Screening of Winter Barley Varieties (*Hordeum vulgare*) for Resistance against Loose Smut (*Ustilago nuda*) and Covered Smut (*Ustilago hordei*) in Germany

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Abstract: Because of the interdiction of chemical seed treatment in organic farming, resistance is a main demand for organic seed production. Only little was known about the resistance of winter barley against loose smut (*Ustilago nuda*) and covered smut (*Ustilago hordei*) in modern varieties in Germany. Due to this, 110 commercially available varieties were tested for resistance against *Uh* by means of artificial infection. 274 varieties (including lines from the world collection of the Federal Centre for Breeding Research on Cultivated Plants, Aschersleben) were tested for resistance against *Un* by artificial infection as well. Against *Uh* seven commercially available varieties showed complete resistance and 23 varieties remained below 0.05%. Overall winter barleys tested for *Un*-resistance, the classified damage (0, > 0–1, > 1–5, > 5–10 and > 10%) was 17.2, 23.4, 39.8, 13.1 and 6.5% for natural infection and 9.5, 0.7, 2.6, 5.1 and 82.1% for artificial infection. Only one commercially available variety showed complete resistance under artificial and natural infection. Without damage under natural infection remained two commercially available varieties.

Keywords: winter barley; loose smut; covered smut; resistance

Up to now organic farmers depend greatly on conventionally bred and produced varieties of barley. A turning point was set in 2004 by EU regulation No. 1452/2003 restricting the use of conventionally propagated seed and planting material for organic agriculture. Concerning smut fungi in barley, conventional seed producer's attention was rarely directed to plant resistance due to the possibility of chemical seed treatment (controlling the diseases completely). A main problem for organic seed producers is that organically produced seeds have to fulfil the same regular phytosanitary requirements like those of conventional origin. For the production of certified seeds not more than five ears infected with *Ustilago hordei* (*Uh*) and/or *U. nuda* (*Un*) are allowed on an area of 150 square meters in Germany (Rutz 1998). Though warm or hot water treatment can give excellent control of *Un* and *Uh* in organic farming (Winter *et al.* 1996), the effect is not sufficient for organic seed production. Even biological control agents (for example Tillecur®) cannot reach the demands of the guidelines reliably. As an effective way to keep the restrictions remains the cultivation of resistant varieties. Main goal of this study was to screen winter barley varieties for their degree of smut resistance in Germany. It started in 2000 (Klause & Spiess 2003) and is sponsored within the Federal Organic Farming Scheme since 2002. Beside this, commercially available varieties were tested for their resistance against covered smut.

MATERIAL AND METHODS

Ustilago hordei. For infection with *Uh*, one gram of spores per kg of seed was used for inoculation by adding the dry spores to the seeds in a glass container (2003) or a plastic bag (2004) and shaking them to disperse the spores. In 2002/03, the screening for *Uh*-resistance took place at two

locations (D-29490 Neu Darchau, D-61118 Bad Vilbel). Plot sizes were 2.4 m^2, resp. 2.25 m^2 in three replications at both sites. In 2004/05, the testing was repeated only for the favourites and the updated range of commercially available varieties near Bad Vilbel. The plot size was increased to eight square meters, three replications again. The number of tested varieties was 91 in 2002/03 and 85 in 2004/05.

Ustilago nuda. Main work with *Un* took place near Bad Vilbel. Inoculation was performed following Poehlmann (1945) by injecting an aqueous teleutospore suspension directly in the ears at the beginning of flowering of each variety individually, when pollen were green or yellowish green. Spores and water were mixed due to the fact, that a slightly dark coloured suspension facilitates the visual control of the inoculation effort, especially for six-rowed varieties (1–2 infected ears per 100 ml of water). To imitate an inoculation under natural conditions by wind as well, plots with the infected early flowering variety IGRI (even used for spore suspension) became interspersed within the plot arrangement. Furthermore, spore-suspension was sprayed all over the field with a backpack sprayer repeatedly. Plot size was 1.5 square meters (rows 1–6). Artificially infected ears (5–8 per plot) were marked and protected by a paper bag and handpicked at harvest. In autumn the artificially inoculated seeds were sown separately (row 1) from those infected by spore drifting via wind (rows 2–6). The number of tested lines was 274 for the entire study period (including varieties from the world collection of the Federal Centre for Breeding Research on Cultivated Plants, Aschersleben).

