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SHORT NOTES

Occurrence of a carboxin-resistant strain of *Ustilago nuda* in Italy

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Summary. Loose smut of barley, caused by *Ustilago nuda* (Jens.) Rostr., is commonly controlled through the use of systemic seed-treatment fungicides. The most widely used active ingredient in these fungicides has been carboxin. However, isolates of *U. nuda* resistant to carboxin have been reported in France. In 1996 and 1997, unsatisfactory levels of loose smut of barley were observed near Perugia (central Italy), despite the treatment of the barley seed with a carboxin-based fungicide. An isolate of *U. nuda* (97-255) was collected from this field to determine if the pathogen had developed resistance to carboxin. Germination tests on carboxin-amended agar media indicated that isolate 97-255 was more resistant to carboxin than a wild type isolate of *U. nuda* collected in Canada. In tests in which isolate 97-255 was inoculated onto barley cv. Regal, the percentage of smutted plants arising from the inoculated seed was not reduced by a carboxin seed treatment. This is the first report of resistance to carboxin in populations of *U. nuda* from Italy.

Key words: fungicide resistance, loose smut, barley, *Hordeum vulgare*.

Introduction

Ustilago nuda (Jens.) Rostr., the causal agent of loose smut of barley (*Hordeum vulgare* L.) is a common world-wide seed-transmitted pathogen (Punithalingam and Waterston, 1970; Vánky, 1994). The mycelium localized in the embryo spreads systemically and asymptomatically in the developing plant and during flowering, the inflorescence is largely replaced by sori containing teliospores of the fungus. The percentage of yield loss caused by *U. nuda* is considered to be equal to the percentage of smutted heads in the barley crop (Semeniuk and Ross, 1942). Losses attributable to loose smut of barley in modern times are generally <1%, but losses of 15 to 25% can occur (Punithalingam and Waterston, 1970; Menzies *et al.*, 1997) if proper control practices are not used. This disease can be effectively controlled through the use of certified seed, smut-free seed (as determined using an embryo infection test [Rennie, 1982]), resistant host cultivars, or by applying systemic seed-treatment fungicides (Bailey *et al.*, 2003).

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Arguably the most effective and widely used active ingredient in systemic seed-treatment fungicides active against *U. nuda* since the 1970s has been carboxin (5,6-dihydro-2-methyl-1,4-oxathin-3-carboxanilide) (Von Schmeling and Kulka, 1966). However, carboxin-resistant field isolates of *U. nuda* were detected in fields of barley cv. Viva in France in 1984 and 1985 (Leroux, 1986; Leroux and Berthier, 1988; Newcombe and Thomas, 1991). These isolates were originally collected in fields in which the effectiveness of a Vitavax SS (active ingredient: carboxin) seed treatment was unsatisfactory. They were initially detected as being carboxin-resistant through a teliospore germination assay (Leroux, 1986; Leroux and Berthier, 1988) and then confirmed as resistant by an *in planta* assay (Newcombe and Thomas, 1991).

In 1996 and 1997, fields of barley near Perugia (central Italy), grown from seed treated with carboxin on the farms by the growers, showed unsatisfactory levels of loose smut (Cappelli, unpublished data) and the harvested seed had >10% infection as determined using the embryo test.

In this manuscript, we summarize the results obtained in an investigation on the carboxin resistance of a strain of *U. nuda* collected in Italy.

Materials and methods

An isolate of *U. nuda*, 97-255, was collected from a barley field near Perugia in central Italy, and compared with a carboxin-sensitive isolate, 72-66, from Canada, and a carboxin-resistant isolate, Viva (Newcombe and Thomas, 1991) from France, using the teliospore germination assay of Leroux (1986) and Leroux and Berthier (1988). Teliospores of each isolate were streaked onto half-strength potato dextrose agar amended with 0, 0.10, 0.25, 0.50, 0.75, 1.0 and 1.25 μg ml^{-1} of carboxin. The cultures were incubated at 20°C in a controlled environment chamber and examined for teliospore germination at 24 h. This procedure was repeated twice.

An *in planta* study was conducted with isolates 72-66, Viva and 97-255. The three *U. nuda* isolates were increased simultaneously to equalize their viability and physiological status. Twenty spikes of barley cv. Regal (a universal suscept for *U. nuda*) were inoculated with each isolate at anthesis by injection of a water suspension of teliospores (1×10^{-3} g of teliospores l^{-1}) into the florets (filling the florets) using a 5 ml syringe with a 21–24 gauge, 2.5 cm needle. The inoculated seeds were harvested at maturity and uninoculated seeds of cv. Regal were added to bring the weight of the seed to 60 g. Ten grams of the seed was removed and kept separate as a control. The remaining 50 g was treated with carboxin (Vitavax SS) at a rate of 240 ml 100 kg^{-1}, the recommended rate in Canada and Italy. Each carboxin-treated seed lot was found to be properly treated with the fungicide (was coated with the minimum recommended label rate) when tested by Gustafson (Calgary, Alberta, Canada). For each of the three isolates, fungicide-treated and untreated seeds were sown in soil beds in greenhouses and grown to the flowering stage. Plants were scored as infected if they produced one or more infected spikes. This procedure was repeated three times. Differences in the percentage of smutted heads between the fungicide-untreated and treated seed lots for each isolate were compared using χ^2 analysis.

