

EPIDEMIC OUTBREAKS OF STRIPE RUST CAUSED BY *PUCCINIA STRIIFORMIS* ON NATURAL POPULATION OF *LOLIUM PERENNE* IN TURKEY

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Abstract

Stripe rust caused by *Puccinia striiformis* was found for the first time on *Lolium perenne* L., in Turkey. Based on surveys conducted during two years, an epidemic level of the infection was observed. Disease rate and severity were measured in 20 local areas in the Malatya region within eastern Anatolia district of Turkey. Disease rates on flag, 2nd and 3rd leaves were 9.45%, 29.75% and 75.95% in 2003, and 12.10%, 35.70% and 91.75% in 2004, respectively. Disease severities on same leaves were 0.20%, 2.81% and 27.50% in 2003, and 0.41%, 3% and 30.09% in 2004, respectively. Ten accessions of the ryegrass were tested to the stripe rust under natural conditions to determine their resistance levels. Based on the averages of the upper three leaves, 7 degrees of the disease were observed in accessions with 3 resistance levels were determined in accessions. In suitable years, yellow rust appears as a restrictive agent on the natural populations of ryegrass while infected ryegrass population may institute the permanent infection sources of the disease in the district. This study exposes the host status of the ryegrass to the yellow rust disease.

Introduction

Stripe rust caused by *Puccinia striiformis* is a serious disease on cereals and grasses. With golden-yellow pustules in long, narrow stripes on leaves, leaf sheaths, glumes and awns, it can be easily differentiated from other cereal rusts. In general, symptoms of the yellow rust are not displayed in early stages of seedlings by hosts. Up to now, the function of telial stage could not be cleared and nor found an alternate host of the pathogen. Stripe rust is a micro cyclic rust disease causing important economic losses on some important members of Gramineae family.

Based on host genus infected by the pathogen, five formae speciales of the stripe rust were named by Eriksson and Henning (Line, 2002). *Puccinia striiformis* Westen. f. sp. *tritici* Eriks & Henn., causes yellow rust infection on wheat. It is one of the most damaging diseases of wheat in many district of the world (Line & Qayoum, 1992). Yellow rust has traditionally been associated with cereal production in the cool, temperate regions of Asia, Europe, North America, South America, the Middle East, Africa and Australia (Wellings & McIntosh, 1990; Mamluk, 1992; McIntosh & Brown, 1997; Boshoff *et al.*, 2002; Chen *et al.*, 2002; Line, 2002). In some regions of Turkey, epidemic outbreaks were also reported on cultivated wheat in past (Karaca, 1965; Canihos *et al.*, 1997). *P. striiformis* f.sp. *hordei*, causes stripe rust on barley and is the most destructive disease in many barley growing areas in the world (Dubin & Stubbs, 1984; Line, 2002). In addition to cereals, stripe rust also infects many grass species. Formae speciales of the pathogen associated with grasses are in the following. *P. striiformis*. f.sp. *poae* on blue grass (*Poa pratensis*); *P. striiformis*. f.sp. *dactylis* on orchard grass (*Dactylis glomerata*) (Hardison, 1984; Stubbs, 1985); *P. striiformis*. f.sp. *elymi* on (*Elymus* spp.); *P. striiformis*. f.sp. *agropyri* on (*Agropyron* spp.) (Line, 2002).

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As well as wild and cultivated cereals, these species reported were also most important hosts of the pathogen. On the other hand, the concept of the *formae speciales* was not accepted for the stripe rust pathogen by some old investigators. According to those researchers *P. striiformis* had no different forms and all stripe rust diseases' occurring on different hosts were produced by *P. striiformis*. Line (2002), based on old literature, pointed out that the host range of yellow rust includes about 320 species, known to be naturally or artificially infected. There is no research reporting the host status of the ryegrass to the stripe rust infection similar to grass species described above.

Survey investigations associated with this study were conducted on Malatya province within eastern Anatolia district in Turkey. The altitudes of the surveyed areas were between 1000-1300 m above sea level. The aim of this study was to investigate the current positions of the stripe rust on *L. perenne* by measuring the infection rate and infection severity on upper three leaves. To screen the resistance levels of some accessions to stripe rust was another goal of the current study.

Materials and Methods

Identification of the pathogen: The causal agent of the rust disease observed on *L. perenne* was identified based on macroscopic and microscopic properties. The sizes, colors and shapes of sori and pustules, and their lining on the leaf blades and leaf sheet were mainly macroscopic properties whereas shapes, colors, ornamentation and dimensions of uredospore and teliospore were microscopic properties examined for identification of the pathogen as described by Wiese (1977).

Disease assessments: Disease rate of the stripe rust on *L. perenne* was measured separately on each leaf of the upper three leaves and determined as percentage. Within Malatya province, 8, 7 and 5 local areas were chosen randomly in Malatya centre, Darende and Kuluncak counties, respectively. At least 100 number plants randomized per area were determined in 1 m² square chamber, the flag leaf and leaves 2nd and 3rd were counted as infected and non-infected leaves. Then infection rate of each leaf position was determined as percentage.

