Fazekas et al. 2014; Dardani et al. 2023; Şenyurt et al. 2025). However, resistant isolates generally occurred at low frequencies in the populations examined. European sensitivity monitoring conducted by FRAC has similarly shown that most *Monilinia* isolates examined remained sensitive to anilinopyrimidines, although isolates with reduced sensitivity values have occasionally been detected (FRAC 2025). In 2024, a few outliers with higher EC50 values were found in France and Switzerland, but no information on the host plant or *Monilinia* species was provided

In July 2024, diseased apricot fruit were collected from an orchard in Grône, Switzerland, where brown rot had occurred despite previous applications of cyprodinil. Therefore, the objectives of this study were to determine the species composition of *Monilinia* isolates obtained from the affected orchard, to characterize their sensitivity to cyprodinil *in vitro*, and to determine whether reduced *in vitro* sensitivity was associated with reduced efficacy of cyprodinil in detached-apricot fruit assays.

Materials and methods

Fungal Isolates and Culture Conditions

As part of a resistance study, *Monilinia* spores were collected with cotton swabs from symptomatic apricot fruit and fruit mummies in an orchard in Grône, Switzerland, in July 2024. Insufficient disease control had previously been observed in this plot despite cyprodinil applications. One *M*. *laxa* isolate was also collected from an apricot twig canker in Switzerland. Five isolates from cherry fruit collected in Zolling, Germany, and ten isolates from plum fruit collected in Velbert, Germany, were included for comparison in the study (Table 1). These fruit had never been treated with fungicides. Single-spore isolation was performed on 2% malt extract agar. Petri dishes were incubated at 20°C. The isolates obtained were transferred onto V8 agar (2% agar, 15% vegetable juice, and 0,3% $CaCO_3$) for conidial production. Plates were incubated at 21°C and 65% relative humidity for 14 days under a 16-h light/8-h dark photoperiod.

Table 1
Number of *Monilinia* isolates collected from different plant parts, crops, and locations

Crop					Nº of isolates		
	Plant part	Year	Country	Location	*M. laxa*	*M. fructigena*	*M. polystroma*
Apricot	Fruit	2024	Switzerland	Grône	8	5	1
Plum	Fruit	2024	Germany	Velbert	1	9	0
Cherry	Fruit	2024	Germany	Zolling	1	3	1
Apricot	Twig	2024	Switzerland	unknown	1	0	0

In vitro Sensitivity to Cyprodinil

The *in vitro* sensitivity assay in 96-well plates was adapted from the method described by Spiegel & Stammler (2006). Conidial suspensions were prepared in double-concentrated DOB liquid medium (MP Biomedicals™). Conidial density was adjusted to 2×10^4 conidia/mL. The active ingredient cyprodinil was used as the formulated compound Unix 75 WG (design code A8779A; Syngenta Crop Protection AG, Basel, Switzerland). Deionized, autoclaved water was used for dilutions and served as the negative control. A 50 µL fungicide solution and a 50 µL spore suspension were mixed in each well. The following final concentrations were used in the microtiter assays: 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, and 10 mg/L cyprodinil. Growth of the isolates was measured using a photometer at 405 nm after five days of incubation in darkness at 18°C. Growth was also assessed visually. Three replicate wells were used for each isolate and fungicide concentration. EC_{50} values were calculated by probit analysis (Weber, 1980).

Monilinia Species Identification

Species were identified based on morphological characteristics and confirmed by molecular analysis. Genomic DNA was extracted from seven-day-old cultures using the NucleoSpin Plant II Kit (Macherey–Nagel, Duren, Germany). The ribosomal ITS1-5.8S-ITS2 region was amplified using primer pair ITS1 and ITS4 (White et al. 1990; van Leeuwen et al. 2002) and Applied Biosystems™ AmpliTaq Gold™ DNA-Polymerase. Amplified products were visualized on a 1% agarose gel stained with ethidium bromide. PCR products were purified with the NucleoSpin Gel and PCR Clean-up Kit (Macherey–Nagel, Dueren, Germany) and Sanger sequenced at Eurofins Genomics (Ebersberg, Germany) with the same primers used for the PCR amplification.

