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Short Notes

Virulence of *Puccinia triticina* and *Puccinia tritici-duri* on durum wheat in southern Spain, from 2020 to 2022

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Summary. Leaf rust is a major wheat disease in southern Spain, where durum wheat is an important crop. Until the 2019/2020 season, this disease was effectively managed, as the most widely planted cultivars in southern Spain had effective resistance genes. A problem arose in the spring of 2020, when every farm field and durum wheat trial examined displayed leaf rust symptoms. Leaves had few but large uredinial pustules, different from those of the normal leaf rust caused by *Puccinia triticina*, and telia developed rapidly after only a few days. The symptoms clearly fitted with *P. tritici-duri*, another wheat leaf rust species already reported in the western Mediterranean Basin. This species is not new in southern Spain but has never been observed at such high severity on almost every durum wheat cultivar grown in that region. Leaf rust severity was assessed in durum wheat field trials in the 2020, 2021 and 2022 growing seasons in four provinces of southern Spain. During 2020 and 2021, 13 single pustule isolates of leaf rust were also collected from different cultivars of durum wheat. Inoculation of the isolates on a differential host set showed that four different races were present, two being of *P. tritici-duri*. Only cultivar Calero showed consistent resistance to the races of *P. tritici-duri* employed in this study.

Keywords. Leaf rust, virulence, resistance, telia.

INTRODUCTION

Durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) is an especially important crop in the Mediterranean Basin, sown on 13.14 Mha. In Spain, it is an important crop in the southern region of Andalusia, with a sown area of 180 Kha (in 2019–2020), representing approx. 60% of the durum area in the country (MAPA, 2022). Leaf rust, caused by *Puccinia triticina* Eriks., is one of the main biotic constraints of wheat in Spain. From 1998 to 2005, leaf rust outbreaks on durum wheat were quite frequent in Andalusia, which forced farmers to apply fungicides, and breeders to develop resistant cultivars. In the spring of 2020, leaf rust uredia (or pustules) were observed in west Andalusia on previously resistant durum wheat cultivars, such as the popular Don Ricardo known to harbour the leaf rust resistance genes *Lr27+Lr31*. The pustules were scarce but larger than usual, with a tendency to rapidly form telia on the abaxial sides of the pustules. The atypical symptoms observed were consistent with leaf rust caused by *Puccinia tritici-duri* V. Bourgin that had already been reported in Morocco, Portugal, and Spain. This species has *Anchusa azurea* Mill. (*Boraginaceae*) as the alternate host, instead of *Thalictrum speciosissimum* (*Ranunculaceae*), the alternate host of *P. triticina* (Ezzahiri and Roelfs, 1992; Anikster *et al*., 1997).

The objectives of the present study were to determine the severity of this newly emerged leaf rust species on a group of important Spanish durum wheat cultivars, and to characterize the virulence spectra of isolates of *P. triticina* and *P. tritici-duri* collected in four locations of southern Spain in 2020, 2021 and 2022.

MATERIALS AND METHODS

Five durum wheat cultivars popular in southern Spain, namely Amilcar (susceptible check), Don Ricardo, Athoris, Euroduro, and Calero, were sown in field trials, each with three replications (plots each of 8 rows and measuring 1.4 × 5 m) in the locations of Ecija (near Seville), Conil de la Frontera (near Cadiz), Cordova, Escacena del Campo (near Huelva), and Jerez de la Frontera (near Cadiz). Natural leaf rust infections occurred during all three seasons the experiments were conducted. Leaf rust severity was assessed using the modified Cobb scale at the time of maximum infection (Roelfs *et al*., 1992). Samples of infected durum wheat leaves were obtained from four locations (Conil, Escacena, Ecija, and Cordova), in 2020 and 2021. Infected leaves were collected from plant breeder plots and commercial fields. Single pustule isolates (six in 2020, seven in 2021) were inoculated and grown on the susceptible check Atil/Local Red. A set of 20 near isogenic lines of

Figure 1. Symptoms of *Puccinia tritici-duri* in durum wheat field trials in southern Spain, showing large but sparse pustules (left panel).

the bread wheat cultivar Thatcher was used to determine the virulence spectrum and nomenclature of the 13 collected single pustule isolates (Kolmer and Hughes, 2017). The susceptible checks Thatcher and Atil/Local Red, the cultivar Gatcher, two additional Thatcher isogenic lines, plus 14 durum wheat cultivars with known resistance genes were also inoculated. Four plants of each genotype were grown in trays (60 × 40 × 10 cm) containing (3:1 peat:sand), in a greenhouse of the University of Seville (Spain). The plants were inoculated at two different stages, onto fully developed but young leaves (the first leaf (seedling) and the fifth leaf of each plant), by blowing a mix of uredospores and talcum powder (1:50) (approx. 0.2 mg per genotype). Inoculated plants were placed in a dark dew chamber for 14 h at 20°C, and 100% relative humidity. Twelve days after inoculation, the plants were evaluated for rust development. Infection types were scored using the 0–4 scale described by Stakman *et al*. (1962).

