# Purifying virulence races of common bunt (*Tilletia caries*) to identify resistance genes in wheat

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## Introduction

Breeding wheat with resistance to common bunt (*Tilletia caries*) is an important part of a strategy to control the disease in organic farming. Many wheat varieties are described as resistant to the disease, and several major genes are described able to control the disease. However, there is a lack of consistency in field trials assessing wheat varieties for their susceptibility to the disease (Blažkova and Bartoš 1997).

No doubt, genetic markers will be a powerful tool to identify resistance genes in breeding lines, also when it comes to resistance against bunt in wheat, but in order to develop markers, reliable phenotypic assessments are needed. To get there, we need tools to distinguish between different resistances in wheat lines.

Most trials published on susceptibility of wheat varieties to bunt are done by adding spores to a seed sample before sowing, at assessing the frequency of of infected heads. This method is a more or less adapted method of trials testing for the effect of seed treatments (EPPO 1997). When testing for resistance, the virulence of the spores are of crucial importance, since a trial will not be able to identify resistance genes, if the spores used are virulent to the gene. Often, the origin and virulence of the spores in trials are more or less unknown, and most spore samples found in practical farming are genetic diverse. The may therefore be a mixture of virulent and avirulent spores against the resistances in question. Using such spores in a trial may result in data difficult to interpret, as a low infection level can be a result of a low frequency of virulent spores in the spore sample. Next year, the composition of spores may have changed, which will lead to a different result in the same varieties.

In this situation, I felt a need to work with less diverse spore samples in my bunt trials, and to achieve this, I developed a simple technique to develop more uniform and well described spores. Already Weston (1932) and Roemer og Bartholly (1933) showed that susceptibility of a variety increased when it was infected by spores from the same variety compared with spores from other varieties. Woolman (1930) showed that only up to 4 spores were able to infect the wheat meristem. Therefore, working with spores from a single plant will decrease diversity in a spore collection, and if this plant is resistant, it will after a few regenerations of such a variety be selective to a specific race, virulent to the resistance gene in question.

## Materials and methods

Spores were collected in the ORGSEED project in 2001-5 by Bent Nielsen. Spores were collected from a broad range of fields grown with different varieties in different regions of Denmark. It is likely that they pretty much represent the genetic diversity of the pathogen present in Danish agriculture by the time of collection. The collection was maintained on susceptible varieties until 2011 and hereafter on a composite cross population.

From 2012 and onwards, resistant varieties were infected with spores collected on their own variety if infected spikes were found. In this way, a collection of 98 virulence races were build up specific to the 98 wheat varieties.

In 2013-14 and in 2014-5, spores from selected varieties were used to infect other varieties.

The varieties were sown in 0.5 rows without replication with 5g seed per row.

## Results and discussion

Some of the varieties that had low infection in previous trials (Steffan 2014 , Steffan *et al.* 2014), and therefore were recorded as resistant, could indeed be infected if they were re-inoculated with the spores from the few infected plants of the variety. For example, varieties with the resistance gene Bt10 had no or low infection when infected with the diverse spore collection, and it was concluded that Danish spores were avirulent to Bt10. However, when varieties with Bt10 were inoculated with spores from their own variety, they turned out to be highly susceptible. The spore collection is therefore a mixture of virulent and avirulent spores against Bt10. The same is the case for Bt2, Bt7, Bt13 and BtZ.

When varieties were inoculated with spores that had been purified on other varieties, they were normally either more resistant or more susceptible compared with inoculation with the spores mixture. It is likely that varieties that react in a similar way to different origins of spores may have the same resistance genes. However, this is not always the case. Some (or maybe most) varieties carry more than on gene affecting the susceptibility, and certainly most virulence races of the pathogen are virulent to more than one resistance gene. For example, spores virulent to Bt10 are in this study also virulent to BtZ, and I have been unable to distinguish between these two genes.

Some varieties have so far been resistant I all studies, and even if a few plants were infected, spores from these plants have been unable to create a high infection level. Some of these varieties may have a resistance gene to which no virulence have been found, and some varieties may have a combination of genes. I have been unable to develop virulence races specific to the resistance genes Bt3, Bt4, Bt5, Bt6, Bt8, Bt9, Bt11, Bt12.

It is possible that the varieties NGB9014, NGB-9015, Tambor, Kuban, Begra, Maribos, Fold, Monopol, Tarso, Torrild, Cardos, Kranich, Türkis, Gluten, Folke have Bt7, since they react in a similar way to the 7 different sources of spores used in 2014-5.

It is possible that the varieties Format, Curier, Complet, Solstice, Bussard, Paroli, Dream, Butaro, Ochre, PG3540 and Hereward have Bt2.

