European Journal of Plant Pathology (2006) 115:357–361
DOI 10.1007/s10658-006-9016-5

Short Communication

Rhamnus lycioides in Tunisia is a new aecial host of oat crown rust

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Accepted 11 April 2006

Key words: aeciospores, pathogenic variability, *Pc*-gene lines, *Puccinia coronata* f. sp. *avenae*

Abstract

Puccinia coronata was not previously described on *Rhamnus* spp. in Tunisia. Three sites in the northwest of Tunisia, where *Rhamnus* is reported to be abundant, were surveyed for the presence of pycnia and aecia of oat crown rust caused by *Puccinia coronata* f. sp. *avenae*. Two *Rhamnus* species (*R. lycioides* and *R. alaternus*) were encountered in the sites. Pycnia with viable pycniospores and aecia with viable aeciospores were found on *R. lycioides*. However, no characteristic structures of crown rust were found on *R. alaternus*. Aeciospores collected from leaves of *R. lycioides* were used to inoculate oat plants usually susceptible to oat crown rust. Typical uredinia containing oat crown rust urediniospores appeared on the leaves of these plants. Moreover, the sixteen *Pc*-gene differential oat lines, used by oat researchers to study the virulence pattern in oat crown rust populations, were artificially inoculated with aeciospores from *R. lycioides*. These inoculated lines showed resistance/susceptibility similar to the registered resistance level of these lines to crown rust under field conditions in Tunisia. These results indicate that *R. lycioides*, a common and endemic part of the vegetation in the northwest of Tunisia, is a new aecial host of oat crown rust. The aeciospores produced on this forest plant could constitute the source of the virulence diversity already detected via the *Pc*-gene line trials.

Oat crown rust, caused by *Puccinia coronata* f. sp. a*venae*, is considered the most widespread and damaging disease of oats (*Avena sativa*) in Tunisia. The most important oat crown rust resistance sources used elsewhere are based on a set of 16 *Pc*-gene lines exhibiting race-specific resistance (Chong et al., 2000). These 16 single gene oat lines, with seedling resistance genes *Pc38*, *Pc39*, *Pc40*, *Pc45*, *Pc46*, *Pc48*, *Pc50, Pc51*, *Pc52*, *Pc54*, *Pc56, Pc58*, *Pc59*, *Pc62*, *Pc64* and *Pc68* are used to reveal the diversity in virulence phenotypes of oat crown rust. Trials conducted in Tunisia on these *Pc*-gene lines during three cropping seasons (2000, 2001 and 2002) showed that the majority of the lines were susceptible to Tunisia's oat crown rust populations (Allagui et al., 2002). The limited durability of the *Pc*-gene resistance is strongly related to frequent changes in virulence phenotypes of the pathogen. In North America and northern Europe, *R. cathartica* is the most important aecial host for the sexual stage of *Puccinia coronata* serving not only as a source of primary inoculum, but also as the origin of new pathogenic races through genetic recombination (Simon, 1985; Leonard, 2002). *Rhamnus cathartica* has not been reported as a native forest plant in Tunisia (Pottier-Alapetite, 1979). The objective of this investigation was to examine the *Rhamnus* species in the regions where oats are usually infected and to determine if these species are aecial hosts for oat crown rust.

A phytoecologic Tunisian map (Schonenberger and Gounot, 1965) was used to localize the areas of *Rhamnus* species. Three sites where *Rhamnus* is abundant in the northwest of the country were selected to determine the presence of pycnia and aecia on the leaves. These sites, about 60 km from each other were : (1) El Guelta Essafra (close to a nursery of forest plants, 17 km from Tabarka), (2) A site 33 km off the Nefza – Beja road (Aouled Ahmed locality) and (3) Sedjnen (near the INRAT station). Surveying was done in these areas during March, April and May 2003 and 2004. Leaves, flowers, fruits and stems of *Rhamnus* plants were examined to identify the species according to the Pottier-Alapetite (1979) and Bonnier (1964) descriptions. Particular fungal structures such as pycnia and/or aecia collected from the leaves were also analysed microscopically in the laboratory and compared to the Viennot-Bourgin (1949) rust classification.

Two *Rhamnus* species were found in the areas surveyed: *R. lycioides* (common name: Ktam) and *R. alaternus* (common name: Ibaggas). These evergreen shrubs or small trees are dioecious plants characterized by deciduous leaf-like pustules, simple leaves, regular flowers having little or no developed petals and fused sepals. The fruit is a drupe with 2–4 seeds. *Rhamnus lycioides* is less than 3 m in height in El Guelta Essafra and shorter (50 cm to 2 m) in Aouled Ahmed. Their leathery and glabrous leaves are pointed at the tip with only one prominent midrib on the abaxial side, from which smaller lateral veins branch off in a network pattern. This species opens its yellowish to greenish sessile flowers in May–June. Their immature fruits are green becoming yellowish green at maturity. *Rhamnus alaternus* is less than 4 m tall having erect stems, tough leaves with 2–3 prominent lateral veins on the abaxial leaf surface, small flowers on small bracts and red fruits turning black during ripening.