Disease severity was measured as disease index (DI), on each leaf of upper three leaves, flag leaf and the 2nd and 3rd leaves. Within Malatya province, 8, 7 and 5 local areas were determined in Malatya centre, Darende and Kuluncak counties, respectively. Twenty plants per local area were randomly determined in 1 m² square chamber and the infected area of each leaf within samples was estimated as percentage according to 0-12 scale. Then DI value for each leaf position of the top three leaves was calculated using the equation of weighted means (DI = $\sum ai/\sum n$), where *a* is the percent leaf area diseased for each leaf measured, *i* is the infected leaf frequency, and *n* is the total leaf number within the sample.

The scale 0-12 used in this study was modified from the original scale of COOB (Chester, 1950) and described as follows: 0. Scale = 0% infection, 1. Scale = 1% infection, 2. Scale = 2- 5% infection 3. Scale = 6- 10% infection, 4. Scale = 11- 20% infection, 5. Scale = 21- 30% infection, 6. scale = 31-40% infection, 7. Scale = 41-50% infection, 8. Scale = 51- 60% infection, 9. Scale = 61-70% infection, 10. Scale = 71- 80% infection, 11. Scale = 81-90% infection, 12. Scale = 91- 100% infection

In field experiments, reaction degrees of each accession to stripe rust were measured as DI as described above, on upper 3 leaves, and then used their average values. In this assessment, 20 plants were randomly chosen per plot at the growth stage (GS) 85 (Zadoks, *et al.*, 1974). After variance analyses, DI value of each accession was compared with the 0-12 scale values, as described before and its resistance levels were determined.

Accessions and reaction experiments: To determine the reaction degrees to stripe rust, 10 accessions were derived from Malatya centre, Darende and Kuluncak counties with 4, 3 and 3, respective numbers in 2003. Seeds of them were sown in plots, 1 x 2 m, scattering by hand. Experiments were established in an area where its surrounding had been covered by the naturally growing susceptible ryegrass population. Sowings planned according to Randomized Complete Block Design, were done on 10 September, 2003 and covered superficially by a hand rake. The germination of seeds, development of seedlings and inoculation of accessions were managed under natural conditions. However, a second inoculation prepared artificially was conducted on plants between GS 51 and 55 (Zadoks *et al.*, 1974). For this purpose, fresh urediniospores of stripe rust were derived from earlier naturally infected plants. Using a fine brush, urediniospores were brushed from sorus on infected leaves and adjusted to 10^5 spores per ml. After addition of 0.5g talcum powder and ten drops of Tween 20 per 1000ml, spore suspension was sprayed on plants.

Results and Discussion

During surveys conducted on ryegrass (*L. perenne*), an important rust disease was observed with typical manifestation. Golden-yellow pustules in long and narrow stripes were intensively observed on leaves and to some extent on leaf sheaths. Urediniospores were yellow to orange in colour, more or less spherical, echinulate and 28-34 μ m in diameter. Teliospores were dark brown and two celled; basal cell not symmetrical and longer than distal one. They were common on leaf blades but rarely on leaf sheaths and first observed until end of June. Based on macroscopic and microscopic characteristics described above, the disease was identified as stripe rust (Wiese, 1977).

According to measurements performed during 2003 and 2004, disease levels were variable between years. Compared with the first year, disease rate and severity were higher in the second year. Disease rates on flag and 2nd and 3rd leaves were 9.45%, 29.75% and 75.95% in 2003 and 12.10%, 35.70% and 91.75% in 2004, respectively. Disease severities values measured as Disease Index (DI) on same leaves were 0.20%, 2.81% and 27.50% in 2003 and 0.41%, 3% and 30.09% in 2004, respectively (Table 1). In some minor populations, in addition to lower leaves, upper leaves were also severely infected and dried by the severe rust pustules at the head formation or flowering stage of *L. perenne*. Compared with the uninfected ones, seeds were not filled or poorly formed in ears of severe by infected plants. It was considered that grain losses described were likely a result of this rust disease. Grain losses caused by the stripe rust on other grass species were previously reported on the blue grass (*P. pratensis*) and orchard grass (*D. glomerata*) (Röbbelen & Sharp, 1978; Stubbs, 1985; Line & Qayoum, 1992). Associated with the disease rate and severity, great heterogeneity was observed in plant populations. This heterogeneity may have demonstrated in differences in virulence of pathogen strains or in resistance of plant germplasms. The results of the reaction experiments (Table 2) may support these hypotheses to some extent. Infection was generally less on the upper leaves and became dense in the lower ones. This may imply that ryegrass population in district is resistant to some extent to the stripe rust. Because, filling of grain in Gramineae species are accomplished mostly by the flag leaf or upper two leaves (Thorne 1966). However, it may form a permanent inoculum source of the disease. Since the host specialisation of stripe rust is generally low, an inoculum derived from one host can be infective on other hosts as reported by Chen *et al.*, (1993).

Table 1. Disease rate (DR) values of stripe rust on upper three leaves of 100 plants per area.

