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T.C. : REINHARDSBRUNN SYMPOSIUM COLLECTION

Monitoring and tracking changes in sensitivity to zoxamide fungicide in *Plasmopara viticola* in Italy

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Abstract

Plasmopara viticola is controlled by fungicides with different modes of action, which include zoxamide. Zoxamide was developed in 1998 by Dow AgroSciences LIC (Indianapolis, IN) and has been commercialized since 2001 by Rohm and Haas Company. This fungicide is highly effective against oomycetes, used for foliar application in vine crops, potato, and other vegetables to control oomycete-induced diseases. Zoxamide acts by causing mitotic arrest by binding to β-tubulin, inhibiting tubulin polymerization and cell division of the pathogen. In the past three decades, the management of fungicide resistance has emerged as a significant concern for both growers and regulatory authorities; this is primarily due to the substantial increase in resistance, in terms of occurrence and spread, towards most fungicide groups. The monitoring of the effectiveness of fungicides is the only way to accurately identify as soon as possible the onset of resistance. In this study, we were interested in tracking the changes in the sensitivity of zoxamide to *P. viticola* populations collected during a 6-year monitoring (2017–2022) in two Italian locations: the autonomous province of Trento and the autonomous region of Friuli-Venezia Giulia. Bioassays on leaf discs were carried out, and EC_{50} and MIC values were elaborated. From our results, zoxamide showed for a long period of time high sensitivity, but over the last years we observed a change, as in 2022 most of the samples tested in these two regions showed MIC > 100 mg L^{-1}.

Keywords Zoxamide · *Plasmopara viticola* · Monitoring · Italy

Introduction

Downy mildew caused by *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni is a disease affecting grapes worldwide, exerting a strong impact on production. In Italy, there is a wide range of products that can be used to control *P. viticola.* At present, this includes 23 approved active ingredients, covering 14 different modes of action.

Zoxamide RH-7281(3,5-dichloro-N-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide) is one of the fungicides utilized in the control of *P. viticola*; its activity is due by arresting nuclear division and destroying the microtubule cytoskeleton of oomycete pathogens (Young and Slawecki 2001). To date, sixteen zoxamide-based products are approved in Italy (Table 1). Despite considerable progress in understanding the biology and aetiology of plant pathogens, plant disease management has not significantly changed for the last 50 years, relying primarily on fungicides (Beckerman et al. 2023). The utilization of single-site fungicides has led to the emergence of a new issue for the control of downy mildew: fungicide resistance that can be defined as the acquired and heritable reduction in the sensitivity of the pathogen to a specific molecules.

In order to avoid the risk of fungicides resistance, a proactive approach using different disease control tactics is the most effective. The degree of success of anti-resistance strategies is strongly influenced by the timing of the start of the monitoring activity (Brent 2012); therefore, it is crucial to plan multiyear monitoring to assess fungicide sensitivity. The objective of this study was to track the zoxamide sensitivity changes towards *P. viticola* populations in two Italian regions where the fungicide was employed for several years, in order to best manage the potential onset of resistance.

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Table 1 Authorized zoxamide-based products

Registration number	Product	Company	Registration date	Authorization expiration	Active substances
12,202	Zemix R WG	Gowan Italia	18/10/07	30/06/34	Copper oxychloride 25% Zoxamide 5.,88%
13,134	Amaline	Nufarm Italia	05/10/11	30/06/34	Tribasic copper sulphate 19,1% Zoxamide 2,85%
14,062	Zoxium 240 SC	Gowan Italia	10/05/12	30/06/34	Zoxamide 21.8%
14,062	Agron R WG	Gowan Italia	27/08/08	30/06/34	Copper oxychloride 25% Zoxamide 5.88%
15,361	Lieto	SIPCAM OXON	28/01/14	30/06/34	Cymoxanil 33% Zoxamide 33%
15,419	Electis R flow	Gowan Italia	14/06/12	30/06/34	Tribasic copper sulphate 19,1% Zoxamide 2,85%
15,744	Reboot	Gowan Italia	28/01/14	30/06/34	Cymoxanil 33% Zoxamide 33%
15,907	Presidium one	Gowan Italia	17/02/16	30/06/34	Dimethomorph 16.36% Zoxamide 16.35%
16,104	Electis Trio WDG	Gowan Italia	19/05/15	30/06/34	Cymoxanil 2.8% Zoxamide 3.6% Fosetyl-aluminium 35%
16,288	Ampexio	Syngenta Italia	08/11/16	30/06/34	Mandipropamid 25% Zoxamide 24%
16,335	Lieto SC	Sipcam Italia	18/04/17	30/06/34	Zoxamide 3.2% Cymoxanil 2.5%
16,889	Electis ZR WDG	Gowan Italia	25/01/17	30/06/34	Zoxamide 5.88% Copper oxychloride 25%
17,075	Movida	Gowan Italia	26/10/17	30/06/34	Zoxamide 21.8%
17,248	Zorvec vinabel	Corteva Agriscience Italia	10/02/20	03/03/28	Zoxamide 30% Oxathiapiprolin 4%
17,643	Astro R Flow	Gowan Italia	17/09/20	30/06/34	Zoxamide 2.85% Tribasic copper sulphate 19.1%
17,728	Cymzeta	SIPCAM OXON	04/12/20	30/06/34	Cymoxanil 33% Zoxamide 33%

