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Master Thesis

# Comparing the pathogenicity of Austrian isolates of *Tilletia caries* on wheat (*Triticum aestivum*)

submitted by

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# Affidavit

I hereby declare that I have authored this master thesis independently, and that I have not used any assistance other than that which is permitted. The work contained herein is my own except where explicitly stated otherwise. All ideas taken in wording or in basic content from unpublished sources or from published literature are duly identified and cited, and the precise references included.

I further declare that this master thesis has not been submitted, in whole or in part, in the same or a similar form, to any other educational institution as part of the requirements for an academic degree.

I hereby confirm that I am familiar with the standards of Scientific Integrity and with the guidelines of Good Scientific Practice, and that this work fully complies with these standards and guidelines.

Vienna, 12 April 2022 Elisabeth Ritzer *(manu propria)*

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## Abstract

Common bunt of wheat, caused by both *Tilletia caries* (DC.) Tul. (syn. *Tilletia tritici)* and *Tilletia foetida* (Wallr.) Liro (syn. *Tilletia laevis*) is a seed-borne disease with a high potential for reproduction. Instead of regular grain filling, the pathogen produces bunt balls filled with teliospores, which lead to yield and quality loss. The teliospores contain trimethylamine, which causes an unpleasant fish-like smell. Especially in organic agriculture, bunt infections cause severe problems. Because chemical seed dressings are unavailable for organic agriculture, resistant cultivars would be desired. Unfortunately, only a few registered cultivars incorporating broad resistance to common bunt are on the market. During the last 15 years, an increase in common bunt incidence in Austria has been observed. Additionally, there is evidence that more aggressive races of common bunt can overcome current resistance sources.

The testing of eight different isolates of common bunt from various regions in Austria on a set of differential lines and other wheat genotypes under high disease pressure in fall-planted field trails revealed three major virulence patterns in the tested isolates. The isolates *Harmannsdorf* and the *IFA Housekeeping* were the least aggressive and showed a similar virulence pattern together with *Sitzendorf* when visualized by biplot analysis. The isolates *Thening, Hinzenbach* and *Loosdorf* show similar virulence to the tested genotypes. Loosdorf scored highest in CB incidence among all the isolates and was considered the most aggressive isolate. *Gerhaus* and *IFA Aggressive* demonstrated similar reactions and a unique aggressiveness against ‘Tillexus’, ‘Tillstop’, ‘Tilliko’, ‘Tillsano’ and M822102 *(Bt10*). All tested isolates overcome the resistance genes *Bt2, Bt3, Bt7, Bt9, Bt10, Bt13* and to some extent *Bt4* and *Bt8.* In contrast, *Bt1, Bt11 and Bt12* showed very high resistance, and *Bt5*, *Bt6* and *Btp* showed negligible infection rates (less than 5% common bunt incidence). The isolate *Loosdorf* showed the highest representativeness and discriminativeness; therefore, using this isolate for testing new cultivars is recommended. The continuous adaption of the common bunt population needs to be considered in sustainable resistance breeding programs.



Key words: common bunt, *Triticum aestivum*, differential set, *Bt*-genes

# Zusammenfassung

Der Gewöhnliche Steinbrand des Weizens wird verursacht durch *Tilletia caries* (DC.) Tul. (syn. *Tilletia tritici)* und *Tilletia foetida* (Wallr.) Liro (syn. *Tilletia laevis*). Der Brandpilz ist ein samenbürtiger Schaderreger mit hohem Vermehrungspotential. Anstatt der regulären Kornfüllung, kommt es zur Bildung von sogenannten Brandbutten, die mit Teliosporen gefüllt sind und zu Qualitätsverlusten führen. Die Sporen beinhalten Trimethylamin, welches einen intensiv fischigen Geruch verursacht. Der Gewöhnliche Steinbrand verursacht vor allem in der biologischen Landwirtschaft immer wieder große Probleme. Da dort keine chemischen Beizmittel erlaubt sind, wären resistente Sorten sehr gefragt. Momentan sind nur wenige resistente Sorten in Österreich zugelassen Außerdem wurde in den letzten fünfzehn Jahren in Österreich ein Anstieg beim Auftreten des Pathogens verzeichnet. Es gibt auch Anzeichen dafür, dass aggressivere Rassen auftreten können und die momentan eingekreuzten Resistenzen durchbrechen. Im Experiment wurden vierzig Weizengenotypen mit acht verschiedenen Steinbrandisolaten, die an unterschiedlichen Orten in Österreich gesammelt worden waren, inokuliert. Die Weizensorten wurden im Herbst ausgesät und die Bedingungen glichen einem hohen Befallsdruck. Bei den meisten Weizengenotypen wurden ähnliche Reaktionen auf die unterschiedlichen Isolate festgestellt. Manche Isolate hingegen zeigten besonders starke Interaktionen mit bestimmten Weizensorten. Die Isolate *Harmannsdorf* und *IFA Housekeeping* waren die schwächsten Isolate. *Sitzendorf* war etwas aggressiver, zeigte aber trotzdem ähnliche Virulenzmuster. Auch die drei Isolate *Thening*, *Hinzenbach* und *Loosdorf* verhielten sich sehr ähnlich zueinander. *Loosdorf* war das Isolat mit dem höchsten gemessenen Krankheitsscore. Die Weizensorten ‚Tillexus', ‚Tillstop', ‚Tilliko', ‚'Tillsano' und M822102 (*Bt10*) schienen besonders anfällig gegenüber *IFA Aggressive* und *Gerhaus*. Alle getesten österreichischen Isolate sind virulent gegenüber den Resistenzgenen *Bt2, Bt3, Bt7, Bt9, Bt10, Bt13* und teilweise gegen *Bt4* und *Bt8*. Im Gegensatz dazu zeigten *Bt1, Bt11* und *Bt12* stabile Resistenz gegenüber den Isolaten. *Bt5*, *Bt6* und *Btp* hatten ebenfalls vernachlässigbare Infektionsraten (<5%). Für weitere Resistenztests wäre das Isolat *Loosdorf* am geeignetsten, da es in unserem Experiment den höchsten repräsentativen Charakter für alle Isolate aufwies und am besten die Unterschiede zwischen den Genotypen aufzeigte.

## Glossary

| | |
|---|---|
| AEA | average-environment axis |
| AEC | average-environment coordination |
| AMMI | additive main effect and multiplicative interaction |
| ANOVA | analysis of variance |
| BBCH | code to evaluate morphological growth stages of plants |
| Bt | common bunt resistance gene |
| BOKU | University of Natural Resources and Life Sciences |
| CBI | common bunt incidence [%] |
| FA | stability measure based on fitted AMMI model |
| GEI | genotype-by-environment interactions |
| GGB | genotype plus genotype-by-block of environment |
| GGE | genotype plus genotype-by-environment effect |
| GxI | genotype by isolate interaction |
| IFA | Institute of Biotechnology in Plant Production |
| ME | megaenvironment |
| MET | megaenvironment trial |
| n | Number of individuals |
| p | p-value |
| PCA | principal component analysis |
| PCR | polymerase chain reaction |
| PH | plant height |
| rFA | rank of stability measure based on fitted AMMI model |
| rY | rank of yield |
| SSI | simultaneous selection index for yield and stability |
| SVD | single value decomposition |
| TSS | total sum of squares |
| Y | yield |

# Introduction

## The success story of wheat (*Triticum aestivum*.)

Humans started to cultivate wheat in the fertile crescent area during the Neolithic age. The diploid *Triticum monococcum* (AA) and tetraploid *T. dicoccum* (AABB*)* were among the first crops domesticated by humans. The world germplasm of wheat species evolved along ancient human migration paths. *Triticum* species played a significant role in the rise of human civilization and the transition from hunter-gatherer tribes to sedentary agricultural communities. Migrating farmers spread the crop eastwards to Asia and westwards to Europe and North Africa. In the 16$^{th}$ century, *Triticum* species were brought to America and Australia. The local communities domesticated the wheat populations grown in different environments, and genetically distinct landraces evolved. (Balfourier et al. 2019; Bonjean and Angus 2001).

Modern bread wheat (*Triticum aestivum*) originates from two polyploidization events. The first event took place around 0.5 million years ago. A cross between *Triticum urartu* (AA genome) and an *Aegilops speltoides*-related species (BB genome) resulted in *Triticum turgidum ssp. diccocoides* (AABB*).* The DD-genome originates from *Aegilops tauschii,* and the two species fused to hexaploid *Triticum aestivum* (AABBDD = 42 chromosomes) around 10 000 years ago (El Baidouri et al. 2017; Bonjean and Angus 2001).

*T. aestivum* has a complex polyploid genome of around 17 Gbps (Miedaner 2014), about five times bigger than the human genome. More than 85% of the genome contains repetitive DNA. In 2018 the first fully annotated wheat genome was sequenced, and 107 891 high-confidence gene models spread over 21 chromosomes (1n) were detected, and more than four million markers were described (Appels et al. 2018).

Today, wheat is among the most important crops used for human consumption, animal feed, and industrial purposes. Common wheat has a high nutritional value and plays an essential role in human nutrition worldwide. It is grown on every continent and has adapted to various climatic conditions. Other wheat subspecies like tetraploid durum (for products like pasta) and spelt play only a minor role globally (Miedaner 2014; Bonjean and Angus 2001). In 2020, world wheat production was about 760 M tons; therefore, it's the second most important cereal after corn (1.15 Bil tons). The Top 5 producing countries are China (113 M tons), India (80 M tons), the USA (58 M tons), Russia (51 M tons) and France (36 M tons). Austria produced 1.65 M tons in 2020 (FAOSTAT 2022), and remarkably, 16.8% of the Austrian winter wheat was grown organically (AGES 2021b). Organic production has continuously increased in Austria since the mid-90s (Figure 1) (AGES 2021b; Huber and Buerstmayr 2006).

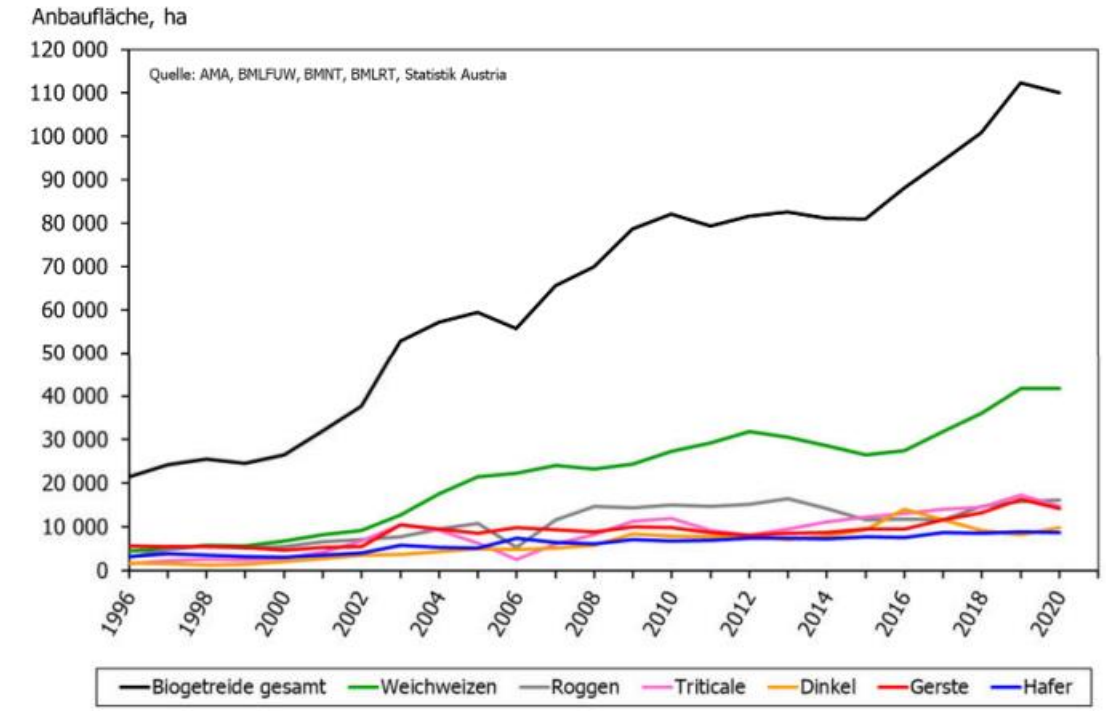


*Figure 1: Acreage of organic wheat in Austria (in hectares) from 1996 to 2020: A steady increase in the organic production area, especially for bread wheat (green line), was observed over the last 24 years (AGES 2021b)*

With the increasing population and increasing demand for animal products in countries like China and India, the need for wheat is ever-growing. Currently, it provides on average 15% to 20% of total daily calorie consumption globally (Balfourier et al. 2019). Climate change, development of resistance in pest populations, and limitations in fertile soil availability are the major challenges for future agriculture. Some models show that with each degree Celsius more in average temperature, the global yield of wheat will decrease by 6% (Asseng et al. 2015). Breeding for drought, heat tolerance and resistance breeding gained more interest in the last years and will have a tremendous impact on the security of supply in the future (Miedaner 2014; Bonjean and Angus 2001). Furthermore, the fundamental changes in agriculture towards more sustainable and environmentally friendly production lead to the resurgence of many seed- and soilborne diseases like common bunt (Matanguihan et al. 2011).

## Common bunt in wheat

Common bunt, caused by *Tilletia laevis* Kuhn [*T. foetida* (Wallr.) Liro.] and *T. tritici* (Bjerk.) Wint. *[T. caries* (DC.) Tul. is a severe disease in wheat and causes yield and quality losses in several world regions (Al-Maaroof et al. 2016; Chen et al. 2016; El-Naimi et al. 2000; Goates and Bockelman 2012; Matanguihan et al. 2011). *Tilletia caries, Tilletia laevis* and *Tilletia controversa* belong to the most dangerous diseases for organic wheat cultivation (Bauer et al. 2013; Wilcoxson and Saari 1996). In conventional agriculture, bunts can be controlled effectively by chemical seed treatments, which are not allowed in organic production. Common bunt also infects spelt (*Triticum aestivum* subsp. *spelta*), einkorn (*Triticum monococcum),* durum (*Triticum durum*) and some triticale (*Triticosecalee*) genotypes (AGES 2021a; Wilcoxson and Saari 1996).

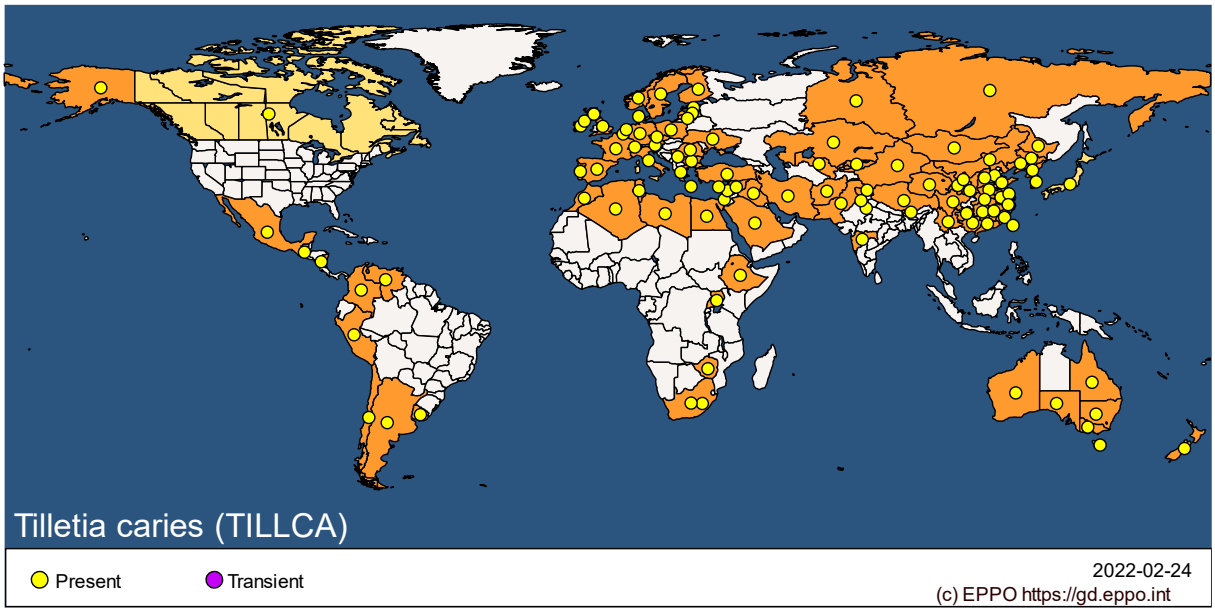


*Figure 2: Global distribution of Tilletia caries according to the EPPO: no data was available for the countries in white, countries in orange represent the presence of T. caries without detailed information, and countries in yellow indicate the widespread distribution of T.caries. https://gd.eppo.int/taxon/TILLCA/distribution.*

## Historical overview

Bunt diseases have probably been associated with wheat since the beginning of wheat cultivation (Gaudet et al. 2012; Wilcoxson and Saari 1996). Their center of origin (fertile crescent) overlaps with their host's roots. This assumption is supported by the high frequency of resistance genes found in wheat genotypes from the Near East (Bonman et al. 2006; Gaudet et al. 2012). Centers for common bunt resistance genes were found in South-Central Asia, Southern Europe and Western Asia, with the highest frequencies in Serbia, Montenegro, Macedonia, Turkey and Iran (Bonman et al. 2006). Documentations of periodic outbreaks of bunts started in ancient times, for example, by Theophrastus of Eresos (300 BC) and later by Plinius in his 18$^{th}$ book "Naturalis Historia" 100 BC (Gaudet et al. 2012; Spieß 2016). They even documented that seed dressing can enhance plant health (Spieß 2016). Unfortunately, the knowledge got lost with the decay of the ancient culture. In the following centuries, plant diseases were seen as the punishment of God for lewd conduct (Gaudet et al. 2012).

Common bunt was one of the most severe diseases on wheat in the Middle Ages. The pathogen was a significant initiator of famines, and periodical endemics are well reported (Spieß 2016; Gaudet et al. 2012). Around 1650 saltwater treatment was discovered by accident in England. A ship carrying wheat seeds sank on its way to Bristol. The seeds were salvaged and planted. While the rest of the country was ravaged by bunt, the fields planted with seeds after the saltwater exposure remained healthy. Although sometimes ineffective, saltwater treatments were applied in the following centuries (Gaudet et al. 2012). In 1755 Tillet was the first who proved that common bunt is an infectious disease and transmitted by spores (Gaudet et al. 2012; Matanguihan et al. 2011; Wilcoxson and Saari 1996). Due to his experiments, Tillet is seen as a pioneer and one of the founders of plant pathology. The genus *Tilletia* was named after him to honor his work (Matanguihan et al. 2011; Wilcoxson and Saari 1996). In the 1800s, Prevost further examined and described the infectious nature of common bunt (Gaudet et al. 2012). Almost 100 years later, the "father" of plant pathology Anton de Bary confirmed the findings of Tillet (Mathre 2000).

Common bunt continued to be a severe problem worldwide until the first effective seed treatments were developed (Spieß 2016; Hoffmann 1982; Wilcoxson and Saari 1996). In some parts of the USA and Canada, bunts were the most damaging pathogens and caused enormous production losses. In some years, losses between 25% to 50% were not uncommon (Gaudet et al. 2012). Similar losses were recorded in Asia, Australia and Europe (Gaudet et al. 2012; Matanguihan et al. 2011; Wilcoxson and Saari 1996). Since Tillet´s discovery, various seed treatments have been explored to control common bunt. Substances like salt brine, lime, lime mixtures, Salpeter, copper-containing compounds, formaldehyde and mercury compounds were tested. Those were either ineffective or detrimental to health and the

environment (Spieß 2016; Matanguihan et al. 2011; Wilcoxson and Saari 1996). Despite its toxicity, mercury was applied until the 1980s. Several severe poisoning events occurred after consuming treated seeds in Iraq and caused death or long-term health problems to thousands of people (Bakir et al. 1973).

Mercury treatments were replaced by synthetic fungicides. A game-changer was the development of polychlorobenzenes. Especially hexachlorobenzene (HCB) enabled farmers to combat seed- and soil-borne pathogens simultaneously (Matanguihan et al. 2011; Wilcoxson and Saari 1996). Common bunt lost importance because it was easily controlled by these fungicides in conventional agriculture, and the disease almost disappeared (Wilcoxson and Saari 1996). In western countries, where proper control practices are used in conventional agriculture, losses were reduced to less than 1%. However, with the expansion of organic agriculture, common bunt regained impact (Spieß 2016; AGES 2021a). Also, in developing countries, losses from 7% up to 90% per year were reported depending on region and cultivar (Gaudet et al. 2012; Mamluk 1998; Al-Maaroof et al. 2016; Goates 1996). Because of the toxicity and the disgusting odor of trimethylamine, the grain and straw of highly infested fields can´t be fed to animals and are mostly burned or delivered to biogas plants (Spieß 2016).

### Physiological Races of common bunt

Smut fungi belong to the phylum Heterobasidiomycota of the order *Tilletiales* They are obligate parasites (biotrophs) and are highly specialized to their hosts (Goates 1996; Wilcoxson and Saari 1996). There are three different designations for common bunt races: the T-form for *T. tritici,* the L-form for *T. laevis* and D for *T. controversa* (Gaudet et al. 2012; Goates 2012; Metzger and Hoffmann 1978). All three species are closely related, and *T. laevis* and *T. tritici* have similar germination requirements, symptoms and life cycles (Matanguihan et al. 2011; Blažková and Bartoš 2002; Wilcoxson and Saari 1996).

The existence of physiologic common bunt races was discovered in the 1920s. Usually, the pathogenicity of obligate biotrophs like common bunt follows Flor´s gene-for-gene hypothesis (Matanguihan et al. 2011). The theory states that for each resistance gene in the plant host, there is a corresponding avirulence gene in the pathogen (Goates 2012; Matanguihan et al. 2011). The resistance genes of common bunt and dwarf bunt are named *Bt*-genes. Currently, 16 major *Bt*-genes are classified and described. Furthermore, numerous unclassified resistance genes were detected (Bonman et al. 2006).

Common bunt races are distinguished by inoculating wheat genotypes carrying single CB resistance genes. Races are considered virulent if the plant host shows more than 10% disease incidence. Different races were described based on this method, and unique virulence patterns are classified as common bunt races (Goates 2012; Hoffmann and Metzger 1976;

Goates 1996; Wilcoxson and Saari 1996). In the 1920s, the first described bunt races, T1 and L1, were only virulent against *Bt7*. However, with the release of new resistant cultivars over time, the common bunt population evolved, and more specialized bunt races occurred. Those new races overcame almost all *Bt*-genes in the subsequent years except *Bt11* (Gaudet et al. 2012; Goates 2012).

The original differential set contained accessions carrying the genes *Bt1* to *Bt10* and has been used since the 1940s (Matanguihan et al. 2011). Hofmann and Metzger further developed this set of monogenic differential lines (Hoffmann and Metzger 1976; Hoffmann 1982). Later, the differential set was expanded by adding *Bt11* to *Bt15* (Goates 1996). The differentials *Bt1* to *Bt13* are hexaploid winter wheat cultivars. *Bt14* and *Bt15* originate from durum wheat. PI173437, known as the carrier of the resistance gene *Btp,* was also added to the differential set. *Btp* shows a reaction to common bunt independent of the other differentials (Goates 2012).

The global common bunt population is very distinct, and many studies evaluated the local race compositions (Al-Maaroof et al. 2016; Goates 2012; Hoffmann and Metzger 1976; Veisz et al. 2000; SOOD and SINGH 1985; Blažková and Bartoš 2002). In the US, 36 pathogenic races of *T. caries*, 15 races of *T. foetida*, and 19 races of *T. contraversa* were identified in 2012 based on their reaction to the differential set (*Bt1* to *Bt13* and *Btp*). In this screening, common bunt races virulent to *Bt8* and *Bt12* were described first in the US (Goates 2012). *Bt14* and *Bt15* were excluded from their study because they showed great sensitivity to temperature and a high variation in virulence reactions. Another study conducted in Ukraine found twelve distinct common bunt races. Some shared the same virulence patterns as the common bunt population described in the US (Babayants et al. 2006). Virulence to *Bt8* was detected for the first time in India (SOOD and SINGH 1985). There are also bunt isolates, which are not virulent to any of the differentials (Babayants et al. 2006; Goates 2012).

