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Article

Picolinamide Fungicides for Controlling Cercospora-leaf Spot (CLS) of Sugar Beet

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Abstract. Studies were initiated to find new effective fungicides to use under field conditions to identify novel options for improved disease management in sugar beet. 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. The disease is now dominant in almost all sugar beet growing areas of Europe, including Hungary. The epidemic spread of this disease can cause up to 50% yield loss. The use of fungicides has been being an integral part of the control of CLS of sugar beet, due to lack of efficacy from non-chemical alternatives. In recent decades, the emergence of resistant strains in *C. beticola* Sacc. populations have reduced the efficacy of some fungicides (Quinoline inside Inhibitors (QoI) – FRAC Group 11; and DeMethylation-Inhibitors (DMI) – FRAC Group 3) because of repeated use over many years. Resistance of CLS to fungicides belonging to different groups of active substances has been described in many countries of the world, including Hungary. The picolinamides are a new distinct group of fungal respiration inhibitors (Inhibition of Complex III) belonging to Quinoline inside Inhibitors (QiI). Two novel fungicides from the group of picolinamides (QiI – FRAC Group 21) – fenpicoxamid and florylpicoxamid– were tested and evaluated over two seasons, in vegetation period of 2020 and 2021 for controlling CLS of sugar beet in Hungary. Both fungicides were applied as straight formulated products 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 product difenoconazole. The results consistently showed all tested dose rates of fenpicoxamid and florylpicoxamid provided effective control against CLS of sugar beet in addition to a clear dose response curve. Disease severity, in terms of area under the disease progress curve values (AUDPC), was significantly correlated with yield decrease, while no significant context existed between disease severity and sugar content of the roots. Additionally, the results showed in two investigated years, the efficacy of both picolinamide fungicides applied at 75 g ai/ha provided significantly better % control on CLS of sugar beet than the DMI fungicide difenoconazole applied at 100 g ai/ha dose rate. Fenpicoxamid is already registered in Europe in cereals and label extension is planned in sugar beet and other crops. Florylpicoxamid is not yet registered in Europe but is beginning to be approved in countries across the globe in a range of crops and is continuously under evaluation for potential markets.

Keywords: fungicides; new fungicides; picolinamides; fenpicoxamid; florylpicoxamid; *Cercospora beticola*; CERCBE

1. Introduction

1.1. The Importance of the Disease

This study's targeted pathogen is Cercospora-leaf spot (CLS) caused by *Cercospora beticola* Sacc. [1] which plays the most prominent role in current sugar beet producing areas. The success of the sugar beet cultivation depends a lot on the efficiency of the disease management against the Cercospora leaf spot, the speed of research and development of new fungicide actives, because the resistance conditions change rapidly. 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. Its main damage is the very significant loss of sugar yield, which can approach 40-50% in the case of medium or high infection pressure [4-5], through the loss of foliage of the sugar beet and the resulting leaf change. By increasing the proportion of impurities, it also complicates the processes of sugar extraction, thereby causing higher processing costs and less extractable amount of sugar [4]. Furthermore, the root yield of infected plants is more prone to rotting in prisms during winter storage [6]. For example, in the late 1980s and early 1990s, severe cercospora-leaf spot outbreaks caused significant economic damage to sugar beet farmers in southern Germany [7]. In Hungary, this fungal disease most often causes the most serious yield losses in sugar beet stands, regardless of the cultivation area [8]. In our country, it can be expected to appear on sugar and fodder beets in the middle of summer, in the months of June and July. In case of early infection, it can cause 2-3 changes of leaves, in which case it can cause 15-25% yield loss, 0.5-1.5% sugar content loss, and 25-35% sugar loss. A 10-20% yield loss and a 5-10% deterioration in germination may occur with seed production. The damage caused by different races can be between 2 and 40% sugar content loss, depending on their infectivity and the susceptibility of different sugar beet varieties. In the northern countries of Europe, its damage is not significant [9]. The cercospora-leaf spot is one of the most common and most serious damaging foliar disease of sugar beet. Control against it forms the backbone of plant protection in most of the sugar beet-growing countries of the world, both in Europe, the United States, Japan and Russia [5]. It is typically a Mediterranean disease, but it appears and then multiplies in all beet-growing areas, where the summer rainfall is around 200 mm, and the average temperature exceeds 19-20°C. Many races have developed in different geographical zones, which were quickly transferred from one continent to another with the seed [9]. The integrated pest management (IPM) in case of controlling CLS includes good tillage practice, resistance breeding focuses on the use of genetic resources for improving plant defense against CLS and timely implementation of fungicide treatments [2]. The aim of establishing good agricultural practices (GAP) is to reduce the amount of initial infectious material in the following season by following the crop rotation, soil cultivation (by ploughing down infected plant residues) and avoiding sowing directly next to the previous year's sugar beet-fields. Adhering to a 2-3-year crop rotation by excluding possible host plants and removing the cut diseased beet heads from the area reduces the sources of infection in order to protect sugar beet crops in the following years [10-11]. Deep ploughing accelerates the decomposition of infected heads in the soil, leading to the death of the fungus [12]. Epidemiological models have been developed to predict the appearance and severity of the disease and to monitor its development in order to properly time fungicide treatments [13-16]. For example, a prediction model based on the number of hours of high relative humidity and critical mean temperature has been successfully used to determine fungicide spray schedules in the United States [17]. Fungicide treatments should be applied early, preventively, targeting primary infections to avoid the development of conidial populations that can infect new, unprotected leaves. The use of contact and systemic fungicides alternately or in tank mixtures can delay the development of resistant pathogen strains [2; 8]. Although many studies have addressed the applicability of various bacteria and fungi as biopesticides against cercospora-leaf spot, including *Trichoderma* spp. and the results of studies with *Bacillus subtilis* [18-19], we currently do not know of any successful research results. In addition, as an alternative solution, the presence of several microbial groups correlates with the frequency of disease occurrence in sugar beet areas, so these microbes can be useful as biological markers in predicting disease outbreaks [20].