No	2003								2004							
	Leaves															
	Flag		2 nd		3 rd		Flag		2 nd		3 rd		DR		DI	
Malatya	1	11	0.3	32	2.2	70	19.2	19	0.7	49	4.5	100	39.7			
	2	9	0.2	26	1.7	72	20.8	12	0.4	38	3.1	86	20.7			
	3	7	0.0	28	2.6	71	21.5	15	0.6	43	4.6	95	27.9			
	4	4	0.0	19	0.8	63	13.8	13	0.4	34	1.7	94	27			
	5	6	0.5	30	2.5	77	27.7	16	0.8	37	1.3	98	34.3			
	6	8	0.0	24	3.1	75	26.2	11	0.0	31	1.6	88	23.4			
	7	12	0.3	32	1.7	84	32.3	5	0.0	29	1.5	84	22.5			
	8	20	0.1	38	4.0	86	33.8	8	0.1	39	3.5	92	26.1			
Darende	9	19	0.2	42	5.0	92	34.6	7	0.5	35	2.1	93	28.8			
	10	5	0.0	17	1.2	65	18.5	3	0.0	27	3.1	80	21.6			
	11	2	0.0	22	1.7	69	21.5	6	0.3	36	3.5	94	38.8			
	12	13	0.4	35	3.0	86	29.2	12	0.4	40	3.6	96	35.2			
	13	5	0.1	29	2.6	77	26.2	13	0.4	33	3.1	97	37.9			
	14	18	0.8	47	6.0	90	35.4	9	0.1	36	4.5	81	27.0			
	15	11	0.5	38	4.1	76	30.8	13	0.6	35	2.7	89	33.4			
Kuluncak	16	2	0.0	19	1.3	62	26.2	15	0.7	31	2.2	96	28.8			
	17	4	0.0	23	2.7	71	27.7	20	0.8	47	5.0	100	43.3			
	18	9	0.0	27	3.5	79	38.5	15	0.3	23	1.6	85	25.2			
	19	14	0.5	36	4.6	80	36.9	9	0.2	20	1.7	87	27.7			
	20	6	0.0	31	2.5	74	29.2	21	0.8	51	6.0	100	32.4			
Average		9.45	0.20	29.75	2.81	75.95	27.50	12.1	0.41	35.7	3.0	91.75	30.09			

Table 2. Average DI values of upper three leaves of each accession, their groups and scale levels to stripe rust under field experiments.

Access No	DI + SE	Scale values
L 1 -Malatya centre	$23.26 \pm 1.28\text{cd}^*$	5
L 2 -Malatya centre	$27.47 \pm 1.24\text{d}$	5
L 3 -Malatya centre	$28.38 \pm 1.07\text{d}$	5
L 4 -Malatya centre	$17.33 \pm 1.04\text{bc}$	4
L 5 -Darende	$19.05 \pm 1.22\text{c}$	4
L 6 -Darende	$8.14 \pm 0.43\text{ab}$	3
L 7 -Darende	$26.53 \pm 1.25\text{d}$	5
L 8 -Kuluncak	$13.88 \pm 1.12\text{b}$	4
L 9 -Kuluncak	$5.55 \pm 0.36\text{a}$	3
L 10 Kuluncak	$24.73 \pm 1.65\text{cd}$	5
Average	19.43	4

*It is significant at $p<0.05$.

In field experiments, 7 different severity values of the disease were displayed by accessions. However, when DI values were matched with the 0-12 scale, a total of three resistance level were observed. According to the average of DI values, the maximum infection level, displayed by the L3 accession in fifth scale, was 28.38% while the minimum disease level, exhibited by the L9 accession in the third scale, was 5.55%. In experiments, disease levels and scale values of other accessions were between these two categories (Table 2).

As a result, the stripe rust infection appears as a restrictive agent on the natural production of susceptible ryegrass in district in epidemic years. On the other hand infected population of this plant, due to perennial properties, may form the permanent infection sources of the stripe rust in this region. According to reports, pathogen survives during the summer on wild grasses at high elevations, and spores from those grasses are the primary sources of inoculum for fall-planted wheat. Line (2002) reported many susceptible hosts for stripe rust in the grass species based on results of earlier researchers. More species were also added in later years (Halisky *et al.*, 1962; Hendrix *et al.*, 1965). According to these authors, *Aegilops*, *Agropyron*, *Bromus*, *Elymus*, *Hordeum*, *Secale* and *Triticum* were the most important genera including host species of the yellow rust. They also stressed that grass species described could be sources of stripe rust inoculum. Tollenaar & Houston (1966) reported, based on 40 years of collections that stripe rust occurred on wild *Hordeum*, *Elymus* and *Sitanion* species in the San Francisco area, the coastal mountains and valleys from San Francisco to the Oregon border and the Sierra Nevada. The climate in some region of Turkey also appears very suitable for stripe rust, since epidemic outbreaks have been reported on wheat earlier (Karaca, 1965; Canihos, *et al.*, 1997). However, ryegrass has not been consider as an important host for stripe rust, similar to above grass species up to now. Therefore, the current study may become important since the host status of the ryegrass was firstly investigated in a region of Turkey.

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