Detached Fruit Assay

Commercially grown, mature but firm apricot fruit (6 untreated and 6 treated apricots for each isolate in each experiment) were washed in water, surface-disinfected in 1% sodium hypochlorite for 2 min, rinsed with sterile deionized water, and air dried. Sixteen hours before inoculation, the fruit were sprayed to runoff using a hand mister with either deionized water (untreated) or 0,9 g/L of Unix 75 WG (design code

A8779A; Syngenta Crop Protection AG, Basel, Switzerland). The concentration was selected based on the recommended use rate of Vangard 75 WG (design code A8779A) in the US, as cyprodinil is not registered in Switzerland as a single, active ingredient for the control of fruit. For inoculation, apricots were pierced at three equidistant points to a depth of 1 cm with a sterile needle and inoculated with a 30-µl droplet of conidia suspension (10^5 spores/mL). Three cyprodinil-sensitive and three cyprodinil-resistant *M. laxa* isolates were used. One sensitive *M. polystroma* and one *M. fructigena* strain with reduced sensitivity were also included. Untreated apricot fruit inoculated with sterile water were used as negative controls. Fruits were incubated in plastic boxes at 22°C for 2 days at 100% humidity (lids closed), followed by 2 days at 70% humidity (lids open). The presence or absence of *Monilinia* signs and symptoms was visually assessed 4 days after inoculation. Lesion diameter was measured to the nearest millimeter using a standard ruler. The entire experiment was repeated twice. Each experiment had six replicates (apricots). Each replicate consisted of three inoculation sites.

Data analysis

A statistical model was developed to examine the effects of isolate and fungicide treatment (and their combination) on lesion size. The method of least squares was used to estimate model terms associated with the factors. Data of the two independent experiments for each experimental response were combined and evaluated with two-way ANOVA, followed by Tukey's honestly significant difference test. Calculations were performed with Python (3.14) and tests were performed with $\alpha = 0.05$.

Results

Monilinia Species Collected and *in vitro S*ensitivity to Cyprodinil

A total of 30 single-spore isolates were obtained in this study. Morphological identification and ITS sequence analysis identified 11 isolates as *M. laxa*, 17 as *M. fructigena*, and two as *M. polystroma* (Table 1). ITS sequences showed 100% identity to reference sequences of the respective species in GenBank. The number of *Monilinia* species collected from each crop and location is shown in Table 1. Of the 14 isolates collected from the apricot plot in Switzerland, eight were *M. laxa*, five were *M. fructigena*, and one was *M. polystroma*. The cyprodinil sensitivities of the three species from the different crops and locations are shown in Fig. 1. Seven of eight (87,5%) *M. laxa* isolates found in the Swiss apricot plot showed reduced sensitivity to cyprodinil (EC_{50} values > 3 mg/L). The *M. fructigena* isolates showed varying levels of sensitivity. Two German *M. fructigena* isolates had comparatively elevated EC_{50} values of 0,447 and 0,819 mg/L, whereas the remaining German isolates had EC_{50} values < 0,15 mg/L.

Detached Fruit Assay on Apricot

A detached apricot fruit assay was conducted to assess *in vivo* efficacy of formulated cyprodinil against resistant isolates. Three sensitive (EC_{50} value in the *in vitro* sensitivity assay < 0,1 mg/L) and three

resistant *M. laxa* (EC_{50} values > 3 mg/L) were chosen for the assay, and one *M. fructigena* and one *M. polystroma* isolate were also included.

Brown rot symptoms on the untreated fruit that were sprayed with deionized water began to develop at the site of inoculation and quickly enlarged. Sporulation occurred on some but not all lesions of the fruit. This varied by isolate (data not shown). All control fruit remained asymptomatic. Treatment with 0,9 g/L Unix 75 WG (A8779A) suppressed lesion development caused by the three cyprodinil-sensitive *M. laxa* isolates, whereas lesions were only slightly inhibited following inoculation with the three resistant isolates. The *M. fructigena* isolate with reduced *in vitro* sensitivity was more strongly inhibited compared to resistant *M. laxa* isolates. Treatment reduced lesion development caused by the cyprodinil-sensitive *M. polystroma* isolate (Table 2).

Table 2
Effect of Unix 75 WG treatment (active ingredient: cyprodinil) on lesion development caused by *Monilinia* isolates differing in *in vitro* cyprodinil sensitivity on detached apricot fruit.