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) [8; 21].

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 [22-24]. Other factors are the "polycyclic nature" of the pathogen, the high rate of spore formation and the frequently used spraying programs in large areas, which also significantly contribute to the development of resistance to fungicides [25]. Alternating sprays of active substances belonging to different groups is used to suppress the selection of resistant strains. As an additional option, systemic fungicides (such as triazoles) are used in a tank mixture with a contact agent to achieve higher efficiency, reduce costs and prevent resistance [26].

1.2.1. Resistance

Benzimidazoles (MBC): First in Greece, already in 1973, the benzimidazole resistance observed in *Cercospora beticola* populations was described [27], then in several other places of production, for example in the United States [28], in China [29] and India [30]. 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 [31]. Davidson et al. [32] and Trkulja et al. [33], identified a glutamic acid and alanine amino acid change in the target β -tubulin gene sequence at codon 198 (called E198A), which was associated with high benzimidazole resistance in several populations of *Cercospora beticola*. Triazoles (DMIs): Although the triazoles were initially thought to have a moderate resistance risk [34], resistance to *Cercospora beticola* has now been detected in Europe [35], Morocco [36], Canada [37] and the United States [23-24; 38]. Resistance to triazoles can be observed with a near continuum, both between high and low EC₅₀ values [39]. *Cercospora beticola* isolates with an EC₅₀ greater than 1 ppm caused significantly more severe disease in sugar beet after a triazole treatment than isolates with an EC₅₀ below 1 ppm. Based on these, 1 ppm was hypothesized as a reasonable threshold for DMI resistance [40-41]. In a 2017 survey, 25.9% of *Cercospora beticola* isolates tested were resistant (EC₅₀ > 1 ppm) to tetraconazole, while 47.1% of the same isolates were resistant to another triazole, difenoconazole, suggesting, that there is no complete cross-resistance to triazoles [42-43]. The mechanism of resistance to triazoles is generally more complex than that to benzimidazoles or strobilurins. Triazoles target fungal lanosterol 14 α -demethylase CYP51, a cytochrome P450 enzyme. It catalyzes a key step in ergosterol biosynthesis in the fungus. Without the biosynthesis of sterol-ergosterol in the cell membrane, the growth of fungal cells can be inhibited. Resistance can arise not only through modification of the CYP51 target site, but also through CYP51 overexpression, increased active efflux of triazoles, and multiple copies of the target CYP51 gene [44-45]. Recently, non-synonymous polymorphisms in CbCYP51 have also been discovered to be associated with triazole resistance [46-47]. Strobilurins (QoI): Similar to other fungi [48], the strobilurin-resistant isolates of *Cercospora beticola* detected so far replaced glycine with alanine at codon 143 (designated G143A) [46; 49; 50-51]. Resistance monitoring studies in Europe [49;51], Morocco [36], Japan [52], Canada [53] and the United States [23; 54] indicated a rapid and stable development of resistance to strobilurins (QoI). Cross-resistance exists among all the active substances belonging to this group [21]. In a 2017 report in the United States, it was shown that 89.1% of *Cercospora beticola* isolates were resistant to pyraclostrobin, so its use is no longer recommended for control of CLS [42].

