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A picolinamide fungicide for controlling Cercospora-leaf spot (CLS) of sugar beet

Akos F. Biró[1*], *Andy J* Leader[2], *Andrea* Hufnagl[2], *Gábor* Kukorelli[1], and *Zoltán* Molnár[1]

[1] Széchenyi István University of Győr, Albert Kazmer Faculty of Agricultural and Food Sciences of Mosonmagyaróvár, Department of Plant Science, Hungary
[2] Corteva Agriscience LTD, Global Disease management, USA

Abstract. Cercospora-leaf spot (CLS) of sugar beet, caused by Cercospora beticola Sacc., is a major foliar disease of sugar beet in all sugar beet growing areas, worldwide, causing up to 50% yield loss. The disease is now dominant in almost all sugar beet growing areas of Europe, including Hungary. The use of fungicides has been being an integral part of the control of CLS of sugar beet. In recent decades, resistance of C. beticola to fungicides belonging to different groups of active substances has been described in many countries worldwide, including Hungary. The picolinamides are a new distinct group of fungal respiration inhibitors (QiI – FRAC Group 21) promise to be a good alternative in the management of fungicide resistance in crops. The florylpicoxamid fungicide were tested and evaluated over two seasons, in vegetation period of 2020 and 2021 for controlling CLS of sugar beet in Hungary. This fungicide was applied as straight formulated product at a range of dose rates, and they showed very effective control of CLS compared to the untreated control check plots and the reference fungicide products difenoconazole and epoxiconazole. All tested dose rates of florylpicoxamid provided effective control of against CLS of sugar beet. The area under the disease progress curve values (AUDPC) was significantly correlated with yield decrease, but AUPDC did not correlated with sugar content of the roots. Additionally, the results showed in two investigated years, the efficacy of florylpicoxamid for the control on CLS of sugar beet crop.

1 Introduction

1.1 The importance of the disease

This study's targeted disease is Cercospora-leaf spot (CLS) caused by *Cercospora beticola* Sacc. [1] which plays the most prominent role in current sugar beet producing areas. The disease was first described by Saccardo in Italy in 1876 on Swiss chard (*Beta vulgaris subsp. cicla*), but it has now been identified worldwide wherever sugar beet is grown [2]. The Cercospora-leaf spot (CLS) of sugar beet causes severe damage in warm, humid growing areas [3] mostly. The success of the sugar beet cultivation depends a lot on the efficiency of

* Corresponding author, biroakos1@yahoo.com

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the disease management against the Cercospora leaf spot. In Hungary, the CLS epidemics usually take place end of June and finish in the middle of September, when cooler temperatures stop the disease progression [4].

1.2 Fungicides used in the control of CLS until the presents

The use of fungicides was and still is an integral part of the control against CLS (*Cercospora beticola*, Sacc.), primarily due to the lack of effectiveness of non-chemical alternatives. Two main types of chemicals are available to treat the disease: protective fungicides with broad-spectrum activity and systemic fungicides, which target the fungus at a specific location. Among the former, the ethylene-bisdithiocarbamate (EBDC, Fungicide Resistance Action Committee = FRAC Group M03) fungicide, a copper-based fungicide (FRAC Group M01), is the most used. The three main groups of systemic fungicides used globally are benzimidazoles (MBC = Methyl Benzimidazole Carbamates; FRAC group 1), triazoles (DMI = DeMethylation Inhibitors; FRAC group 3) and strobilurins (QoIs = Quinone outside Inhibitors; FRAC 11. group) [5]. In recent decades, the effectiveness of these groups of active ingredients has been continuously impaired by the appearance of resistant strains in *Cercospora beticola* populations. CLS-resistance has been detected after widespread and repeated applications of the same fungicide classes [6-7]. The speed of research and development of new fungicide actives because the resistance conditions change rapidly. The use of contact and systemic new fungicides with distinct mode of action (specific site of linkage), alternately or in tank mixtures can delay the development of resistant pathogen strains [2].