### The life cycle and symptoms of common bunt

Disease severity of common bunt infections can fluctuate significantly between different locations and seasons (Weinhappel M. 2016; Babayants et al. 2006; Veisz et al. 2000; Gaudet et al. 2012). The major damage is attributable to yield loss and loss of quality. The second name, “stinking smut,” originates from the characteristic smell of decaying fish caused by trimethylamines. These substances can be detrimental to human and animal health and, if consumed in high amounts, can even cause death (Pospischil et al. 2017; Spieß 2016).

Bunt balls are shattered during the threshing of infected wheat fields, and the released spores contaminate the healthy kernels, harvesting equipment, and the soil. Besides the unpleasant smell of the spores, they can also cause allergic reactions in men handling infected grains and contaminated harvesting and threshing equipment (Gaudet et al. 2012). If seeds are stored

under dry conditions, spores can survive easily for up to 20 years (Spieß 2016). They can also survive several years in the soil depending on plowing depth and microbial activity (Bauer et al. 2013; Weinhappel M. 2016).

Usually, no symptoms or small leaf spots occur during early growth stages. On resistant genotypes, chlorotic leaf spots are often the only unspecific symptom (Spieß 2016). The first specific evidence of common bunt infection is visible after the pollination phase (Mathre 2000). Diseased heads are slimmer than healthy ones, and the infected heads can be recognized by a greasy dark green color (Mathre 2000; Wilcoxson and Saari 1996). Plant growth can be stunted (Goates 2012; Mathre 2000), and diseased plants can stand more erect due to the lighter weight of their heads. However, these symptoms may not be observed in all genotypes (Spieß 2016). More specific symptoms are the greenish-blueish coloring of infected ears and the “flaring-out” of the spikelets due to the filling with teliospores (Spieß 2016; Mathre 2000). Anthers may be withered and stay inside the spelt (Spieß 2016; Goates 2012; Goates and Bockelman 2012)). Awns can be shortened or missing. The kernel is replaced by a sorus (or so-called bunt ball) filled with dark-brown to black teliospores. Bunt balls appear darker and are lighter in weight than regular wheat kernels, but shape and size are comparable to regular grains. The odor alone is a strong indicator for common bunt infection (Atkinson et al. 1984; Mathre 2000).

*Figure 3: Diseased heads of Triticum aestivum: Instead of regular grain filling, bunt sori filled with black teliospores are produced. Pictures provided by Magdalena Ehn*

Traditionally, *Tilletia* species are distinguished by spore morphology. *T. caries* spores have a reticular surface and a diameter of 14µm to a maximum of 25µm. In contrast, *T. laevis* spores are smoother and slightly smaller, with a diameter of 13µm to 22µm (Pieczul et al. 2018; Mathre 2000; Gaudet et al. 2012; Matanguihan et al. 2011; Wilcoxson and Saari 1996). *T. laevis* spores showed faster germination rates than *T. caries* and were more competitive in a mixed inoculum (Dumalasová and Bartoš 2008). The color of the teliospores can vary from different shades of brown to black. The *Tilletia* species infecting wheat can also be distinguished by molecular methods like PCR or LAMP (Pieczul et al. 2018; Mathre 2000). Because common bunt and dwarf bunt are closely related, it can be challenging to differentiate between them when high numbers of isolates are compared (Wilcoxson and Saari 1996). However, dwarf bunt has different climatic requirements, is restricted to regions with permanent snow cover, and is primarily soil-borne. Due to its climatic preferences, dwarf bunt is only described on winter wheat (Goates 1996; Wilcoxson and Saari 1996). *T. laevis* is more prevalent in Southern Europe, whereas *T. caries* is more common in the northern and mid-European countries (Matanguihan et al. 2011).

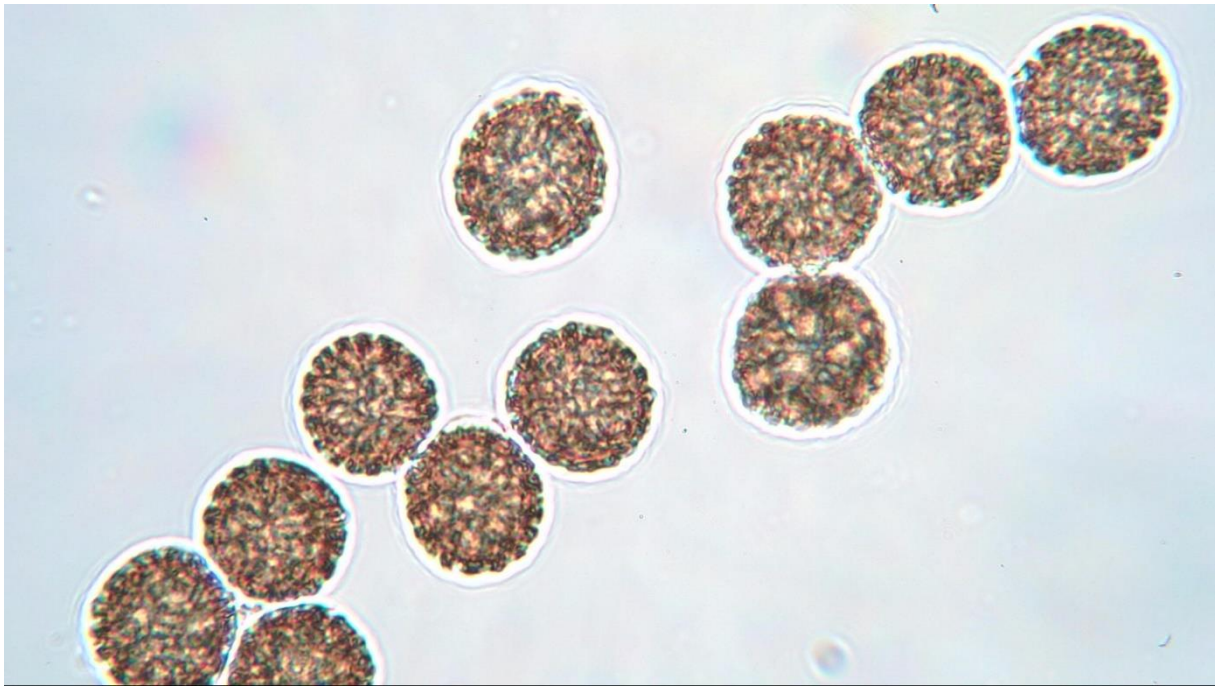

*Figure 4: Teliospores of T. caries. Picture provided by Magdalena Ehn*

Advances in molecular biology enable differentiating between similar species based on their genetic background instead of relying only on the cryptic morphology of spores. Despite the known limitations, most federal diagnostic labs still practice seed washing and morphology-based identification methods. Recently, a set of genes unique for each species was developed as candidate genes for differentiation using real-time PCR. Diagnostics remains challenging due to the ability of different *Tilletia* species to hybridize. However, it´s crucial to distinguish between *Tilletia* species because some are quarantine diseases in some parts of the world (Nguyen et al. 2019). Molecular methods also allow finding race-specific differences and help reveal the genetic background of avirulence genes.

Common bunt thrives on winter wheat cultivars because the fungus prefers cool soil temperature for germination and development (Goates and Bockelman 2012; Wilcoxson and Saari 1996). Different optimum temperatures for infections are found in the literature and range between 5°C to 15°C with an optimum at 6°C to 7°C (Spieß 2016; Hoffmann 1982; Mathre 2000; Wilcoxson and Saari 1996). Optimal soil moisture between 40% and 50% favors seed and spore germination (Mathre 2000; AGES 2021a; Wilcoxson and Saari 1996). Common bunt requires neutral to acidic pH in soils, and clay-based soils with high humus content favor the infection (Goates 1996). Optimal winter hardening in wheat requires similar conditions as common bunt needs for infection and development of the disease (Veisz et al. 2000). Optimal conditions for overwintering depend on wheat genotype and environment and can be negatively influenced by bunt infection (Veisz et al. 2000).

A Hungarian study tested the frost resistance of different wheat cultivars containing *Bt1* to *Bt10* resistance genes. They compared artificially inoculated to uninoculated cultivars over six years. The decreased frost resistance of the *Bt*-lines was not correlated with varietal susceptibility. For example, *Bt10*, *Bt5*, and *Bt8* suffered more significant frost kill in young plants but showed good resistance to common bunt, whereas *Bt7* was highly susceptible to the bunt infection but showed good frost tolerance. *Bt6* demonstrated good bunt resistance and showed a high level of frost resistance. Overall, the inoculated plants suffered more than uninoculated under cold conditions (Veisz et al. 2000). The expression of some of the *Bt*-genes seems to be temperature-dependent (Wilcoxson and Saari 1996). For example, *Bt8* was suppressed at low temperatures (Gaudet et al. 2012).

## Reproduction

Meiosis occurs within the teliospore prior to germination. Afterwards, the germinating teliospores form a germ tube (basidium) and the haploid nuclei wanders into the pormycelium. One nucleus migrates into each primary sporidium, where mitosis takes place. The promycelium bears a cluster of eight to sixteen filiform hyaline and haploid basidiospores (primary sporidia) at the apex. Genetically compatible primary sporidia fuse in pairs (anastomosis) and form characteristic H-shaped structures. Haploid nuclei come together and form a dikaryon (two separate nuclei). The germinating H-shaped structure produces sickle-shaped, hyalin secondary sporidia (secondary basidiospores), dikaryotic infection hyphae or vegetative hyphae. Also the secondary sporidia can develop to vegative or infection hyphae (Mathre 2000; Wilcoxson and Saari 1996). At this point, a race between mycelium and host starts. The infection hyphae must penetrate the floral primordia before internodal elongation for successful pathogen establishment (Veisz et al. 2000; Mathre 2000). After successful infection, the pathogen establishes itself right behind the apical meristem, and the fungus grows with little interference inside the host until ear differentiation. However, the pathogen competes with its host for nutrients at an early growth stage. During severe infection, a drop in the cell sap concentration leads to increased frost kill (Veisz et al. 2000).

Hyphae accumulate in the wheat ovaries, and the cells of the wheat plant are destroyed (Mathre 2000). Common bunt sporulates in the endosperm tissue, and the hyphal mass is rapidly transformed into spores inside the pericarp, forming bunt balls. One sorus (bunt ball) contains up to 5 M spores (Spieß 2016). Young teliospores are still dikaryotic. The nuclei fuse and form a diploid nucleus (Figure 5) (Mathre 2000).

Also, latent infection is possible if the mycelium infects older seedlings (>2cm). In this case, the pathogen cannot penetrate the rudimental structure of the ears (Spieß 2016).

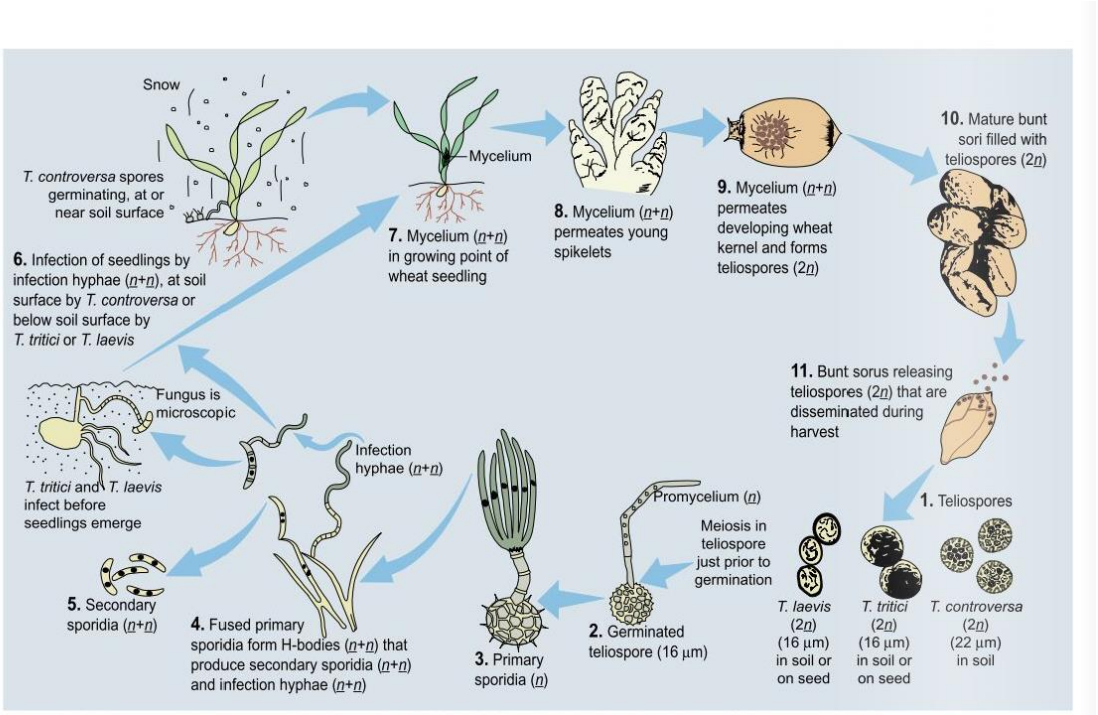


*Figure 5: Disease cycle of* common bunt *(T. caries and T. laevis) and dwarf bunt (T. controversa) (source: (Wilcoxson and Saari 1996))*

## Resistance mechanism

The initial infection stage involves the recognition of the pathogen by the host plant through the interaction of resistance genes and avirulence genes. They encode specific effectors or elicitors. Incompatible interactions usually result in the release of reactive oxygen species, nitric oxide, salicylic acid and jasmonic acid by the infected plant. Those substances activate the expression of other plant defense mechanisms to interfere with the fungal infection and result in a hypersensitive response at the infection site (Gaudet et al. 2006). A survey on gene expression on the carrier of *Bt10* inoculated with race T-1 revealed the upregulation or downregulation of genes responsible for cellular metabolism, development, abiotic/biotic stress response and the expression of transcription factors. Especially signaling pathways regulated by jasmonic acid and salicylic acid were responsible for activating defense-related genes in incompatible host-pathogen interaction (Lu et al. 2005; Gaudet et al. 2006). If wheat genotype and bunt are compatible, the fungus can penetrate the coleoptile, invade the first embryonic leaf, and establish mycelia below the apical growing point. In incompatible interactions, the mycelium can´t grow further than the coleoptile (Hoffmann 1982; Gaudet et al. 2006). Thickening of the epidermis, gelatinization between cuticle and plasma membrane, expression of cell wall defense-related phenolics and callose production were observed at the infection site and are typical defense mechanisms of plants (Gaudet et al. 2012; Gaudet et al. 2006). Furthermore, pathogen-related (PR) proteins, lipases and chitinases are essential factors interfering with the fungal infection. They are either fungistatic or fungi toxic (Gaudet et al. 2012).

### Strategies to control common bunt

The prevention of common bunt infection should be prioritized because symptoms occur late in the growing stage when curative measurements are no longer possible.

Although *Tilletia caries* and *T. tritici* are soil-borne, the primary infection source is contaminated seeds (El-Naimi et al. 2000). Therefore, the cultivation of uncontaminated certified seeds is crucial, especially for organic farmers. The threshold value in Austria for certified untreated seeds is ten spores/kernel. If the number of spores per kernel exceeds 300, it is not allowed to place the seeds on the market (Weinhappel M. 2016). Clean planting material is the most effective measure to control common bunt spread.

Different countries have established different thresholds for maximum numbers of spores found on seeds for cultivation. For example, threshold values range from zero in Denmark to 20 spores/ kernel in Germany in certified seeds. (Matanguihan et al. 2011; Waldow and Jahn 2007). In Austria a maximum of five diseased plants on an area of 150m² is allowed for certified seeds (Bundesamt für Ernährungssicherheit 2020) The number of bunted ears increases with increasing inoculum dose, and plant vigor decreases, especially in susceptible cultivars (Dumalasová and Bartoš 2008). Establishing suitable thresholds for susceptible (one spore/seed) and resistant cultivars (20 spores/seed) was recommended by Waldow and Jahn for certified seeds (Waldow and Jahn 2007)

The use of high-quality seeds with good germination capacity, thousand-seed mass, and high nitrogen and phosphorus content also enhances the seedlings' germination speed and robustness. Slow development of plants increases the probability of successful penetration of the growing point (Gaudet et al. 2012). If farmers use farm-saved seeds, they should test them for common bunt infestation in specialized laboratories (AGES 2021a). During the flowering phase of the wheat plants, a first infestation control should be done by cutting open the ears. It´s essential to cut them because only partial infestation of the ear can occur and may not be detected by visual inspection alone. During this stage, the bunt sori are unripe and still soft (Spieß 2016).

*Figure 6: Diseased head of Triticum aestivum observed in the experimental field. bunt sori filled with teliospores were observed after cutting the head open*

Furthermore, crop rotation plays a significant role in avoiding high disease pressure, especially for soil-borne diseases (AGES 2021a). The number of spores in the soil decreases every year by planting other crops different from wheat and wheat relatives, as the pathogen is highly specialized on its host. Crops like wheat, spelt, emmer, einkorn and triticale should be avoided after infestation (Spieß 2016). As a rule of thumb, a pause of three to five years is recommended to decrease spore viability (AGES 2021a).

Common bunt´s optimum infection temperature lies within 5°C to 10°C (Fuentes-Davilla et al. 2002; Hoffmann 1982; Wilcoxson and Saari 1996), and the infection is coupled with the slow development of the wheat plant in cold conditions during winter (Hoffmann 1982). Early sowing in autumn in dry soil can help to prevent infestation (Goates and Mercier 2011; Wilcoxson and Saari 1996). The project “CARIES” conducted by the Austrian agency for health and food security (AGES) and the Bio Forschung Austria tested mechanisms of infection and the spread of common under Austrian conditions over three years. They found significant differences between the two testing locations and sowing time. Early sown winter wheat showed minor infestation compared to late sowing dates. The project also proved the exponential increase of infection rates if contaminated seeds were used for the following growing period (Weinhappel M. 2016).

The regular mowing of the boundary ridge before the grasses can produce seeds is essential because they can be a source of infection. Intensive organic fertilization enhances the antagonistic activity of the soil microbiome and reduces spore numbers. Some microbes produce toxic metabolites, which inhibit spore germination (Becker and Weltzien 1993; Spieß 2016). After harvesting, shallow tillage is recommended because teliospores in the upper soil layer and on the surface are more likely to germinate in the following months (AGES 2021a).

In general, all practices that favor fast germination and plant growth (sowing time, depth of sowing, soil conditions) help the seedlings escape infestation (Spieß 2016).

### Chemical treatments

In conventional agriculture, chemical treatments of seeds are very effective (Al-Maaroof et al. 2016; Goates and Mercier 2011). It´s recommended to use seed treatments in susceptible cultivars if only a single spore is found (Spieß 2016). A considerable advantage of seed dressing is that it simultaneously controls seed- and soil-borne spores. Examples of effective chemical fungicides are Mancozeb, Diathane, Dividend and Lamardor. According to the phytosanitary register of Austria (BAES 2022) in 2022, the following fungicides are allowed for conventional production in Austria: Fludioxonil, Difenoconazole, Tebuconazole, Prothioconazole and Sedaxane. Different combinations of those active agents are available for conventional farmers in Austria (https://psmregister.baes.gv.at/psmregister/faces/main).

However, fungicides which were efficient but detrimental to the environment have been banned from the phytosanitary register. The use of chemical treatments faces many environmental problems like pollution of waterways and adverse side effects on non-target organisms (El-Naimi et al. 2000).

### Alternative treatments for common bunt control

In field trials with skimmed milk powder, hucket (local skimmed milk from Western Asia) and wheat flour, a reduction of common bunt infection up to 96% was found (El-Naimi et al. 2000). The effectiveness was equal to chemical treatments. Also, *Sinapis alba* (yellow mustard) flour showed promising results in inhibiting spore germination and reducing disease development (Waldow and Jahn 2007). There is a product named Tillecur® with yellow mustard as the active ingredient, but it´s not available in Austria (BAES 2022).

Another option for organic farmers is the use of biocontrol agents. Some microorganisms produce antibiotic substances, which can also be exploited for plant protection. So far, they are less effective compared to chemical fungicides. The fungus *Muscodor albus* showed promising results when seeds were covered with it´s spores in a field study. *M. albus* produces small volatile compounds that inhibit other fungis' spore germination (Goates and Mercier

2011). Cerall® is based on the soil bacteria *Pseudomonas chloroaphis* and is the only bio-fungicide available against common bunt in Austria (BAES 2022).

Physical methods like hot-air treatment (Thermoseed®) or steam (Steamlab®) are available for organic agriculture. However, they never prevailed due to their high costs. The company "Westrup" from Denmark developed a seed brushing machine that achieves satisfactory results in bunt control (Spieß 2016).

The Danish project "SåGodt" aimed to improve the toolbox to control common bunt and evaluated different products for their potential use in organic agriculture. Sonosteam® combines high-pressure steam and ultrasound and showed 100% effectiveness in killing single spores. However, the treatment did not affect intact bunt sori. E-vita® and E-pura® are electron beam treatments with high efficacy. However, they conflict with the ban on ionizing radiation in organic farming. Furthermore, the project included testing of two polysaccharides originating from brown algae and saponins extracted from various plants like quinoa and corncockle. Those treatments showed ambiguous results, and more work needs to be done to guarantee high efficiency. Panoramix® based on *Trichoderma spp.* significantly reduced bunt infection. Furthermore, vinegar and citric acid were tested, but are not recommended due to adverse effects on plant vigor, if not applied correctly (Borgen 2021).

### Resistance Breeding

Even if common bunt can be controlled easily with chemical treatments, the disease can spread fast under organic conditions. The cultivation of resistant wheat accessions remains a cornerstone in bunt control for environmental and economic reasons. So far, it´s the most effective way of disease management for organic farmers and low-input farms (Ciucă 2011; Matanguihan et al. 2011). Farmers in developing countries often don't have access to certified and treated seeds for every planting season. The high costs and the distribution of the treated material are also significant constraints for farmers in these countries (El-Naimi et al. 2000).

Because most modern breeding programs are designed for conventional agriculture, little effort is put into common bunt resistance. Only a few resistant or less susceptible cultivars are registered worldwide, and there is excellent potential in resistance breeding. However, for the development of locally adapted wheat genotypes, it is essential to investigate the race-specific virulence patterns of the pathogen for every region. Especially for organic farmers who find infestations in their fields, cultivating resistant cultivars is critical to avoid further spread and yield loss (Spieß 2016). Because most resistant cultivars are developed under conventional conditions, they often do not fulfill the requirements for organic farms and lack essential traits needed for low input cropping systems (Matanguihan et al. 2011; AGES 2021b).

In the Austrian catalog of varieties, there is no fully resistant cultivar listed. Only three cultivars are described as moderately resistant: Tillexus (*Bt10*), Tilliko (*BtZ*) and Tillsano (*Bt5*) (AGES 2021b; Die Saat 2021; Cultivari 2019; Oberforster and Plank M. 2021; Borgen et al. 2019). Those three cultivars were bred and approved under organic conditions (AGES 2021b).

In Canada, intermediate resistance to common bunt is required by the registration testing system of the Prairie Recommending Committee for Grain for new wheat cultivars because common bunt is considered a Priority1 disease (Chen et al. 2016; Goates and Bockelman 2012). However, in Austria, common bunt resistance is not a necessity for the admission of new cultivars (AGES 2021b). Adapting the cultivar registration system would be desirable, and common bunt resistance should be included in those requirements (AGES 2021b).

