

## Development of Methods for Bunt Resistance Breeding for Organic Farming

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**Abstract:** An international winter wheat assortment was evaluated for resistance to *T. caries* and *T. controversa* under organic farming conditions. 98 genotypes were screened for resistance to *T. caries* and 29 genotypes for resistance to *T. controversa* by artificial inoculation during one season. A set of differential cultivars was included for testing virulence/avirulence against particular resistant genes. For *T. caries* no disease symptoms were observed for 9 genotypes, 25 genotypes showed low infection (0.1%–1% infection) and 14 genotypes were highly susceptible (40.5%–70.6% diseased spikes). Screening for resistance to *T. controversa* resulted in only 1 resistant genotype (0.0% infection), one genotype showed low infection (0.5% diseased spikes) and 11 genotypes were highly susceptible (39.0%–85.3% infection). Resistance genes *Bt12* and *Bt13* conferred resistance to both species: *T. caries* and *T. controversa*.

**Keywords:** *Tilletia caries*; *Tilletia controversa*; resistance; winter wheat; *Bt* genes

Increased awareness of environment and economic advantages enhanced organic farming in Austria since joining the European Union. Accordingly the expansion of organic farming and the associated use of untreated seed, bunt diseases are on the rise. Common bunt of wheat, caused by *T. caries* (DC.) Tul. and dwarf bunt of wheat, caused by *T. controversa* Kühn, are major seed and soil-borne diseases in Austria. In addition to loss of grain yield, bunt reduces grain quality because it imparts a fishy odour to wheat and its products. Contamination with *T. caries* occurs all over Austria, whereas dwarf bunt, is limited to higher-altitude areas. A possibility for the decrease of bunt is the cultivation of resistant varieties.

The objectives of this study were: (1) Evaluation of potential genotypes for *T. caries* and *T. controversa* resistance and (2) Recommendations for breeding programs.

### MATERIAL AND METHODS

Experiments were conducted during the season 2005/2006 at two locations that commonly provide optimal conditions for bunt incidence.

Most of the genotypes obtained from abroad have been already evaluated for bunt resistance and several genotypes were identified as resistant.

*Tilletia caries*. An international assortment consisting of 98 resistant and susceptible winter wheat genotypes was evaluated for resistance to *T. caries* at the experimental field of Untermallebarn, 30 km northwest of Vienna, at 190 m above sea level. The average temperature and annual precipitation were 9.4°C and 642 mm.

The soil type is loess. The seeds were artificially inoculated (1 g teliospores per 100 g seeds) with a bulk of teliospores collected from naturally infected spikes in Austria. Therefore the seeds were

mixed with a suspension of teliospores, dissolved in aqueous methylcellulose. Inoculation method used is similar to GOATES (1996). The experimental layout was a randomized complete block design with 6 blocks. Two row plots of 2.5 m length were sown at two different times (14<sup>th</sup> and the 25<sup>th</sup> of October 2005). At maturity 1 m of each row was evaluated for common bunt severity by counting the total number of spikes and the number of diseased spikes. Disease severity was expressed in percent bunt infected spikes.

*Tilletia controversa*. The resistance against *T. controversa* was studied on 29 winter wheat genotypes at the experimental field of Goepfritz, 100 km northwest of Vienna, at 580 m above sea level. The average temperature and annual precipitation were 5.9°C and 646 mm. The soil type is clay. The experimental design was a randomized complete block design with 3 blocks. Sowing time was the 11<sup>th</sup> October 2005. Plots consisted of double rows of 2.5 m length which were spray inoculated with teliospores suspension twice. Teliospores collected from naturally infected spikes in Austria were applied in a concentration of 4.4 g teliospores per liter. Inoculation was achieved using the method described by GOATES (1996).

At maturity 1 m of each row was scored, counting diseased and healthy spikes.

The field data were analyzed as randomized complete block design using the GLM procedure of SAS/STAT.

## RESULTS

An international assortment consisting of 98 resistant and susceptible winter wheat genotypes was evaluated for resistance to *T. caries*. 29 genotypes of them were also tested for reaction to *T. controversa*.