1.2.1 Resistance of C. beticola to fungicides

Benzimidazoles (MBC): First in Greece, already in 1973, the benzimidazole resistance observed in Cercospora beticola populations was described [8], then in several other places of production, for example in the United States [9], in China [10] and India [11]. Subsequently, DMI-type (triazole) fungicides were introduced to treat resistant populations. In Hungary, stable 100% resistance to the active ingredient benomyl was demonstrated much later [4]. Triazoles (DMIs): Although the triazoles were initially thought to have a moderate resistance risk [12], resistance to Cercospora beticola has now been detected in Europe [13], Canada [14] and the United States [7]. Resistance to triazoles can be observed with a near continuum, both between high and low EC50 values [15]. Strobilurins (QoI): Similar to other fungi [16], the strobilurin-resistant isolates of Cercospora beticola detected so far replaced glycine with alanine at codon 143 (designated G143A) [17]. Resistance monitoring studies in Europe [17], Japan [18], and the United States [7] indicated a rapid and stable development of resistance to strobilurins (QoI). Cross-resistance exists among all the active substances belonging to this group [5].

1.3 The picolinamides (QiI – FRAC Group 21)

QiI fungicides act at the quinone inside (Qi) site of the inner membrane of complex III. Picolinamide chemistry delivers a novel biochemical mode of action for the cereal fungicide market involving inhibition of mitochondrial complex III via binding to the Qi ubiquinone binding site30 rather than to the Qo site targeted by the strobilurin class of fungicides and, as such, no target-site-based cross-resistance to strobilurin fungicides would be anticipated [19] [20-21]. The first member of the group of picolinamides is fenpicoxamid used for the control of foliar diseases. It was introduced by Dow Agrosciences in 2016. A second-generation molecule inspired by UK-2A necessitates a nonmacrocyclic structure with fewer stereogenic

centres to enable cost-effective large-scale production via total synthesis. The florylpicoxamid (Ref: X12485659; also known as: XDE 659; XR-659, Adavelt™ active), is a new active ingredient in the picolinamide class of fungicide chemistry, introduced in 2019 [19-20; 22]. Florylpicoxamid was the first strong broad spectrum picolinamide fungicide with activity against 21 different plant pathogenic fungi within the phyla Ascomycota and Basidiomycota [19]. The representative uses in the World are for Cereals; Vines; Fruits; Nuts; Vegetables; Oilseed rape; Sugar beet; Lentils; Ornamentals for control of a broad range of diseases such as *Septoria spp.*, Powdery Mildews, *Botrytis spp.*, Anthracnose, *Alternaria spp.*, Scab, *Monilinia spp.* [22]. Florylpicoxamid was also discovered for controlling CLS caused by *Cercospora beticola* (CERCBE) [23] patented as a highly effective 'Compound I' against CERCBE based on field testing [24].

2 Objectives

Based on the importance of the above-mentioned aspects, the objective of this current study was to find new effective fungicides and identify novel options for improved disease management in sugar beet. The present study entitled "A picolinamide fungicide for controlling Cercospora-leaf spot (CLS) of sugar beet" was undertaken involving a picolinamide fungicide in 10 trials, with the following objectives as key questions (KQs):

KQ1: What is the dose response of florylpicoxamid on CERCBE?

KQ2: Which dose rates of florylpicoxamid providing superior or equivalent control to standards as difenoconazole and epoxiconazole on CERCBE?

KQ3: Are the tested products safe to the sugar beet compared to the standards?

3 Materials and methods

Ten field trials were established in order to determine the efficacy performance of new invented straight fungicide products from Corteva Agriscience™ to control *Cercospora beticola* (EPPO code: CERCBE [25]) in sugar beet. In Hungary these 10 trials were split between two years. Six trials were conducted in 2020 and four trials in 2021. Trials were conducted in the South-East EPPO zone, in Hungary. The trials were carried out by Akos Biro, follow the EPPO standards and are officially recognized by the competent authorities to carry out field trials in accordance with the principles of Good Experimental Practice (GEP) [26]. The layout and design of the trials were detailed below in **3.2 Trial design**.