Marker-assisted selection (MAS) is beneficial for resistance breeding because molecular techniques can be applied to seeds or seedlings. They reduce time and cost for developing new resistant cultivars because usually, bunt symptoms are only visible late in the growing phase (Gaudet et al. 2012). Mutations and recombination of avirulent genes in the bunt population increase the difficulty of obtaining long-term efficiency if only single *Bt*-genes are introduced. The use of markers also allows gene pyramiding of several resistance genes for durable resistance in wheat varieties (Gaudet et al. 2012). Molecular markers closely linked to common bunt resistance genes are desired for gene pyramiding. Much research has been conducted on marker development, and some markers are already available and used in breeding. For example, markers linked to *Bt9*, *Bt10* and *Bt12* were developed (Steffan et al. 2017; Muellner et al. 2020; Laroche et al. 2000). The resistance QTLs of the cultivars ‘Blizzard’ and ‘Bonneville’ were mapped on the chromosomes 1A, 1B and 7A (Wang et al. 2009; Muellner et al. 2021). Genome-Wide Association Studies (GWAS) can be used to identify SNPs associated with resistance located on different chromosomes (Mourad et al. 2018; Bhatta et al. 2019). Diversity of resistance genes provides durable resistance to common bunt because combinations of resistance genes are difficult to overcome by the pathogen. For example, the wheat line PI178383 carries *Bt8, Bt9* and *Bt10* and was effectively exploited in the US for more than 20 years (Gaudet et al. 2012; Wilcoxson and Saari 1996). Molecular markers were also used to screen the Romanian winter wheat germplasm for *Bt10* (Ciucă and Săulescu 2008). Numerous more studies have been conducted, and the abovementioned are only some examples of the use of molecular tools.

Bonman et al. (2006) tested more than 10 000 common wheat accessions, and they found that resistance is connected to other traits like geographic distribution, awnedness, glume and kernel color. Furthermore they found a high frequency of resistant landraces originating from Southern Europe, Western Asia and Southcentral Asia - mainly from Turkey, Iran, Macedonia, Serbia and Montenegro (Bonman et al. 2006). Therefore, they suggest screening especially

genotypes from these regions as resistance sources. Another study used GGE biplot analysis to screen 200 different wheat genotypes collected in provinces of Turkey for common bunt resistance, and they found 59 resistant lines (Akçura and Akan 2018). New sources of resistance with unknown genes or different gene combinations were identified in landraces of the National Small Grains Collection (USDA-ARS) (Goates and Bockelman 2012). Screening for new resistance sources is essential to broaden the genetic diversity in wheat breeding programs

Another promising approach is the integration of resistance genes from wheat-related species like *Aegilops spp*. and *Agropyron spp*. by interspecific hybridization and repeated selections afterward. In Ukraine, cultivars resistant to various pathogenic fungi like *Fusarium spp*, powdery mildew, rusts and bunts were developed by hybridization with *Aegilops cylindrica, Aegilops variabilis, Triticum erebuni* and *Triticum tauschii.* New and effective *Bt*-genes were identified (Babayants et al. 2006). Also, lines derived from crosses with triticale and *T. monococcum* are potential new sources for resistance (Onicica and Săulescu 2008). The intercrossing of wheat with its relatives helps to diversify the genetic basis for resistance. The exploitation of locally occurring common bunt races with unique virulence patterns assists in targeting and elucidation of *Bt*-genes in wheat and supports the development of regionally adapted cultivars.

## Research Questions:

The objective of this study was to evaluate the virulence of eight isolates of common bunt collected in different areas of Austria on forty wheat genotypes. For the evaluation, the wheat genotypes were artificially inoculated with each of the eight isolates, and the host-pathogen interaction was examined under field conditions in two replications. The test panel included the common bunt differential set to test isolate-specific virulence to different resistance genes.

1. Are there specific genotype by isolate interactions?
2. Which isolate can overcome which *Bt*-gene?
3. How do isolates differ in their aggressiveness?
4. Are resistance sources currently used in pre(breeding) projects effective against a range of Austrian isolates?
5. How strong is spore-carryover at sowing?

# Material and Methods

## Location

The experimental field was located in Tulln (N 16°02,497' E 48°18,454') close to the Interuniversity Department for Agrobiotechnology (IFA) Tulln (Figure 7). The preceding crop on this site was winter wheat (*Triticum aestivum*).


*Figure 7: Location of the experimental field (double red lines in the middle) and the IFA (red circle on the top right side) in Tulln*

## Climatic Conditions

For the growing period of 2020/21, the average temperature in Tulln was at 9.6°C. It was slightly lower than the average temperature (11.6°C) measured over 30 years (1991 – 2020). 2020/21 was very dry. Total precipitation during the growing period was only 377mm. The mean rainfall measured over the last 30 years for Tulln was 685.1mm ((Meteostat 2022); Figure 22). The soil temperature decreased from approximately 8°C on the sowing date (15$^{th}$ of November) to 4°C at the end of November 2020 ((BOKU 2022); Figure 23).

## Isolates

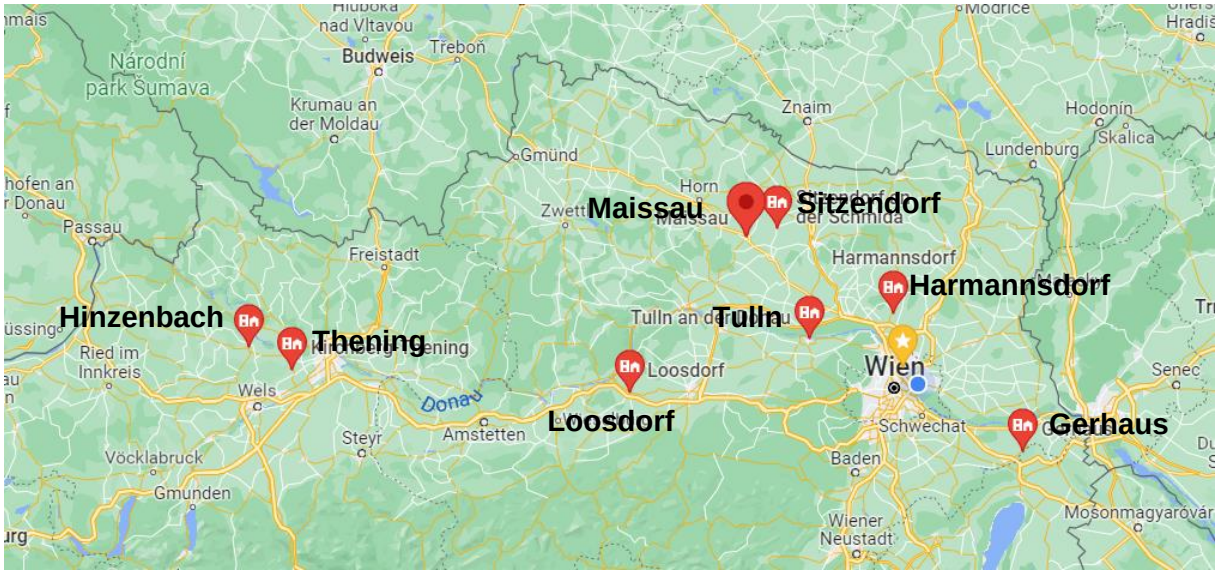


*Figure 8: Isolates were collected from wheat fields in Burgenland, Upper and Lower Austria*

The IFA provided the isolates *IFA Housekeeping* and *IFA Aggressive*. *IFA Housekeeping* contains a race mix of *T. caries* spores collected at three different locations in Eastern and Western Austria (Muellner et al. 2020). *IFA Aggressive* was collected in 2018 on the wheat cultivar 'Tilliko,' which is described to be tolerant against common bunt (Die Saat 2021). The IFA Aggressive spores originally were collected on the farm of Christian Hameter in Maissau and were forwarded to the IFA by Christian Gladysz (Saatbau Linz). DI Michael Oberforster (Department for sustainable plant production, AGES) provided the other six isolates. They were gathered in different wheat-growing regions of Austria (Figure 8; Table 1).

*Table 1:Origins of isolates*

| Isolate origin | Provided by |
|---|---|
| IFA Housekeeping | IFA |
| IFA Aggressive (Maissau) | IFA |
| Loosdorf | AGES |
| Gerhaus | AGES |
| Thening | AGES |
| Hinzenbach | AGES |
| Sitzendorf | AGES |
| Harmannsdorf | AGES |

## Wheat genotypes

The test panel consisted of forty wheat genotypes, including fourteen genotypes of the CB differential set. The differential set consists of wheat accessions, which carry specific bunt resistance (*Bt*) genes (*Bt1* to *Bt13* and *Btp*). They were used in previous studies to determine pathogenic races of common bunt and dwarf bunt (Goates 2012; Hoffmann and Metzger 1976). The differentials containing *Bt14* and *Bt15* were excluded because their expression depends on environmental conditions and is unstable (Goates 2012). *BtZ* originates from a translocation from the wheat relative *Agropyron intermedium* (Wilcoxson and Saari 1996) and is an additional major resistance gene. In this trial the cultivar 'Tilliko' was included as carrier of *BtZ* (Borgen et al. 2019). Furthermore, the test panel included regionally adapted resistant cultivars, single resistance QTL/gene donors from breeding projects and promising exotic resistance sources. The susceptible cultivars 'Capo' and 'Aurelius' were included in the field trial as highly susceptible check cultivars to determine disease pressure and aggressiveness of the different bunt isolates. A complete list of the lines comprised in the test panel can be found in the appendix (Table 12).

## Inoculation of seeds

Seeds of each genotype were artificially inoculated with bunt teliospores collected from infected spikes from eight different Austrian locations (Table 1). Bunt balls were crushed and teliospores obtained through sieving. The spore samples were sieved using 500µm and 125µm Retsch sieves to remove plant residuals from the spores.

For seed inoculation, a solution of 0.05% methylcellulose in water was prepared and mixed with the spores. 2g of methylcellulose (ROTH Nr 8421.2, M ~ 3000 g/mol, 3300-4500 mPa s) were dissolved in 1L deionized water. The suspension was homogenized overnight at room temperature. A spore suspension was prepared for each of the eight isolates separately. Approximately 4.5g of teliospores were mixed with 30ml of the 0.05% methylcellulose solution in an Erlenmeyer flask. The mixture was stirred using a magnetic stir bar to homogenize the spore-methylcellulose suspension until a viscous liquid with a spore concentration of 0.15 mg/ml was obtained.

20g of seeds of each of the forty wheat accessions were separately inoculated with each isolate. The inoculation was performed with a machine designed for seed dressing of small grain samples (Figure 9). With an electric pipette, 0.6 ml of the isolate suspension were pipetted onto the seeds in a can (approx. 0.18g of spores/20g of seeds). The cans were closed and shaken for approximately one minute until the seeds were fully coated with the spore solution. The high spore concentration was essential to ensure high disease pressure and prevent disease escape due to insufficient inoculum application. After inoculation, the seeds

were filled into paper bags and stored at room temperature until sowing. After each isolate, all the material was rinsed with hot water and cleaned to avoid cross-contamination.


*Figure 9: Picture on the left: seed dressing machine / right: methylcellulose - spore suspension*

For sowing, 10g of seed per genotype and isolate were used for one plot. Each genotype-isolate combination was sown in two replications in a randomized complete block design. Seeds were planted with the sowing machine “Wintersteiger plomatic ERS” at a depth of two to three cm in 1.6 m double rows per plot with 17 cm distance between the rows of one plot. The distance between plots was 33 cm. Sowing was done late in the season, on the 15th of November 2020, because of bad weather conditions and to favor bunt infection.

After sowing of each isolate, eleven plots with uninoculated seeds of the highly susceptible cultivars ‘Capo’, ‘Aurelius’ and ‘Midas’ were sown to “clean” the sowing machine from spore residues and test spore carry-over between plots after each isolate. The team of IFA Tulln conducted field management and plant protection measurements. A summary of agricultural practices applied in the experimental field can be found in the additional material (Table 11).

## Trait Assessment

Trait assessment started at the end of May 2021 and ended in July 2021. The plots were checked at least every third day.

The evaluation of resistant or susceptible reactions of each wheat line to each bunt isolate was done by assessing a total of 150 spikes per plot (75 plants/row) and by counting how many of them were diseased. Resistance was assumed if no diseased heads were found in the first row (first 75 plants). The ears were cut longitudinally using a standard garden shear and

recorded as diseased if at least one bunted kernel was found inside the ear. The mean percentage of diseased heads for each genotype and isolate was calculated afterward.

A scheme used in previous studies (Szunics 1990) was used to evaluate the genotypes in terms of their reaction to common bunt (Table 2).

*Table 2: Types of resistance (Szunics 1990)*

| Type of resistance | Infected ears [%] |
|---|---|
| Very resistant | 0.0 |
| Resistant | 0.1–5.0 |
| Moderately resistant | 5.1–10.0 |
| Moderately susceptible | 10.1–30.0 |
| Susceptible | 30.1–50.0 |
| Very susceptible | 50.1–100.0 |

The isolates were also evaluated by their virulence/ avirulence reaction to the **differential set,** according to Hoffmann and Metzger (1976). Differentials with lower than 10% CB incidence were considered resistant, whereas all differentials with >10% CBI showed a susceptible response to different isolates. This evaluation scheme was used to compare the results with other studies, which used the same scheme (Hoffmann and Metzger 1976).

Additionally, traits like flowering time, date of heading and plant height of the wheat accessions were recorded. Date of heading and date of flowering were scored as days after 1$^{st}$ of May. The heading date was recorded as the day when approximately 50% of the ears in the respective plot reached BBCH55. The flowering date was recorded when about 50% of heads in a plot were flowering (BBCH65). Scoring of heading started on the 29$^{th}$ of May and ended on the 18$^{th}$ of June. Flowering started on the 5$^{th}$ of June for early genotypes and ended on the 22$^{nd}$ of June for late genotypes.

After plant growth was completed, plant height was determined as the average height of plants per plot measured from ground to the top (excluding awns) in five-centimeter intervals.

## Statistical analysis

The program R (version 4.0.5 (2021-03-31)) and R Studio were used for statistical analysis and visualization of the data (R Core Team 2021).

### Data visualization

The relationships between common bunt and other traits (plant height, date of flowering, date of heading) were visualized with scatterplots (Figure 20). Differences between genotypes and isolates were displayed using boxplots (Figure 11/Figure 12). Violin plots were used to visualize data distribution (Figure 21). To observe specific genotype-isolate interactions, a heatmap (Figure 9) and GGE biplots (Figure 10-16) were created.

### Analysis of variance (ANOVA)

Analysis of variance (ANOVA) was performed to determine the effects of isolates, genotypes and their interactions by fitting a linear model of the form:

$$t_{ijk}=\mu+c_k{:}b_j+a_i+b_j+(ab)_{ij}+e_{ijk}$$

with $t_{ij}$ being the phenotypic observation of common bunt incidence on genotype *i* inoculated with isolate *j*, $\mu$ denoting the overall mean CB incidence, $c_k{:}b_j$ representing the replicates (=blocks) nested within the individual isolates, $a_i$ referring to the genotypic effect of the $i^{th}$ accession, $b_j$ representing the effect of the $j^{th}$ isolate, which can be regarded as the environmental effect, $(ab)_{ij}$ being the interaction effect between genotype and isolate, and $e_{ij}$ describing the error term. All factors except the error term were treated as fixed.

### Phenotypic correlations

A Shapiro-Wilk test revealed that none of the morphological traits (plant height, date of flowering, date of heading) had a normal distribution. Therefore, Spearman´s rho correlation coefficient was calculated for all relationships between common bunt incidence and other traits. Spearman´s rho can take values between +1 and -1. If rho is close to zero, the relationship between the two traits is weak. Rho of +1 means a perfect positive association of ranks, whereas -1 means a perfect negative association between ranks.

### AMMI-Stability

AMMI stability was calculated because it is a common method to explore genotype-by-environment interactions (GEI). AMMI captures large portions of the interaction sum of squares and separates the main and interaction effects. The model combines ANOVA (isolate and genotype main effects) and principal component analysis (PCA) for the interactions. For genotype stability, the Stability Measure based on Fitted AMMI model (FA) was calculated (Raju 2002).

The different isolates were evaluated as environments. ANOVA alone fails to detect some of the interaction components, and with PCA alone, it's challenging to separate significant genotypes and isolate main effects (Zobel et al. 1988; Raju 2002). AMMI assists in clarifying the genotype by isolate interaction by identifying patterns and relationships of genotypes and isolates (environments) (Zobel et al. 1988). The interaction effects can be visualized by biplot analysis where PCA scores are plotted against each other or PC1 against common bunt incidence (Badu-Apraku et al. 2020).

ANOVA separates the variance into three components: genotype deviations from the grand mean, isolate deviations from the grand mean, and GxI deviations from the grand mean. Subsequently, multiplication effect analysis divides GxI deviations into different interaction principal component axes (PCA) (Figure 14) (Raju 2002).

The AMMI model, according to (Gauch and Zobel 1996), is defined as

$$Y_{ij} = \mu + g_i + e_j + n \sum k=1 \lambda_k * \alpha_{ik} * \gamma_{jk} + e_{ij},$$

where $Y_{ij}$ is the mean CB incidence of the $i$th genotype treated with the $j$th isolate, $gi$ referring to the genotypic effect of the $i$th genotype minus the grand mean, $\lambda_k$ is the square root of the eigenvalue of the PCA axis $k$, $\alpha_{ik}$ and $\gamma_{jk}$ are the principal component scores for PCA axis $k$ of the $i$th genotype and the $j$th isolate, respectively and $e_{ij}$ is the residual term. The isolate and genotypic PCA scores are expressed as unit vector times the square root of $\lambda_k$; i.e., isolate PCA score = $\lambda_k$ 0.5 $\gamma_{jk}$; genotype PCA score = $\lambda_k$ 0.5 $\alpha_{ik}$.

### GGE biplot analysis for pathogen – genotype interactions

GGE biplot analysis is an excellent data visualization tool and can be applied to different agronomic and genetic data. The G stands for genotype and GE for Genotype-by-environment-effect and has been used since 1971. The tool was continuously expanded for environment and genotype evaluation, megaenvironment evaluation, and pathogen-genotype interactions (Yan and Falk 2002; Akçura and Akan 2018). GGE biplot allows graphically extracting information from all kinds of two-way data and complex genotype x environment interactions (GEI) (Yan and Falk 2002; Badu-Apraku et al. 2020; Yan et al. 2007). Suppose ANOVA shows

significant effects for the interaction term; the biplot assists in displaying the nature of the interactions (Badu-Apraku et al. 2020). For this experiment, isolates were treated as environments. Superior genotypes were the most susceptible genotypes, and their stability, when tested in different environments (isolates), indicates specific susceptibility to certain isolates. A stable genotype is defined by unchanged performance regardless of isolate treatment (Badu-Apraku et al. 2020).

Different views of GGE biplots have been constructed, facilitating genotype and isolate comparisons and finding specific virulence patterns based on mean CB incidence (Yan and Falk 2002). Isolates with similar virulence patterns were combined into megaenvironments (MEs). Megaenvironments are defined as locations (isolates) that share the same set of winning (susceptible) genotypes (Yan et al. 2007). Within these megaenvironments, genotypes can be evaluated for their performance and stability (Yan et al. 2007). The “discriminating power vs. representativeness” GGE biplot was constructed to assess the best test isolate. The “ideal” isolate should be both: discriminating between the genotypes and representative for all isolates. (Yan et al. 2007). Using host-by-pathogen biplots can be helpful to find meaningful genotype by isolate interactions (genotype by isolate specificity), to distinguish between horizontal and vertical resistance, to elaborate which germplasm carries resistance genes to which isolate and to group the isolates and genotypes according to their responses. Biplots are also a valuable tool to investigate disease response over several years (Yan and Falk 2002).

The data was environment (isolate)-centered, meaning that the biplots consist of the genotype main effects and genotype by isolate interactions (Yan and Falk 2002). The data was then subjected to SVD (singular value decomposition), resulting in various principal components (PCs). SVD is used to find the best representation of the data in the two-dimensional space of the biplots (Kroonenberg 2008). The biplots were constructed by plotting the first two PCs (PC1 and PC2), representing the data's main variability (Kroonenberg 2008; Yan et al. 2007). Subsequent PCs are considered as residues (Yan and Falk 2002). The following formula estimates each element displayed in the GGE biplot:

$$Y_{ij}=\mu+e_j+\sum n=1N*\lambda_n*\gamma_{in}*\delta_{jn}+\varepsilon_{ij}$$

Where, $Y_{ij}$ = mean response of *i*th genotype (*i* = 1,…,I) treated with the *j*th isolate *(j* = 1,..,J), $\mu$ = grand mean, $e_j$= isolate deviations from the grand mean, $\lambda_n$ = the eigen value of PC analysis axis, $\gamma_{in}$ and $\delta_{jn}$ = genotype and isolate PCs scores for axis n, $N$= number of PCs retained in the model and $\varepsilon_{ij}$= residual effect~ N (0,$\sigma^2$).

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# Results

## Isolates

Eight isolates collected in different Austrian regions were tested on forty wheat accessions, breeding lines and cultivars to compare their aggressiveness and detect possible isolate-specific virulence patterns. Significant differences were found between the isolates by performing an ANOVA (Table 5) with a posthoc test (Table 6) to compare the isolates against each other. The overall mean value of common bunt incidence across all isolates was calculated by including the whole panel and was 10.44% ± SD 1.53%. The average incidence caused by each isolate is shown in red in Figure 10. Median values of all isolates were low because more than half of the tested genotypes showed resistance (<5% CB incidence). The median, minimum, maximum, mean and standard deviation of CB incidence per isolate can be found in Table 3. The distribution of the data is visualized in Figure 21. *Loosdorf* was the most aggressive isolate (12.50%), followed by *IFA Aggressive* (12,08%) and *Gerhaus* (11.58%). *Harmannsdorf* (8.26%) and *IFA Housekeeping* (8.33%) were the least aggressive isolates in our experiment. *Thening* (11.07%), *Sitzendorf* (10.04%) and *Hinzenbach* (9.71%) showed intermediate aggressiveness.

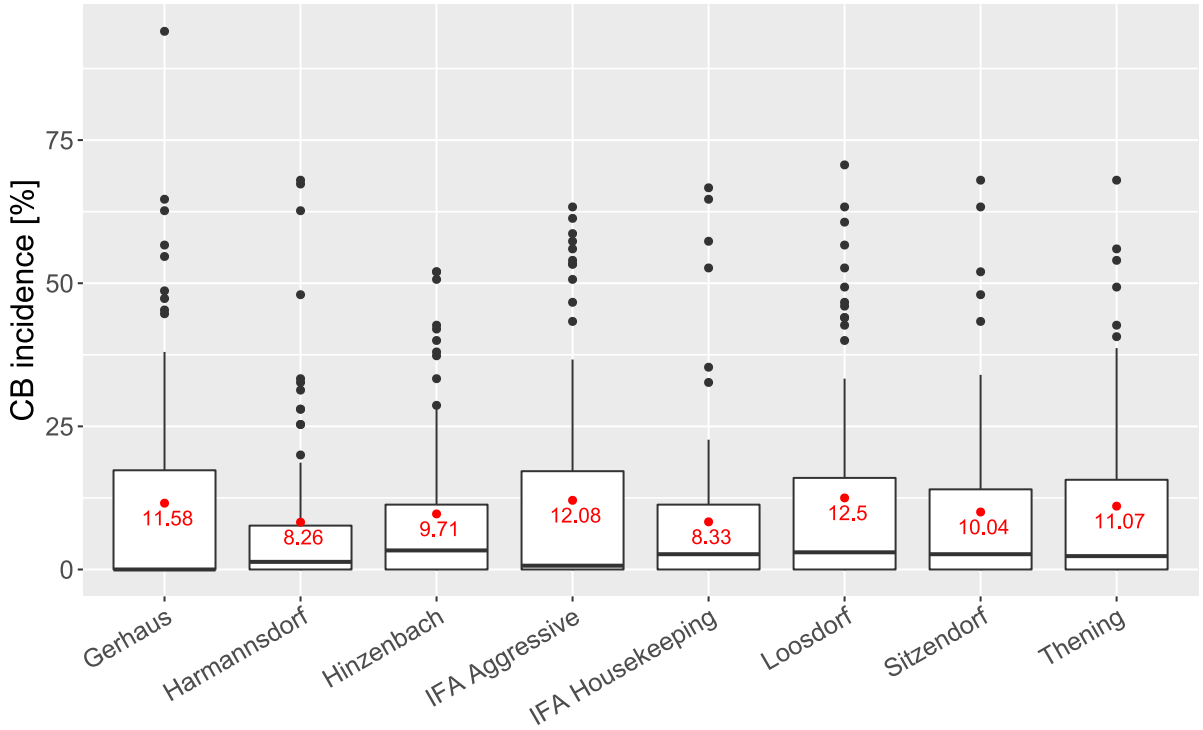


*Figure 10: Common bunt incidence [%] of eight Austrian isolates. Red numbers and dots display the mean value of common bunt incidence in %*

*Table 3: Mean, standard deviation (SD), maximum value (MAX), minimum value (MIN) and median of common bunt incidence caused by different Austrian isolates. Values are displayed in %.*

| | Mean | SD | MAX | MIN | MEDIAN |
|---|---|---|---|---|---|
| **IFA Housekeeping** | 8.26 | 13.86 | 58.69 | 0 | 2 |
| **IFA Aggressive** | 12.08 | 19.17 | 62.33 | 0 | 1.51 |
| **Loosdorf** | 12.5 | 18.32 | 67 | 0 | 4.5 |
| **Gerhaus** | 11.58 | 19.31 | 63.67 | 0 | 1.67 |
| **Thening** | 11.07 | 15.8 | 49.33 | 0 | 3 |
| **Hinzenbach** | 9.71 | 14.55 | 52.67 | 0 | 3.34 |
| **Sitzendorf** | 10.04 | 14.81 | 58 | 0 | 2.01 |
| **Harmannsdorf** | 8.26 | 14.92 | 67.67 | 0 | 1.5 |
| **Total Mean** | **10.44** | | | | |
| **SD-Total Mean** | **1.53** | | | | |

The isolates were also evaluated in terms of their virulence/ avirulence reaction to the differential set according to (Hoffmann and Metzger 1976). Differentials with lower than 10% CB incidence were considered resistant, whereas all differentials with >10% CBI showed a susceptible response to different isolates. The only isolates sharing the same patterns on the differentials were *Hinzenbach* and *Harmannsdorf*. Those isolates were virulent against *Bt2, Bt3, Bt7* and *Bt13.* All Austrian isolates overcame the resistance genes *Bt2*, *Bt7* and *Bt13*. Conversely, the resistance genes *Bt1, Bt4 – Bt6, Bt11, Bt12* and *Btp* were resistant to all Austrian isolates. *Bt3, Bt8, Bt9* and *Bt10* were susceptible to some isolates (Table 4).