1.3. The Picolinamides (QiI – FRAC Group 21)

A novel fungicide group with a new class of chemistry. 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 [55-57]. The first member of the group of picolinamides is fenpicoxamid for the control of foliar diseases. It was introduced by Dow Agrosiences in 2016. The natural product UK-2A was transformed post fermentation to fenpicoxamid (Inatreq™ active), a new crop protection active ingredient for disease control in wheat and bananas [56]. It acts as a contact and residual protectant with limited systemic activity but some translaminar activity. The representative uses in Europe were for cereals for control of *Zymoseptoria tritici* (*Mycosphaerella graminicola*). Fenpicoxamid is also registered for use on banana for the control of Black Sigatoka (*Mycosphaerella fijiensis*). The fenpicoxamid (Ref: XDE-777; also known as: X772777; XR-777; UK 2A procide; Antibiotic UK 2A procide; GF-2925; lyserphenvalpyr; Inatreq™ active) possesses these characteristics and is a member of a novel picolinamide class of fungicides derived from the antifungal natural product UK-2A [55; 58]. A second-generation molecule inspired by UK-2A necessitates a nonmacroyclic structure with fewer stereogenic centers to enable cost-effective large-scale production via total synthesis. The florylpicoxamid (Ref: X12485659; also known as: XDE 659; XR-659, Adavelt™ active), a new active ingredient in the picolinamide class of fungicide chemistry was introduced in 2019 [56-58]. Florylpicoxamid the first strong broad spectrum picolinamide fungicide with activity against 21 different plant pathogenic fungi within the phyla Ascomycota and Basidiomycota [57]. 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. [58].

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™ on *Cercospora beticola* (EPPO code: CERCBE [59]) 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) [60]. The layout and design of the trials were detailed below in paragraph 3.2 Trial design.

Table 1. A list and details of conducted field trials in 2020 and 2021.

Trial number	Country code	Trial location	Variety	Appl. date	Appl. Crop BBCH	Initial CERCBE % inf. sev.
EA20F9B001F-AB01	HUN	Jászberény	Smart Belamia	20June 20 28June 20 03 Aug 20	38 38-39 39	1.5
EA20F9B001F-AB02	HUN	Jászberény	Smart Djerba	21June 20 28June 20	38 38-39 39	2.5

				03 Aug 20		
EA20F9B001F- AB03	HUN	Jászberén y	Balaton	22June 20 08 Aug 20	38 39	3
EA20F9B002F- AB01	HUN	Jászberén y	Smart Belamia	20June 20 28June 20 03 Aug 20	38 38-39 39	1.5
EA20F9B002F- AB02	HUN	Jászberén y	Smart Djerba	21June 20 28June 20 03 Aug 20	38 38-39 39	2.5
EA20F9B002F- AB03	HUN	Jászberén y	Balaton	22June 20 08 Aug 20	38 39	3
EA21F9B001F- AB01	HUN	Jászberén y	Smart Belamia	24 Aug 21 07 Sep 21	39 39	0
EA21F9B001F- AB02	HUN	Jászberén y	Smart Djerba	24 Aug 21 07 Sep 21	39 39	1.25
EA21G1C001F- AB01	HUN	Jászberén y	Smart Belamia	24 Aug 21 07 Sep 21	39 39	0
EA21G1C001F- AB02	HUN	Jászberén y	Smart Djerba	24 Aug 21 07 Sep 21	39 39	1.25

3.1. Trial Sites and Used Cultivars

Trial sites were selected on the basis of known pest pressure, favourable agronomical and environmental factors, in areas representative of those where the crop is grown commercially and where *Cercospora beticola* (CERCBE) is an abundant disease. For further trial site and application details see Table 1 above. Trials were conducted during 2020 and 2021, in Jászberény, at the Northern Great Plain area in Hungary. 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. In this area, cercospora-leaf spot (CLS) is the most important foliar disease of sugar beet due to the relatively warm and dry summers prevailing in the region. 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. 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.