For both diseases the mean values of % infected spikes and the corresponding standard deviations are listed in ascending order of % diseased spikes in Table 1.

Screening of winter wheat germplasm for resistance to *T. caries* resulted in a broad range in variation from as low as 0.0 percentage of diseased spikes up to 70.6 percentage of diseased spikes for the cultivar HeinesVII. No infection for *T. caries* was found in Boneville, Eltan, Golden Spike, PI 178383, PI 560606 sel blaw, PI 560795 sel bcors, PI 560841 sel bcl, PI 362695 and Weston.

For *T. controversa* disease severity varied between 0.0 to 85.3 percentage of diseased spikes.

No infection for *T. controversa* was found in the differential line PI 119333, carrying *Bt12*. Low infection levels for *T. controversa* were observed for PI 178383 (1.6%, 0.0% *T. caries*), Thule-III – *Bt13* (0.5%, 2.9% *T. caries*) Weston (2.8%, 0.0% *T. caries*) and PI 560603 sel blaw (4.4%, 0.0% *T. caries*). The highest disease level after inoculation with *T. controversa* was evaluated for the Austrian cultivar Capo with 85.3% infected spikes.

The virulence against particular resistance genes was determined by testing cultivars with defined *Bt* genes (provided by Dr. B. Goates). Lines carrying *Bt7* and *Bt2* were susceptible to common bunt. Low *T. caries* incidence, ranging between 0.2–1.5 percentage infected spikes, was evident on the differential lines carrying *Bt4*, *Bt5*, *Bt6*, *Bt8*, *Bt9*, *Bt10*, *Bt11* and *Bt12*.

Virulence of *T. controversa* was tested only for *Bt8*, *Bt9*, *Bt10*, *Bt12*, *Bt13* and *Bt15*. *T. controversa* revealed virulence against the resistance genes *Bt9*, *Bt8*, *Bt10* and *Bt15*. No infection level for *T. controversa* showed the differential cultivar, carrying resistance gene *Bt12*. Cultivars having *Bt13* showed only 0.5 percentage infected spikes.

## DISCUSSION

This experiment gives an overview of sources of resistance to *T. caries* and *T. controversa*.

Large variation in bunt incidence for both isolates was observed. Genotypes ranged from highly resistant (0.0% diseased spikes) up to very susceptible (70.6% *T. caries*; 85.3% *T. controversa* diseased spikes).

In this study the most resistant cultivars to *T. caries* were Boneville, Eltan, Golden Spike, PI 178383, PI 560603 sel blaw, PI 560795 sel bcors, PI 560841 sel bcl, PI 326695 and Weston. Boneville, Eltan and Golden Spike are highly resistant to both species: *T. caries* and *T. controversa*. In addition to these, PI 178383, PI 560603 sel blaw, PI 560795 sel bcors, PI 560841 sel bcl, PI 326695 are land races with poor agronomic characters but are extremely resistant as well. Some of these have shown immunity in multiple tests (GOATES, person. commun.). Resistance of Weston for *T. caries* was already described by BLAŽKOVÁ and BARTOŠ (1997) carrying the resistance genes *Bt8*, *Bt9* and *Bt10* derived from the resistance source PI 178383 (HOFFMANN 1982) which also applies to the European resistant cultivar Stava. For Stava it is not well known if there are all three resistance

Table 1. Mean values and standard deviations of percentage of diseased spikes for genotypes infected with *T. caries* and *T. controversa* in ascending order of % diseased spikes