*Table 4: Virulence formula: summary of the reaction of the differential set to Austrian CB isolates*

| | **virulent against *Bt*-genes (10.1-100% CBI)** | **avirulent against *Bt*-genes (0-10% CBI)** |
|---|---|---|
| **IFA Housekeeping** | 2,7,9,13 | 1,3,4,5,6,8,10,12,p |
| **IFA Aggressive** | 2,3,7,10,13 | 1,4,5,6,8,9,11,12,p |
| **Loosdorf** | 2,3,7,8,9,10,13 | 1,4,5,6,11,12,p |
| **Gerhaus** | 2,7,10,13 | 1,3,4,5,6,8,9,11,12,p |
| **Thening** | 2,7,9,10,13 | 1,3,4,5,6,8,11,12,p |
| **Hinzenbach** | 2,3,7,13 | 1,4,5,6,8,9,10,11,12,p |
| **Sitzendorf** | 2,3,7,8,13 | 1,4,5,6,9,10,11,12,p |
| **Harmannsdorf** | 2,3,7,13 | 1,4,5,6,8,9,10,11,12,p |

## Genotypes

The test panel included nine registered cultivars, fourteen differential lines (bunt resistance genes *Bt1-13* and *Btp*) and seventeen experimental lines or resistance donors. Table 2 displays the evaluation scheme used to define the type of resistance based on the percentage of infected ears. All wheat accessions developed well during the growing period, and a sufficient amount of plants was available for evaluation in each plot.

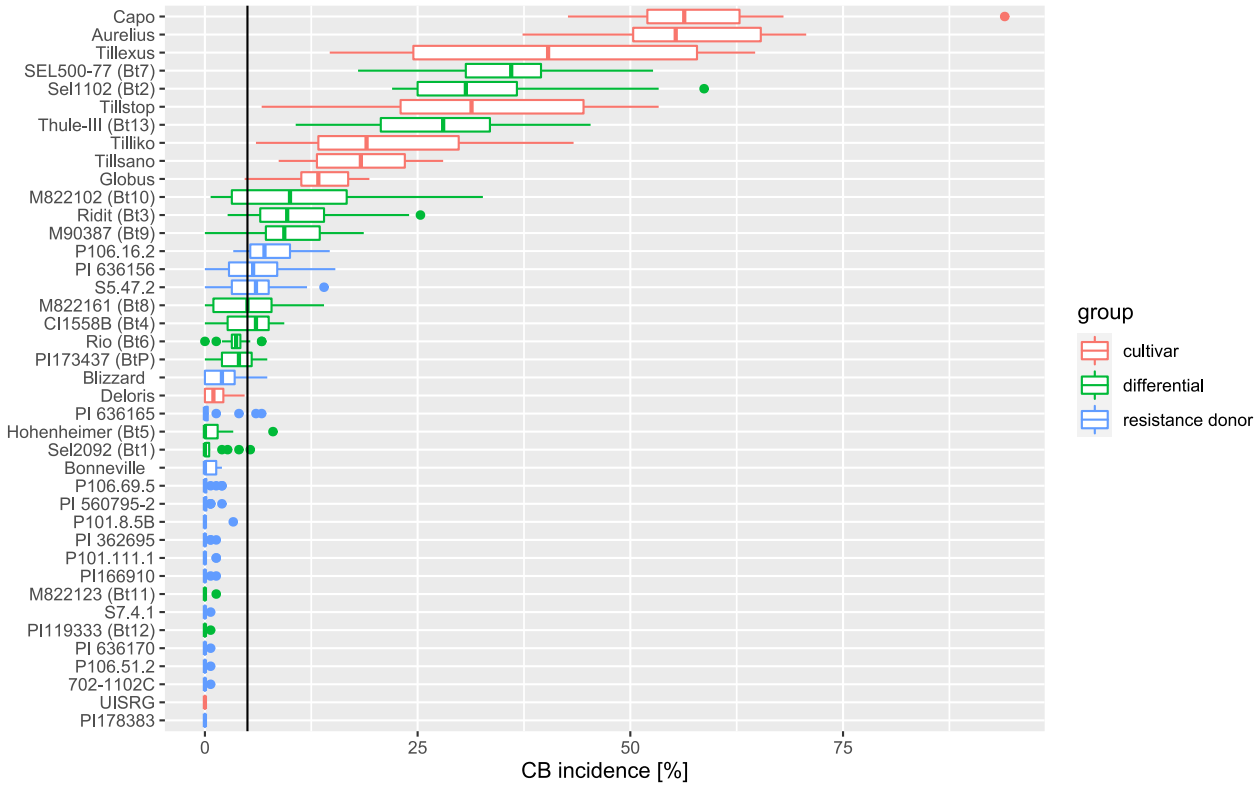


*Figure 11: Boxplots demonstrating* common bunt *incidence [%] of tested wheat accessions: Genotype groups are represented by different colors. Genotypes are ordered by their mean CB incidence from most susceptible on top to least susceptible at the bottom. The black line at 5% separates resistant (<5%* common bunt *incidence, left) from susceptible (>5%* common bunt *incidence, right) accessions. Outliers are represented as dots.*

Common bunt incidence is expressed as the percentage of infected heads in the total number of scored heads and classified by the scheme of Szunics (Szunics 1990) (Table 2). 'Aurelius' (57.30%) and 'Capo' (58.45%) scored highest, and both were classified as very susceptible (>50% disease score) to common bunt. They were included in the panel as highly susceptible check cultivars. High common bunt incidence was also found in the cultivars 'Tillexus' (40.71%), 'Tillstop' (31.58%), 'Tilliko' (21.67%) and 'Tillsano' (18.58%). All of them were either moderately susceptible or susceptible. The only resistant cultivars were 'Deloris' with 1.39%, and 'UISRG' with 0.00% mean common bunt incidence. In the genotype group "resistance donor," only the breeding lines P106.16.2 (7.83%), PI636156 (5.96%), and S5.47.2 (5.46%) showed mean common bunt incidence higher than 5%, while all other genotypes in the group had lower incidence levels. None of the resistance donors scored higher than 10%, and

therefore all of them were considered moderately to very resistant. Mean values of all tested genotypes can be found in the heatmap Figure 13.

Genotypes designated to be carriers of the resistance genes *Bt7* (35.79%), *Bt2* (32.75%), *Bt13* (27.87%), *Bt10* (12.83%), *Bt3* (11.00%) and *Bt9* (10.42%) also showed moderate to high susceptibility. In contrast, the carriers of the resistance genes *Bt12* (0.04%), *Bt11* (0.08%), *Bt1* (0.88%), *Bt5* (1.33%), *Btp* (3.59%) and *Bt6* (3.79%) were resistant to common bunt infection. *Bt4* (5.09%) and *Bt8* (5.38%) were moderately resistant. An overview of the differentials can be found in Figure 12.

Some of the differential lines showed high variability. For example, SEL500-77 (*Bt7)* ranged from 26.67% incidence with *IFA Housekeeping* to 49.67% under the *Loosdorf* treatment indicating varying susceptibility to different isolates.

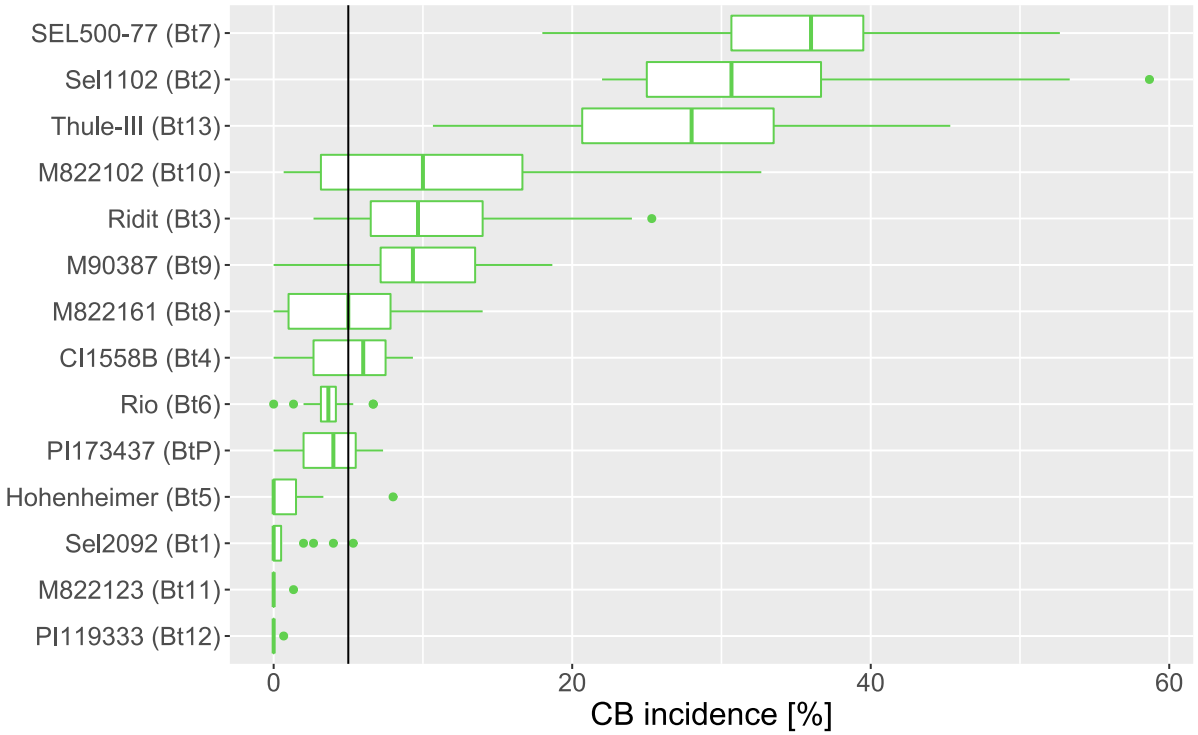


*Figure 12: Boxplots of* common bunt *incidence [%], including only the genotypes of the differential set. Genotypes are ordered by their mean CB incidence [%], starting with the most susceptible on top to the least susceptible at the bottom. The black line represents the threshold of 5%. All genotypes with less than 5% CB incidence are considered resistant Outliers are represented as dots.*

| genotype | IFA Housekeeping | IFA Aggressive | Loosdorf | Gerhaus | Thening | Hinzenbach | Sitzendorf | Harmannsdorf | mean |
|---|---|---|---|---|---|---|---|---|---|
| PI119333 (*Bt12*) | 0.33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.04 |
| M822123 (*Bt11*) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.67 | 0.08 |
| Sel2092 (*Bt1*) | 0 | 0 | 0 | 0 | 3.33 | 3.67 | 0 | 0 | 0.88 |
| Hohenheimer (*Bt5*) | 0 | 0.67 | 5 | 2 | 0 | 1.33 | 0 | 0 | 1.13 |
| PI173437 (*Btp*) | 6 | 0 | 4.67 | 1.33 | 4.67 | 6.67 | 3.67 | 1.67 | 3.59 |
| Rio (*Bt6*) | 5.5 | 1.67 | 3 | 3.67 | 5.5 | 3 | 4.67 | 3.33 | 3.79 |
| CI1558B (*Bt4*) | 2.67 | 1.34 | 5.34 | 3.34 | 7.33 | 7.33 | 7 | 6.33 | 5.09 |
| M822161 (*Bt8*) | 3.67 | 1 | 10.67 | 0 | 3.34 | 7.67 | 13.34 | 3.33 | 5.38 |
| M90387 (*Bt9*) | 11 | 9 | 18 | 6.67 | 14 | 9 | 8.34 | 7.33 | 10.42 |
| Ridit (*Bt3*) | 4 | 10.33 | 11.33 | 4.33 | 7.33 | 11 | 19 | 20.67 | 11 |
| M822102 (*Bt10*) | 6.33 | 32.67 | 14.67 | 26 | 13.33 | 5.67 | 2.67 | 1.33 | 12.83 |
| Thule-III (*Bt13*) | 12.33 | 19.67 | 31.67 | 45 | 35.33 | 21.33 | 27.33 | 30.33 | 27.87 |
| Sel1102 (*Bt2*) | 27.67 | 56 | 43.34 | 21.33 | 34.33 | 25.67 | 24.33 | 29.33 | 32.75 |
| SEL500-77 (*Bt7*) | 26.67 | 35.33 | 49.67 | 33 | 33.34 | 40 | 38.67 | 29.67 | 35.79 |
| PI178383 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| UISRG | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| PI 636170 | 0 | 0 | 0 | 0 | 0.33 | 0 | 0 | 0 | 0.04 |
| P106.51.2 | 0.33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.04 |
| S7.4.1 | 0.33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.04 |
| 702-1102C | 0 | 0 | 0 | 0 | 0 | 0 | 0.335 | 0 | 0.04 |
| PI166910 | 0.33 | 0 | 0 | 0 | 0 | 0 | 0.67 | 0 | 0.13 |
| P101.111.1 | 0 | 0 | 0.67 | 0 | 0.67 | 0 | 0 | 0 | 0.17 |
| PI 362695 | 0 | 0 | 0.67 | 0 | 0.34 | 0 | 0 | 0.34 | 0.17 |
| P101.8.5B | 1.67 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.21 |
| P106.69.5 | 0 | 0 | 0.67 | 0 | 1 | 0 | 0 | 0.33 | 0.25 |
| PI_560795-2 | 1.33 | 0 | 0 | 0.34 | 0 | 0 | 0.34 | 0 | 0.25 |
| Bonneville | 2 | 0 | 0.67 | 0 | 0 | 1 | 0 | 1 | 0.58 |
| PI_636165 | 6.34 | 2 | 0 | 0 | 0 | 0.67 | 0 | 0 | 1.13 |
| Deloris | 1 | 2.34 | 1.34 | 3 | 1 | 1.33 | 0.67 | 0.4 | 1.39 |
| Blizzard | 1 | 2.67 | 4.33 | 2.34 | 2 | 1.67 | 1.34 | 2 | 2.17 |
| S5.47.2 | 1.33 | 4.33 | 7 | 0 | 6 | 7.67 | 10.67 | 6.67 | 5.46 |
| PI_636156 | 10.67 | 2 | 7.67 | 3 | 2.67 | 5.67 | 13 | 3 | 5.96 |
| P106.16.2 | 13.67 | 6 | 10.33 | 4 | 7.33 | 6.67 | 9 | 5.67 | 7.83 |
| Globus | 17.33 | 13.67 | 15.33 | 14.67 | 16 | 12 | 13.67 | 5.33 | 13.5 |
| Tillsano | 11.67 | 24 | 22.67 | 18.33 | 27.67 | 18.33 | 15.33 | 10.67 | 18.58 |
| Tilliko | 7.33 | 36.33 | 16 | 34.67 | 29.67 | 23 | 17 | 9.33 | 21.67 |
| Tillstop | 13.67 | 50 | 50 | 41.33 | 31.33 | 30.67 | 26.33 | 9.33 | 31.58 |
| Tillexus | 14.67 | 62.33 | 48.34 | 63.67 | 47.34 | 41.33 | 28.67 | 19.33 | 40.71 |
| Aurelius | 58.69 | 54 | 67 | 51 | 49.33 | 52.67 | 58 | 67.67 | 57.3 |
| Capo | 62 | 55 | 55 | 75.34 | 55.34 | 52 | 57.57 | 55.34 | 58.45 |
| mean per isolate | 8.29 | 12.06 | 12.63 | 11.46 | 11 | 9.93 | 10.04 | 8.26 | 10.46 |

*Figure 13: Heatmap showing common bunt incidence in % for susceptible lines (>5% CB incidence) (y-axis) tested with eight different isolates (x-axis). Common bunt x genotype interaction is displayed with varying intensities of color.*

The heatmap (Figure 13) summarizes the abovementioned observations and shows genotype interactions with different isolate treatments. ‘Capo’ and ‘Aurelius’ displayed high CB incidence consistently across all isolates. ‘Tillexus’ showed high susceptibility, especially when treated with *IFA Aggressive* and *Gerhaus*. The isolates *Loosdorf*, *Thening* and *Hinzenbach* showed strong virulence against ‘Tillexus’ too. ‘Tillstop’ showed a similar pattern but lower overall susceptibility than ‘Tillexus’. ‘Tillexus’ is a carrier of the resistance gene *Bt10*, ‘Tillsano’ carries *Bt5* and ‘Tilliko’ carries *BtZ* (Oberforster and Plank M. 2021)..These three cultivars harboring three different resistance genes also showed varying levels of susceptibility in the experiment. The Bt5 differential ‘Hohenheimer’, carrier of the same resistance gene as ‘Tillsano’, showed resistance to all isolates and had a mean CB incidence of 1.13%, whereas Tillsano had 18.85% mean CB incidence. The same pattern was found for the differential M822102 (*Bt10), which* was less susceptible (12.83%) compared to ‘Tillexus’ (40,71%).

*IFA Housekeeping* and *Harmannsdorf* were the least aggressive isolates among the isolates and showed similar virulence patterns. *Gerhaus* and *IFA Aggressive,* the most virulent isolates, also showed virulence patterns similar to each other. They exhibited lower virulence to *Btp* and *Bt8* than other isolates. However, they were very virulent against *Bt10, Bt2, Bt7, Bt13* and the resistance sources of ‘Tillexus’, ‘Tilliko’ and ‘Tillstop’.

## ANOVA and posthoc test

*Table 5: Results of two-way ANOVA for common bunt incidence, DF = degrees of freedom; SumSQ = Sum of Squares, Mean SQ = mean Square*

| | DF | SumSQ | Mean SQ | F-value | p-value |
|---|---|---|---|---|---|
| **Isolates** | 7 | 1485 | 212 | 17.04 | $<2*10^{-16}$ |
| **Genotype** | 39 | 154527 | 3962 | 319.13 | $<2*10^{-16}$ |
| **Replication within isolates** | 8 | 153 | 19 | 1.56 | 0.137 |
| **GxI** | 273 | 17829 | 65 | 5.24 | $<2*10^{-16}$ |
| **Residuals** | 312 | 3820 | 12 | | |

A two-way ANOVA showed highly significant effects of isolates *(F(7) = 17.04, p < 0.0001*), and genotypes *(F(39) = 319.13, p < 0.0001*) as well as highly significant interactions between genotypes and isolates *(F(237) = 5.24, p < 0.0001) (*Table 5). No significant effect was found for the replicates (=blocks) nested within the isolates.

A Tukey HSD test at p = 0.05 probability level revealed that the highest differences in mean CB incidence occurred between *Loosdorf - Harmannsdorf* (+4.24%) and *Loosdorf - IFA Housekeeping* (+4.17%). Significant differences were also found between *Gerhaus - Harmannsdorf* (+3.33%), *Gerhaus - IFA Housekeeping* (+3.26%), *IFA Aggressive - Harmannsdorf* (+3.82%) and *IFA Aggressive - IFA Housekeeping* (+3.76%). All significant differences between the isolates are displayed in Table 6.

*Table 6: Tukey HSD posthoc results: only isolate combinations with significant differences (p <0.05) are shown and ordered decreasingly by their p-values.*

| | difference [%] | p-value |
|---|---|---|
| **Loosdorf-Harmannsdorf** | 4.24 | $9.63*10^{-12}$ |
| **Loosdorf-IFA Housekeeping** | 4.17 | $1.98*10^{-11}$ |
| **IFA Aggressive-Harmannsdorf** | 3.82 | $9.67*10^{-12}$ |
| **IFA Housekeeping-IFA Aggressive** | -3.76 | $2.00*10^{-9}$ |
| **Harmannsdorf-Gerhaus** | -3.32 | $1.77*10^{-7}$ |
| **IFA Housekeeping-Gerhaus** | -3.26 | $3.42*10^{-7}$ |
| **Thening-Harmannsdorf** | 2.81 | $2.13*10^{-5}$ |
| **Loosdorf-Hinzenbach** | 2.79 | $2.46*10^{-5}$ |
| **Thening-IFA Housekeeping** | 2.74 | $3.77*10^{-5}$ |
| **Sitzendorf-Loosdorf** | -2.46 | $3.71*10^{-4}$ |
| **IFA Aggressive-Hinzenbach** | 2.38 | $6.92*10^{-4}$ |
| **Sitzendorf-IFA Aggressive** | -2.04 | $6.95*10^{-3}$ |
| **Hinzenbach-Gerhaus** | -1.88 | $1.91*10^{-2}$ |
| **Sitzendorf-Harmannsdorf** | 1.78 | $3.21*10^{-2}$ |
| **Sitzendorf-IFA Housekeeping** | 1.72 | $4.59*10^{-2}$ |

## Additive Main Effects and Multiplicative Interaction analysis (AMMI).

AMMI stability analysis is traditionally used to find the most stable high-yielding genotype in different environments. It is a useful tool to evaluate and visualize the interactions between genotypes and environments. The AMMI results were interpreted differently for my analysis to find the most susceptible and unstable genotypes. The isolates were treated as environments. The AMMI ANOVA revealed significant effects of environments (isolates) (*F(7)=11.13, p<0.001*), genotypes (*F(39)=323.58*, *p<0.0001)* and a significant interaction effect *(F(237)=5.33, p<0.0001).* No significant effect for replication was found (Table 13)

Genotypes with high AMMI yield ranks (rY) were highly susceptible, and stable genotypes were susceptible or resistant across all isolates. In other words, they showed the same response to different bunt isolates. In the AMMI model, wheat accessions were ranked by their susceptibility (rank yield, rY) and performance (rank of stability, rFA) regarding to the common bunt isolates (environments). The stability measure based on fitted AMMI model (FA) is an indicator for the stability of the wheat genotypes. It considers all significant interaction principal components (PCs) in the AMMI model. All resistant genotypes showed low FA values and obtained lower ranks because they were equally resistant to all the isolates.

In contrast, high FA values indicated a strong genotype by isolate interaction. The ranks were summed up, and the simultaneous selection index for yield and stability (SSI) score was obtained. Those findings were further investigated using biplot analysis. Especially the cultivars and differentials ‘Tillexus’, ‘Tillstop’, Sel1102 (Bt2), M822102 (Bt10), ‘Tilliko’ and Thule III (Bt13) had high FA values and were considered unstable. In contrast, genotypes with low rFA (rank of genotype based on stability) and low rY (rank of genotype based on CB incidence) showed consistent resistance against all the isolates. These results are displayed in the AMMI 1 biplot (Figure 14).