3.2. Trial Design

All 10 trials were conducted to GEP (Good Experimental Practices, EPPO PP 1/181 (4)) and followed the appropriate EPPO [60] standards: PP 1/001 (4): Foliar diseases on sugar beet, 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, fenpicoxamid (= GF-3308; at 50 g a.s./L, EC) was tested at 50, 75, 100 and 150 g a.s./ha (equivalent to 1.0, 1.5, 2.0 and 3.0 L/ha) and 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 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. 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 two applications were carried out because of lower level of disease infection, in 2021. The first application took place on 24th August and the second on 7th September, the third application was not required because of lower levels of infection as disease development was later in the season than in 2020. The treatments in all trials were applied using a Euro-Pulve knapsack precision plot sprayers equipped with low drift flat fan nozzles (ALBUZ AVI TWIN 11002) delivering water volumes 400 L/ha (0.64 L/plot).

3.5. Trial Assessment

Disease severity was recorded as a percentage of visual diseased foliage on whole plot (% 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 PP 1/1 (4) guideline prescriptions. 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):

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

Equation 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 [61].

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 $p < 0.05$ level. The data from all trials were then subsequently combined to generate a mean control percentage value calculated with Abbott's formula as follows (Equation 2):

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

Equation 2 Where X – the severity of infection in untreated plot; Y – the severity of infection in treated plot [62].

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 Figure 1-2 and Figure 2, 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 3 and Figure 4).

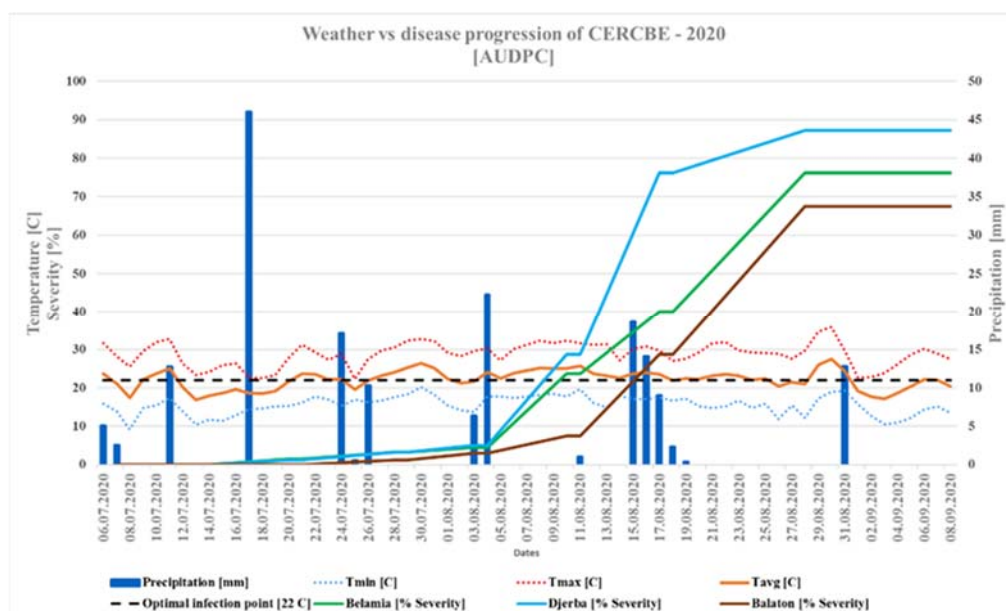


Figure 1. Disease progression of CLS in terms of weather conditions in case of three used varieties ('Smart Belamia', 'Smart Djerba' and 'Balaton') in 2020. That trial season was quite favorable for *Cercospora beticola* infection and an epidemic in this area of Hungary.