Genotype	Donor	<i>T. caries</i>		<i>T. controversa</i>	
		mean (%)	S.D.	mean (%)	S.D.
Boneville	USA	0.0	0.0		
Eltan	USA	0.0	0.0		
Golden Spike	USA	0.0	0.0		
PI 178383	Canada	0.0	0.0	1.6	2.8
PI 560603 sel blaw	USA	0.0	0.0	4.4	4.0
PI 560795 sel bcors	USA	0.0	0.0		
PI 56084 sel bcl	USA	0.0	0.0		
PI 362695	USA	0.0	0.0		
Weston	Austria	0.0	0.0	2.8	4.8
Ute	USA	0.1	0.3		
0028162-II	Romania	0.1	0.4		
Rio, <i>Bt6</i>	USA/differential	0.2	0.4		
Hohenheimer, <i>Bt5</i>	USA/differential	0.2	0.4		
PI 211657	USA	0.2	0.4		
Winridge	USA	0.2	0.5	2.9	4.0
Globus	Denmark	0.3	0.7	66.9	25.8
Kmor	USA	0.4	0.6		
Wasatch	Canada	0.4	0.9	7.1	1.3
Franklin	Germany	0.4	1.0		
Tambor	Austria	0.4	0.8	76.3	1.8
PI 560603sel wcows	USA	0.4	1.0		
PI 173437, <i>Btp</i>	USA/differential	0.4	1.1	9.8	6.3
M82-2123, <i>Bt11</i>	USA/differential	0.5	0.8		
Bellatrix	Austria	0.5	0.8	39.0	6.7
M 90-387, <i>Bt9</i>	USA/differential	0.5	1.3	57.2	16.0
Sprague	USA	0.6	1.4	5.3	3.0
Tommi	Germany	0.6	1.1	74.1	14.2
DW-Red	USA	0.6	1.5		
PI 560601	USA	0.6	0.9	11.2	7.0
Promontory	USA	0.7	1.1		
Stava	Sweden	0.8	2.0	21.4	14.1
Hansel	USA	0.9	2.1		
PI 560603 sel wcors	USA	1.0	1.4		
Penta	Denmark	1.0	2.1		
PI 119333, <i>Bt12</i>	USA/differential	1.1	2.4	0.0	0.0

Table 1 to be continued

Genotype	Donor	<i>T. caries</i>		<i>T. controversa</i>	
		mean (%)	S.D.	mean (%)	S.D.
Magnifik	Sweden	1.2	1.1		
M82 2161, <i>Bt8</i>	USA/differential	1.3	2.8	23.9	26.3
M82 2102, <i>Bt10</i>	USA/differential	1.4	1.8	23.6	25.5
CI 1558B, <i>Bt4</i>	USA/differential	1.5	1.7		
Breuhl	USA	2.2	4.9		
Greti R	Romania	2.5	3.0		
0028761-II	Romania	2.7	5.4		
Thule-III, <i>Bt13</i>	USA/differential	2.9	4.2	0.5	0.7
0027162-31	Romania	3.2	3.3		
Sel. 2092, <i>Bt1</i>	USA/differential	3.3	2.9		
Ridit, <i>Bt3</i>	USA/differential	3.5	1.5		
Toronit	Switzerland	5.8	7.2		
Amigo	Germany	7.2	2.1	20.2	20.1
Cheyenne	USA	8.4	16.8		
Sel. 50077, <i>Bt7</i>	USA/differential	13.6	3.3		
Fatima-2	Hungary	13.6	3.2		
WW_BIO_30	Austria	14.5	5.8		
WW_BIO_121	Austria	15.7	5.6		
Titlis	Switzerland	15.8	4.6		
WW_BIO_99	Austria	17.5	12.4		
Lewjain	USA	19.3	29.5		
Piotta	Switzerland	19.4	8.4		
WW_BIO_22	Austria	20.5	6.6		
Levis	Switzerland	20.5	12.8		
Adomir	Austria	22.6	13.3	10.3	
Zinal	Switzerland	23.1	16.5		
UA0110594	Ukraine	25.1	11.0		
WW_BIO_66	Austria	25.6	14.7		
UA0102692	Ukraine	25.6	13.1		
SE-306/03	Austria	26.0	6.8		
Arolla	Switzerland	27.0	17.0		
Lona	Switzerland	28.1	7.4		
Capo	Austria	29.9	13.7	85.3	8.7
UA0105858	Ukraine	30.8	18.7		
Z068-9545	Austria	31.1	17.9		
UA0102689	Ukraine	32.7	10.6		