Overall, ‘Tillexus’, ‘Tillstop’, Sel1102 (*Bt2)*, ‘Capo’, ‘Aurelius’, ‘Tilliko’, Thule III *(Bt13),* Sel500-77 *(Bt7),* M822102 *(Bt10)* and Ridit *(Bt3*) showed the highest SSI (Simultaneous Selection Index for yield and stability) values and were considered susceptible and unstable (Table 7).

*Table 7: Results of AMMI stability analysis ordered by SSI (ascending): Genotypes were ranked based on their stability (Stability Measure Based on Fitted AMMI model (rFA)) and their susceptibility (rY); SSI displays the sum of the ranks. Low SSI ranks indicate resistance to all isolates; High ranks indicate unstable responses across isolates as well as high susceptibility*

| Genotype | FA | rank FA (rFA) | rank CB incidence (rY) | SSI (sum of ranks) |
|---|---|---|---|---|
| **PI178383** | 18.05 | 8 | 1.5 | 9.5 |
| **UISRG** | 18.05 | 9 | 1.5 | 10.5 |
| **PI636170** | 17.99 | 7 | 5 | 12 |
| **702-1102C** | 18.51 | 10 | 5 | 15 |
| **P101.111.1** | 15.33 | 5 | 10 | 15 |
| **P106.51.2** | 19.60 | 11 | 5 | 16 |
| **PI362695** | 16.81 | 6 | 11 | 17 |
| **PI119333 (*Bt12*)** | 19.60 | 12 | 5 | 17 |
| **P106.69.5** | 14.02 | 4 | 14 | 18 |
| **S7.4.1** | 19.60 | 13 | 5 | 18 |
| **Hohenheimer (*Bt5*)** | 8.29 | 2 | 17 | 19.5 |
| **Blizzard** | 6.44 | 1 | 20 | 21 |
| **Deloris** | 9.31 | 3 | 19 | 22 |
| **M822123 (*Bt11*)** | 21.04 | 15 | 8 | 23 |
| **PI166910** | 20.54 | 14 | 9 | 23 |
| **P101.8.5B** | 27.64 | 17 | 12 | 29 |
| **PI560795-2** | 24.90 | 16 | 13 | 29 |
| **Bonneville** | 31.37 | 18 | 15 | 33 |
| **Sel2092 (*Bt1*)** | 32.97 | 19 | 16 | 35 |
| **Rio (*Bt6*)** | 38.16 | 20 | 22 | 42. |
| **PI636165** | 73.67 | 25 | 17.5 | 42.5 |
| **PI173437 (*Btp*)** | 67.52 | 23 | 21 | 44 |
| **CI1558B (*Bt4*)** | 67.81 | 24 | 23 | 47 |
| **M90387 (*Bt9*)** | 39.81 | 21 | 28 | 49 |
| **S5.47.2** | 98.60 | 27 | 25 | 52 |
| **Globus** | 60.35 | 22 | 31 | 53 |
| **M822161 (*Bt8*)** | 162.76 | 30 | 24 | 54 |
| **P106.16.2** | 100.99 | 28 | 27 | 55 |
| **PI636156** | 144.63 | 29 | 26 | 55 |
| **Tillsano** | 98.59 | 26 | 32 | 58 |
| **Ridit (*Bt3*)** | 269.04 | 32 | 29 | 61 |
| **M822102 (*Bt10*)** | 713.78 | 38 | 30 | 68 |
| **SEL500-77 (*Bt7*)** | 260.06 | 31 | 37 | 68 |
| **Thule-III (*Bt13*)** | 619.33 | 35 | 34 | 69 |
| **Tilliko** | 688.52 | 36 | 33 | 69 |
| **Aurelius** | 489.37 | 34 | 39 | 73 |
| **Capo** | 371.00 | 33 | 40 | 73 |
| **Sel1102 (*Bt2*)** | 706.56 | 37 | 36 | 73 |
| **Tillstop** | 1147.51 | 39 | 35 | 74 |
| **Tillexus** | 2015.03 | 40 | 38 | 78 |

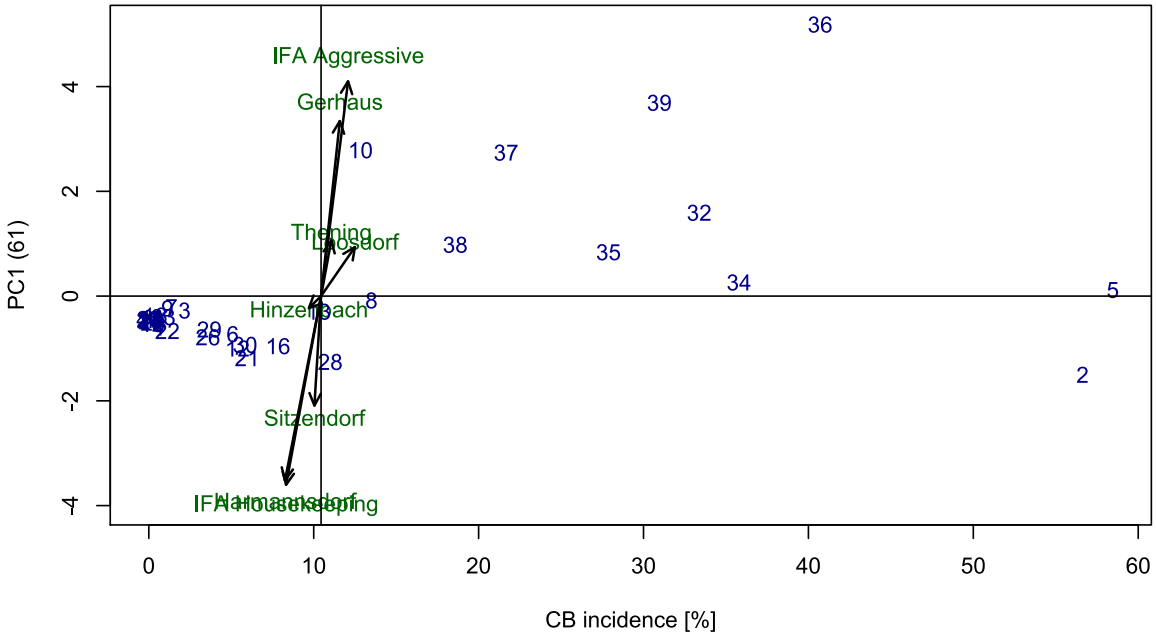


*Figure 14: AMMI1 biplot for common bunt incidence [%] of forty wheat genotypes displayed as numbers (blue) inoculated with eight isolates (green). GxI PC1 scores were plotted against CB incidence [%]. The black perpendicular line marks the grand mean. The corresponding names of the genotypes can be found in Table 8*

*Table 8: List of genotypes ordered by alphabet. Numbers correspond to the numbers in the biplots (Figure 14 to Figure 18)*

| No. | Genotype | No. | Genotype | No. | Genotype | No. | Genotype |
|---|---|---|---|---|---|---|---|
| **1** | 702-1102C | **11** | M822123 (*Bt11*) | **21** | PI_560795-2 | **31** | S7.4.1 |
| **2** | Aurelius | **12** | M822161 (*Bt8*) | **22** | PI_636156 | **32** | Sel1102 (*Bt2*) |
| **3** | Blizzard | **13** | M90387 (*Bt9*) | **23** | PI_636165 | **33** | Sel2092 (*Bt1*) |
| **4** | Bonneville | **14** | P101.111.1 | **24** | PI119333 (*Bt12*) | **34** | SEL500-77 (*Bt7*) |
| **5** | Capo | **15** | P101.8.5B | **25** | PI166910 | **35** | Thule-III (*Bt13*) |
| **6** | CI1558B (*Bt4*) | **16** | P106.16.2 | **26** | PI173437 (*Btp*) | **36** | Tillexus |
| **7** | Deloris | **17** | P106.51.2 | **27** | PI178383 | **37** | Tilliko |
| **8** | Globus | **18** | P106.69.5 | **28** | Ridit (*Bt3*) | **38** | Tillsano |
| **9** | Hohenheimer (*Bt5*) | **19** | PI 362695 | **29** | Rio (*Bt6*) | **39** | Tillstop |
| **10** | M822102 (*Bt10*) | **20** | PI 636170 | **30** | S5.47.2 | **40** | UISRG |

Genotypes with low PC1 scores were placed close to the horizontal zero line. These wheat accessions were considered stable (susceptible or resistant across all the isolates). Wheat accessions with high PCA1 scores were more responsive and indicated different interactions when treated with different isolates. Genotypes found far on the left side of the AMMI1 biplot (Figure 14) were less susceptible than genotypes placed on the right.

Genotypes that cluster together on the plot behaved similarly. ‘Capo’ (5) is positioned far-right and has shown to be highly susceptible to all isolates (close to zero line). ‘Aurelius’ (2) was more susceptible to those isolates placed below the zero line (*Sitzendorf, IFA Housekeeping, Harmannsdorf*).

Similar virulence patterns of the isolates *IFA Aggressive* and *Gerhaus* on ‘Tillexus’ (36), ‘Tillstop’ (39), ‘Tilliko’ (37), and M822102 (*Bt10*) (11) were detected. The isolates *Thening*, *Loosdorf* and *Hinzenbach* also clustered close together as well as *Sitzendorf*, *Harmannsdorf* and *IFA Housekeeping*. Long vectors of isolates indicate specific virulence patterns because some genotypes were more susceptible to those isolates. Short vectors signify less genotype by isolate interaction.

## Visual investigation of genotype-isolate interactions by GGE and GGB biplot.

A GGE biplot combines wheat genotypes and isolates into a single scatter plot. Each genotype and isolate is positioned according to its scores on the first two principal components. The biplot assists in identifying virulence patterns among the isolates and reveals strain-specific genotype interactions.

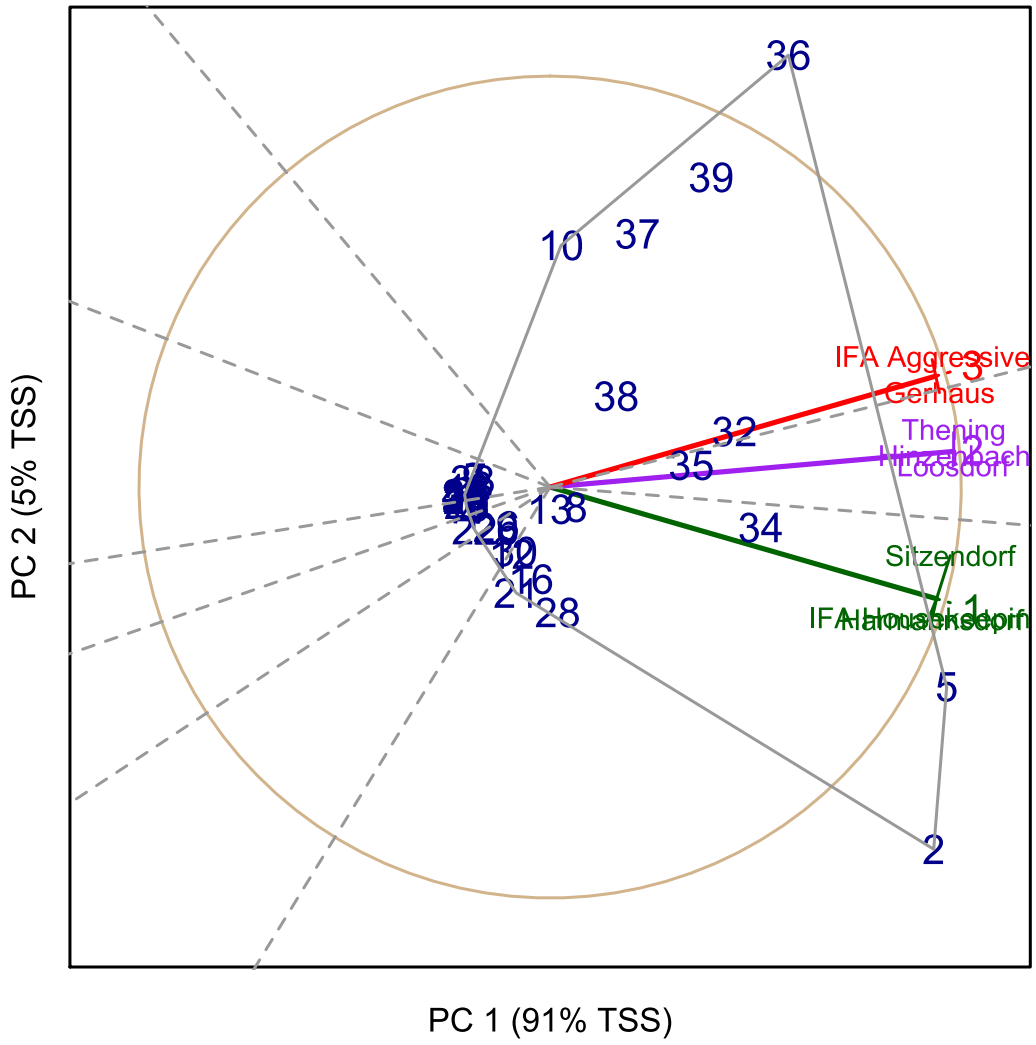


*Figure 15: Which-won-where biplot. Block of environment vectors are represented with solid lines in different colors (red, purple and green). The isolates are separated by dashed lines originating from the biplot center. TSS = Total Sum of Squares.* Gentoypes are represented as numbers 1-40. The list of genotypes and their corresponding numbers can be found in *Table 8*

A large portion of the variance in common bunt incidence is explained by PC1 (91%) and PC2 (5%). The polygon in Figure 15 is drawn along the genotypes located farthest from the biplot origin so that all other wheat genotypes are contained inside that polygon. Genotypes inside

the sector showed less variation in their response to the isolates than the genotypes on the vertex of the polygon. The biplot origin displays the grand mean. From the biplot origin, perpendicular lines are splitting the polygon into sectors. The genotypes located at the vertices were either the most or the least susceptible to some or all of the isolates. Genotypes situated close to the biplot origin were less specific in susceptibility. The vertex genotype in each sector comprising similar isolates was the most susceptible to the isolates in this respective segment ("winning genotype"). The genotypes far away from the origin were more discriminating between isolates than those close to the center. In contrast, if a genotype is linked to the polygon vertex, where no isolate drops in the same sector, this indicates that such a genotype was highly resistant to all isolates.

If isolates cluster together in one sector, it can be assumed that the genotypes behaved similarly when inoculated with one of these isolates. The eight isolates were grouped into three megaenvironments (ME) based on their similarity in virulence patterns: ME1 consisted of *IFA Housekeeping and Harmannsdorf;* ME2 of *Hinzenbach, Thening* and *Loosdorf; and* ME3 consisted of *IFA Aggressive* and *Gerhaus*. Overall, all isolates clustered together at one side of the biplot, indicating that most genotypes performed similarly to different isolate treatments. Isolates located in the same sector in Figure 15 were not significantly different.

To further investigate GxI interaction, the different megaenvironments were examined separately using GGB biplots.

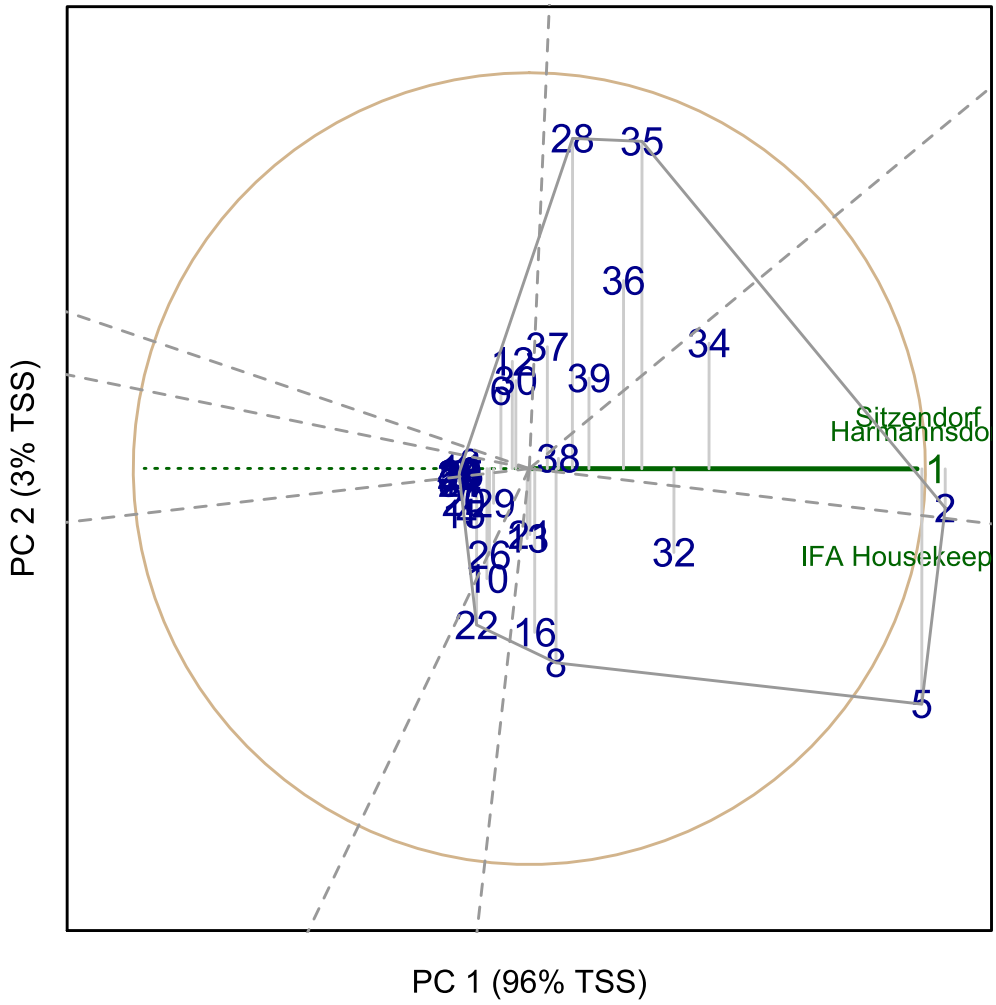


*Figure 16: Genotype by Block of Environment (GGB) biplot of megaenvironment (ME) 1: The megaenvironment consists of the isolates Harmannsdorf, Sitzendorf and IFA Housekeeping (green).* Gentoypes are represented as numbers 1-40. The list of genotypes and their corresponding numbers can be found in *Table 8*. *TSS = Total sum of squares*

Almost all variance observed in common bunt incidence was explained by the first two principal components (PC1 96% and PC2 3%) in ME1 (Figure 16). The thick horizontal lines in the GGB biplots (Figure 16 to Figure 18) indicate the average incidence across all the isolates in the respective megaenvironment. The further on the right a genotype was positioned, the higher its common bunt incidence. Again, all genotypes clustering to the biplot origin were considered resistant to the respective isolates. If genotypes were placed close to the horizontal line, they behave stable inside the megaenvironment. The projections of the genotypes indicate the individual genotype's specific reaction to an isolate within the megaenvironment.

In ME1 (Figure 16), ‘Aurelius’ (2) and ‘Capo’ (5) scored highest. However, ‘Capo’ (5) was more susceptible to *IFA Housekeeping*. SEL500-77 (*Bt7*) (34) and Sel1102 (*Bt2*) (32) also showed varying susceptibility to the isolates comprised in ME1. Isolates *Sitzendorf* and *Harmannsdorf*

showed high virulence to the differentials Thule III (*Bt13*) (35) and Ridit (*Bt3)* (28). Virulence of the isolates comprised in ME1 against 'Tillexus' (36), 'Tillstop' (39), 'Tillsano' (38) and 'Tilliko' (37) was significantly lower than in the other two MEs.

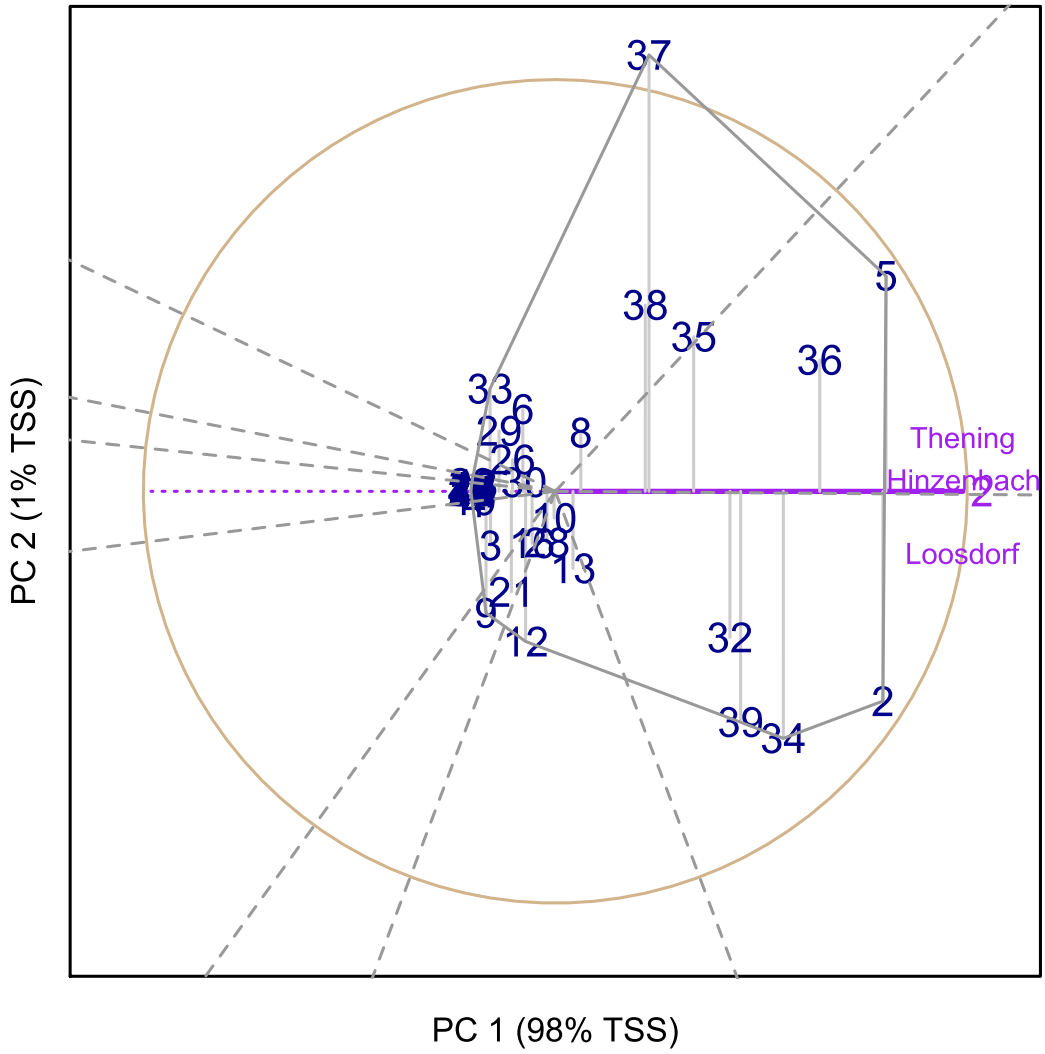


*Figure 17: Genotype by Block of Environment (GGB) biplot of megaenvironment (ME) 1: The megaenvironment consists of the isolates Harmannsdorf, Sitzendorf and IFA Housekeeping (green). Gentoypes are represented as numbers 1-40. The list of genotypes and their corresponding* numbers can be found in *Table 8*. *TSS = Total sum of squares*

Almost all variance observed in common bunt incidence was explained by PC1 (98%) and PC2 (1%) in ME2 (Figure 17). Inside ME2, the isolate *Loosdorf* showed strong virulence to 'Aurelius' (2), SEL500-77 (*Bt7*) (34), 'Tillstop' (39) and Sel1102 (*Bt2*) (32). *Loosdorf* was the most aggressive isolate and showed moderate virulence against a lot of genotypes, including the differentials M822102 (*Bt10*) (11), CI1558B (*Bt4*) (6), M90387 (*Bt9*) (13) and 'Ridit' (*Bt3*) (28). In contrast, *Thening* had strong virulence to 'Capo' (5), 'Tillexus' (36), Thule III (*Bt13*) (35), and 'Tillsano' (38) and was much more potent in infecting 'Tilliko' (37) than any of the other tested isolates in this ME.