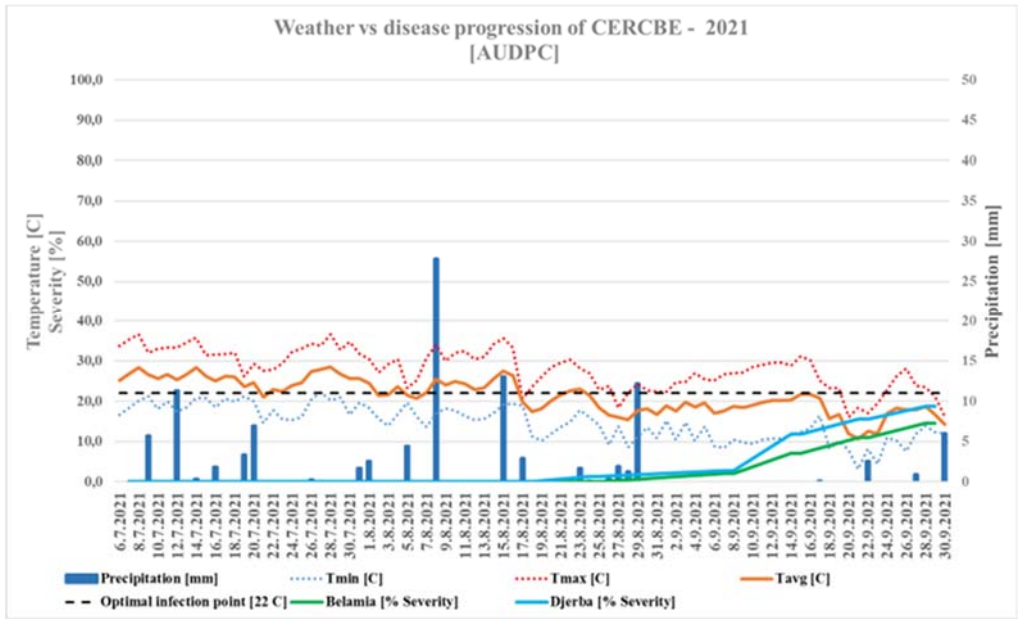


Figure 2. Disease progression of CLS in terms of weather conditions in case of three used varieties ('Smart Belamia', and 'Smart Djerba') in 2021. That trial season was not favorable for *Cercospora beticola* infection. The first symptoms appeared almost one month later than generally expected in Hungary. The infection level stayed at low level until the harvest.

4.2. Efficacy of Fungicides

Ten small plot field trials were established in order to evaluate the efficacy for the control of the CLS (caused by CERCBE) in sugar beet. The trials were conducted in Hungary.

4.2.1. Efficacy for Fenpicoxamid (Inatreq™)

Across the 10 trials conducted in Hungary, fenpicoxamid (=GF-3308) applied at 150 g a.s./ha between BBCH 38 and 39 of sugar beet provided 86.59% control of CERCBE. Applied in the same trials at 100 g a.s./ha fenpicoxamid (=GF-3308) provided 84.2% control, at 75 g a.s./ha fenpicoxamid (=GF-3308) provided 76.45% control and at 50 g a.s./ha fenpicoxamid (=GF-3307) gave 61.01% control of CERCBE. Fenpicoxamid (=GF-3308) applied at 75 g a.s./ha, provided significantly better control (76.45%) 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). Fenpicoxamid (=GF-3308) applied at 100 and 150 g a.s./ha provided significantly better control (84.2-86.59%) than the reference products difenoconazole or epoxiconazole (Table 2).

4.2.2. Efficacy for Florylpicoxamid (Adavelt™)

Across the same 10 trials conducted in Hungary, florylpicoxamid (=GF-3840) applied at 150 g a.s./ha between BBCH 38 and 39 of sugar beet provided 90.93% control of CERCBE. 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 calculated by Abbott's formula) for picolinamides against CLS versus reference fungicide actives in 2020 and 2021.