Source: Italian health ministry (access October 2023)

Material and methods

Sample collection

The samples collected for the monitoring studies were originating from three provinces of the region Friuli-Venezia Giulia (FVG), Pordenone (PN) Udine (UD), and Gorizia (GZ), located in the lowland area (Fig. 1), and from the Province of Trento (Fig. 2).

104 *P. viticola* populations were generated out of leaf samples showing typical symptoms of downy mildew being collected from the same number of commercial vineyards in FVG and Trento and then studied in leaf disc tests to determine EC_{50} and MIC values. Samples were dispatched to the laboratory from 2017 to 2022. Each sample constitutes around 25–40 leaves and considered to be representative for each vineyard.

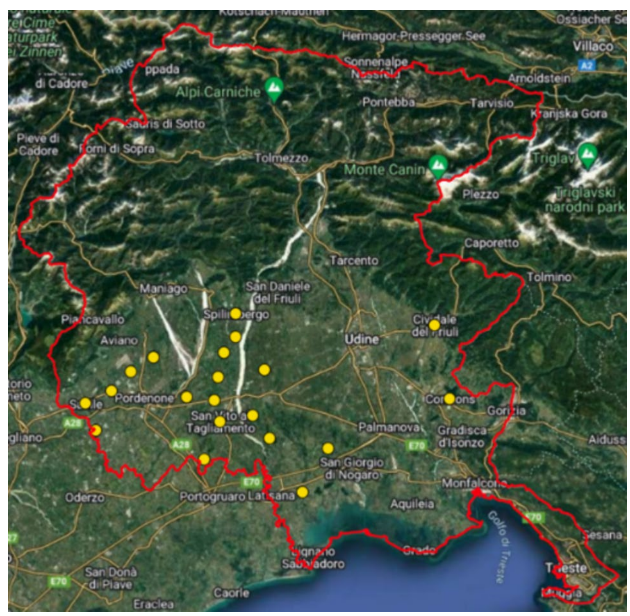


Fig. 1 Map of Friuli-Venezia Giulia, yellow dots indicate the sampling location (imagine elaborated with QGIS 3.32)

Biological assays

Bioassays were carried out utilizing grape leaf discs (cv. Chardonnay), being treated with three concentrations (1, 10, and 100 mg L^{-1}) of zoxamide (Zoxium 240 SC) 24 h before inoculation. For each concentration tested, an untreated control was included. Fifteen leaf discs were soaked in fungicide suspensions of each concentration for 45 min. The inoculation was conducted by spraying a sporangial suspension (5×10^4 spores mL^{-1}) onto the adaxial surface of leaf discs, which were then incubated at 23 °C and a 12-h photoperiod. The sporulation was assessed 8–10 days after the inoculation by evaluating the percentage of sporulated leaf area, the EC_{50} values (mg L^{-1}) were calculated by probit analysis, and minimum inhibitory concentration (MIC) was derived (Figs. 3 and 4).

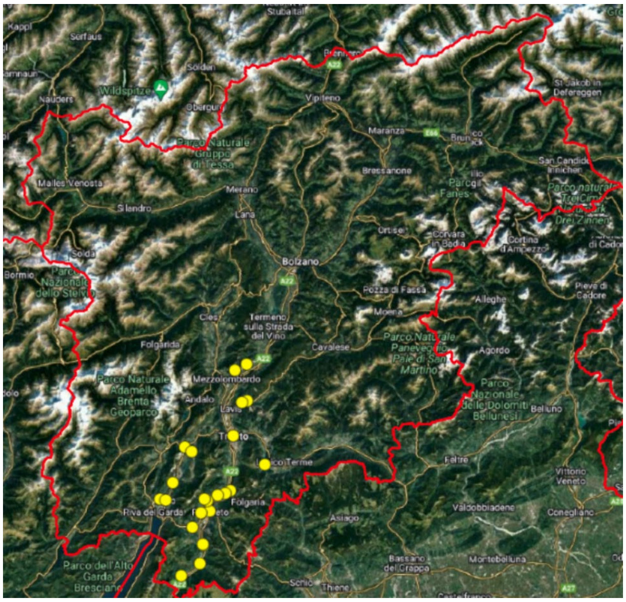


Fig. 2 Map of Trentino-Alto Adige, yellow dots indicate the sampling location (QGIS 3.32)