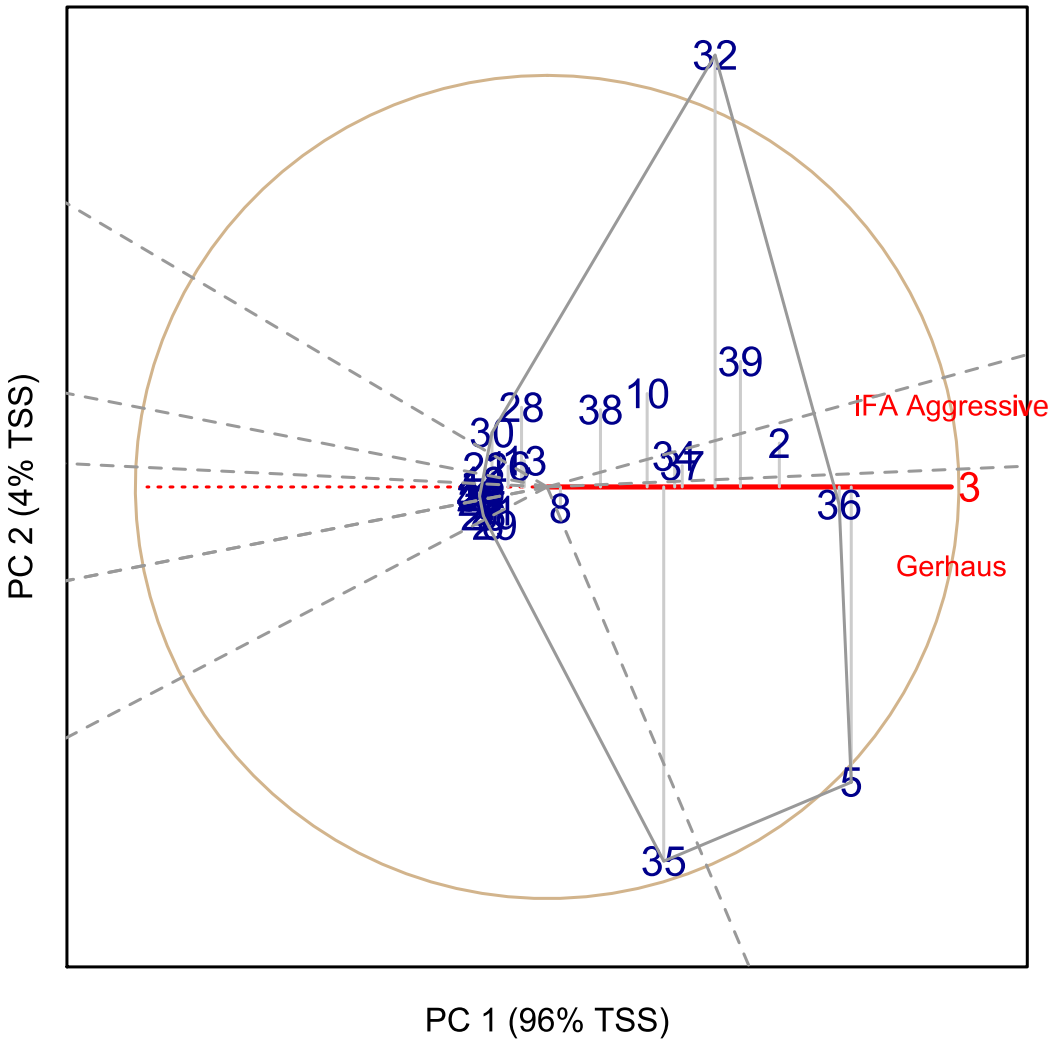


*Figure 18: Genotype by Block of Environment (GGB) biplot of megaenvironment 3: The megaenvironment consists of the isolates Gerhaus and IFA Aggressive (red).* Gentoypes are represented as numbers 1-40. The list of genotypes and their corresponding numbers can be found in *Table 8*. *TSS = Total sum of squares*

All the variance observed in common bunt incidence was explained by PC1 (96%) and PC2 (4%) in ME3 (Figure 18). *Gerhaus* and *IFA Aggressive* were similarly virulent to 'Tillexus' (36), 'Aurelius' (2), 'Tilliko' (37) and SEL50077 (*Bt7*) (34) because they are located close to the red line (mean CB incidence across isolates comprised in the megaenvironment). However, they differ in virulence to 'Capo' (5) and Thule-III (*Bt13*) (35). Those genotypes were more susceptible to *Gerhaus*. In contrast, Sel1102 (*Bt2*) (32) was more susceptible to *IFA Aggressive*. Nevertheless, the overall virulence pattern of the two isolates was very similar.

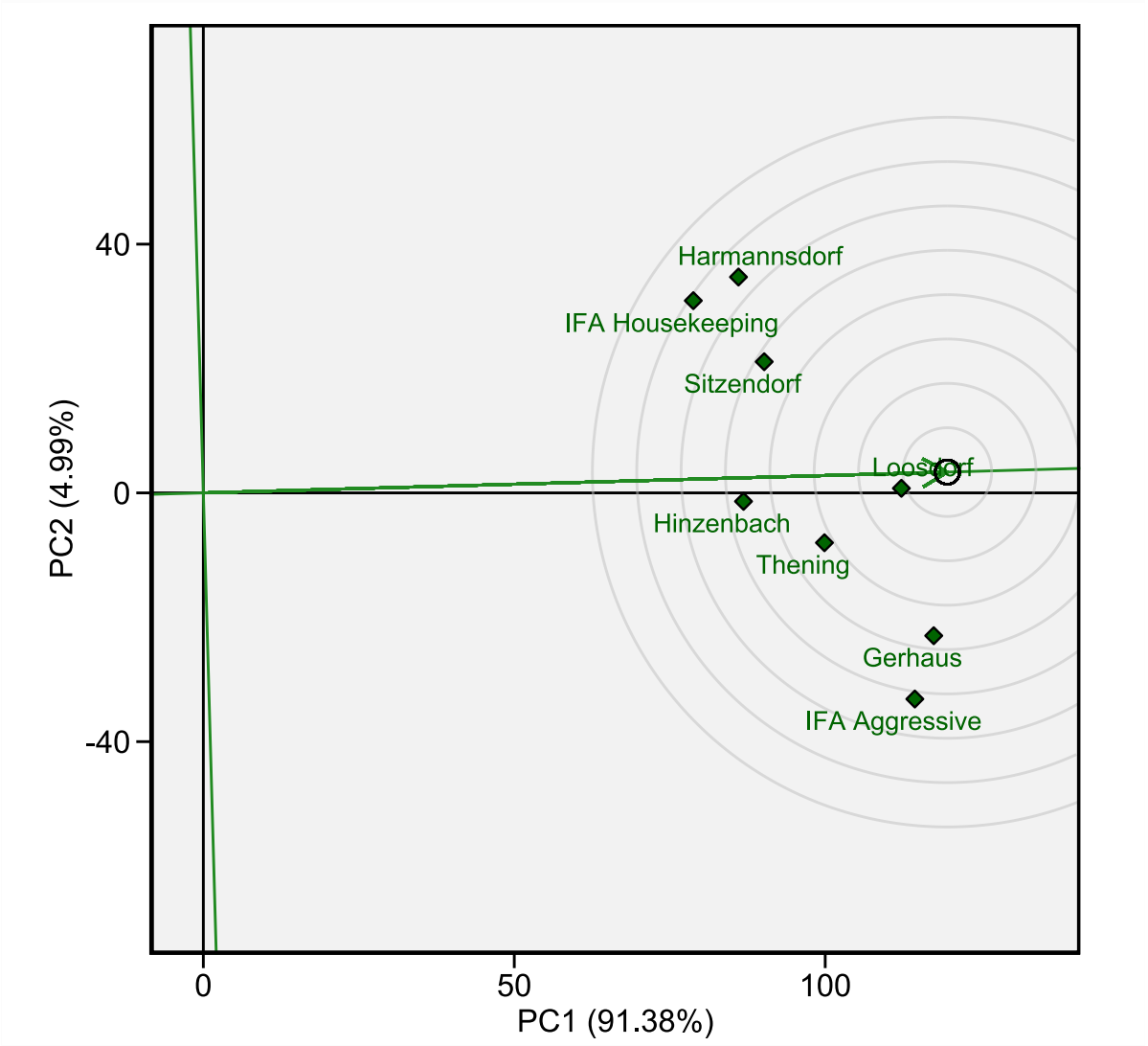


*Figure 19: Ranking of Environments GGE biplot for isolate comparison with ideal isolate (inner circle). The biplot shows G+GxI interaction effects of eight isolates (green) with forty different genotypes*

Usually, the ranking of the environment GGE biplot is used to find the ideal test environment for successful breeding and the selection of superior genotypes. Essential features for an ideal environment are a high ability to distinguish between genotypes (discriminativeness) and the power of the environment to represent all other environments (representativeness). For this experiment, the results were interpreted as isolates (environments) that best distinguished between genotypes and represented the avirulence/ virulence reaction of all the isolates against the genotypes. Isolates positioned further away from the ideal isolate (inner circle) showed more specific virulence patterns. In general, isolates closer to biplot origin like *Hinzenbach* had a lower ability to discriminate between the genotypes.

A large portion of the variance in common bunt incidence levels is explained by PC1 (91.38%) and PC2 (4.99%) in the GGE biplot (Figure 19). The green horizontal line represents the average environment axis (AEA). The axis passes through the inner circle, representing the mean across all isolates. All isolates are far from the biplot origin, demonstrating a high ability

to discriminate between genotypes, whereas a position close to the AEA indicates high representativeness. The inner circle expresses the average incidence across environments. The angle between the isolate position and the AEA describes isolate representativeness. For example, *IFA Aggressive* was positioned far away from the AEA, indicating specific virulence patterns (genotype by isolate interaction). According to the biplot (Figure 19), *Loosdorf* would be the ideal test isolate because its furthest away from biplot origin (high discriminating ability) and close to the AEA (high representativeness).

## Phenotypic correlations

A Shapiro-test was performed for each observed trait (plant height, date of heading, flowering) to test for normality. The null hypothesis of normal distribution had to be rejected for all traits at a significance level of α = 0,05. Therefore, spearman's rank correlation test was performed to test for correlation between morphological traits and common bunt incidence. Figure 20 visualizes significant negative correlations with small correlation coefficients between all morphological traits and common bunt (Table 9).

*Table 9: Spearman's rank correlation:* common bunt *[%) tested against plant height, date of heading and flowering date*

| model | Correlation coefficient | p-value |
|---|---|---|
| **plant height** | -0.27 | $5.22*10^{-12}$ |
| **date of heading** | -0.12 | 0.0024 |
| **flowering date** | -0.09 | 0.0167 |

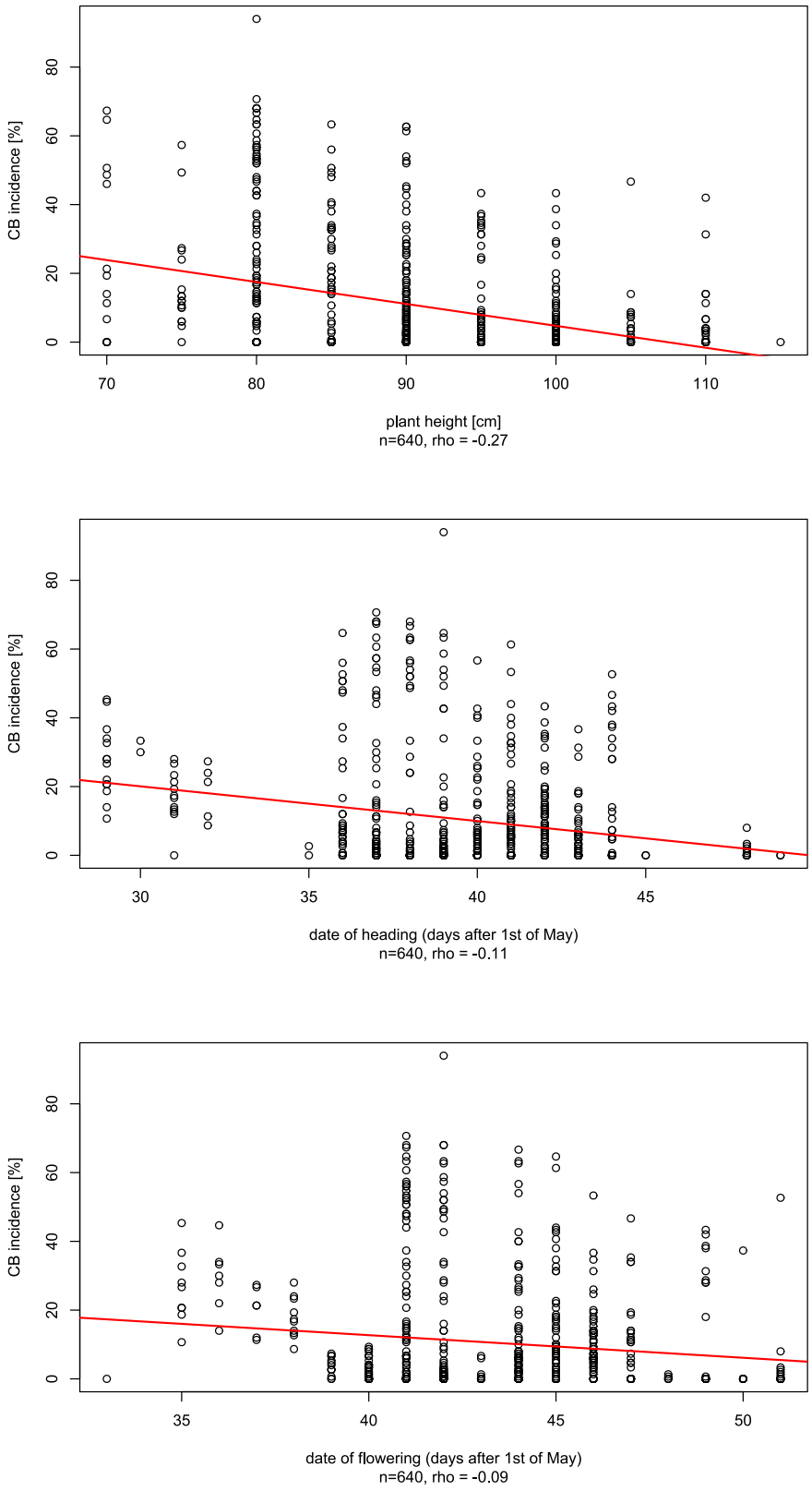


*Figure 20: Scatterplots with regression lines (red) and regression coefficients showing the relationship between common bunt incidence [%] and plant height [cm], date of heading and date of flowering (both in days after 1st of May), respectively.*

# Discussion

## Isolates

This study aimed to contribute to the knowledge about the Austrian bunt population and find suitable resistance sources for Austrian breeding programs. Furthermore, the findings of this study contribute to the understanding of the current composition of the common bunt population in local common bunt isolates.

### Evaluation including the whole test panel

The testing of eight different isolates of common bunt from various regions in Austria on a set of differential lines and other wheat genotypes under high disease pressure revealed for many tested lines largely similar virulence patterns, with some specifities for some isolates, leading to significant genotype x isolate interactions. For evaluation, a scheme developed by Szunics (1990), AMMI stability and GGE biplot analysis were used.

Biplots and AMMI analysis were instrumental in visualizing the interactions between isolates and genotypes. The isolates were positioned into three sections in the GGE biplot (Figure 15), respectively, with different genotypes winning in each sector. The genotypes plotted in the same section as the group of isolates were most susceptible to these isolates and may carry corresponding virulence/ avirulence genes (Yan and Falk 2002). According to their pathogenicity patterns, the isolates were separated into three different megaenvironments (Figure 16, Figure 17, Figure 18). Differences were found even between the isolates comprised within the respective ME.

All tested isolates share virulence against the resistance genes *Bt2, Bt7* and *Bt13*. The isolates *Harmannsdorf, IFA Housekeeping* and *Sitzendorf* were the least aggressive isolates and clustered together (ME1). The isolates *Thening*, *Hinzenbach* and *Loosdorf* also showed similarities in virulence to the tested genotypes and were comprised to ME2. Of all isolates, *Loosdorf* was most virulent and scored highest in disease incidence. *Gerhaus* and *IFA Aggressive* (ME3*)* demonstrated a unique and relative high aggressiveness against 'Tillexus', 'Tillstop', 'Tilliko', 'Tillsano' and M822102 (*Bt10*).

Overall, Austrian isolates overcame the resistance genes *Bt2, Bt3, Bt7, Bt9, Bt10, Bt13* and to some extent *Bt4* and *Bt8.* In contrast, *Bt1, Bt11 and Bt12* showed high resistance (<1% CBI) to all tested isolates, and *Btp, Bt5* and *Bt6* showed low infection rates (less than 5% common bunt incidence). The ranking of environment GGE biplot (Figure 19) revealed the isolate *Loosdorf* as the ideal test environment (race composition) because it had the highest ability to distinguish between genotypes (discriminativeness) and the highest power to represent the avirulence/virulence reactions of all isolates tested in this study.

### Evaluation of isolates including only the differentials

Additionally, the isolates were analyzed by including only the differentials as it was done in previous studies (Goates 2012; Hoffmann and Metzger 1976; Matanguihan and Jones 2011). If CB incidence was higher than 10%, the isolates were considered virulent to the respective differential, according to Hoffmann and Metzger (Hoffmann and Metzger 1976). The only isolates that shared the same virulence pattern were *Harmannsdorf* and *Hinzenbach* (virulent to *Bt2, Bt3, Bt7* and *Bt13*). All the other isolates showed distinct reactions to the differential set. All tested isolates share virulence to *Bt2*, *Bt7* and *Bt13* and avirulence to *Bt1, Bt4, Bt5, Bt6, Bt11, Bt12* and *Btp*. *Loosdorf* was the most virulent race composition and overcame the resistance genes *Bt2, Bt3, Bt7, Bt8, Bt9, Bt10* and *Bt13*. *Loosdorf* and *Sitzendorf* were unique in their virulence to *Bt8* in this experiment.

### Geographic influences

In Figure 8, the collection sites of the spore samples are displayed. The isolates collected in *Hinzenbach* and *Thening* cluster together in the biplot analysis and are also in geographic vicinity. Interestingly. *Gerhaus* and *IFA Aggressive* showed strong similarities in the experiment. However, their collection sites are far afield from each other. Those findings indicate that race compositions can differ enormously within a few hundred kilometers. Because most isolates were collected in the eastern part of Austria, the results only give an overview of the common bunt population for the corresponding collection sites. Isolate samples collected from other regions in Austria would be interesting too.

### Comparison with other studies

The virulence spectrum of common bunt was and is being evaluated worldwide. In most studies, the threshold for resistant cultivars is set at 10% CBI. Since the 1940s, monogenic differential wheat genotypes have been used to evaluate common bunt races. These genotypes are supposed to carry single major resistance genes (so-called *Bt*-genes). The genetic background of bunt isolates can be assessed by exploring the virulence characteristics on these wheat accessions (Hoffmann and Metzger 1976; Goates 2012). The differential set was advanced over time, and more resistance genes were added. Today, it consists of 16 wheat lines and is used globally (Goates and Bockelman 2012).

In the US two similar studies tested local common bunt races on the differentials (Matanguihan and Jones 2011; Goates 2012), and for the first time, virulence to *Bt8* and *Bt12* was described in the US populations, whereas *Bt11* remained resistant to all the tested races. New races were designated, and race T-34 was the first defined race with virulence to nine differentials. (Goates 2012).

In Iraq, lines carrying *Bt1, Bt2, Bt4, Bt7, Bt10, Bt13, Bt1*4 and *Bt15* showed high infection levels to the local common bunt population, while those carrying *Bt3, Bt5, Bt6, Bt9, Bt11* and *Bt12* showed low infestations (Al-Maaroof et al. 2016).

In 2002 isolates from different European countries were tested by Blazkova and Bartos. Virulence to *Bt1, Bt2* and *Bt7* was most frequent in their study, and no virulence to *Bt3, Bt5, Bt6, Bt8, Bt9, Bt11, Bt12* and *Bt13* was found. Some isolates showed virulence to *Bt4* and *Bt10* (Blažková and Bartoš 2002). A similar study in Austria screened 98 wheat genotypes for *T. caries* and *T. controversa* resistance. When inoculated with *T.caries*, the differenentials carrying *Bt5, Bt6, Bt9, Bt11* and *Btp* were highly resistant. Some diseased spikes were found on *Bt1, Bt3, Bt4, Bt8, Bt10* and *Bt12* (1.1-3.5% CBI). Virulence was found on the carriers of *Bt2* and *Bt7* (>10% CBI) (Huber and Buerstmayr 2006). Several years of field testing in Romania proved stable resistance of *Bt5, Bt10, Bt11* and *Bt12* to the local bunt population (Ittu et al. 2006). A Hungarian study found resistance to *Bt4*, *Bt5, Bt6, Bt8, Bt9* and *Bt10*, whereas *Bt1, Bt2, Bt3,* and *Bt7* were susceptible to the local Hungarian bunt population. The authors described variation over several years due to changes in the race composition (Veisz et al. 2000). In a field trial conducted in the Czech Republic from 2019 to 2021, virulence to *Bt1* to *Bt7* was found. *Bt8* to *Bt13* and *Btp* were resistant to the Czech isolates (Dumalasová 2021).

None of the described races shared the same virulence pattern as the Austrian isolates tested for this thesis. Virulence to *Bt1*, *Bt2* and *Bt7* is widespread in the bunt population worldwide. The isolates tested in my study showed much higher variability in virulence reactions than previous studies conducted in Austria (Huber and Buerstmayr 2006; Hagenguth 2016). These differences indicate that the local bunt isolates may differ in their aggressiveness and virulence. Increased virulence to an increasing number of differentials was found in recent studies (Borgen 2014; Babayants et al. 2006; Dumalasová 2021). Strikingly, no isolate in my study overcame *Bt1*, which was described as susceptible in almost all the literature, except the Austrian studies (Hagenguth 2016; Huber and Buerstmayr 2006).

The differences in aggressiveness of pathogenic races support the gene-for-gene hypothesis and indicate the presence of specific virulence factors or genes in aggressive isolates. Another important aspect are the synergistic or antagonistic effects of virulence factors on wheat resistance genes. The interactions of different virulence factors may explain some of the virulence patterns. Hybridization of non-virulent bunt races can lead to virulent bunt races (Hoffmann and Metzger 1976).

In the 1930s, experiments showed that the susceptibility of wheat lines increased when they were reinoculated with spores collected from the same variety (Roemer and Bartholly 1933). Further trials showed that the diversity in the spore collection will decrease after several rounds of reinoculation The company Agrologica in Denmark has aimed to purify bunt races since 2010. These bunt races are preferably consistent in their reaction to the resistance genes. The purified races can be used to infect interesting wheat varieties and determine which resistance genes they carry (Borgen 2015)

In the first purifying step, Borgen (2015) used a bulk spore sample to inoculate the wheat accessions, including the differentials. In the next step spores from the respective wheat accession were collected and used to reinoculate seeds of the same wheat genotype. The infection level increased by more than 50% compared to the bulk spore sample. Probably, virulence was present in the bulk spore sample at low frequency, and the race composition became adapted to the wheat genotype after reinoculation. For example, suppose a race virulent to *Bt1* is of interest. Several rounds of collecting spores from a single plant of a *Bt1* carrier and reinoculation of *Bt1* carriers in the next generation will select for races virulent to the respective resistance gene. Using this method on the differential set revealed virulence to *Bt1, Bt2, Bt3, Bt4, Bt5, Bt7, Bt8, Bt10* and *Bt13*, and avirulence against *Bt6*, *Bt9*, *B11* and *Bt12* in the Danish bunt population by Anders Borgen (2014). If only the bulk spore sample had been used, he would only have identified virulence to *Bt1, Bt3* and *Bt4*. The study proved that virulence against most *Bt*-genes was present in Denmark at low frequencies. The introduction of single resistant wheat genotypes enhances race-specific co-evolution of common bunt to overcome the respective resistance gene probably due to selection for better adapted common bunt races or by mutation (Borgen 2014).