Years																
		2020					2021					Combined years (2020 & 2021)				
Treatments	Dose rate [g a.s./ha]	AUDPC*	Statistics**		% Control***	Stat**	AUDPC*	Statistics**		% Control***	Stat**	AUDPC*	Statistics**		% Control***	Stat**
			Incl UTC	Exc. UTC				Incl UTC	Exc. UTC				Incl UTC	Exc. UTC		
		(n=6 trials)					(N=4 trials)					(n=10 trials)				
<i>fenpicoxamid</i>	50	433,83	a	a	53,98	f	55,56	a	a	71,56	e	282,53	a	a	61,01	e
<i>fenpicoxamid</i>	75	312,42	a	a	67,26	cde	31,50	a	c	83,33	c	151,89	a	a	76,45	c
<i>fenpicoxamid</i>	100	235,50	a	a	75,80	bcd	18,16	a	d	90,50	b	111,30	a	a	84,20	b
<i>fenpicoxamid</i>	150	196,21	a	a	79,96	ab	6,56	a	e	96,54	a	120,35	a	a	86,59	ab
<i>florypicoxamid</i>	50	357,83	a	a	61,80	ef	47,25	a	b	76,00	d	233,60	a	a	67,48	d
<i>florypicoxamid</i>	75	260,96	a	a	72,00	bcd	26,69	a	c	86,06	c	167,25	a	a	77,63	c
<i>florypicoxamid</i>	100	190,77	a	a	79,40	ab	16,19	a	d	91,48	b	120,94	a	a	84,23	b
<i>florypicoxamid</i>	150	130,42	a	a	86,68	a	5,25	a	e	97,32	a	80,35	a	a	90,93	ab
<i>difenoconazole</i>	100	329,42	a	a	63,96	de	50,31	a	ab	73,84	de	217,78	a	a	67,91	d
<i>epoxiconazole</i>	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	-	-	-	195,13	a	-	-	-	631,03	a	-	-	-

*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 transformation

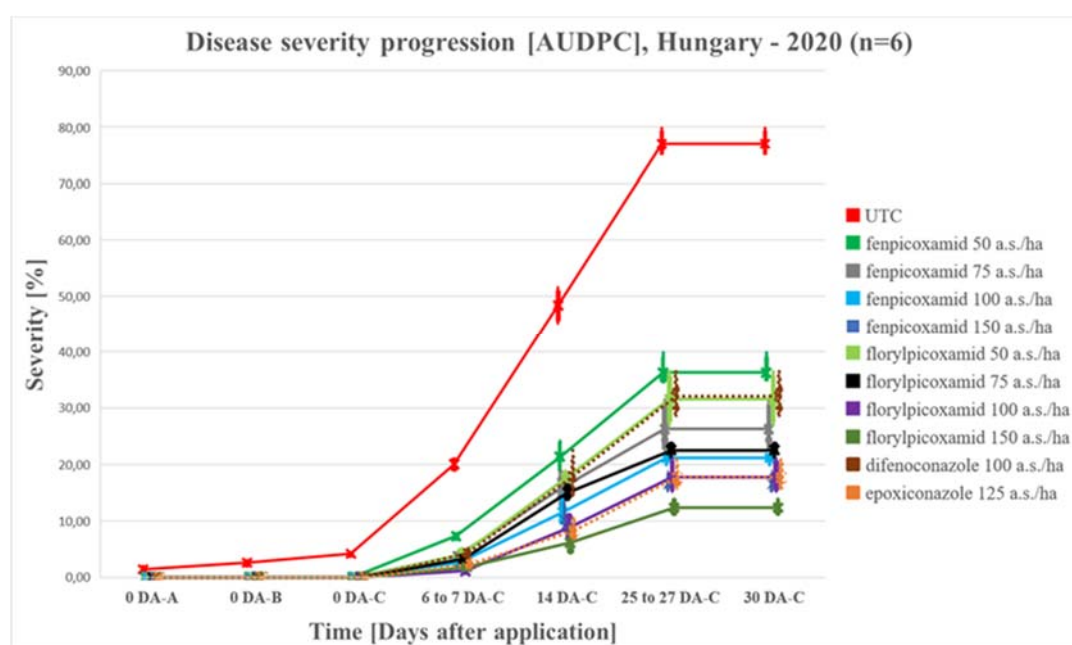


Figure 3. Regarding the six conducted trials in 2020, the best performing treatments could control the CLS up to three-four weeks after last fungicide application. The treatments gave a clear dose response in case of fenpicoxamid and florypicoxamid. Both picolinamides provided significantly better or comparable efficacy to reference products depending on the rates respectively.