Results

Table 2 shows the EC_{50} and MIC values of the *P. viticola* populations coming from FVG and Trento. The zoxamide baseline was determined after 6 years of monitoring and shows a range of EC_{50} values from 0.00004 to 0.47.

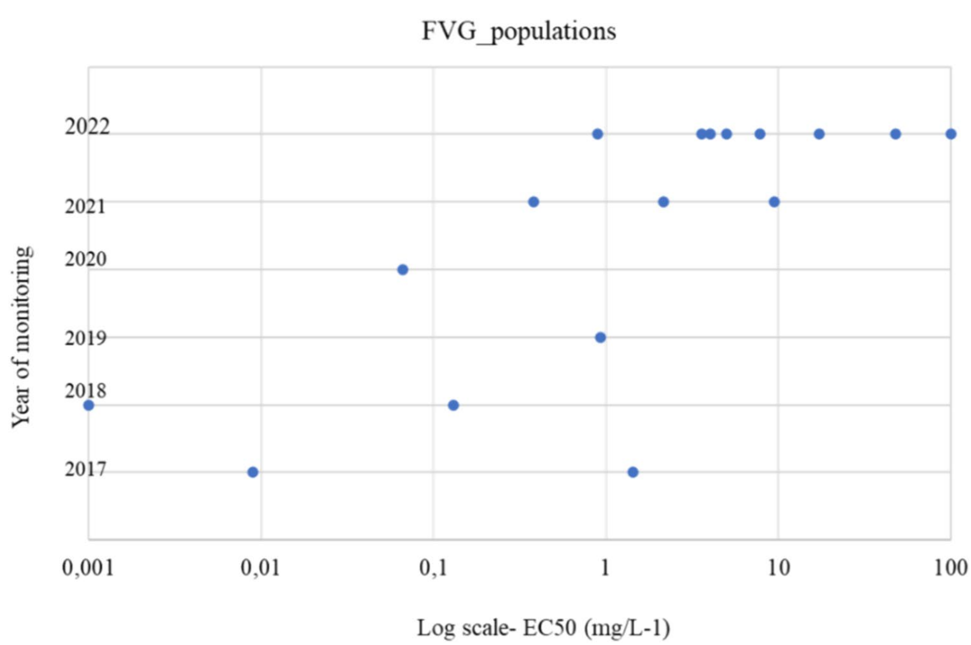
Friuli-venezia giulia region

In the season 2017, only two populations were tested for zoxamide (448, 455), which showed an EC_{50} value and MIC of 0.009/ < 10 mg L^{-1} and 1.43/100 mg L^{-1}, respectively. In 2018, again only two populations were tested (538, 567), showing EC_{50} values and MICs of 0.13/ < 10 mg L^{-1} and 0.001/ < 10 mg L^{-1}, respectively. Population 610 tested in 2019 showed an EC_{50} and MIC of 0.93 / < 10 mg L^{-1}. In 2020, three populations tested, 654, 655, and 679, showed EC_{50} and MIC values of 0.066/ < 100 mg L^{-1}, 0.001/ < 1 mg L^{-1}, and 0.001/ < 1 mg L^{-1}, respectively. Three populations studied in 2021 showed EC_{50} values ranging from 0.38 to 9.44 mg/l, while MIC values were < 100 to > 100 mg L^{-1}. In 2022, eight populations investigated showed EC_{50} values ranging from 0.89 to 100 mg/l, while MIC values were for all samples > 100 mg/l.

Trento province

Populations originating from Trento in 2017 showed an EC_{50} range between 0.001 and 0.15 mg L^{-1}; MIC values were between < 1 to < 100 mg L^{-1}.