The co-evolution between resistance genes and bunt races was already proven in the 1950s in the US when the first resistant cultivars were released. Each resistant variety was subsequently attacked and overcome by common bunt races, which were unknown before. The release of resistant varieties incorporating different resistance genes changed the dynamics in the natural population. Those observations also support the gene-for-gene model, where each dominant common bunt resistance gene (*Bt*) has a corresponding avirulence (avr) gene in the pathogen (Matanguihan et al. 2011; Wilcoxson and Saari 1996). Changes in race composition over the years were reported in several studies (Gaudet et al. 2012; Huber and Buerstmayr 2006; Veisz et al. 2000; AGES 2021a)

Traditionally, if a genotype's disease incidence was less than 10%, the resistance gene it carries was considered effective against common bunt infection (Metzger and Hoffmann 1978). In consideration of the results of the Danish study, a threshold of 5% CBI for resistant genotypes may be more suitable to give safe recommendations to breeders and farmers. The evaluation

scheme of (Szunics 1990) categorizes the wheat accessions using a six-point scale, which allows a more detailed description of the virulence/ avirulence reactions. Under Austrian conditions, the resistance genes *Bt1, Bt5, Bt6, Bt11, Bt12* and *Btp* showed less than 5% incidence and may be exploited for Austrian breeding programs.

For evaluation of isolates, it´s essential to consider that the spores most likely represent a population of common bunt genotypes. Studies showed that resistant varieties turned susceptible by reinoculation with spores collected from the few infected plants of the respective variety (Borgen 2015). In Denmark, the common bunt population was considered avirulent to *Bt10*. However, after inoculation with spores from the same variety, the *Bt10* carriers showed high susceptibility. Similar reactions were found for *Bt2, Bt7, Bt13* and *BtZ.* Low infection rates on resistant wheat accessions do not necessarily represent the absence of virulent races. Even if only a tiny number of virulent spores can infect some plants of a resistant variety, the pathogen can multiply rapidly in the following years. For example, *Bt4* and *Bt8* showed more than 5% and less than 10% incidence in the Austrian setting and were classified as moderately resistant. However, the infection rate indicates that virulent spores are present in low amounts. As a process of natural selection, those bunt races infectious to *Bt4* and *Bt8* would propagate, and in the next generation, more spores virulent to *Bt4* and *Bt8* would be present. By these mechanisms, the common bunt population can adapt rapidly to the release of new resistant cultivars (Borgen 2014, 2015; Hoffmann and Metzger 1976).

## Genotypes

Bunt symptoms occurred in almost all genotypes except PI178383 and ‘UISRG’ (0.0% CBI). Overall, a considerable variation in common bunt incidence ranging from 0.0% in highly resistant genotypes to 75.34% in the highly susceptible cultivar ‘Capo’ was observed. However, all genotypes with a common bunt incidence lower than 5% were classified as highly resistant because the high inoculum dose simulates a situation of extreme disease pressure not expected to occur under natural infection conditions.

Even when highly susceptible wheat cultivars are treated with high inoculum doses, not all plants are infected. On the contrary, not all resistant genotypes are entirely immune and genotypes are often considered as resistant with disease scores up to 10% (Goates 1996). 22 of 40 genotypes showed less than 5% diseased plants, most of them belonging to the “genotype group” resistance donors. The highly susceptible cultivars ‘Capo’ and ‘Aurelius’ developed high disease levels under all isolate treatments, indicating that all isolates were viable and infectious. Additionally, most of the differentials were infected by Austrian isolates. Only the carriers of *Bt1, Bt11* and *Bt12* showed negligible infection levels. In Europe, virulence to *Bt12* was first detected in Denmark in 2018 (Borgen et al. 2019).

Most genotypes showed stable susceptibility or resistance reactions to all isolates. Only some wheat accessions showed distinct interactions with specific isolates. AMMI and biplot analysis were valuable tools to unravel those particular interactions. According to the AMMI model, 'Tillexus', 'Tillstop', M822102 (*Bt10*), Sel1102 (*Bt2*). 'Tilliko' and Thule-III (*Bt13*) were unstable and showed distinct interactions with the isolate treatments. It is hypothesized that lines with similar responses may carry the same resistance gene. *Bt10* and *BtZ* showed identical reactions to different races, and they were hard to distinguish when tested in other studies (Borgen et al. 2019). This pattern was also found in this experiment. 'Tillexus' (carrier of *Bt10*) and 'Tilliko' (*BtZ*) were very susceptible to the isolates *IFA aggressive* and *Gerhaus*. The bad performance of 'Tillexus' compared to the differential M822102 (both carriers of *Bt10*) may be caused by minor or non-specific resistance genes present in the differential or other unknown factors influencing infestation. A recent study compared Czech isolates to Austrian isolates. The trial included the cultivars 'Tillexus', 'Tilliko', 'Tillsano', 'Deloris' and 'UISRG' among others, and they found significantly higher susceptibility of 'Tillexus', 'Tilliko' and 'Tillsano' to the Austrian isolate. Only minor virulence was found when treated with the Czech isolate (Dumalasová 2021). The abovementioned cultivars were classified/ registered as moderately resistant to common bunt (Die Saat 2021; Cultivari 2019). However, their resistance is not reliable under Austrian conditions.

The differential 'Hohenheimer' (*Bt5)* showed good resistance, whereas the cultivars 'Globus' and 'Tillsano' (both carriers of *Bt5*) (Borgen A., personal communication) were moderately susceptible. 'Hohenheimer' may also carry some undescribed minor resistance genes or other unknown resistance mechanisms.

The resistance donor 702-1102C and the differential M90387 are both described as carriers of *Bt9* (FALLBACHER et al. 2020). However, 702-1102C was highly resistant to all isolate treatments. In contrast, the differential M90387 (*Bt9*) was moderately susceptible, especially to the isolates *Loosdorf* (18% CBI) and *Thening* (14% CBI). In conclusion of these observations, the hypothesis seems valid that several wheat genotypes in the differential set probably carry more than one gene influencing resistance.

In a Lithuanian study, differences in common bunt incidence were found in genotypes carrying the same resistance genes. The authors suggest that environmental influences and partial resistance cause the differences. The genetic background of the wheat accession plays a significant role in the expression of the resistance genes. Modifying genes like transcription activators may account for the inconsistent expression of the resistance genes in different genetic backgrounds of the wheat accessions (Ruzgas and Liatukas 2009).

Interestingly, the disease response of highly susceptible cultivars has been constant over the years, whereas moderately susceptible lines showed high variation. Researchers concluded that possible unknown factors relevant for initial infection or environmental influences affected some of the lines. However, the underlying mechanisms are not well understood yet (Hoffmann 1982; Matanguihan et al. 2011; Goates and Bockelman 2012).

Winter hardiness can vary tremendously among the wheat accessions. Less CBI may be detected if a genotype is more prone to frost kill. In infected susceptible genotypes, higher mortality was proven after frost exposure (Veisz et al. 2000). Also, wheat genotypes with higher tillering are less affected because low inoculum doses of common bunt could not penetrate all tillers (Liatukas and Ruzgas 2008).

Previous studies showed that combinations of two resistance genes were also overcome rapidly (Hoffmann 1982; Matanguihan et al. 2011). The resistance donors PI166910 (*Bt7, Bt9, Bt11*) and PI178383 (*Bt8, Bt9, Bt10*) carry at least three major resistance genes. They showed high resistance to the Austrian isolates tested in this study. Especially, gene combinations of more than two resistance genes offer durable resistance. For example, combinations of *Bt1.2.5*, *Bt3.9.10, Bt4.5, Bt4.7, Bt8.9, Bt8.9.10* and *Bt12.13* showed complete resistance in a Lithuanian study (Liatukas and Ruzgas 2008). Only a minority of modern wheat cultivars carry a combination of effective resistance genes, and very few are suitable for low-input farming systems. Often highly resistant wheat accessions are susceptible to lodging, other diseases or have low yielding capacity. Transferring resistance genes into high-performing cultivars is a time- and labor-consuming task and takes several years (Liatukas and Ruzgas 2008). Combinations of traits like improved nutrient use efficiency, weed competitiveness, and disease resistance are desired in organic cultivars. Furthermore, a combination of race-specific and other unspecific resistance genes could aid in maintaining resistance over a long period. Broad genetic diversity could prevent the selection of more virulent races (Matanguihan et al. 2011; Gaudet et al. 2012).

All genotypes in the experiment with infection levels close to zero are considered resistant and probably carry multiple resistance genes. Those wheat accessions can be promising resistance sources and should be further investigated. The cultivars ‘Blizzard’ and ‘Bonneville’ have shown good resistance to many European isolates for more than 20 years (Blažková and Bartoš 2002; Huber and Buerstmayr 2006). So far, also *Bt11* and *Bt12* are highly resistant to the Austrian bunt population. They can be exploited as parents in combination with high-yielding susceptible wheat cultivars (Al-Maaroof et al. 2016).

Some of the resistance donors originate from experiments at IFA Tulln. For example, lines P106.51.2 and P106.69.5 carry *Bt12* from the resitance donor PI119333 (Muellner et al. 2020).

Furthermore, the breeding lines S5.47.2 and S7.4.1 are offspring of the resistance donors ‘Bonneville’ and ‘Blizzard’. PI119333, ‘Blizzard’ and ‘Bonneville’ carry resistance QTLs on chromosomes 1A, 1B, 4B, 7A and 7D (Muellner et al. 2021; FALLBACHER et al. 2020). These breeding lines are promising for developing new highly resistant and well-adapted cultivars and for further investigations of the genetic background of common bunt resistance.

## Phenotypic correlations

Significant differences among the wheat accessions for plant height, date of heading and date of flowering under common bunt were found. Correlation coefficients among the phenotypic traits and common bunt were low, indicating that common bunt resistance is independent of the tested phenotypic features. Therefore, traits like plant height are not suitable as selection criteria for common bunt resistance. Low phenotypic correlations between common bunt and other traits were reported previously (Mourad et al. 2018).

## Limitations of this study

Data of multiple years would be desired to decide whether the isolates can be classified reliably as in this study. At least three years of field trials are recommended to reduce effects like disease escape or environmental influences (Matanguihan and Jones 2011).

Traditionally, trials screening for resistant wheat varieties are conducted by adding spores to seeds before sowing and assessing the number of infected heads at the end of the season. Usually, the underlying virulence of the spores is unknown, and the spore samples collected from fields are genetically diverse. Therefore, those samples may be a mix of avirulent and virulent spores in respect to the wheat genotype. The interpretation of the obtained data is challenging because a low infection level may result from a low number of virulent spores in the spore sample. If the same experiment is repeated in the following year, the composition of spores differs, and different results may be found on the same varieties. For further investigations, the development of uniform races would enhance repeatability.

As mentioned above, if wheat accessions are infected with a mix of different races and spores are collected for reinoculation in the following years, the infection level will likely increase. Selection to more virulent races due to natural selection was shown previously (Borgen et al. 2019). In this experiment, isolates were collected from different genotypes, which influences the race composition tremendously. For example, *IFA Aggressive* was collected from the cultivar ‘Tilliko’ (carrier of *Bt10)*. Based on the knowledge from other studies (Borgen 2014, 2015), it was no surprise that the isolate *IFA Aggressive* was especially virulent to carriers of *Bt10* because the isolate composition adapts according to the wheat accession. Furthermore,

the inoculum dose should be standardized because a dose-effect was reported, especially on susceptible cultivars (Dumalasová and Bartoš 2008).

On the tested field, wheat was the preceding crop. This circumstance is not ideal because spores can survive in the soil for several years. Also, replicating the experiment on the exact location is not recommended (Weinhappel M. 2016). Uninoculated, highly susceptible genotypes should be included in subsequent trials as negative controls to test spore contamination of the soil, supplementary to the susceptible cultivars planted for testing of spore carry-over. Common bunt races with known virulence patterns could be included as positive controls (Matanguihan and Jones 2011)

Tests at different locations simultaneously could reveal underlying environmental impacts. Climatic factors like frost, snow cover and air temperature can considerably affect common bunt severity (Veisz et al. 2000; Liatukas and Ruzgas 2009).

The seed mainly originated from previous field trials, where admixture with other wheat genotypes may have happened. For example, Thule-III (*Bt13*) and SEL500-77 (*Bt7*) showed two different phenotypes: one with long spikes, which showed high susceptibility and the other with more compact, shorter spikes, which showed moderate susceptibility. Both phenotypes showed susceptibility to all the isolates. However, only the phenotype occurring in higher abundance was included in the evaluation. Seed sterilization is recommended before inoculation to avoid cross-contamination with spores already present on the planting material (Matanguihan and Jones 2011).

Eleven rows with uninoculated seeds of highly susceptible genotypes (‘Capo’, ‘Aurelius’, ‘Midas’) were sown to clean the sowing machine and prevent cross-contamination. However, continuous spore-carry over was observed from the first to the last row. Therefore, better cleaning methods need to be elaborated.

It´s also worth mentioning that artificially inoculated test environments are necessary for breeding. However, they differ from the natural occurrence of the disease.

## Outlook

Despite the effective use of seed treatments in conventional agriculture, resistance breeding has gained importance in many areas of the world (Goates and Bockelman 2012; Al-Maaroof et al. 2016). In recent years, a growing interest in sustainable agriculture has led to a shift in agricultural practices and an increase in the prevalence of seed- and soil-borne pathogens (Matanguihan et al. 2011). Exploiting genetic resources is an environmentally-friendly way to fight common bunt (Ciucă 2011) and is also attractive for conventional and low-input farms. Additionally, farmers in developing countries would profit from cultivars harboring resistance loci from such sources because they often can't afford chemical fungicides and rely on farm-saved seeds (Matanguihan et al. 2011; Al-Maaroof et al. 2016). At the moment, 16 resistance genes are described, and most of them are already ineffective against certain common bunt races. Only a few of the resistance genes are mapped and used for breeding. Advanced molecular methods, like marker-assisted selection and mapping of resistance genes, will speed up the breeding process (Ciucă 2011). So far, no fully resistant cultivar is available in Austria. Therefore, combining cropping of less susceptible cultivars with other control measures and seed testing is still necessary to avoid further spreading of the disease.

A significant constraint for resistance breeding is the appearance of more aggressive physiological races over time. New cultivars should combine several resistance genes for durable resistance. Good examples for durable resistance are the cultivars 'Bonneville' (Souza et al. 1995) and 'Blizzard' (Sunderman et al. 1991), which were released in the 90s and they are still highly resistant to European and Canadian common bunt isolates (Dumalasová and Bartoš 2006; Wang et al. 2009; Muellner et al. 2021). The reaction of the local bunt population after the release of resistant cultivars needs to be observed, and an establishment of a pathogen surveillance program would be advantageous. The selective pressure of resistant wheat genotypes will lead to new combinations of virulence genes in the local bunt population (Hoffmann and Metzger 1976). The control of common bunt will be a continuous effort and requires systemic breeding programs. Knowledge of the current status of common bunt races occurring in an area is vital in developing sustainable strategies (Hoffmann and Metzger 1976). The classification of the races should be continuously updated because race-specific virulence patterns enable specific targeting of resistance genes in wheat cultivars. Purifying Austrian common bunt races on resistant wheat varieties would be a great achievment, and sequencing those specific races may be interesting. Examination of common bunt variability and evolution across regions and time is essential for successful disease management. More studies about the pathogen's genetic variability and population biology are necessary to understand the host-pathogen interaction fully and to be able to predict future evolution processes. For holistic management, resistance breeding together with crop rotation, sowing dates, and seed treatments need to be combined.

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# Publication bibliography

AGES (2021a): Gewöhnlicher Steinbrand und seine Bedeutung in der BIO-Landwirtschaft. Edited by Austrian Agency for Health and Food Security. Available online at https://www.ages.at/themen/landwirtschaft/saat-und-pflanzgut/forschung/gewoehnlicher-steinbrand-und-seine-bedeutung-in-der-bio-landwirtschaft/, updated on 13.04.21, checked on 15.12.21.

AGES (2021b): Österreichische Beschreibende Sortenliste 2021 Landwirtschaftliche Pflanzenarten. Schriftenreihe 21/2021, ISSN 1560-635X. Available online at https://bsl.baes.gv.at/fileadmin/BSL/pdfVersion/Beschreibende_Sortenliste/12_Getreide_im_biologischen_Landbau_BSL2021.pdf, checked on 06.02.22.

Akçura, M.; Akan, K. (2018): Assessment of the reactions of pure lines selected from Turkish bread wheat landraces against bunt disease (Tilletia foetida ) with the GGE-biplot method. In *Plant Genet. Resour.* 16 (4), pp. 325–333. DOI: 10.1017/S1479262117000363.

Al-Maaroof, M.; Emad; Ali, R. M.; Mahmood T.M.; Aziz, H. A. (2016): SEARCHING FOR RESISTANCE SOURCES TO WHEAT COMMON BUNT DISEASE AND EFFICIENCY OF BT GENES AGAINST TILLETIA TRITICI AND T. LAEVIS POPULATIONS. In *AgricultForest* 62 (1). DOI: 10.17707/AgricultForest.62.1.20.

Appels, R.; Eversole, K.; Feuillet, C.; Keller, B.; Rogers, J.; Stein, N. et al. (2018): Shifting the limits in wheat research and breeding using a fully annotated reference genome. In *Science (New York, N.Y.)* 361 (6403). DOI: 10.1126/science.aar7191.

Asseng, S.; Ewert, F.; Martre, P.; Rötter, R. P.; Lobell, D. B.; Cammarano, D. et al. (2015): Rising temperatures reduce global wheat production. In *Nature Climate Change* 5 (2), pp. 143–147. DOI: 10.1038/nclimate2470.

Atkinson, T. G.; Martens, J. W.; Seaman, W. L. (1984): Diseases of field crops in Canada. An illustrated compendium. Harrow, Ontario: The Canadian Phytopathological Society.

Babayants, L. T.; Babayants, O. V.; Baranovskaya, V. L.; Dubinina, L. A. (2006): Tilletia caries and resistance of wheat to this pathogen in Ukraine. In *Czech Journal of Genetics and Plant Breeding* 42 (Special Issue), pp. 33–36. DOI: 10.17221/6228-CJGPB.

Badu-Apraku, B.; Fakorede, B.; Akinwale, R.; Annor, B.; Adewale, S.; Toyinbo, J.; Akintibu, Samson (2020): Application of the GGE Biplot as a Statistical Tool in the Breeding and Testing of Early and Extra-Early Maturing Maize in Sub-Saharan Africa. In *Crop Breed Genet Genom.* 2 (3), e200012. DOI: 10.20900/cbgg20200012.

BAES (2022): Pflanzenschutzmittel-Register. Bundesamt für Ernährungssicherheit. Wien. Available online at https://psmregister.baes.gv.at/psmregister/faces/main, updated on 10.02.22, checked on 10.02.22.

Bakir, F.; Damluji, S. F.; Amin-Zaki, L.; Murtadha, M.; Khalidi, A.; al-Rawi, N. Y. et al. (1973): Methylmercury poisoning in Iraq. In *Science (New York, N.Y.)* 181 (4096), pp. 230–241. DOI: 10.1126/science.181.4096.230.

Balfourier; B.; Robert S; De Oliveira R; Rimbert H; Kitt J; Choulet F (2019): Worldwide phylogeography and history of wheat genetic diversity. In *Science Advances* 5 (5), eaav0536. DOI: 10.1126/sciadv.aav0536.

Bauer, R.; Benno Voit; B. Killermann; K. Hülsbergen (2013): Nachweis über die Dauer der Infektionsfähigkeit von Steinbrand- (Tilletia caries) und Zwergsteinbrandsporen (Tilletia controversa) im Boden und Stallmist in Biobetrieben. In.

Becker, J.; Weltzien, H. C. (1993): Bekämpfung des Weizensteinbrandes (Tilletia caries (D.C.) Tul. & C. Tul.) mit organischen Nährstoffen / Control of common bunt of wheat (Tilletia caries (D.C.) Tul. &. C. Tul.) with organic nutrients. In *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz / Journal of Plant Diseases and Protection* 100 (1), pp. 49–57. Available online at http://www.jstor.org/stable/43386137.

Bhatta, M.; Morgounov, A.; Belamkar, V.; Wegulo, S. N.; Dababat, A. A.; Erginbas-Orakci, G. et al. (2019): Genome-Wide Association Study for Multiple Biotic Stress Resistance in Synthetic Hexaploid Wheat. In *International journal of molecular sciences* 20 (15). DOI: 10.3390/ijms20153667.

Blažková, V.; Bartoš, P. (2002): Virulence pattern of European bunt samples (Tilletia tritici and T. laevis) and sources of resistance. In *Cereal Research Communications* 30 (3), pp. 335–342. DOI: 10.1007/BF03543427.

BOKU (2022): Wetterdaten UFT. Available online at https://dnw-web.boku.ac.at/dnw/wetter_form_uft.php, updated on 31.01.22, checked on 31.01.22.

Bonjean, A. P.; Angus, W. J. (2001): The World Wheat Book: A history of wheat breeding.

Bonman, J. M.; Bockelman, H. E.; Goates, B. J.; Obert, D. E.; McGuire, P. E.; Qualset, C. O.; Hijmans, Robert J. (2006): Geographic Distribution of Common and Dwarf Bunt Resistance in Landraces of Triticum aestivum subsp. aestivum. In *Crop Sci.* 46 (4), pp. 1622–1629. DOI: 10.2135/cropsci2005.12-0463.

Borgen, A. (Ed.) (2014): Virulence pattern in Danish races of common bunt (Tilletia caries) Common Bunt. SOLIBAM Congress 2014, ‘Diversity strategies for organic and low input

agricultures and their food system'. Nantes, France, 7.-9. July. Available online at https://agrologica.dk/filer/publikationer/Nantes2014-virulence2.pdf, checked on 17.02.22.

Borgen, A. (2015): Purifying virulence races of common bunt (Tilletia caries) to identify resistance genes in wheat. Agrologica. Mariager, Denmark. Available online at https://agrologica.dk/filer/publikationer/Virulence_Vingsted2015.pdf, checked on 18.02.22.

Borgen, A. (2021): Seed treatments to control common bunt. Agrologica. Available online at https://agrologica.dk/filer/publikationer/SeedTreatments_Eucarpia2021.pdf, checked on 25.02.22.

Borgen, A.; Backes, G. Spieß, H.; Müller, C. (Eds.) (2019): Identifying resistance genes in wheat against common bunt (Tilletia caries) by use of virulence pattern of the pathogen.

Bundesamt für Ernährungssicherheit (2020): Sorten- und Saatgutblatt: Sondernummer 65. BAES 28, p. 12. Available online at file:///C:/Users/elsed/Downloads/Feldanerkennung_Getreide_2020.pdf, checked on 3/30/2022.

Chen, J.; Guttieri, M. J.; Zhang, J.; Hole, D.; Souza, E.; Goates, B. J. (2016): A novel QTL associated with dwarf bunt resistance in Idaho 444 winter wheat. In *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik* 129 (12), pp. 2313–2322. DOI: 10.1007/s00122-016-2783-2.

Ciucă, M. (2011): A preliminary report on the identification of SSR markers for bunt (Tilletia sp.) resistance in wheat. In *Czech Journal of Genetics and Plant Breeding* 47 (Special Issue), S142-S145. DOI: 10.17221/3269-CJGPB.

Ciucă, M.; Săulescu, N. (2008): Screening Romanian winter wheat germplasm for presence of Bt10 bunt resistance gene, using molecular markers. Available online at https://www.semanticscholar.org/paper/ca2655dd2d7010061fa372c25c2d803e4a2fd707.

Cultivari (2019): Tilliko. Qualitätsweizen für besondere Standorte. Cultivari gGmbH. Darzau. Available online at https://www.cultivari.de/sorten/tilliko/, updated on 3/25/2019, checked on 24.02.22.

Die Saat (2021): Tilliko. RWA - Raiffeisen Ware Austria. Korneuburg. Available online at https://www.diesaat.at/wp-content/uploads/2020/02/sortenpass-winterweizen-tilliko-bio.pdf, updated on Mai 2021, checked on 24.02.22.

Dumalasová, V. (2021): Ecobreed Project and common bunt field inoculation trials at the crop research institute. 72nd Plant Breeders Conference, 22-24 November 2021. Crop Research Institute, Praha-Ruzyně, Czech Republic. Aigen/Ennstal. Available online at http://www.saatgut-

austria.at/MEDIA/Ecobreed%20project%20and%20common%20bunt%20field_Dumalasova.pdf, checked on 02.03.22.

Dumalasová, V.; Bartoš, P. (2006): Resistance of winter wheat cultivars to common bunt, Tilletia tritici (Bjerk.) Wint. and T. laevis Kühn. In *J Plant Dis Prot* 113 (4), pp. 159–163. DOI: 10.1007/BF03356173.