The varieties Korrund, Aron, Karat, Tulsa, Xenos, Tataros, Erbachshofer Braun and Indigo have in some trials shown resistance, but in 2014-15, they have been susceptible to most or all virulence races.

**Infecting 65 varieties of winter wheat with 7 sources of common bunt inoculum**
**"*Own spores*" means spores harvested previous year on the tested variety**

| | Vr: Own spores | Vr2: Complet | Vr: 10 (Weston) | Vr:1 (Pi554108) | Vr: 4 (Cardos) | Vr: 5 (Hohenheim) | Vr: 13 (Thule III) | Vr: 8 (Pi554120) |
|---|---|---|---|---|---|---|---|---|
| Pi554-121 (Bt3) | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | | 0,0 | 0,0 |
| Ridit (Bt3) | 11,1 | 0,0 | 5,0 | 3,4 | 0,0 | 0,0 | 0,0 | 0,0 |
| PI 554-116 (Bt3 | 33,3 | 0,0 | 0,0 | 11,1 | 7,1 | 0,0 | 0,0 | 0,0 |
| Nebred (Bt4) | 16,7 | 0,0 | 0,0 | 14,3 | 0,0 | 3,7 | 0,0 | 2,3 |
| PI-554-115 (Bt4) | 66,7 | 0,0 | 4,8 | 0,0 | 14,8 | 3,8 | 0,0 | 0,0 |
| Carlton | 0,0 | 6,7 | | 0,0 | 60,0 | 0,0 | 7,8 | 0,0 |
| PI-554-117 (Bt6) | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| Erythrosperum 5221 (Bt14) | 0,0 | 2,6 | 0,0 | 3,0 | 9,8 | 0,0 | 0,0 | 4,8 |
| Promesse (Bt5) | 0,0 | 0,0 | 0,0 | 2,7 | 0,0 | 0,0 | 0,0 | 0,0 |
| Hohenheimer (Bt5)Ci-11458 | 50,0 | 27,6 | 16,7 | 0,0 | 0,0 | 6,9 | 0,0 | 12,5 |
| Lars | 0,0 | 8,3 | 35,3 | 0,0 | 0,0 | | 2,4 | 6,3 |
| Triple Dirk | 0,0 | 3,2 | 13,0 | 0,0 | 0,0 | 4,8 | 0,0 | 0,0 |
| Hypnos | 0,0 | 2,9 | 18,2 | 3,7 | 3,4 | 9,7 | | 0,0 |
| Skagen | 4,5 | 37,8 | 20,0 | 9,1 | 0,0 | 3,3 | 1,6 | 0,0 |
| Skotte | 0,0 | 26,7 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| PI-554-110 (Bt8) | 0,0 | 0,0 | 20,0 | 33,3 | 40,0 | 0,0 | 0,0 | 0,0 |
| PI-554-111 (Bt8) | 50,0 | 0,0 | | 100,0 | 20,0 | 47,1 | 0,0 | 0,0 |
| Thule III (Bt13) | 71,4 | 0,0 | 0,0 | 0,0 | 0,0 | 2,6 | 30,4 | 13,3 |
| Inna | 22 | 0,0 | 84,0 | 0,0 | 0,0 | 0,0 | 4,2 | 0,0 |
| Nemchinovskaya 25 (BtZ) PI | 0,0 | 3,4 | 55,6 | 3,8 | 7,1 | 0,0 | 0,0 | 2,9 |
| Pi173437 (Btp) | | 2,0 | 18,8 | 0,0 | 0,0 | 0,0 | 0,0 | 13,3 |
| Weston | 33,3 | 0,0 | 52,6 | 0,0 | 0,0 | 0,0 | 0,0 | 13,6 |
| PI-554-118 (Bt10) | 45,5 | 0,0 | 53,6 | 0,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| PI-554-109 (Bt10) | 33,3 | 0,0 | 50,0 | 10,5 | 7,1 | 15,8 | 0,0 | 0,0 |
| Intaler | 47,4 | 15,7 | 9,1 | 13,8 | 2,4 | 10,5 | 1,8 | 2,0 |
| Lutescens 6028 (PI 591884) | 0,0 | 12,5 | 25,0 | 30,0 | 10,0 | 17,1 | 4,0 | 4,8 |
| Penta | 20,0 | 9,5 | 0,0 | 0,0 | 0,0 | 4,8 | 0,0 | 0,0 |
| PI-554-103 (Bt2) | 25,0 | 8,8 | 0,0 | 45,0 | 0,0 | | 0,0 | 0,0 |