3.1 Trial sites and used cultivars

Trial sites were selected on the basis of known disease pressure, favorable agronomical and environmental factors, in areas representative of those where the crop is grown commercially and where *Cercospora beticola* (CERCBE [25]) is endemic. For further trial site and application details see **Table 1** below. Trials were conducted during 2020 and 2021, around Jászberény, at the Northern Great Plain area in Hungary. There were chosen two fields per variety, distanced with 10 km between each other. In 2020 season, six adjacent fields were selected with three different varieties of sugar beet for the trials. In 2021 trial season, four adjacent fields were selected with two different varieties of sugar beet for the experiments. Usually, 5–7 fungicide spray applications per growing season are required for satisfactory disease control. The selected fields were previously cropped to cereals (winter wheat and winter barley) and received a pre-planting fertilization of 680 kg/ha 15N–15P–15K, during both years of the experiment. During the seasons, crop was irrigated three times in 2020 and twice in 2021, receiving an average 25-30mm of precipitation at each irrigation timing. The

sugar beet varieties selected for the trials were 'Smart Belamia KWS', 'Smart Djerba KWS' and 'Balaton' cultivars that are susceptible (based on pre-experience) to CLS to varying degrees.

Table 1. A list and details of conducted field trials in 2020 and 2021. Conditions at first applications.

Trial number	Country code	Trial location	Variety	Appl. date	Appl. Crop BBCH*	Initial CERCBE** % inf. sev.
EA20F9B001F-AB01 EA20F9B002F-AB01	HUN	Jászberény	Smart Belamia	20June 20 28June 20 03 Aug 20	38 38-39 39	1.5
EA20F9B001F-AB02 EA20F9B002F-AB02	HUN	Jászberény	Smart Djerba	21June 20 28June 20 03 Aug 20	38 38-39 39	2.5
EA20F9B001F-AB03 EA20F9B002F-AB03	HUN	Jászberény	Balaton	22June 20 08 Aug 20	38 39	3
EA21F9B001F-AB01 EA21G1C001F-AB01	HUN	Jászberény	Smart Belamia	24 Aug 21 07 Sep 21	39 39	0
EA21F9B001F-AB02 EA21G1C001F-AB02	HUN	Jászberény	Smart Djerba	24 Aug 21 07 Sep 21	39 39	1.25

Notes: * Crop stage at application timings indicated with BBCH scale [27];
** Infection severity level [%] of Cercospora beticola (CERCBE) at first application timing.

3.2 Trial design

All 10 trials were conducted to GEP (Good Experimental Practices, EPPO PP 1/181 (4)) and followed the appropriate EPPO [26] standards: PP 1/001 (4): Foliar diseases on sugarbeet, PP 1/135 (4): Phytotoxicity assessment, PP 1/152 (4): Design and analysis of efficacy evaluation trials, PP 1/181 (4): Conduct and reporting of efficacy evaluation trials including GEP, PP1/225(2) - Minimum effective dose. The layout and design of the trials were detailed below. The trials were of a randomized complete block design (RCBD) with 4 replicates and a plot size 16 m² (2m x 8m). A plot included 4 rows where the two middle rows were evaluated. The crop density was between 54 and 70 plants per plot depending on the variety, field and year. The different varieties and year meant different fields for the trials.

3.3 Test items

In both years, florylpicoxamid (= GF-3840; at 100 g a.s./L, EC) was tested at 50, 75, 100 and 150 g a.s./ha (equivalent to 0.5, 0.75, 1.0 and 1.5 L/ha) in sugar beet for the control of CERCBE. The reference products included were difenoconazole (= Score 250 EC; 250 g a.s./L) applied at 100 g a.s./ha and epoxiconazole (= Opus 125 EC; 125 g a.s./L) applied at 125 g a.s./ha.