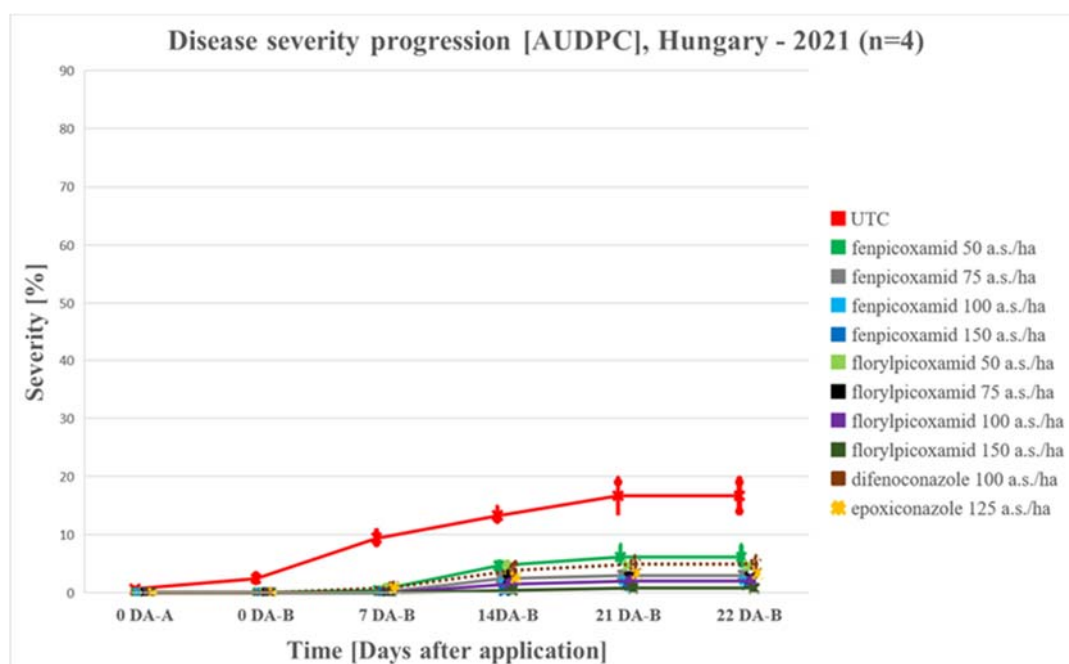


Figure 4. In 2021, the infection level stayed at low level until the harvest. Only two applications were needed. All treatments were able to control CLS easily providing three weeks residual protection after the last fungicide application.

5. Discussion and Conclusions

This two years study presented has introduced the key issues of sugar beet cultivation and new solutions to control CLS within sustainable disease management programs. All over Hungary, and in Jászberény, where the trial sites were established, *Cercospora* leaf spot (CLS) is the key foliar disease of sugar beet. 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. Historically, in this area near Jászberény, the sugar beet fields generally were highly infected irrespective of the cultivar's resistance level in previous years. At first year of this study the disease pressure was high as expected based on previous experience, but in the second year the disease pressure stayed at low level until the harvest, epidemic was not formed. In 2021, only two applications were necessary instead of planned three applications. Under high disease pressure conditions and on very sensitive varieties, straight picolinamides provided strong control, and proved to deliver efficient disease management comparable or higher to the current straight products leading the sugar beet fungicide market. The tested picolinamide fungicides, as fenpicoxamid and florylpicoxamid, feature a curative and protectant effect for control of foliar diseases in cereal crops with translaminar activity [63]. Florylpicoxamid was discovered for controlling several fungal diseases in several crops and as an example it has efficacy for controlling CLS caused by *Cercospora beticola* (CERCBE) [64] patented as a highly effective 'Compound I' against CERCBE based on field testing [65]. Fenpicoxamid and florylpicoxamid also proved to be safe to the crop sugar beet in all trials. As first members of a new class of Qil fungicide group, with no cross resistance with current chemistries registered in sugar beet market, picolinamides, especially fenpicoxamid 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, fenpicoxamid and florylpicoxamid applied at 75 g a.s./ha provide optimum overall control and should be considered as effective against CERCBE in sugar beet. They should be inserted in a spraying program in combination with other actives from different mode of action fungicide groups. They have three weeks long-lasting control against CERCBE. The tested picolinamide fungicides, fenpicoxamid or florylpicoxamid delivered comparable or superior CLS control to other existing fungicide actives in sugar beet market based on current results compared with previous publications, such as strobilurins as trifloxystrobin, pyraclostrobin and DMI fungicide as difenoconazole [66-67] or market leader DMI+morpholine fungicide ready-to-use combination as difenoconazole+fenpropidin [68]. Moreover, the lower amount of active substance per hectare

compared to current market references support sustainable use. Overall, it can be declared that fenpicoxamid or florylpicoxamid from the group of picolinamides can be good candidates for providing a new solution for the management of this tough controllable CLS disease of sugar beet, caused by *Cercospora beticola*.

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