Results and discussion

A qualitative difference was observed between the Viva and 97-255 isolates on the one hand and the carboxin-sensitive 72-66 isolate on the other. After incubation on agar with a carboxin concentration of 0.1 μg ml^{-1} for 24 h, teliospores of isolates Viva and 97-255 had germinated, developed a long promycelium and showed subsequent mycelial growth, while those of isolate 72-66 germinated to form a short promycelium, but then did not develop further. At a carboxin concentration of 1.0 μg ml^{-1}, the teliospores of isolates Viva and 97-255 germinated, produced a long promycelium and initiated some branching at the end of the promycelium, but teliospores of 72-66 did not germinate.

Significant differences in the percentage of smutted heads of carboxin-treated and untreated plants were observed for *U. nuda* isolate 72-66 in all runs of the *in planta* experiments (Table 1). These results confirmed the results of Newcombe and Thomas (1991) who found isolate 72-66 to be sensitive to carboxin seed treatment.

Significant differences in the percentage of smutted heads of carboxin-treated and untreated plants were observed for *U. nuda* isolate Viva in three of the four runs of the *in planta* experiments (Table 1). Isolate Viva was reported to be resistant

Table 1. Percentage of plants with smutted heads arising from seed of barley cv. Regal infected with *Ustilago nuda* isolates 72-66, Viva or 97-255, untreated or treated with carboxin (Vitavax SS).

Isolate of *U. nuda*	No. of uninfected plants, untreated	No. of infected plants, untreated	Smutted plants, untreated %	No. of uninfected plants, carboxin treated	No. of infected plants, carboxin treated	Smutted plants, carboxin treated %	χ^2
Run 1							
72-66	298	20	6.3	550	3	0.5	$P<0.0001$
Viva	316	22	6.5	600	10	1.6	$P<0.0001$
97-255	213	12	5.3	429	34	7.3	$P<0.3221$
Run 2							
72-66	200	9	4.3	344	1	0.3	$P<0.0006$
Viva	178	16	8.3	334	11	3.2	$P<0.0098$
97-255	141	4	2.8	274	16	5.52	$P<0.1953$
Run 3							
72-66	182	14	7.1	448	3	0.7	$P<0.0001$
Viva	164	13	7.3	338	25	6.1	$P<0.5582$
97-255	155	21	11.9	284	41	12.6	$P<0.8245$
Run 4							
72-66	146	14	8.8	444	3	0.7	$P<0.0001$
Viva	192	13	6.3	505	11	2.1	$P<0.0045$
97-255	201	13	6.1	329	10	3	$P<0.0730$

to carboxin by Newcombe and Thomas (1991). Isolate Viva was not as sensitive to carboxin seed treatment as isolate 72-66, as can be seen in the third run of these experiments, the lower levels of significance of Viva in runs 3 and 4, and the results of the teliospore germination tests. The unexpected carboxin sensitivity *in planta* of isolate Viva may be caused by a reduction (or loss) of resistance caused by long term storage and its occasional increase on untreated seed, but also by the incomplete dominance of inheritance of carboxin resistance in *U. nuda*, as suggested by Newcombe and Thomas (2000).

There were no significant differences for the percentage of smutted heads between carboxin-treated and untreated plants inoculated with *U. nuda* isolate 97-255 in all *in planta* experiments (Table 1), indicating a resistance (or insensitivity) to carboxin.

Carboxin-resistant isolates of *U. nuda* were first reported from France in 1984 and 1985 (Leroux, 1986; Leroux and Berthier, 1988; Newcombe and Thomas, 1991). The current study is the first report of carboxin resistance in Italian populations of this pathogen; however, its presence prior to this study cannot be excluded. The carboxin-resistant strains of the pathogen may have been imported with infected barley seed from other countries, but it is also possible that resistance to carboxin in the Italian population of *U. nuda* may have arisen *de novo* under selection pressure from the common or incorrect use of carboxin-based fungicide seed treatments.

Generally, the risk of *U. nuda* developing resistance to carboxin has not been considered high and appropriate disease management strategies must be utilized in order to keep fungicide resistance under control in pathogen populations. There are other fungicide-active ingredients that can be used as seed treatments for the control of *U. nuda* on barley. A number of these are triazole-type chemicals (tebuconazole, triadimenol, propiconazole, difenoconazole, triticonazole) belonging to the ergosterol demethylation inihibitors (DMIs). However, resistance to the DMIs has been reported for a number of ascomycete and basidiomycete fungi (Russell, 1995), including *U. maydis* (Wellmann *et al.*, 1996). It is likely that if DMIs were used as the

sole fungicide seed treatment for control of *U. nuda* on barley, resistance to them would arise in the pathogen population. To avoid the loss of fungicide seed treatments as an effective tool to control loose smut of barley, a fungicide rotation scheme (applying different seed treatment fungicides from year to year) in which carboxin seed treatments are used in rotation with DMI seed-treatments would be highly recommended. The choice of the various fungicides in such a scheme would have to be made with care, however, as there are reports of cross resistance of carboxin resistant isolates of *U. nuda* and *U. hordei* with other fungicide active ingredients (Ben-Yephet *et al.*, 1974; Leroux and Berthier, 1988). Finally, growers should be persuaded to employ only commercial fungicide mixtures and to use appropriate machinery for seed treatments on their own farms for control of *U. nuda*.

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