| PI-554-097 (Bt2) | 57,1 | 61,3 | 5,9 | 31,6 | 0,0 | 0,0 | 0,0 | 0,0 |
| Format | 76,9 | 44,4 | 0,0 | 22,2 | 0,0 | 4,7 | 0,0 | 0,0 |
| Curier | 54,5 | 41,9 | 11,8 | 0,0 | 0,0 | 2,4 | 0,0 | 0,0 |
| Complet | 43,8 | **69,7** | 0,0 | 6,3 | 0,0 | 0,0 | 0,0 | 0,0 |
| Solstice | 75,0 | 40,3 | 0,0 | 12,5 | 2,4 | 4,2 | 0,0 | 0,0 |
| Bussard | 77,3 | 63,4 | 0,0 | 12,5 | 2,3 | 0,0 | 0,0 | 0,0 |
| Paroli | 80,8 | 53,3 | 0,0 | 14,8 | 2,0 | 4,8 | 0,0 | 0,0 |
| Dream | 61,5 | 28,1 | 0,0 | 16,7 | 0,0 | 2,3 | 0,0 | 0,0 |
| Butaro (154) | 37,5 | 64,7 | 7,1 | 25,0 | 0,0 | 3,0 | 2,6 | 0,0 |
| Ochre | 50,0 | 30,0 | 0,0 | 24,2 | 0,0 | 1,7 | 0,0 | 0,0 |
| PG3540 | 50,0 | 10,0 | 0,0 | 15,0 | 0,0 | 0,0 | 0,0 | 0,0 |
| Hereward | 60,0 | 14,8 | 12,0 | 4,2 | 0,0 | 0,0 | 0,0 | 7,7 |
| PI-554-108 (Bt1) | | | 0,0 | | 0,0 | | 0,0 | |
| PI-554-101 (Bt1) | 100 | 11,1 | 0,0 | 37,5 | 7,7 | 12,5 | 0,0 | 0,0 |
| Bill | 16,7 | 0,0 | 3,8 | 0,0 | 2,0 | 25,0 | 0,0 | 2,8 |
| NGB9014 | 57,1 | 3,2 | 15,6 | 0,0 | 0,0 | 30,0 | 10,0 | 0,0 |
| NGB-9015 | 33,3 | 22,5 | 23,1 | 4,2 | 0,0 | 18,0 | 4,7 | 0,0 |
| Tambor | 0,0 | 31,6 | 12,5 | 0,0 | 2,0 | 19,7 | 4,0 | 3,0 |
| Kuban | 11,1 | 18,5 | 12,1 | 3,0 | 32,8 | 11,9 | 0,0 | 0,0 |
| Begra | 0,0 | 27,8 | 44,4 | 4,5 | 17,6 | 27,5 | 5,4 | 7,3 |
| Maribos | | 12,1 | 21,4 | 3,2 | 37,5 | 8,6 | 2,3 | 10,0 |
| Fold | 16,7 | 12,2 | 14,6 | 5,6 | 8,6 | 9,8 | 0,0 | 4,3 |
| Monopol | 50,0 | 90,9 | 0,0 | 40,0 | 11,1 | 3,4 | 0,0 | 0,0 |
| Tarso | 7,1 | 23,4 | 34,4 | 16,0 | 28,6 | 6,3 | 8,7 | 2,1 |
| Torrild | 14,3 | 38,5 | 35,0 | 13,9 | 14,3 | 17,5 | 2,1 | 3,2 |
| Cardos | 17,6 | 26,2 | 57,9 | 31,3 | **16,0** | | 8,9 | 0,0 |
| Kranich | 19,0 | 35,0 | 10,5 | 33,3 | 23,7 | 22,7 | 11,7 | 0,0 |
| Türkis | 45,0 | 40,0 | 25,0 | 11,1 | 42,2 | 28,6 | 6,8 | 0,0 |
| Gluten | 28,6 | 16,7 | 46,2 | 34,6 | 61,0 | 22,5 | 14,3 | 0,0 |
| Folke | 37,0 | 32,6 | 25,0 | 33,3 | 70,6 | 24,3 | 5,6 | 0,0 |
| PI-554-114 (Bt7) | 50,0 | 19,2 | 22,2 | 30,0 | 0,0 | 25,0 | 15,2 | 3,7 |
| PI-554-100 (Bt7) | 33,3 | 28,6 | 45,7 | 4,5 | 0,0 | 35,5 | 10,9 | 9,1 |
| Korrund | 20,0 | 25,0 | 55,6 | 0,0 | 4,2 | 17,0 | 14,8 | 35,7 |
| Aron | 33,3 | 50,0 | 55,6 | 15,8 | 2,9 | | | |
| Karat | 84,6 | 55,6 | | 12,5 | 0,0 | | | 20,0 |
| Tulsa | 21,1 | | 54,8 | 42,9 | 51,1 | 34,4 | 36,2 | 45,7 |
| Xenos | 70,7 | 62,0 | 66,7 | 39,4 | 18,4 | | 13,2 | 26,3 |
| Tataros | 12,5 | 34,6 | 52,0 | 26,7 | 29,7 | 26,2 | 16,7 | 26,3 |
| Erbachshofer Braun | 55,6 | 75,0 | 45,5 | 55,0 | 46,4 | 57,1 | 12,5 | 0,0 |
| Indigo | 76,6 | 68,0 | 77,5 | 88,2 | 75,7 | 59,0 | 30,0 | 4,4 |