No characteristic structures of crown rust were found on the numerous *R. alaternus* plants observed (almost 100 trees per site). On *R. lycioides*, brown to dark circular spots were found on the upper surface of the leaves. The diameter of these spots varied from 1 to 5 mm (Figure 1). On a binocular microscope, 1–4 black points were seen on the surface of each spot. Microscopic observation showed that these points were pycnia (30 μm diam) containing typical pycniospores (Figure 2). In addition, orange-yellow pustules with aecia were found, mainly on the abaxial side of the leaves as well as on young shoots and foliar buds. Many young leaves with numerous aecia were hypertrophied in a blister-like manner (Figure 3). The aecia were surrounded by peridia and contained typical aeciospores formed in chains (catenulate spores). The aeciospores were yellow, strongly verrucose, 22.5–32.5 $\times$ 15–20 μm having a cell wall 2.5 μm thick. Cytoplasm and cell walls of these aeciospores showed a great morphological similarity to previously published descriptions of these spores (Weber and Davoli, 2002; Littlefield et al., 2005). Figure 4 illustrates aeciospores before and after germination in distilled water for 4 h at 20 °C. Some aecia were found on *R. lycioides* leaves at the beginning of March, but primary infection occurred in April. In late May, as the weather becomes dry and warmer, the infected organs darken before withering and death. Several *R. lycioides* plants were highly infected, but these infected plants were relatively rare compared to healthy ones. For example, only three infected plants were found in an area of almost 40 m^2 at the Aouled Ahmed site. These results suggest the presence of both resistant and susceptible plants of *R. lycioides*.

Aeciospores collected in a mixture from the leaves of several *R. lycioides* plants using a spore collector (Geoff Harms, University of Minnesota) were used within 2 days to inoculate the seedling stage (two true leaves) of 16 *Pc*-gene oat lines and the susceptible oat variety Av.95 (Table 1). These aeciospores were suspended in 250 ml distilled

Figure 1. Pycnia on *R. lycioides* leaves. Bar = 1 cm.

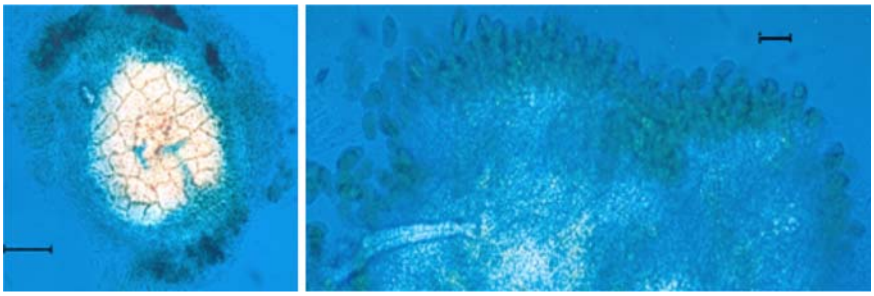

Figure 2. Microscopic observation in lactophenol cotton blue stain of the pycnia (left) and pycniospores (right) from *R. lycioides* leaves. Bar (left) = 10 μm, bar (right) = 1 μm.

water mixed with a drop of Tween-20 surfactant. The concentration of aeciospores was around 82,000 ml^{-1}. A sample (10 ml with three replications) from the spore suspension was incubated for 4 h at 20 °C to test the viability of the aeciospores. This test showed that the average germination of these aeciospores was 19.6%. Plant inoculation performed in May was made with a hand driven atomizer (1 l) by spraying the aeciospore suspension above the seedlings to form numerous droplets on the leaf surface until run-off. After inoculation, seedlings in pots maintained at near-saturation were incubated for 24 h in the dark at 16–20 °C then kept under greenhouse conditions (temperature 20–24 °C, photoperiod 14 h and relative humidity 70%). Infected plants were scored 11 days after inoculation (May 17, 2004) using a scale ranging from 0 to 4 (Chong et al., 2000). On this scale: 0 = no uredinia or other macroscopic signs of infection; ; = no uredinia, but necrotic or chlorotic flecks; 1 = small uredinia surrounded by chlorosis or necrosis; 2 = small to medium-size uredinia in chlorotic areas; 3 = medium-size urdinia in chlorotic areas and 4 = large uredinia without necrosis or chlorosis. This experiment was done to demonstrate the pathogenicity of the aeciospores on various oat genotypes.