3.4 Field trials

Applications were started at early curative timing, targeting primary infections (1-3 lesions per leaf with 1-3% incidence) to avoid the development of conidial populations that can infect new, unprotected leaves, just after the 'closing' of the rows and repeated at intervals of 8–35 days depending on the different disease pressure and progression between the different varieties (Table 1). By the end of the season, three applications were made in 2020 which was a year with high disease pressure than began early in the season. The first application was made on 20th June, the second on 28th June and the third on 3rd August. In the second season (2021) two applications were carried out because of lower level of disease infection. The first application took place on 24th August and the second on 7th September.

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3.5 Trial assessment

Disease severity was recorded as a percentage of visual diseased foliage on whole plot, where each plot was scored for leaf area affected (% Severity). Cercospora leaf spot (CLS) infection was assessed three times at 6-7, 14 and 21-27 days after the last application. The disease infection was recorded following EPPO guideline prescriptions (Figure 1. in EPPO PP 1/001 (4)). Area under the disease progress curve (AUDPC) was calculated for each plot using the sets of recorded severity data. Relative AUDPC (% control based on AUDPC) was calculated as percent of the untreated control (UTC) and averaged over all field trials. Percentage control also was calculated based on Abbott's transformation relative to the infection level (% Severity) present in the untreated control. Area under the disease progress curve (AUDPC) was calculated for the assessment period as follows (**Equation 1**):

$$\text{AUDPC} = \sum_{i=1}^{n} [(Y_{i+1} + Y_i)/2][t_{i+1} - t_i] \qquad (1)$$

Where Y_i is the disease severity at the i^{th} observation, t_i the time (days) at the i^{th} observation and n the total number of observations [27].

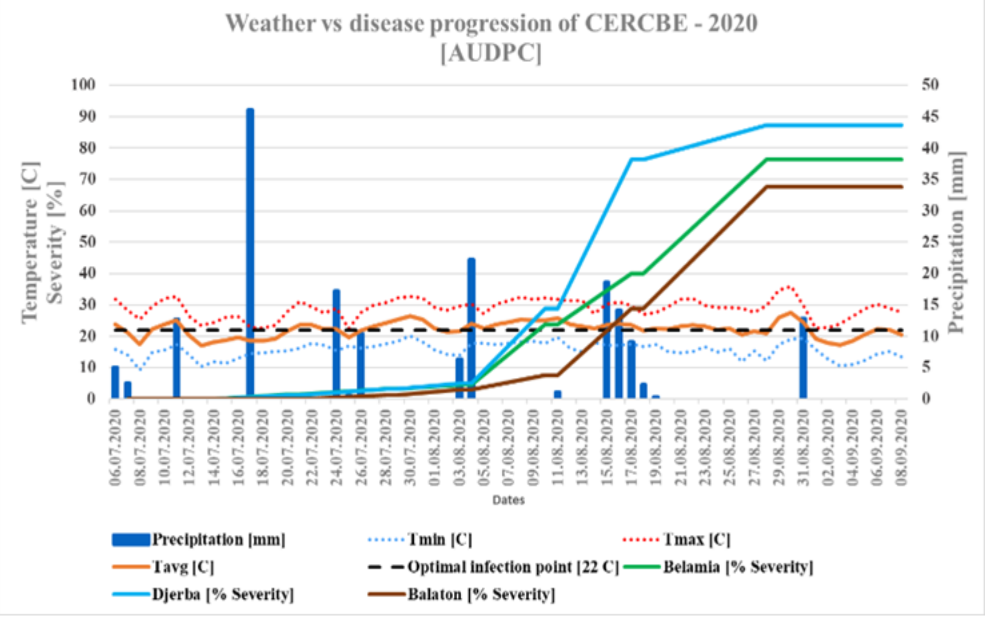

Fig. 1. Disease progression of CLS and weather conditions during experimental trial of sugar beet crop for three varieties ('Smart Belamia', 'Smart Djerba' and 'Balaton') in 2020.