Isolate		***In vitro*** **sensitivity assay**	***In vivo*** **sensitivity assay / Lesion diameter (cm)**	
	Species	**EC50 values (mg/L)**	**Control**	**Unix 75 WG**
Ms24-Cherry-B	*M. laxa*	0,077	3,0 a	0,0 d
Ms24-Plum-B	*M. laxa*	0,031	3,6 a	0,0 d
Ms24-CVS-02/12	*M. laxa*	0,091	3,5 a	0,0 d
Ms24-CVS-02/2	*M. laxa*	6,136	2,9 a	2,2 ab
Ms24-CVS-02/5	*M. laxa*	7,634	3,6 a	3,0 a
Ms24-CVS-02/10	*M. laxa*	> 10,000	1,9 a	1,2 bc
Ms24-CVS-02/7	*M. polystroma*	0,055	3,5 a	0,4 cd
Ms24-CVS-02/14	*M. fructigena*	4,099	2,7 a	1,1 c

z Numbers in control and Unix 75 WG treatment columns followed by the same letter are not significantly different α = 0.05 as determined by analysis of variance. Letters are comparable within column only. Mean separation was conducted using Tukey's HSD test.

Discussion

In our study, we detected a high frequency (7 of 8 isolates) of cyprodinil-resistant *M. laxa* isolates (EC50 values > 3 mg/L) in a Swiss apricot orchard with reported poor disease control. Reports of reduced sensitivity to anilinopyrimidines in *Monilinia* spp. from stone fruit are relatively uncommon. Reduced sensitivity has previously been detected in *M. laxa* isolates from stone fruit in Hungary, Italy, and Türkiye (Fazekas et al. 2014; Dardani et al. 2023; Şenyurt et al. 2025), as well as in one *M. fructigena* isolate in Italy (Abate et al. 2018), and one *M. fructicola* isolate in Spain (Veloso et al. 2026). European sensitivity monitoring conducted by FRAC has similarly shown that most *Monilinia* isolates examined were sensitive to anilinopyrimidines, although isolates with elevated EC50 values have occasionally been detected (FRAC 2025). In 2024, a few outliers with higher EC50 values were found in France and Switzerland, but no information on the host plant or *Monilinia* species was provided.

The reduced *in vitro* sensitivity observed in our study was associated with reduced efficacy of cyprodinil in the detached-fruit assay, indicating that the observed resistance phenotype may also affect the efficacy of cyprodinil against fruit infections under field conditions. This association was particularly evident for *M. laxa*: while the three sensitive isolates were completely inhibited on cyprodinil-treated fruit, all three resistant isolates developed lesions despite treatment. In apricot, *M. laxa* causes not only brown rot of fruit but also blossom and twig blight. Due to methodological limitations of the experimental design, the detached-fruit assay focused exclusively on fruit and did not assess the efficacy of cyprodinil against resistant isolates on blossoms or twigs. Consequently, the extent to which cyprodinil resistance may affect the control of blossom and twig blight cannot be fully assessed from the present study. Further studies addressing these disease stages, as well as monitoring of cyprodinil-resistant isolates under field conditions, are needed. In Switzerland, cyprodinil is registered as a single-active-ingredient product for the control of blossom and twig blight and is also available in combination with fludioxonil. Future studies should therefore evaluate the efficacy of cyprodinil alone against resistant *M. laxa* isolates on blossoms and twigs, as well as the efficacy of the cyprodinil–fludioxonil combination.

Although cyprodinil resistance in *Monilinia* appears to be currently rare in Switzerland, our findings from the apricot orchard in Grône, together with the outliers reported by FRAC, highlight the importance of systematic resistance monitoring in Swiss apricot and other stone fruit orchards. Such monitoring would help to better characterize the distribution of cyprodinil-resistant isolates and provide a valuable tool for the early detection of emerging resistance.

Our results also indicate species-specific differences in cyprodinil sensitivity. While the *M. laxa* isolates tested showed a clear separation between sensitive and resistant phenotypes, *M. fructigena* isolates exhibited a broader range of sensitivity. FRAC monitoring (2025) similarly indicated species-dependent differences in baseline sensitivity, with *M. fructigena* showing partly higher EC50 values than other *Monilinia* species, consistent with our observations. Species-specific variation in morphology, genetic characteristics, and fungicide sensitivity, as also reported for other fungicides (Miessner and Stammler 2010; Pagano et al. 2025), highlights the importance of differentiating among *Monilinia* species in resistance-monitoring studies. Accurate species identification is therefore important for the

interpretation of resistance-monitoring data and the development of appropriate disease-management strategies.

To our knowledge, this is the first report documenting cyprodinil resistance in *M. laxa* from apricot in Switzerland and demonstrating reduced efficacy against resistant isolates in a detached-fruit assay.