Four to seven days after inoculation with aeciospores collected from *R. lycioides*, well-developed uredinia with typical oat crown rust urediniospores appeared on the leaves of the susceptible oat cv. Av 95. The *Pc*-gene oat lines inoculated with these aeciospore populations produced different reaction types 11 days after inoculation (Table 1). Lines *Pc40*, *Pc45*, *Pc46*, *Pc48*, *Pc52*, *Pc54*, and *Pc56* were highly susceptible showing large uredinia without chlorosis or necrosis (infection type 4). Lines *Pc39*, *Pc50*, *Pc51*, *Pc59* and *Pc64* were slightly less susceptible with medium-size uredinia in chlorotic area (infection type 3). Lines *Pc58* and *Pc62* were moderately resistant (infection type 2) with small uredinia. The

Figure 3. Aecia and aeciospores on leaves and stem of *R. lycioides*. Note the blister and deformation produced by the fungus. Bar = 1 cm.

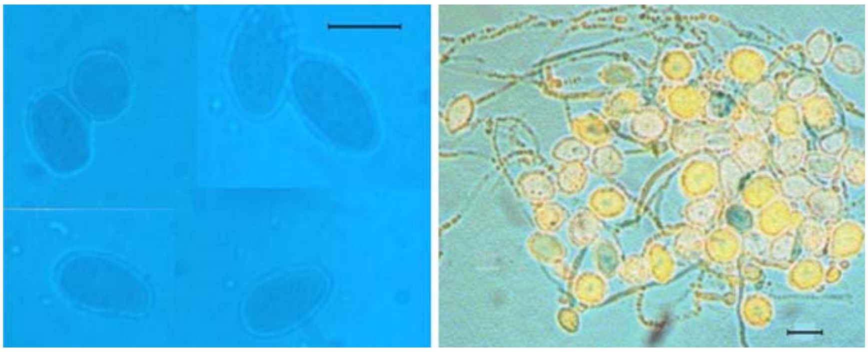

Figure 4. Microscopic observation of aeciospores stained by lactophenol cotton blue, (left) before germination and (right) after germination in distilled water 4 h after incubation at 20 °C. Bar (left) = 15 μm, bar (right) = 20 μm.

lines *Pc38* and *Pc68* were highly resistant to these aeciospores populations (no pustules), although some necrotic flecks developed on the *Pc38* line.

Our survey in 2003 and 2004 in three sites in northwest Tunisia showed the presence of two *Rhamnus* species, *R. lycioides* and *R. alaternus*. Pycnia and aecia were found on *R. lycioides* during the spring (March, April and May). The infected young leaves and twigs showed hypertrophic blister-like deformations. Aeciospores collected from *R. lycioides* produced the typical symptoms of oat crown rust on susceptible oat plants. This result suggests that *R. lycioides* in Tunisia is a new aecial host of oat crown rust involved in the infection of oats. This aecial host may release aeciospores with pathogenic diversity that might overcome oat crown rust resistance genes in commercial oat fields. Unlike *R. cathartica* in other countries, little is known about *R. lycioides* as an aecial host of oat crown rust. In the Mediterranean region, *R. lycioides* can be found in the mountains of North Africa, France, Sicily and Greece, but this species is poorly documented as an aecial host of oat crown rust in these countries mainly because of a lack of surveying the oat crown rust fungus on the forest *Rhamnus* species. Rieuf (1971) indicated that *R. lycioides* was an alternate host of *P. coronata* in Morocco without any information concerning other countries such as Tunisia. Simon (1985) pointed out that *R. frangula* and *R. lanceolata* in the USA were occasionally reported as oat crown rust alternate hosts and Cornu (1880) obtained heavy infection on young oat plants using aeciospores from *R. cathartica* and *R. oloides*. During our survey, no characteristic structures of crown rust were found on *R. alaternus* in Tunisia, although this species together with *R. palaestina* were reported as aecial hosts of oat crown rust in the Palestine region (Rayss and Hebelska, 1942; Rayss, 1951, in Anikster and Wahl, 1979).

A spore suspension from different aecia was used in a mixture in the inoculation experiments in order to confirm the pathogenicity of the aeciospores on oats and the ability to attack differentially the 16 *Pc*-gene oat lines used as reference material in oat crown rust genetic resistance. Our objective was not to select from a single aecium more or less virulent aeciospores to particular

Table 1. Reaction of differential *Pc*-gene oat lines at the seedling stage in the greenhouse to inoculation with aeciospores collected from *Rhamnus lycioides*

Pc-gene lines	Infection type*
Pc38	;
Pc39	3
Pc40	4
Pc45	4
Pc46	4
Pc48	4
Pc50	3
Pc51	3
Pc52	4
Pc54	4
Pc56	4
Pc58	2
Pc59	3
Pc62	2
Pc64	3
Pc68	0
Av 95	4

*Reactions were scored according to 0–4 scale. ; No uredinia, but necrotic or chlorotic flecks.