RESULTS AND DISCUSSION

During the 2019–2020 season, leaf rust infections were observed in the field on all durum wheat cultivars tested except Calero (Table 1). The symptoms observed were considered atypical because of the abundance of telia and because the five host cultivars had previously and repeatedly displayed reactions in farmers' fields ranging from complete resistance (Don Ricardo) to intermediate resistance (Amilcar). Rust severity varied among the cultivars and site-years, ranging from 5 to 65% in the susceptible cultivar Amilcar. The variability can be accounted for by different annual weather patterns, and because the sites such as Conil and Jerez were near the ocean where relative humidities are high day-night thermal oscillations are small. However, the relative rankings of the cultivars remained the same throughout.

Data of infection types of the 20-line differential set showed that the 13 isolates corresponded to four different races (Table 2). Two of the races were *Puccinia triticina* and two were *P. tritici-duri*. *Puccinia triticina* differed

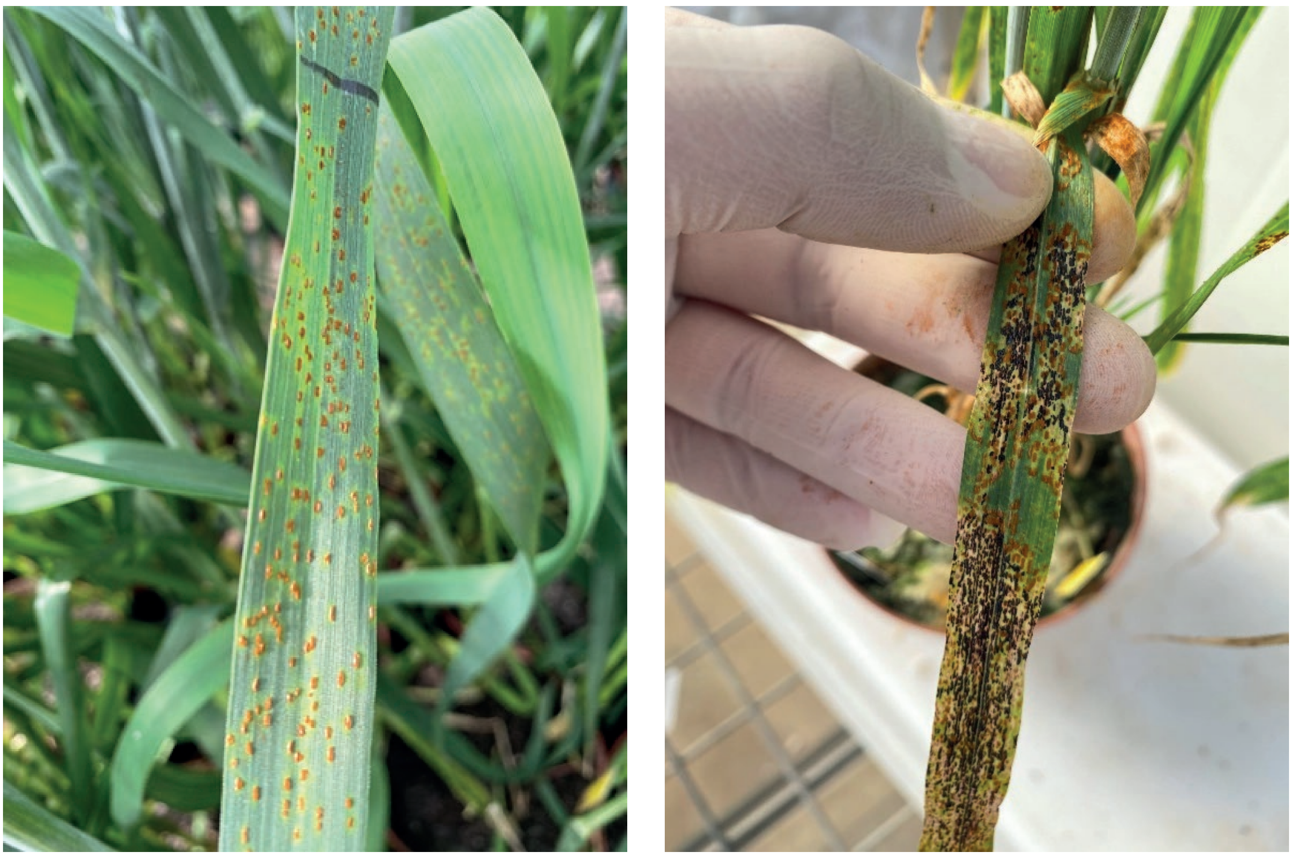

Figure 2. Uredia of *Puccinia triticina* (left) and uedia and telia of *P. tritici-duri* uredia+telia (right) on infected durum wheat leaves in a greenhouse 30 d after inoculation

Table 1. Mean leaf rust severity (%) on five durum wheat cultivars grown in replicated field trials at five locations of southern Spain from 2019/20 to 2021/22.

Cultivar / Location-year	Ecija 2020	Jerez 2020	Conil 2020	Cordova 2020	Conil 2021	Escacena 2022
Amilcar	18 a*	50 a	65 a	5 b	17 a	6 a
Don Ricardo	10 b	23 c	25 c	1 c	7 b	3 a
Athoris	10 b	32 bc	40 b	7 b	10 b	4 ab
Euroduro	18 a	37 b	53 ab	12 a	15 a	11 a
Calero	1 c	1 d	5 d	1 c	1 c	1 b

*Duncan test. Within each column, means accompanied by a common letter are not different ($P \leq 0.05$). Disease severities shown are means of single ratings taken for each of the three replicated plots per cultivar.

from *P. tritici-duri* in its avirulence on *Lr1, Lr3ka, Lr9, Lr17*, and virulence on *Lr18, Lr20*, whereas *P. tritici-duri* gave a constant mesothetic reaction on almost all the Thatcher near isogenic lines, except for those with *Lr24, Lr26* and *Lr28* genes. The mesothetic reaction of *P. tritici-duri* was also common when this pathogen was inoculated onto the additional set of durum wheat cultivars, including cultivars considered resistant to *P. triticina* (Camayo, Storlom). Another difference between the two rust species was the speed with which they developed telia under our greenhouse conditions, with *P. tritici-duri* doing so 26 d after inoculation, much more rapidly than the 45 d for *P. triticina*.