To evaluate the virulence of the inoculated spores of *Un* (originating from the testing location) in 2004 a number of 15 winter barley varieties were artificially infected with the home race of *Un* (DE-61118 Bad Vilbel) and by comparison with spores from three other distant locations in Germany individually. The provenances of the spores and the tested varieties are listed in Table 2.

The degree of resistance for both diseases was recorded at harvest as percentage of infected ears, which is more accurate than counting infested plants (Shchelko 1975).

RESULTS

The results show great variation in susceptibility to smut. Most of the tested varieties or lines were susceptible, but some varieties had a moderate level of resistance and only a few were highly resistant. Resistance against *Uh* goes not together with resistance against *Un*.

Ustilago hordei. Against *Uh* only two currently available commercial varieties showed complete resistance in both test years (Table 1, Alissa, Uschi). Among those varieties first available in the second testing year, another five showed no visible symptoms (Campanile, Maximiliane, Mercedes, Spectrum, Verticale). Out auf 33 varieties with damage below 0.05%, only 23 were commercially available and could be candidates for a covered smut risk minimized organic cultivation: Allegra, Antalya, Bombay, Cabrio, Camera, Carat, Car-

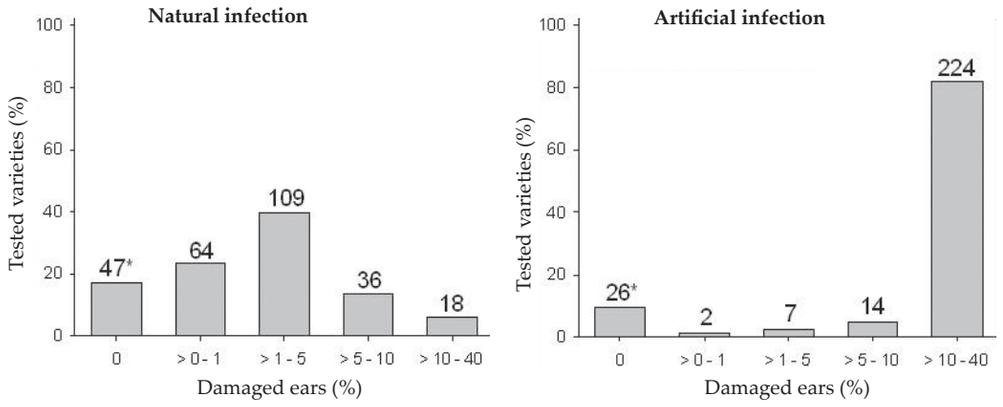

Figure 1. Classified percentage of damaged ears after artificial and natural infection of winter barley varieties with loose smut (*Ustilago nuda*) (*number of tested varieties)

Table 1. Maximum damage (out of all replications and years) of winter barley varieties (*Hordeum vulgare*) tested for resistance against covered smut (*Ustilago hordei*) by means of artificial infection [underlined variety commercially available in July 2006]