3.6 Statistics and data analysis

Mean percentage based on AUDPC of *Cercospora beticola* (CERCBE) infection values of each individual trial were subjected to the analysis of variance (ANOVA), then compared by means of the Tukey's HSD test to highlight treatment differences ($p<0.05$). The data from all trials were then subsequently combined to generate a mean control percentage value calculated with Abbott's formula as follows

$$Control\ [\%] = \frac{(X - Y)}{X} \times 100 \qquad (2)$$

Where X – the severity of infection in untreated plot; Y – the severity of infection in treated plot [29].

The statistical analysis was supported by the ARM software (version: ARM2024.0) from the Gylling Data Management, Inc.

4 Results

4.1 Infection level (AUDPC)

4.1.1 Disease progress in 2020 vs 2021

During the season of 2020, severe CLS developed in the experimental field and the first signs of the disease appeared on the lower leaves at the end of July. Disease incidence was less severe in 2021 than in 2020 due to the less favorable weather conditions in the trial sites. Data on AUDPC, disease progress during the season with weather data (temperature [°C] and precipitation [mm]) for the 2020 and 2021 trials are summarized in Figures 1 to 4, respectively. Of the three cultivars tested ‘Smart Djerba KWS’ was the most sensitive (18.75-87.25% severity of infection), ‘Smart Belamia KWS’ was sensitive (14.5-76.25% severity of infection) and ‘Balaton’ was moderately sensitive (67.5% severity of infection) to CLS in these trials with the higher levels of infection in 2020 trials (see in Figure 1 and 2).

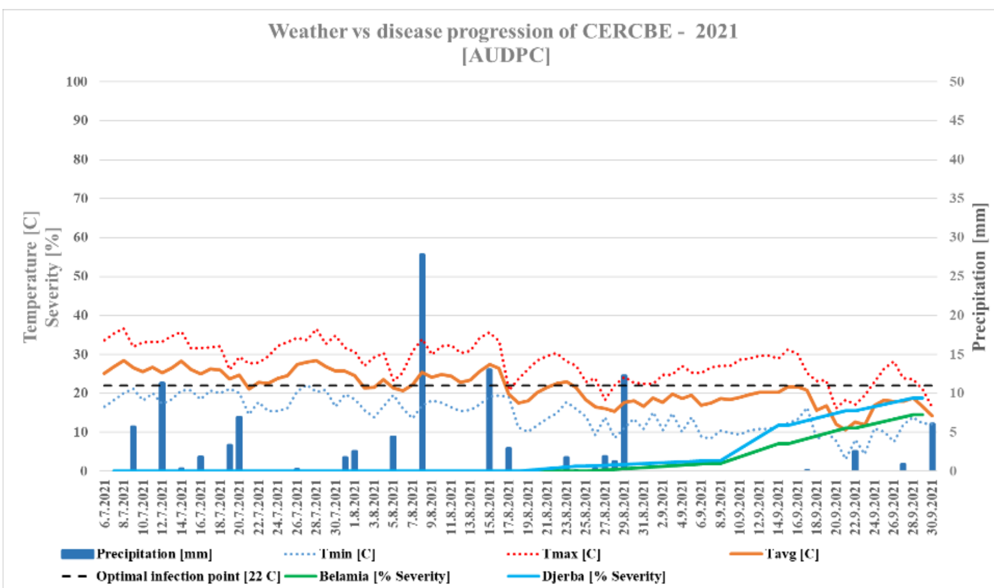

Fig. 2. Disease progression of CLS and weather conditions during experimental trial of sugar beet crop for two varieties (‘Smart Belamia’, and ‘Smart Djerba’) in 2021.