Declarations

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Conflict of interest

There is no conflict of interest in this manuscript, and the manuscript is approved by all authors for publication.

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References

1. Abate D, Pastore C, Gerin D, De Miccolis Angelini RM, Rotolo C, Pollastro S, Faretra F (2018) Characterization of Monilinia spp. populations on stone fruit in South Italy. Plant Dis 102. https://doi.org/10.1094/PDIS-08-17-1314-RE
2. BLV (2026) Pflanzenschutzmittelverzeichnis. Bundesamt für Lebensmittelsicherheit und Veterinärwesen BLV – Pflanzenschutzmittelverzeichnis. https://www.psm.admin.ch/de/produkte. Accessed 29 August 2026
3. Chen F, Liu X, Chen S, Schnabel E, Schnabel G (2013) Characterization of *Monilinia fructicola* strains resistant to both propiconazole and boscalid. Plant Dis 97:645–651.
4. Dardani G, Guarnaccia V, Nari L, Testempasis SI, Karaoglanidis GS, Gullino ML (2023) Identification of pathogens causing brown rot of stone fruit in Cuneo province (Italy) and assessment of

sensitivity to azoxystrobin, cyprodinil, fenhexamid, fludioxonil, and tebuconazole. Phytopathol Mediterr 62:455–465. https://doi.org/10.36253/phyto-14399

5. Egüen B, Melgarejo P, De Cal A (2016) The effect of fungicide resistance on the structure of *Monilinia laxa* populations in Spanish peach and nectarine orchards. Eur J Plant Pathol 145:815–827. https://doi.org/10.1007/s10658-016-0871-4
6. Fazekas M, Madar A, Sipiczki M, Miklós I, Holb IJ (2014) Genetic diversity in *Monilinia laxa* populations in stone fruit species in Hungary. World J Microbiol Biotechnol 30:1879-92. doi: 10.1007/s11274-014-1613-4
7. FRAC (2025) Minutes of the 2025 AP Meeting recommendations for 2025. CropLife International A.I.S.B.L. https://www.frac.info/media/krpors4m/minutes-of-the-2025-ap-meeting-recommendations-for-2025.pdf. Accessed 10 September 2026
8. FRAC (2026) AP fungicides: Introduction and general information. CropLife International A.I.S.B.L. https://www.frac.info/frac-teams/working-groups/ap-fungicides/. Accessed 29 Aug 2026
9. Martini C, Guidarelli M, Di Francesco A, Ceredi G, Mari M (2016) Characterization of thiophanate-methyl resistance in Italian *Monilinia fructicola* isolates. J Plant Pathol 98:453–462.
10. Pagano MM, Oettl S, Deltedesco E, Pii Y, Spitaler U (2025) Variation in Growth, Morphology, and Fungicide Sensitivity Among *Monilinia* Species from South Tyrol's Alpine Orchards. J Fungi 11:690. https://doi.org/10.3390/jof11100690
11. Şenyurt H, Demir D, Eken C (2025) Fungicide resistance and pathogenicity of *Monilinia laxa* isolates from *Prunus* species in Türkiye. Appl Fruit Sci 67:145. https://doi.org/10.1007/s10341-025-01356-0
12. Spiegel J, Stammler G (2006) Baseline sensitivity of *Monilinia laxa* and *M. fructigena* to pyraclostrobin and boscalid. J Plant Dis Prot 113:199–206. https://doi.org/10.1007/BF03356181
13. van Leeuwen GCM, Yen RPB, Holb IJ, Jeger MJ (2002) Distinction of the Asiatic brown rot fungus *Monilia polystroma* sp. nov. from *M. fructigena*. Mycol Res 106:444–451. https://doi.org/10.1017/S0953756202005695
14. Veloso J, Villarino M, Guerra L, et al (2026) Fungicide resistance profile of *Monilinia fructicola* and *Monilinia laxa* isolates from commercial peach and nectarine orchards in the Ebro Valley, Spain. Plant Pathol 75:e70213. https://doi.org/10.1111/ppa.70213
15. Weber E (1980) Grundriss der biologischen Statistik, 8th edn. Gustav Fischer Verlag, Stuttgart, New York
16. White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, New York, pp 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Figures