No.	Variety	Years (*n*)	Maximum of damage (%)	No.	Variety	Years (*n*)	Maximum of damage (%)	No.	Variety	Years (*n*)	Maximum of damage (%)
1	Adlon	1	5.36	38	Cornelia	2	8.05	75	Marylin	1	0.01
2	Advance	2	2.02	39	Cosima	2	0.22	76	Maximiliane	1	0
3	Affair	2	0.07	40	Duet	2	0	77	Mellori	2	1.85
4	Akropolis	1	2.05	41	Dyveke	1	0.23	78	Mercedes	1	0
5	Alissa	2	0	42	Edda	2	1.70	79	Merlot	2	0.71
6	Allegra	2	0.30	43	Elbany	2	0.18	80	Millie	1	6.65
7	Alpaca	1	0	44	Elfe	1	0	81	Mombasa	2	5.95
8	Anastasia	2	1.06	45	Existenz	1	0.05	82	Naomie	1	0.23
9	Angela	1	4.43	46	Fee	2	0.21	83	Nelly	2	1.36
10	Angora	2	4.00	47	Finita	1	0.04	84	Nicola	2	0.43
11	Antalya	1	0.31	48	Fiona	1	0.08	85	Nikel	2	0.75
12	Aquarelle	2	0.09	49	Franziska	2	0.94	86	Palmyra	1	0.04
13	Arkona	1	0.03	50	Gilberta	2	3.00	87	Passion	2	0.10
14	Artist	2	0.58	51	Goldmine	2	1.29	88	Premuda	1	0.65
15	Astrid	2	2.38	52	(GW 2289)	1	0	89	Regina	2	2.38
16	Aviron	2	0.67	53	Hanna	2	5.08	90	Reni	2	0.06
17	Babylone	1	1.95	54	Jasmin	2	2.82	91	Sarah	2	0.06
18	Barcelona	2	2.66	55	Jessica	2	2.91	92	Silke	1	0.78
19	Bavaria	1	1.03	56	Jolante	2	0	93	Spectrum	1	0
20	BB 42	1	45.47	57	Julia	2	2.72	94	ST. 2267	1	0
21	Bistro	1	0.04	58	Jura	1	7.52	95	Stephanie	2	0.38
22	Bombay	2	0.18	59	Kamoto	2	1.57	96	Structura	2	4.00
23	Cabrio	2	0.19	60	Kreta	1	0	97	Svenja	1	1.27
24	Camera	2	0.02	61	Kyoto	2	1.19	98	Tafeno	2	9.44
25	Campanile	1	0	62	Labea	1	1.77	99	Tessy	2	1.67
26	Candesse	2	1.00	63	Landi	1	0.35	100	Theda	2	5.40
27	Caprima	1	0.44	64	Leonie	2	1.67	101	Theresa	2	0.06
28	Carat	2	3.00	65	Loden	2	2.81	102	Tiffany	1	2.34
29	Carola	2	0.11	66	Lomerit	2	1.14	103	Tilia	2	0.45
30	Carrero	2	0	67	Lubeca	1	0.54	104	Traminer	2	1.90
31	Cascaya	1	0.03	68	Ludmilla	2	0.86	105	Uschi	2	0
32	Catania	1	0.21	69	Lunaris	1	12.18	106	Vanessa	2	0.39
33	Cinderella	1	1.36	70	Madeline	1	2.08	107	Venezia	2	0.19
34	Cita	1	0.68	71	Madita	1	0	108	Verena	1	0
35	Clara	2	4.35	72	Madou	2	1.96	109	Verticale	1	0
36	Cleopatra	2	5.33	73	Malwinta	1	0.94	110	Yuka	2	0
37	Corbie	2		74	Marinka	1	4.21				

rero, Cinderella, Cosima, Dyveke, Elbany, Fee, Finita, Landi, Naomie, Nicola, Passion, Reni, Sarah, Stephanie, Theresa, Tilia and Vanessa.

Ustilago nuda. Over all 274 lines tested for *Un*-resistance, the classified damage (0, >0–1, >1–5, >5–10 and >10%) was 17.2, 23.4, 39.8, 13.1 and

Table 2. Percentage of infected ears of winter barley after artificial inoculation with spores of *Ustilago nuda* collected at four distant German provenances

Variety	Percentage of infected ears			
	German provenance of inoculum (with postal code)			
	Neu Darchau (DE-29490)	Hadmersleben (DE-39398)	Bad Vilbel (DE-61118)	Düren (DE-52351)
Astrid	0.0	0.0	0.0	0.0
Carrero	0.0	0.0	0.0	0.0
HJ 171	0.0	0.0	0.0	0.0
Isolde	0.8	0.0	0.0	0.0
Milton	0.0	0.0	0.0	0.0
Ogalitsu	0.0	0.0	0.0	0.0
DM 86	8.3	0.0	0.0	0.4
NS 96515/26	4.2	7.2	0.0	0.0
BB 38	0.6	20.7	0.6	0.4
HOR 11832	2.3	3.4	1.3	0.0
Yuka	19.5	16.3	1.6	0.0
HOR 248	19.2	3.0	3.1	0.4
Grete	11.5	5.0	5.3	0.0
HOR 13453	48.2	30.9	13.1	0.0
DM 12	60.6	0.0	16.2	0.0

6.5% for natural infection by wind and 9.5, 0.7, 2.6, 5.1 and 82.1% for spore injection (Figure 1). Within the group '0% under artificial infection' only Carrero is commercially available at present in Germany, Astrid and Laurena in Austria. Another fully resistant variety in four testing years was Milton, an American hybridation of Keowee and Volbar with known resistance too (Buivids *et al.* 1977). Ogalitsu, likewise a donor of *Un*-resistance in breeding (Tyulina 1971), validated his resistance in two years.