Puccinia tritici-duri has been present in the western Mediterranean Basin for a long time (D´Oliveira and Samborski, 1966; Anikster *et al*., 1997), in countries such as Morocco, Portugal and Spain with traditions of planting durum wheat. Rust-infected *Anchusa azurea* (the alternate host of *P. tritici-duri*) was observed in southern Spain before 1918 (González-Fragoso, 1918), but all *A. azurea* plants inspected at different locations in Andalusia during 2020 and 2021 were free of rust. It is likely that *P. tritici-duri* had coexisted with *P. triticina* for a long time, infecting durum wheat fields. From 1992 to 2005, durum wheat acreage increased in Andalusia and other regions of Spain, due to subsidies from the European Union, and leaf rust became a serious problem beginning in 1998. Until that time, the host resistance mainly relied on two genes globally, namely *Lr72* and *Lr14a*. However, development of virulent races against these two genes made *P. triticina* a major disease in durum wheat worldwide, including Spain, where virulence to *Lr14a* was first reported in 2013 (Martínez-Moreno and Solís, 2019). By 2005, new *P. triticina* resistant cultivars were released in southern Spain, likely providing *P. tritici-duri* with enhanced opportunities to infect durum wheat.

Given the almost general and variable levels of susceptibility observed in the relatively small sets of durum wheat germplasm evaluated in southern Spain against *P. tritici-duri*, and the very limited host options available with known *R*-genes to provide complete resistance to *P. triticina*, breeders and pathologists will need to explore and find new sources of suitable resistance in wider and more diverse sets of germplasm. These resistance discovery activities will have to be conducted under conditions that ensure the exclusive presence of *P. tritici-duri,* without the confounding effects of *P. triticina*. However, before such costly initiatives are taken, it is important to accurately assess the likelihood of *P. tritici-duri* becoming a major and yield-limiting pathogen in southern Spain, and to determine the actual yield losses and economic impacts when this pathogen is present on cultivars that are otherwise resistant to *P. triticina*.

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AUTHOR CONTRIBUTIONS

FMM and ISM conceived the manuscript and designed the research. JNRV and FMM took and analyzed the data and wrote the manuscript. All authors reviewed the manuscript.