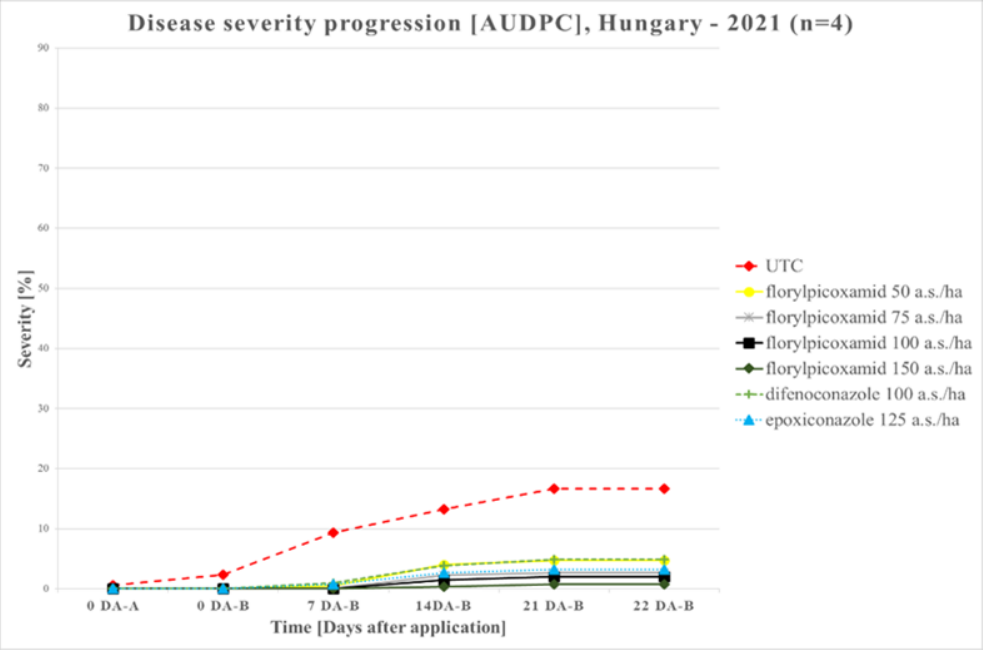

Fig. 3. Efficacy of florylpicoxamid and dose response compared to the reference products (difenoconazole, epoxiconazole) and UTC (untreated check) in 2020.

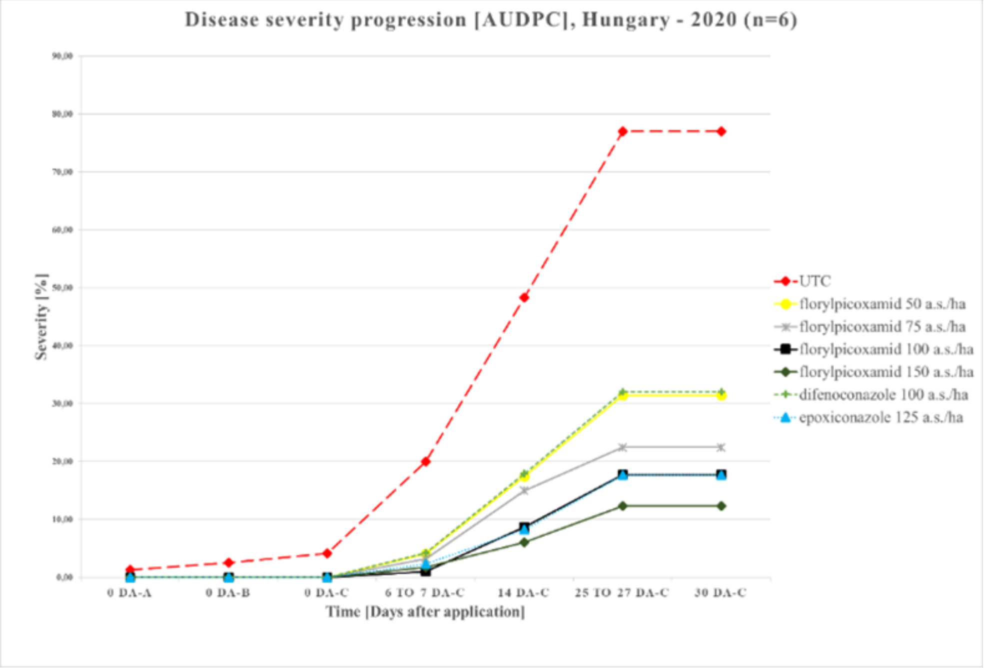

Fig. 4. Efficacy of florylpicoxamid and dose response compared to the reference products (difenoconazole, epoxiconazole) and UTC (untreated check) in 2021.