Table 1 to be continued

Genotype	Donor	<i>T. caries</i>		<i>T. controversa</i>	
		mean (%)	S.D.	mean (%)	S.D.
Arbola	Switzerland	33.9	19.6		
Astardo	Austria	34.1	11.4	60.4	38.3
Valerius	Austria	34.5	5.1	77.8	20.3
Exquisit	Austria	34.7	20.4	65.4	27.9
WW_BIO_73	Austria	34.9	18.0		
Z246-9357	Austria	37.3	9.8		
Sel. 1102, <i>Bt2</i>	USA/differential	38.0	14.6		
UA01110807	Ukraine	38.8	21.2	55.9	13.8
UA0103355	Ukraine	39.3	10.6		
WW_BIO_208	Austria	39.4	14.1		
UA0103677	Ukraine	39.8	13.2	69.2	9.9
Mv-17	Hungary	40.5	20.0	74.8	3.8
WW_BIO_212	Austria	42.1	13.7		
W240-7430	Austria	43.8	10.8		
WW_BIO_31	Austria	45.4	17.8		
Muveran	Switzerland	47.1	23.1		
SE-275/01	Austria	49.2	19.2		
UA0107230	Ukraine	51.3	15.0		
UA0102673	Ukraine	57.0	11.7		
WW_BIO_68	Austria	57.4	12.4		
UA0101831	Ukraine	58.8	14.3		
SE-327/02	Austria	63.7	10.8		
Batis	Germany	65.7	17.9		
WW_BIO_225	Austria	67.5	9.8		
HeinesVII, <i>Bt0</i>	USA/differential	70.6	18.6		
Carleton, <i>Bt15</i>	USA/differential			28.6	
9914664-122	Romania			21.9	16.7

genes present (FISCHER *et al.* 2002). Even the virulence of *T. caries* and *T. controversa* is regulated by the same resistance genes in wheat (GOATES 1996) not all genotypes resistant to *T. caries* were found as resistant to *T. controversa*. Stava (0.8% infected spikes for *T. caries*) and the cultivar Tommi (0.6% infected spikes for *T. caries*) described as resistant for *T. caries* by WÄCHTER *et al.* (2004) were moderately to highly susceptible for *T. controversa*: Stava (21.4% infection) and Tommi (74.1% infec-

tion). This is also the case for the cultivar Globus (*T. caries*: 0.3% infected spikes; *T. controversa*: 66.9% infected spikes). All lines which were resistant to *T. controversa* showed also resistance to *T. caries*, but not vice versa.

Impurity of seed stocks of the genotypes with low *T. caries* infection may have influenced the results slightly. Therefore it is possible that the resistance level of these cultivars was underestimated.

For *T. caries* Austrian cultivars ranged between moderately susceptible WW\_BIO\_30 (14.5% infection) up to highly susceptible WW\_BIO\_225 (67.5% infection).

For the pathogen *T. caries*: good resistance (0.1 to 0.5% infection) was found on *Bt5*, *Bt6*, *Bt9*, *Bt11* and *Bt1p*. Low disease levels (1.1–2.9% diseased spikes) contributed *Bt4*, *Bt8*, *Bt10*, *Bt12* and *Bt13* as well as *Bt1* (3.3% infection) and *Bt3* (3.5% infection). Virulence was noticed for *Bt7* (13.6% diseased spikes) and *Bt2* (38.0% diseased spikes). For the pathogen *T. controversa*: only differentials with *Bt9*, *Bt8*, *Bt10*, *Bt12* and *Bt15* were tested for resistance. *Bt12* (0.0% infection) and *Bt13* (0.5% infection) contributed resistance. High susceptibility was found for *Bt9* with 57.2% infected spikes.

For further studies on resistance testing to *T. controversa* the whole set of *Bt* differentials and all lines which showed resistance to *T. caries*, e.g. the highly common bunt resistant landraces, should be included. Analyzing of the virulence spectrum of Austrian common bunt and dwarf bunt isolates is of high interest.

The presented data are based on one season only and as a consequence of the severe winter in the year 2005 plant stands were not excellent in the resistance test against *T. controversa*, therefore the experiment should be repeated for at least one additional year to ensure the correct reactions to *T. caries* and *T. controversa*.

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