Fig. 3 Distribution of the sensitivity ($\log_{10} EC_{50}$) of 19 *P. viticola* populations to zoxamide, originating from Friuli-Venezia Giulia region



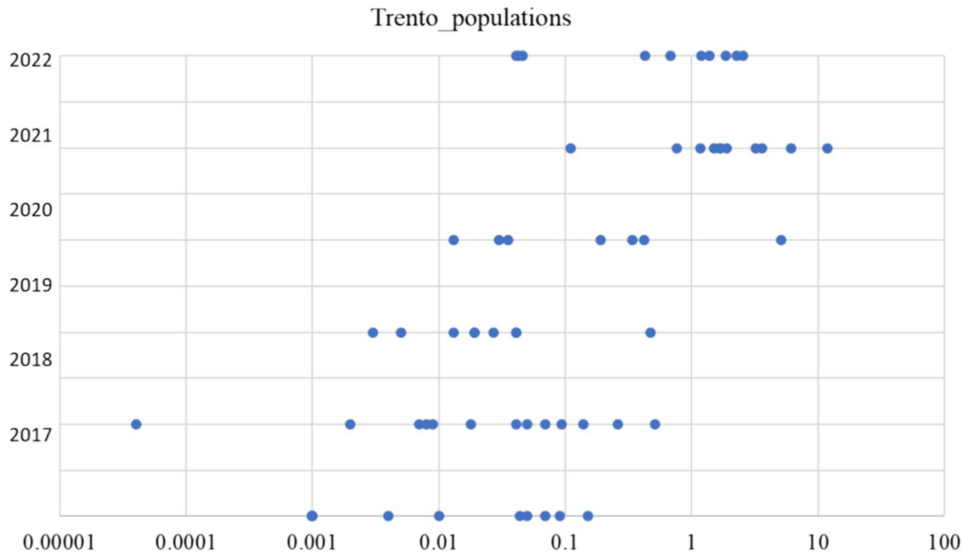


Fig. 4 Distribution of the sensitivity ($\log_{10}$ EC_{50}) of 85 *P. viticola* populations to zoxamide, originating from Trento

In 2018, EC_{50} values did not exceed 0.51 mg L^{-1}, and only two populations (495, 547) showed MIC values >100 mg L^{-1}. In 2019, EC_{50} values did not exceed 0.47 mg L^{-1}, and MIC values were between <1 to <100 mg L^{-1}. In the year 2020, populations showed EC_{50} values being always below 0.42 mg L^{-1}, while the corresponding MIC values were ranging between <10 and <100. Only population (650) showed an EC_{50} of 5.08 mg L^{-1} and a MIC<100. In 2021, EC_{50} values ranged between 0.11 and 11.8 mg L^{-1}; in particular, sample (685) showed the highest EC_{50} with a MIC>100; furthermore, seven samples out of eleven showed a MIC>100 mg L^{-1}. In 2022, EC_{50} values ranged between 0.041 and 2.56 mg L^{-1}. Regarding the MIC values, nine populations out of eleven exhibited values above 100 mg L^{-1}, one population (742) was below <100 mg L^{-1}, and population 748 showed a MIC of <1 mg L^{-1}.

Discussion

Zoxamide is a fungicide considered by the FRAC with a low to moderate risk of resistance. Regular sensitivity monitoring of fungicides is the best way to accurately identify as soon as possible the onset of resistance. In this study, we present a six-year monitoring (2017–2022) conducted in two regions of Northern Italy, Trentino-Alto Adige, in particular the province of Trento, and Friuli-Venezia Giulia, specifically the provinces of Gorizia (GZ), Pordenone (PN), and Udine (UD), where zoxamide was used to control downy mildew.

This study revealed that for the Friuli-Venezia Giulia region, from 2017 to 2020, two samples out of eleven exhibited an EC_{50} less than 0.5 mg L^{-1}. However, starting in 2021, EC_{50} values began to exceed 0.5 mg L^{-1}, and in 2022, EC_{50} values were generally higher than those in previous years, with MICs>100 mg L^{-1} for all samples tested.

Data generated with samples coming from Trento showed high sensitivity towards zoxamide from 2017 to 2020, while in 2021 and 2022 15 out of 22 samples showed EC_{50}>0.5 mg L^{-1} and 16 out of 22 samples showed a MIC>100 mg L^{-1}.

Based on these sensitivity monitoring programs, it is possible to state that *P. viticola* populations coming from Friuli-Venezia Giulia and Trento territories were sensitive to zoxamide over the years; starting from the 2021 and 2022 seasons, populations exhibited changes of sensitivity to zoxamide.