4.2 Efficacy of fungicides

Across the same 10 trials conducted in Hungary, florylpicoxamid (=GF-3840) applied at 150 g a.s./ha between BBCH 38 and 39 [27] of sugar beet provided 90.93% control of CERCBE [25]. Applied in the same trials at 100 g a.s./ha florylpicoxamid (=GF-3840) provided 84.23% control, at 75 g a.s./ha florylpicoxamid (=GF-3840) provided 77.63% control and at 50 g a.s./ha florylpicoxamid (=GF-3840) gave 67.48% control of CERCBE. Florylpicoxamid (=GF-3840) applied at 75 g a.s./ha, provided significantly better control (77.63%) than difenoconazole applied at 100 g a.s./ha (67.91% control), and it gave comparable performance to epoxiconazole at 125 g a.s./ha (81.03% control). Florylpicoxamid (=GF-3840) applied at 100 and 150 g a.s./ha provided significantly better control (84.23-90.94%) than the reference products difenoconazole or epoxiconazole (Table 2).

Table 2 Comparison and summary of efficacy % control (based on the AUDPC and was calculated by Abbott's transformation) for florylpicoxamid against CLS versus reference fungicide actives in 2020 and 2021.

		Years														
		2020					**2021**					**Combined years (2020 & 2021)**				
			Stat**					**Stat****					**Stat****			
Treatments	**Dose rate [g a.s./ha]**	**AUDPC***	**Incl. UTC**	**Exc. UTC**	**% Control*****	**Stat****	**AUDPC***	**Incl. UTC**	**Exc. UTC**	**% Control*****	**Stat****	**AUDPC***	**Incl. UTC**	**Exc. UTC**	**% Control*****	**Stat****
		(n=6 trials)					**(n=4 trials)**					**(n=10 trials)**				
florylpicoxamid	50	375,83	a	a	61,80	ef	42,25	a	b	76,00	d	233,60	a	a	67,48	d
florylpicoxamid	75	260,96	a	a	72,00	bcd	26,69	a	c	86,06	c	167,25	a	a	77,63	c
florylpicoxamid	100	190,77	a	a	79,40	ab	16,19	a	d	91,48	b	120,94	a	a	84,23	b
florylpicoxamid	150	130,42	a	a	86,68	ab	5,25	a	e	97,32	a	80,35	a	a	90,93	ab
difenoconazole	100	329,42	a	a	63,96	de	50,31	a	ab	73,84	de	217,78	a	a	67,91	d
epoxiconazole	125	184,63	a	a	80,05	ab	33,69	a	c	82,49	c	124,25	a	a	81,03	bc
UTC	-	921,63	a	a	-	-	195,13	a	-	-	-	631,03	a	a	-	-

Notes: *AUDPC calculated from disease severity values [% Severity]; **Different letters indicating where are significant differences between treatment means; ***Percent control was calculated from AUDPC values by Abbott transform.

5 Conclusions

Under high disease pressure conditions and on very sensitive varieties, picolinamide fungicides provided control, and proved to deliver efficient disease management comparable or higher to the current straight products leading the sugar beet fungicide market. Florylpicoxamid also proved to be safe to the crop sugar beet in all trials, there was not observed any crop injury during these two trial seasons. As one of the first members of a new class of QiI fungicide group, with no cross resistance with current chemistries registered in sugar beet market [20], picolinamides, especially florylpicoxamid can offer an additional tool for the sugar beet growers, to support successful resistance management in a long term. According to the presented results for the control of CERCBE, florylpicoxamid applied at 75 g a.s./ha provide optimum overall control and should be considered as effective against CERCBE in sugar beet. It should be inserted in a spraying program in combination with other

actives from different mode of action fungicide groups. It has three weeks long-lasting control against CERCBE. Florylpicoxamid can be good candidate for providing a new alternative for the management of CLS disease of sugar beet crop, caused by *Cercospora beticola*.

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