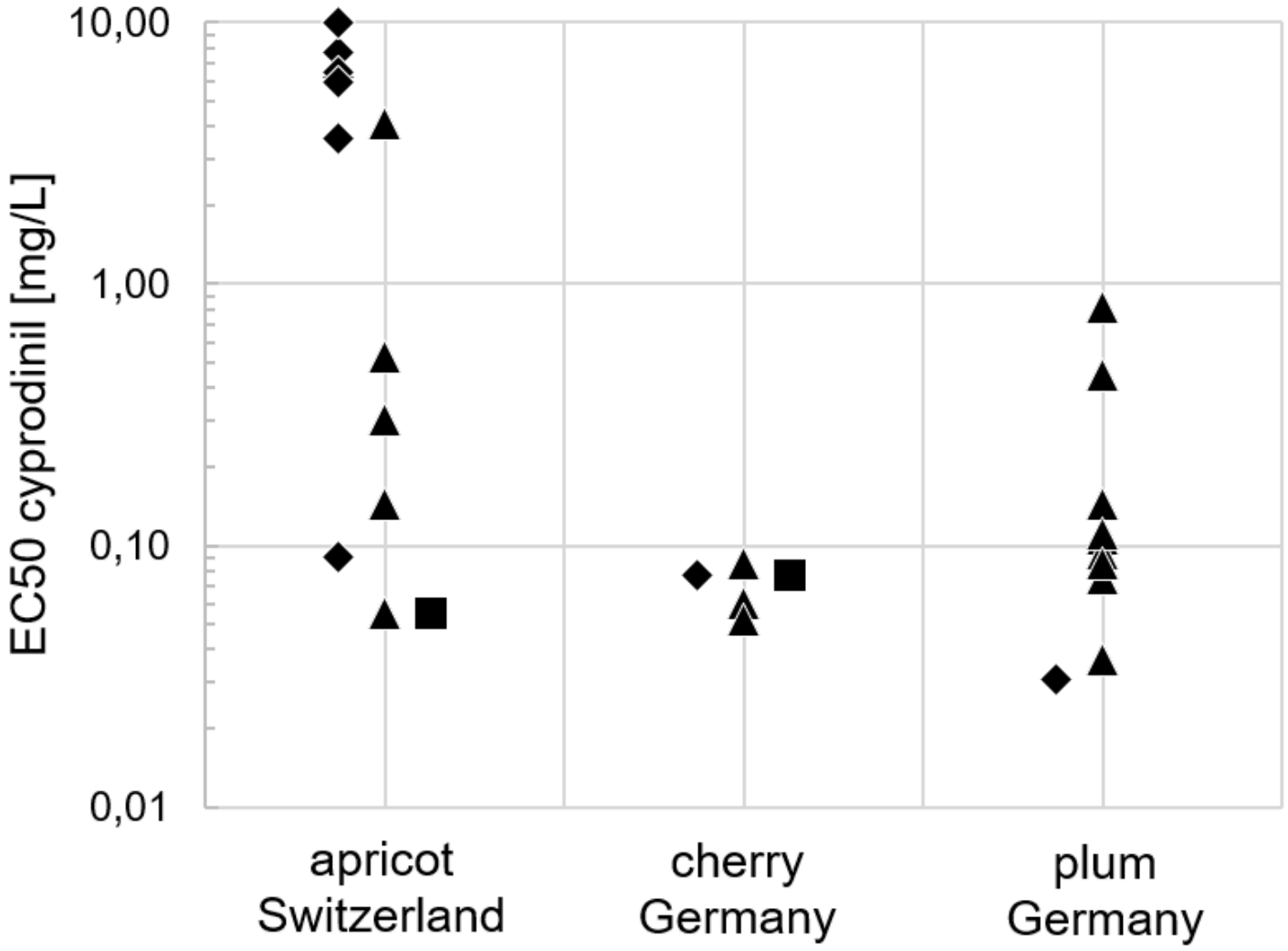


Figure 1

Cyprodinil sensitivity of *M. laxa*, *M. fructigena*, and *M. polystroma* isolates collected from apricot in Switzerland and untreated cherry and plum in Germany. Sensitivity was determined in a 96-well microtiter assay and expressed as values for the concentration of cyprodinil at which fungal development is inhibited by 50% (EC_{50} values in mg/L). Diamonds (♦) = *M. laxa*, triangles (▲) = *M. fructigena*, and squares (■) = *M. polystroma* isolates. The y-axis is shown on a logarithmic scale. Values exceeding the highest concentration tested (>10 mg/L) were plotted at 10 mg/L