As we know the emergence of populations with reduced sensitivity to one fungicide could be caused by the repeated use of the molecule with a single-site mode of action, in order to prevent the onset of these issues, careful monitoring and technical assistance are necessary. Performing bioassays is well known to be time-consuming, but to our strongest belief and long-term experience, this method is necessary to closely understand the in vivo behaviour of the fungicide. Certainly, molecular tools to potentially detect mutations conferring fungicide resistance are very insightful in explaining sensitivity changes and should be added to in vivo methods when appropriate, providing an even more comprehensive overview of the phenomenon of fungicide resistance.

Table 2 EC_{50} (mg L^{-1}) values and MIC of zoxamide for *P. viticola* populations (FVG and TN)

Population ID (FVG)	EC_{50} Zoxamide (mg L^{-1})	MIC Zoxamide (mg L^{-1})	Population ID (FVG)	EC_{50} Zoxamide (mg L^{-1})	MIC Zoxamide (mg L^{-1})	Population ID (FVG)	EC_{50} Zoxamide (mg L^{-1})	MIC Zoxamide (mg L^{-1})	Population ID (FVG)	EC_{50} Zoxamide (mg L^{-1})	MIC Zoxamide (mg L^{-1})
448—'17	0.009	< 10	440—'17	0.09	< 100	598—'19	*	< 1	716—'21	1.51	> 100
455—'17	1.43	< 100	441—'17	0.001	< 100	599—'19	*	< 1	717—'21	3.23	> 100
538—'18	0.13	< 10	442—'17	0.004	< 100	600—'19	*	< 1	719—'21	1.68	> 100
567—'18	0.001	< 10	443—'17	0.07	< 100	601—'19	0.041	< 100	721—'21	3.6	< 100
610—'19	0.93	< 10	410A—'17	0.001	< 10	602—'19	*	< 1	723—'21	0.77	< 100
654—'20	0.066	< 100	411A—'17	0.044	< 100	617—'19	*	< 1	728—'21	1.67	< 100
655—'20	*	< 1	413A—'17	0.001	< 100	618—'19	0.47	< 100	738 -'22	2.56	> 100
679—'20	*	< 1	494—'18	0.008	> 100	619—'19	0.027	< 10	739 -'22	1.38	> 100
700—'21	2.15	> 100	495—'18	0.26	> 100	620—'19	0.041	< 100	740 -'22	0.43	> 100
709—'21	0.38	< 100	496—'18	0.002	< 100	621—'19	0.003	< 10	741 -'22	2.28	> 100
757—'21	9.44	> 100	497—'18	*	< 1	622—'19	*	< 1	742 -'22	0.041	< 100
655 -'22	100	> 100	498—'18	*	< 1	623—'19	*	< 1	743 -'22	0.68	> 100
700 -'22	0.89	> 100	499—'18	0.07	< 10	624—'19	0.019	< 10	744 -'22	0.046	> 100
757 -'22	3.61	> 100	500—'18	0.018	< 10	625—'19	0.013	< 10	746 -'22	0.043	> 100
765 -'22	4.03	> 100	501—'18	0.041	< 10	626—'19	0.019	< 10	748 -'22	*	< 1
771 -'22	48.14	> 100	503—'18	0.007	< 10	648—'20	0.013	< 10	750 -'22	1.86	> 100
772 -'22	5.04	> 100	547—'18	0.009	> 100	650—'20	5.08	< 100	752 -'22	1.2	> 100
775 -'22	7.78	> 100	548—'18	0.14	< 100	651—'20	0.19	< 100			
777 -'22	17.19	> 100	549—'18	0.05	< 100	652—'20	0.34	< 10			
410—'17	0.001	< 100	550—'18	0.094	< 100	667—'20	0.03	< 10			
411—'17	0.001	< 100	551—'18	0.00004	< 10	668—'20	0.035	< 10			
412—'17	0.001	< 100	552—'18	*	< 1	669—'20	*	< 1			
413—'17	0.001	< 100	553—'18	*	< 1	671—'20	*	< 1			
415—'17	0.001	< 10	554—'18	*	< 1	673—'20	0.42	< 10			
416—'17	0.001	< 10	555—'18	*	< 1	685—'21	11.8	> 100			
417—'17	0.15	< 100	556—'18	*	< 1	687—'21	0.11	> 100			
418—'17	0.01	< 10	557—'18	0.51	< 100	688—'21	1.18	< 100			
419—'17	0.05	< 100	558—'18	*	< 1	713—'21	1.9	> 100			
422—'17	0.001	< 100	597—'19	0.005	< 10	714—'21	6.16	> 100			

*star in the table means that EC_{50} values were not calculated because the *P. viticola* sporulation was present only in the untreated control

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