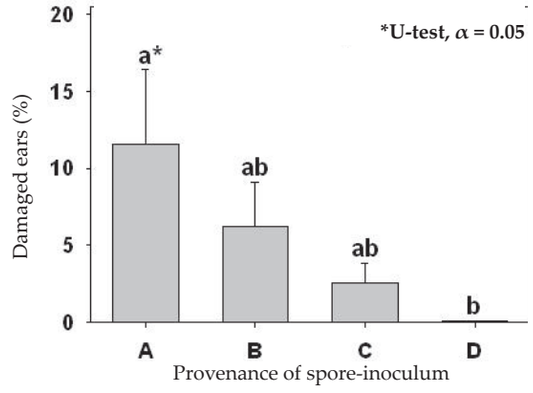

Figure 2. Mean damage of a test-sample of 15 varieties of winter barley after artificial infection with loose smut (*Ustilago nuda*) of various German provenances (A: DE-29490 Neu Darchau, B: DE-39398 Hadmersleben, C: DE-61118 Bad Vilbel, D: DE-52351 Düren)

Without damage under natural infection, Jessica and Annicka for Germany can be added. Below 1% under natural infection remained Elbany, Cabrio, Venezia, Lomerit, Cosima, Barcelona, Nicola and Carat.

The results concerning the virulence of different spore-provenances show Table 2 and Figure 2. Astrid, Carrero, HJ 171, Milton and Ogalitsu remained without visible symptoms after artificial infection with each inoculum and are maybe unfailing donors of resistance for the conditions in Germany.

The inoculum with the highest virulence was collected near Neu Darchau (DE-29490, Figure 2). The spore collections from Hadmersleben (DE-39398) and Bad Vilbel (DE-61118) showed a lower virulence. The spores deriving from Düren (DE-52351) caused the lowest damage. We con-

clude that the virulence of the spores deriving from Bad Vilbel (inoculated during the entire study) was adequate to evaluate the degree of resistance of winter barley varieties in Germany. For testing DE-29490, DE-39398 and DE-61118 in spring barley see also Mueller (2006).

DISCUSSION

Seed quality is a central issue in ecologically/organically oriented farming systems. In winter barley, covered smut and in particular loose smut infection must be at the lowest possible level. Out of all tested varieties of winter barley as far as permitted for trade by the inspecting authority (71 var. in July 2006), not one showed resistance against both diseases. Only the variety Carrero must be highlighted because of the complete resistance against all four tested geographic provenances of spores of *Un* and a moderate resistance against *Uh*.

Ustilago hordei. Whereas only two varieties remained without visible symptoms, a range of 23 varieties below 0.05% of infected ears gives the chance on a risk-minimized cultivation of winter barley varieties for organic farming in Germany. This impact assessment considers the fact that the spores of *Uh* adhere to the surface of the seeds and therefore are to be reached with accepted agents of seed treatment in ecological seed production. For future breeding programs, the breeders have to keep in mind that a high degree of resistance against *Uh* might be associated with a reduced content of amino acids (Peresipkin & Rebenko 1971).

Ustilago nuda. Besides the commercially available variety with full resistance (Carrero) eight varieties showed symptoms when artificially inoculated but stayed healthy under the condition of natural infection (DM 70, DM 58, Cebu, Duet, Murcie, Alpaca, Svenja and Clho 10890). In literature four defence reactions of barley are described and might explain this phenomenon: cleistogamous flowering (Fröier *et al.* 1959; Pedersen 1960), embryo hypersensitive reaction (Niemann 1961), last observed in some Japanese varieties. Cleistogamous flowering prevents the contact of spores and growing fungal hyphae with the susceptible early stage of the seed.

For the latter three types, the defence happens after infection. In the last German evaluation for resistance against *Un* (admittedly in spring barley) three defence reactions were observed: cleistogamous resistance (pseudoresistance) showed Nudinka, Irania, Cerise and Claudia. No cultivar had total embryo resistance (assessed after inoculation of single florets); the best was Villa with 10–30% disease incidence. Emir, Irania and Luna were resistant to disease spread (escape reaction), in that infected seed did not give rise to infected plants (Wicke & Weltzien 1986). Within this study there was no time to proof for types of resistance because the need of elaborate microscopic work. Concerning *Un* resistance in Germany the question remains if a selection of cleistogamic varieties as a strategy for ecologically/organically oriented farming systems will be sufficient or if a breeding for genetically determined resistance should be practised with every emphasis.

With regard for good resistance properties against smut only Allegra, Passion, Reni, Stephanie, Theresa (*Uh*) and Lomerit (*Un*) were available for cultivation out of organic seed production in autumn 2005 (looked up at www.organicXseeds.de).

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