Colour legends: low plant number | 0,0% | <10% | 10-40 | >40%

Spores harvested on the variety Tommi by Svalöf/SLU in 2014 were able to give a high infection in the Tommi, Globus and Segor, and a medium infection was achieved with spores from BOKU, even though these varieties have so far been resistant or shown low susceptibility to spores from Denmark. The variety Quebon could be infected by spores purified from Czech Republic, but was resistant to all other spores tested on other varieties. This indicates that Tommi, Globus and Segor may carry the same resistance gene, which is different to the gene in Quebon, and different to the known Bt-genes.

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**Infecting 34 highly resistant varieties of winter wheat with 4 sources of common bunt inoculum**
**"*Own spores*" means spores harvested previous year on the tested variety**

| | Vr: Own spores | Vr: Tommi Svalöf | Vr: Tommi BOKU | Vr: CCP mixture | Vr: RU11 | Vr: Weihenstephan |
|---|---|---|---|---|---|---|
| Quebon | 24,0 | 0,0 | 6,7 | 0,0 | 39,3 | 6,3 |
| Tommi | 26,7 | 17,1 | 14,3 | 10,3 | | |
| Globus | 0,0 | 55,6 | 14,3 | 0,0 | | |
| Segor | | 43,8 | 0,0 | 13,3 | | |
| Huslop | | 0,0 | 0,0 | 0,0 | | |
| Winridge | 0,0 | 0,0 | 0,0 | 0,0 | | |
| Wasatch | 0,0 | 0,0 | 0,0 | 0,0 | | |
| Sprague | | 0,0 | 0,0 | 0,0 | | |
| PI-554-104 (Bt5) | | 0,0 | 0,0 | 0,0 | | |
| PI-554-112 (Bt9) | | 0,0 | 0,0 | 0,0 | | |
| Pi 554099 (Bt9) | | 0,0 | 0,0 | | | |
| Pi 554099 (Bt9) | | 0,0 | | | | |
| Andrews (Bt-9?) | | 0,0 | 0,0 | 0,0 | | |
| BB118 (Philipps) | 2,9 | | 0,0 | 0,0 | | |
| BB152 (Philipps) | 0,0 | 0,0 | 2,9 | 0,0 | | |
| PI-554-098 (Bt11) | | 0,0 | 0,0 | 0,0 | | |
| PI-554-119 (Bt11) | | 0,0 | 0,0 | 0,0 | | |
| PI-554-105 (Bt4)+5 | | 0,0 | 0,0 | 0,0 | | |
| Hansel (Bt-8+9+10?) | | 2,4 | 0,0 | 0,0 | | |
| PI-119-333 (Bt12) | 0,0 | | | | | |
| PI-119-333 (Bt12) | 7,4 | 0,0 | 0,0 | 0,0 | | |
| PI-554-106 (Bt12) | | 9,1 | 6,5 | 44,4 | | |
| Rio (Bt6) | 0,0 | 0,0 | 8,3 | 0,0 | | |
| Sam | 0,0 | 9,1 | 0,0 | 0,0 | | |
| Golda (Zarya-res.) | | 31,0 | 0,0 | 0,0 | | |
| Bill | | | 10,4 | | | |
| Marin (Ridit-res.) | 43,8 | 27,0 | 0,0 | 4,0 | | |
| PI-554-120 (Bt8) | 50,0 | | 0,0 | | | |
| Yayla | 50,0 | | 0,0 | 33,3 | | |
| Turkey (Pi11610) Bt4) | | 41,2 | 19,0 | 34,8 | | |
| Heines VII (no Bt genes) | | 33,3 | 50,0 | 30,0 | | |
| Rola (Trebelir-res.) | 45,5 | 2,9 | 68,0 | 44,4 | | |
| PI 172201 (Doubbi resistens) | | | 0,0 | | | |
| PI 172201 (Doubbi resistens) | | | 50,0 | | | |
| Doubbi (Bt-14) | 25,0 | | 50,0 | | | |
| Doubbi (Bt-14) | | | 0,0 | | | |

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