Pc-gene oat lines. It was found that reaction of the *Pc*-gene oat lines to inoculation with the populations of aeciospores was not significantly different from the reactions registered in natural urediniospore oat crown rust infections (Allagui et al., 2002; Allagui and Chakroun, 2004). This suggests a virulence similarity between the populations of aeciospores and that of the uedinospores, as seen with the reaction of the *Pc*-gene oat lines. Such virulence similarity on different single-gene oat lines was also reported for uredinial isolates from cultivated oats and aecial isolates from *R. cathartica* in natural habitats (Leonard et al., 2004).

From an epidemiological point of view, urediniospores on volunteer and wild oat plants could initiate oat crown rust epidemics as early as November because early plantings are highly receptive to early oat crown rust attack, particularly in Tunisia's coastal area away from the mountains harbouring the *Rhamnus* species (Allagui et al., 2002). However, in the northwest of Tunisia (Beja and Jendouba), aeciospores are likely to initiate oat crown rust epidemics because the disease frequently appears on commercial oats in April and May when aeciospores are released.

Acknowledgements

We thank D. D. Stuthman (Department of Agronomy and Plant Genetics, University of Minnesota) for reviewing this manuscript.

References

Allagui MB and Chakroun M (2004) Oat crown rust in Tunisia in 2003. Proceedings, 7th International Oat Conference, Helsinki, July 17–23, p 180, in electronic version: http://www.mtt.fi/met/pdf/met51.pdf.

Allagui MB, Ahmed AS and Chakroun M (2002) La rouille couronnée de l'avoine en Tunisie; effet de la date de semis et efficacité des gènes *Pc* de résistance. Annales de l'INRAT 75: 205–221.

Anikster Y and Wahl I (1979) Coevolution of the rust fungi on gramineae and liliaceae and their hosts. Annual Review of Phytopathology 17: 367–403.

Bonnier G (1964) Flore complète illustrée en couleur de France, Suisse et Belgique. Paris – Librairie Générale de l'Enseignement, E. Orlhac. Editeur, 721 pp.

Chong J, Leonard KJ and Salameron JJ (2000) A North American system of nomenclature for *Puccinia coronata* f. sp. *avenae*. Plant Disease 84: 580–585.

Cornu MM (1880) Notes sur quelques parasites des plantes vivantes: Générations Alternantes, Pezizes à sclérotes. Bulletin de la Société Botanique de France 27: 209–210.

Leonard KJ (2002) Oat lines with effective adult plant resistance to crown rust. Plant Disease 86: 593–598.

Leonard KJ, Anikster Y and Manisterski J (2004) Patterns of virulence in natural populations of *Puccinia coronata* on wild oat in Israel and in agricultural populations on cultivated oat in the United States. Phytopathology 94: 505–514.

Littlefield LJ, Marek SM, Tyrl RJ and Winkelman KS (2005) Morphological and molecular characterisation of *Puccinia lagenophorae*, now present in central North America. Annals of Applied Biology 147: 35–42.

Pottier-Alapetite G (1979) Flore de la Tunisie. Angiosperme-Dicotylédone *Apétales-Dialypétales. Faculty of science, El Manar, Tunisia, 651 pp.

Rayss T (1951) Nouvelle contribution à la connaissance des Uridinées de Palestine. Uredineana 3: 154–221.

Rayss T and Habelska H (1942) Rhamnus palaestina Boiss. – a new host of crown rust. Palestinian Journal of Botany Jerusalem 2: 250.

Rieuf P (1971) Parasites et saprophytes des plantes au Maroc. Les Cahiers de la Recherche Agronomique 30: 520.

Shonenberger A and Gounot M (1965) Carte phytoécologique de la Tunisie septentrionale: feuille III: Région de Tabarka – Souk el Arba.

Simon MD (1985) Crown rust. In: Roelfs AP and Bushnell WR (eds.) The Cereal Rusts (pp. 131–172) Academic Press, Orlando, FLVol. 2.

Viennot-Bourgin G (1949) Les champignons parasites des plantes cultivées, Masson & Cie, France, 1850 pp.

Weber RWS and Davoli P (2002) Autophagocytosis of caretonoid-rich lipid droplets into vacuoles during aeciospore ageing in *Puccinia distincta*. New Phytologist 154: 471–479.