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Table 2. Distribution of leaf rust infection phenotypes at seedling and fifth leaf stages on 22 near-isogenic Thatcher lines, Thatcher, Gatcher, Atil/Local Red, and 14 durum wheat cultivars with known *R*-genes, that were inoculated with the four leaf rust races collected in this study.

Resistance gene	Virulence of races and infection type[a]			
	Puccinia tritici-duri 1	*P. tritici-duri* 2	*P. triticina* 1	*P. triticina* 2
Race nomenclature	PBDSS	BBBBB	DBBSJ	DBBTJ
Inoculations	Seedling/5th leaf	Seedling/5th leaf	Seedling/5th leaf	Seedling/5th leaf
Thatcher	4 / 3	X / 2	4 / 4	3 / 3
Thatcher-*Lr1*	4 / 3	X / 3	1 / ;	2 / 1
Thatcher-*Lr2a*	1 / 1	1 / 1	2 / 1	2 / 1
Thatcher-*Lr2c*	3 / 3	X / 1	3 / 3	3 / 3
Thatcher-*Lr3*	3 / 3	X / 2	1 / ;	1 / 1
Thatcher-*Lr9*	1 / 2	1 / 1	0 / 0	0 / 0
Thatcher-*Lr16*	2 / 2	X / 2	1 / 2	1 / 3
Thatcher-*Lr24*	; / ;	; / ;	; / ;	; / 1
Thatcher-*Lr26*	; / ;	; / ;	; / ;	; / 1
Thatcher-*Lr3ka*	1 / 2	1 / 1	0 / 0	0 / 0
Thatcher-*Lr11*	X / 3	1 / 1	1 / 1	1 / 1
Thatcher-*Lr17*	3 / 3	X / 2	; / ;	1 / 1
Thatcher-*Lr30*	2 / 2	1 / 2	2 / 2	2 / 1
Thatcher-*LrB*	4 / 3	2 / 2	3 / 3	3 / 3
Thatcher-*Lr10*	3 / 4	X / 2	3 / 2	3 / 3
Thatcher-*Lr14a*	4 / 3	X / 2	3 / 4	3 / 3
Thatcher-*Lr18*	X / 2	X / 2	2 / 2	3 / 3
Thatcher-*Lr3bg*	3 / 3	X / 1	1 / ;	1 / 1
Thatcher-*Lr14b*	4 / 2	X / 2	3 / 4	3 / 3
Thatcher-*Lr20*	3 / 3	X / 2	3 / 2	3 / 3
Thatcher-*Lr28*	; / ;	1 / ;	1 / ;	1 / 1
Thatcher-*Lr19*	1 / 2	; / 1	; / ;	; / 0
Thatcher-*Lr23*	3 / 3	2 / 2	2 / 2	3 / 3
Gatcher (*Lr27+Lr31*)	X / 3	X / 1	4 / 4	4 / 4
Atil/Local Red	4 / 3	4 / 4	4 / 4	4 / 4
Gallareta (*LrAltar*)	X / X	X / 3	3 / 3	4 / 3
Somateria (*Lr14a*)	X / X	X / X	3 / 4	3 / 3
Camayo (*LrCam*)	X / X	X / X	1 / ;	1 / ;
Colosseo (*Lr14a+*)	X / 3	X / X	4 / 4	3 / 3
Don Jaime (*Lr14a*)	X / X	X / X	4 / 4	3 / 3
Don Ricardo (*Lr27+Lr31*)	X / X	X / X	1 / ;	1 / ;
Don Javier	X / 3	X / X	2 / 2	2 / 3
Storlom (*Lr3*)	X / X	X / X	; / ;	0 / ;
Jupare (*Lr27+Lr31*)	X / X	X / X	1 / ;	1 / ;
Guayacán (*Lr61*)	X / X	X / X	; / ;	; / ;
Aconchi-*Lr19*	X / X	X / X	0 / 0	0 / 0
Aconchi-*Lr47*	X / X	X / X	; / ;	0 / ;
Cirno	X / X	X / X	1 / 1	1 / ;
Calero	1 / 1	1 / 1	1 / 1	; / ;
No. isolates	6	3	2	2

[a] Infection type assessment according to the 0–4 scale of Stakman *et al.* (1962). 0 = no macroscopic signs of infection, ; = no uredinia with hypersensitive necrotic flecks present, 1 = small uredinia surrounded by necrosis, 2 = small to medium-size uredinia surrounded by necrosis, X = mesothetic response, few but big uredinia surrounded by some necrosis, accompanied by small necrotic spots, 3 = medium-size uredinia with or without chlorosis, 4 = large uredinia without chlorosis or necrosis.

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