Dumalasová, V.; Bartoš, P. (2008): Effect of inoculum doses on common bunt infection on wheat caused by Tilletia tritici and T. laevis. In *Czech Journal of Genetics and Plant Breeding* 44 (No. 2), pp. 73–77. DOI: 10.17221/2/2008-CJGPB.

El Baidouri, M.; Murat, F.; Veyssiere, M.; Molinier, M. F. R.; Burlot, L.; Alaux, M. et al. (2017): Reconciling the evolutionary origin of bread wheat (Triticum aestivum). In *New Phytol* 213 (PMID: 27551821), p. 1486. DOI: 10.1111/nph.14113.

El-Naimi, M.; Toubia-Rahme, H.; Mamluk, O. F. (2000): Organic Seed-treatment as a Substitute for Chemical Seed-treatment to Control Common Bunt of Wheat. In *Eur J Plant Pathol* 106 (5), pp. 433–437. DOI: 10.1023/A:1008785021771.

FALLBACHER, I.; EHN, M.; Buerstmayr, M.; Buerstmayr, H. (2020): Common bunt resistance in winter wheat – a cross-chromosome journey: Zenodo.

FAOSTAT (2022): Crops and livestock products. Item: Wheat. Available online at https://www.fao.org/faostat/en/#data/QCL/visualize, checked on 1/24/2022.

Fuentes-Davilla, G.; Goates, Blair J.; Thomas, Julian B.; Nielsen, J.; Ballantyne, B. (2002): Smut diseases. in B.C. Curtis, S. Rajaram and H. Gomez Macpherson (eds.). in B.C. Curtis, S. Rajaram and H. Gomez Macpherson (eds.). In *Bread Wheat: Improvement and Production* (Plant Production and Protection Series No. 30), checked on 27.03.22.

Gauch, H. G.; Zobel, Richard (1996): Ammi analysis of yield trials.In: Genotype-by-environment interaction, kang ms and hg gauch (eds.). In *Boca Raton CRE CRC*, pp. 85–122.

Gaudet, D. A.; Leggett, F.; Lu, Z.-X.; Frick, M.; Puchalski, B.; Despins, T.; Laroche, A. (2006): Compatible and incompatible interactions involving the Bt10 gene in wheat for resistance to Tilletia tritici, the common bunt pathogen. In *Czech Journal of Genetics and Plant Breeding* 42 (Special Issue), p. 14. DOI: 10.17221/6222-CJGPB.

Gaudet, D. A.; Menzies, J. G.; Sharma, I. (2012): Common bunt of wheat: an old foe remains a current threat. In, pp. 220–235.

Goates, B. J. (1996): Common bunt and dwarf bunt.

Goates, B. J. (2012): Identification of New Pathogenic Races of Common Bunt and Dwarf Bunt Fungi, and Evaluation of Known Races Using an Expanded Set of Differential Wheat Lines. In *Plant disease* 96 (3), pp. 361–369. DOI: 10.1094/PDIS-04-11-0339.

Goates, B. J.; Bockelman, H. E. (2012): Identification of New Sources of High Levels of Resistance to Dwarf Bunt and Common Bunt among Winter Wheat Landraces in the USDA-ARS National Small Grains Collection. In *Crop Sci.* 52 (6), pp. 2595–2605. DOI: 10.2135/cropsci2012.01.0060.

Goates, B. J.; Mercier, J. (2011): Control of common bunt of wheat under field conditions with the biofumigant fungus Muscodor albus. In *Eur J Plant Pathol* 131 (3), pp. 403–407. DOI: 10.1007/s10658-011-9817-z.

Hagenguth, J. (2016): Breeding for organic agriculture: evaluation of common bunt resistance in three winter wheat populations derived from M822123, PI178383 and PI560841-bcl. Masterthesis. Universität für Bodenkultur, Wien. Available online at https://epub.boku.ac.at/obvbokhs/download/pdf/1935484?originalFilename=true, checked on 05.03.22.

Hoffmann, J. A. (1982): Bunt of Wheat. In *Plant disease* 66 (1), p. 979. DOI: 10.1094/PD-66-979.

Hoffmann, J. A.; Metzger, R. J. (1976): Current Status of Virulence Genes and Pathogenic Races of the Wheat Bunt Fungi in the Northwestern USA. In *Phytopathology* 66 (5), p. 657. DOI: 10.1094/Phyto-66-657.

Huber, K.; Buerstmayr, H. (2006): Development of methods for bunt resistance breeding for organic farming. In *Czech Journal of Genetics and Plant Breeding* 42 (Special Issue), pp. 66–71. DOI: 10.17221/6235-CJGPB.

Ittu, M.; Saulescu, N. N.; Ittu, G. (2006): Latest in breeding for resistance to common bunt in Romania. In *Czech Journal of Genetics and Plant Breeding* 42 (Special Issue), p. 15. DOI: 10.17221/6223-CJGPB.

Kroonenberg, P. M. (Ed.) (2008): Applied Multiway Data Analysis. Hoboken, NJ, USA: John Wiley & Sons, Inc (Wiley Series in Probability and Statistics).

Laroche, A.; Demeke, T.; Gaudet, D. A.; Puchalski, B.; Frick, M.; McKenzie, R. (2000): Development of a PCR marker for rapid identification of the Bt-10 gene for common bunt resistance in wheat. In *Génome* 43 (2), pp. 217–223. DOI: 10.1139/GEN-43-2-217.

Liatukas, Ž.; Ruzgas, V. (2008): Resistance genes and sources for the control of wheat common bunt (Tilletia tritici (DC.) Tul.). In *Biologija* 54, pp. 274–278. DOI: 10.2478/v10054-008-0056-y.

Liatukas, Ž.; Ruzgas, V. (2009): Effect of air temperature on common bunt (Tilletia caries ) infection in winter wheat. In *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science* 59 (3), pp. 225–232. DOI: 10.1080/09064710802022929.

Lu, Z.-X.; Gaudet, D.; Puchalski, B.; Despins, T.; Frick, M.; Laroche, A. (2005): Inducers of resistance reduce common bunt infection in wheat seedlings while differentially regulating defence-gene expression. In *Physiological and Molecular Plant Pathology* 67 (3-5), pp. 138–148. DOI: 10.1016/j.pmpp.2005.12.001.

Mamluk, O. F. (1998): Bunts and smuts of wheat in North Africa and the Near East. In *Euphytica* 100 (1/3), pp. 45–50. DOI: 10.1023/A:1018343603827.

Matanguihan, J.; Jones, S. (2011): A New Pathogenic Race of Tilletia caries Possessing the Broadest Virulence Spectrum of Existing Races. In *Plant Health Progress* 12. DOI: 10.1094/PHP-2010-0520-01-RS.

Matanguihan, J. B.; Murphy, K. M.; Jones, S. S. (2011): Control of Common Bunt in Organic Wheat. In *Plant disease* 95 (2), pp. 92–103. DOI: 10.1094/PDIS-09-10-0620.

Mathre, D. E. (2000): Stinking smut of wheat. In *PHI*. DOI: 10.1094/PHI-I-2000-1030-01.

Meteostat (2022): Wetterdaten Tulln. Available online at https://meteostat.net/de/place/3X31AV/climate, updated on 31.01.22, checked on 31.01.22.

Metzger, R. J.; Hoffmann, J. A. (1978): New Races of Common Bunt Useful to Determine Resistance of Wheat to Dwarf Bunt 1. In *Crop Sci.* 18 (1), pp. 49–51. DOI: 10.2135/cropsci1978.0011183X001800010013x.

Miedaner, T. (2014): Kulturpflanzen: Botanik -- Geschichte -- Perspektiven. Place of publication not identified: Springer Science and Business Media. Available online at http://search.ebscohost.com/login.aspx?direct=true&scope=site&db=nlebk&db=nlabk&AN=878351.

Mourad, A. M. I.; Sallam, A.; Belamkar, V.; Mahdy, E.; Bakheit, B. Abo E., Atif; Stephen Baenziger, P. (2018): Genetic architecture of common bunt resistance in winter wheat using genome-wide association study. In *BMC plant biology* 18 (1), p. 280. DOI: 10.1186/s12870-018-1435-x.

Muellner, A. E.; Buerstmayr, M.; Eshonkulov, B.; Hole, D.; Michel, S.; Hagenguth, J. F. et al. (2021): Comparative mapping and validation of multiple disease resistance QTL for simultaneously controlling common and dwarf bunt in bread wheat. In *Theoretical and Applied Genetics* 134 (2), pp. 489–503. Available online at https://neuro.unboundmedicine.com/medline/citation/33120433/Comparative_mapping_and_validation_of_multiple_disease_resistance_QTL_for_simultaneously_controlling_common_and_dwarf_bunt_in_bread_wheat_.

Muellner, A. E.; Eshonkulov, B.; Hagenguth, J.; Pachler, B.; Michel, S. Buerstmayr, M.; Hole, David; Buerstmayr, Hermann (2020): Genetic mapping of the common and dwarf bunt

resistance gene Bt12 descending from the wheat landrace PI119333. In *Euphytica* 216 (5). DOI: 10.1007/s10681-020-02614-w.

Nguyen, H. D. T.; Sultana, T.; Kesanakurti, P.; Hambleton, S. (2019): Genome sequencing and comparison of five Tilletia species to identify candidate genes for the detection of regulated species infecting wheat. In *IMA fungus* 10, p. 11. DOI: 10.1186/s43008-019-0011-9.

Oberforster, M.; Plank M. (2021): Susceptibility of winter wheat cultivars to various isolates of common bunt (Tilletia caries). Edited by ÖMKi Hungarian Research Institute of Organic Agriculture, updated on April 2021, checked on 06.02.22.

Onicica, F.; Săulescu, N. (2008): POTENTIALLY NEW SOURCES OF GENES FOR RESISTANCE TO COMMON BUNT (TILLETIA spp. ) IN WINTER WHEAT (TRITICUM AESTIVUM L. ). Available online at https://www.semanticscholar.org/paper/742e1719cb76b96c0491923eedb365c6c7ef91c0.

Pieczul, K.; Perek, A.; Kubiak, K. (2018): Detection of Tilletia caries, Tilletia laevis and Tilletia controversa wheat grain contamination using loop-mediated isothermal DNA amplification (LAMP). In *Journal of microbiological methods* 154, pp. 141–146. DOI: 10.1016/j.mimet.2018.10.018.

Pospischil, E. J.G.; Nielsen, G. D.; Papameletiou, D.; Klein, C. L. (2017): SCOEL/REC/179 Trimethylamine. In *Publ. Sci. Comm. Occup. Expo. Lim. Eur. Union*.

R Core Team (2021): R: A language and environment for statistical computing. Version version 4.0.5. Vienna, Austria:. R Foundation for Statistical Computing. Available online at https://www.R-project.org/., checked on 25.02.22.

Raju, B.M.K (2002): A study on AMMI model and its biplots. In *J. Ind. Soc. Agril. Statist* 55, pp. 297–322.

Roemer, T.; Bartholly, R. (1933): Die Aggressivität verschiedener "Steinbrandherkünfte" [Tlletia tritici. (Bjerk.) Wint.] und ihre Veränderung durch die Wirtssorte. In *Phytopathologische Zeitschrift* (6 (5)), pp. 469–504.

Ruzgas, Y.; Liatukas, I. (2009): Response of Lithuanian Winter Wheat Advanced Lines to Common Bunt (Tilletia tritici (BJERK.) WINT). In.

SOOD, A. K.; SINGH, B. M. (1985): Occurrence and distribution of virulences of Tilletia foetida and T. caries causing bunt of wheat in Himachal Pradesh. In *Indian phytopathology* 38 (CODEN: IPHYAU), p. 699.

Souza, E.; Windes, J. M.; Sunderman, D. W.; Whitmore, J.; Kruk, M.; Goates, B. J. (1995): Registration of 'Bonneville' Hard Red Winter Wheat. In *Crop Sci.* 35 (4), pp. 1218–1219. DOI: 10.2135/cropsci1995.0011183X003500040072x.

Spieß, H. (2016): Weizensteinbrand. eine Broschüre im Rahmen des "Aktionsplans biologische Landwirtschaft Luxemburg". With assistance of Ewald B, Stoll E. Edited by Insititut für biologische Landwirtschaft und Agrarkultur Letzebuerg (IBLA). Munsbach. Available online at https://ibla.lu/_res/uploads/2016/07/Weizensteinbrand.pdf, checked on 1/18/2022.

Steffan, P. M.; Torp, A. M.; Borgen, A.; Backes, G.; Rasmussen, S. K. (2017): Mapping of common bunt resistance gene Bt9 in wheat. In *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik* 130 (5), pp. 1031–1040. DOI: 10.1007/s00122-017-2868-6.

Sunderman, D. W.; Souza, E.; Birzer, Diane; Whitmore, Jim (1991): Registration of 'Blizzard' Wheat. In *Crop Sci.* 31 (2), pp. 491–492. DOI: 10.2135/cropsci1991.0011183X003100020074x.

Szunics, L. (1990): Data on common bunt infection in wheat varieties. In *Novenytermeles* 39, pp. 297–304.

Veisz, O.; Szunics, L.; Szunics, L. (2000): Effect of common bunt on the frost resistance and winter hardiness of wheat (Triticum aestivum L.) lines containing Bt genes. In *Euphytica* 114 (2), pp. 159–164. DOI: 10.1023/A:1003940808041.

Waldow, F.; Jahn, M. (2007): Investigations in the regulation of common bunt (Tilletia tritici) of winter wheat with regard to threshold values, cultivar susceptibility and non-chemical protection measures. In *Journal of Plant Diseases and Protection* 114, pp. 269–275.

Wang, S.; Knox, R. E.; DePauw, R. M.; Clarke, F. R.; Clarke, J. M.; Thomas, J. B. (2009): Markers to a common bunt resistance gene derived from 'Blizzard' wheat (Triticum aestivum L.) and mapped to chromosome arm 1BS. In *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik* 119 (3), pp. 541–553. DOI: 10.1007/s00122-009-1063-9.

Weinhappel M. (2016): CARIES - Forschungsprojekt zu Gewöhnlichem Steinbrand in Österreich. Austrian Agency for Health and Food Security. Wien.

Wilcoxson, Roy D.; Saari, E. E. (1996): Bunt and smut diseases of wheat. Mexico: CIMMYT.

Yan, W.; Falk, D. E. (2002): Biplot Analysis of Host-by-Pathogen Data. In *Plant disease* 86 (12), pp. 1396–1401. DOI: 10.1094/PDIS.2002.86.12.1396.

Yan, W.; Kang, M. S.; Ma, B.; Woods, S.; Cornelius, P. L. (2007): GGE Biplot vs. AMMI Analysis of Genotype-by-Environment Data. In *Crop Sci.* 47 (2), pp. 643–653. DOI: 10.2135/cropsci2006.06.0374.

Zobel, R. W.; Wright, M. J.; Gauch, H. G. (1988): Statistical Analysis of a Yield Trial. In *Agron.j.* 80 (3), pp. 388–393. DOI: 10.2134/agronj1988.00021962008000030002x.

# Appendix

## Additional Material and Methods

*Table 10: Origins of isolates and amount of spores used for the experiment*

| Isolate origin | Provided by | Spores used for inoculation |
|---|---|---|
| **IFA Housekeeping** | IFA | 4,87g |
| **IFA Aggressive** | IFA | 4,63g |
| **Loosdorf** | AGES | 4,51g |
| **Gerhaus** | AGES | 4,56g |
| **Thening** | AGES | 4,15g |
| **Hinzenbach** | AGES | 4,36g |
| **Sitzendorf** | AGES | 4,11g |
| **Harmannsdorf** | AGES | 4,54g |

*Table 11: Measures for plant protection and fertilization*

| | Date of treatment | Substance | Amount |
|---|---|---|---|
| **Herbicide** | 24.04.2021 | Arrat + Dash | 200g/ha + 1l/ha |
| | 04.06.2021 | Puma Extra | 1l/ha |
| **Stem shortener** | 28.05.2021 | Cerone | 0,7l/ha |
| **Fertilization** | 09.03.2021 | NPK 17:6:18+7S | 330 kg/ha |
| | 10.05.2021 | KAS 27%N | 130 kg/ha |

*Table 12: List of all wheat genotypes included in the field trial.*

| Genotype name [1,2,3] | Association[1,2,3] | Genotype group | Resistance gene/ QTL [1,2,3,4,5,6] | Pedigree[2,3,4,5,6] | Origin/ Breeders[2,3,4,5,6] |
|---|---|---|---|---|---|
| **Sel2092 (Bt1)** | PI 554101 | differential | Bt1 | | R. J. Metzger, Oregon State University, USA |
| **Sel1102 (Bt2)** | PI 554097 | differential | Bt2 | | R. J. Metzger, Oregon State University, USA |
| **Ridit (Bt3)** | Cltr 6703 | differential | Bt3 | Turkey/ Florence | E. F. Gaines, Washington Agricultural Experiment Station USA (1923) |
| **CI1558B (Bt4)** | PI 11610 | differential | Bt4 | | J. G. Haney, Branch Agricultural Experiment Station – Fort Hays, USA |
| **Hohenheimer (Bt5)** | Cltr 11458 | differential | Bt5 | | E. L. Kendrick, Washington Agricultural Experiment Station, USA |
| **Rio (Bt6)** | Cltr 10061 | differential | Bt6 | Selection from Argentine, CI 1569 | D. E. Stephens, Sherman County Branch Station, USA and H. M. Woolman, Oregon Agricultural Experiment Station, USA 1930 |
| **SEL500-77 (Bt7)** | PI 554100 | differential | Bt7 | Selection from Cltr 13561 | Dr. R. J. Metzger, Oregon State University, USA |
| **M822161 (Bt8)** | PI 554120 | differential | Bt8 | PI 173438 / Elgin | Dr. R. J. Metzger, Oregon State University, USA |
| **M90387 (Bt9)** | PI 554099 | differential | Bt9 (Steffan et al. 2017) | Elgin / PI 178383 | Dr. R. J. Metzger, Oregon State University, USA |
| **M822102 (Bt10)** | PI 554118 | differential | Bt10 (Laroche et al. 2000) | Elgin / PI 178383 | Dr. R. J. Metzger, Oregon State University, USA |
| **M822123 (Bt11)** | PI 554119 | differential | Bt11 | Elgin / PI 166910 | Dr. R. J. Metzger, Oregon State University, USA |
| **PI 119333 (Bt12)** | PI 119333 | differential | Bt12 (Muellner et al. 2020) | | Elazig, Turkey (1936) by H. L. Westover and F. L. Wellman, USDA – Bureau of Plant Industry, USA |
| **Thule-III (Bt13)** | PI 181463 | differential | Bt13 | Thule II / Sammet | Swedish Seed Association, Sweden |
| **PI 173437 (Btp)** | PI 173437 | differential | Btp | | Hakkari, Turkey (1948) by Jack. R. Harlan, USDA-ARS, USA |
| **Tillliko** | | cultivar | BtZ [5,6] | (HS-JulaRe.Z145=(Jubilar x Hess.Landweizen) x Renan) x (Zarya x Tambor) | Karl-Josef Müller, Die Saat |
| **Tillexus** | | cultivar | Bt10 [5,6] | | Saatzucht Donau |
| **Tillstop** | | cultivar | | | Saatzucht Donau |
| **Capo** | | cultivar | | Martin/Pokal | Probstdorfer Saatzucht |
| **Aurelius** | | cultivar | | | Saatbau Linz |
| **P106.51.2** | | resistance donor | Bt12 (Muellner et al. 2020) | PI 119333/ Rainer | IFA Tulln (personal communication) |
| **S5.47.2** | | resistance donor | *QBt.ifa-1AL* (Muellner et al. 2021) | Rainer/ Blizzard | IFA Tulln (personal communication) |
| **P101.8.5B** | | resistance donor | *QBt.ifa-1AL* (Muellner et al. 2021) | Bonneville/ Rainer | IFA Tulln (personal communication) |

| | | | | | |
|---|---|---|---|---|---|
| **S7.4.1** | | resistance donor | *QBt.ifa-1BS* (Muellner et al. 2021) | Rainer x Bonneville | IFA Tulln (personal communication) |
| **P101.111.1** | | resistance donor | *QBt.ifa-1BS* (Muellner et al. 2021) | Bonneville / Rainer | IFA Tulln (personal communication) |
| **P106.69.5** | | resistance donor | Bt12 (Muellner et al. 2020) | PI 119333/ Rainer | IFA Tulln (personal communication) |
| **702-1102C** | | resistance donor | Bt9 (FALLBACHER et al. 2020) | | IFA Tulln (personal communication) |
| **P106.16.2** | | resistance donor | *QBt.ifa-4B* (Muellner et al. 2020) | PI 119333 / Rainer | IFA Tulln (personal communication) |
| **Blizzard** | PI 512302 | cultivar | *QBt.ifa-1AL, QBt.ifa-1BS, QBt.ifa-7AL* (Muellner et al. 2021) | A68203W-E-1-3-3/A68203W-A-1-6-1 ; A68203W = Utah 216c-12-10/Cheyenne/5/PI 476212/4/Burt/3/Rio/Rex//Nebred | University of Idaho and USDA-ARS, (Sunderman et al. 1991) |
| **Bonneville** | PI 557015 | cultivar | *QBt.ifa-1AL*, *QBt.ifa-1BS, QBt.ifa-7AL,* Muellner et al. 2021) | A774125W-16-3-1/A7470W-11-2 = Utah216c-12-10/Cheyenne/5/PI 476212/4/Burt/3/Rex/Rio//Nebred/6/ Kiowa/Utah222a-437-2//Dm/3/PI476212/ MT6619/4/McCall/ El Gaucho/3/Kiowa/Utah233-3-10/Burt | Idaho Agric. Exp. Station and USDA-ARS (Souza et al. 1995) |
| **Dimenit** | PI166910 | resistance donor | Bt 7,9,11 | | Landrace collected in Tokat, Turkey (1948) by Harlan, J. R., USDA-ARS |
| **VIII/25-A** | PI 362695 | resistance donor | | | Landrace collected in Montenegro (1971) |
| **TU86-07-01-4** | PI 560795 | resistance donor | | | Landrace collected in Hakkari, Turkey (1989) by Atchley et al. |
| **PI 636156** | PI 636156 | resistance donor | | Selection from PI 560795 | Goates, Blair J., USDA-ARS, Idaho, USA (2004) |
| **PI560845-sel-wcl** | PI 636170 | resistance donor | | Selection from PI 560845 | Goates, Blair J., USDA-ARS, Idaho, USA (2004) |
| **PI560841-sel-wco** | PI 636165 | resistance donor | | Selection from PI 560841 | Goates, Blair J., USDA-ARS, Idaho, USA (2004) |
| **Globus** | | cultivar | Bt 5 (Borgen et al. 2019) | NORD 92-147//Astron/CWW 4442 | Saatzuchtgesellschaft Nordsaat GmbH, Germany |
| **PI178383** | **PI 178383** | resistance donor | Bt 8,9,10 (Metzger et al. 1977 | | Landrace from Hakkari, Turkey collected by J. R. Rodney (USDA-ARS) 1949 |
| **Tillsano** | | culitvar | Bt5 (Oberforster and Plank M. 2021) | Miroir/Genius/Gallio [5,6] | Probstdorfer Saatzucht |
| **UI SRG** | PI 660546 | cultivar | | Utah 100'*2/ 'Boundary | Idaho Agricultural Experiment Station, USA (Chen et al. 2012) |
| **Deloris** | PI 631447 | cultivar | | Arbon/Hansel//PI 470329/3/Weston/ NE7060 | Utah Agricultural Experiment Station (UAES), USA (Hole et al. 2004) |

[1]Goates BJ (2012) Identification of New Pathogenic Races of Common Bunt and Dwarf Bunt Fungi, and Evaluation of Known Races Using an Expanded Set of Differential Wheat Lines. Plant Dis 96:361–369. doi: 10.1094/PDIS-04-11-0339

[2]Agricultural Research Service of the US Department of Agriculture (USDA) (2022) Germplasm Resource Information Network. https://npgsweb.ars-grin.gov/gringlobal/search, Accessed: 30.03.22

[3]GRIN Czech Release 1.10.3 (2022): https://grinczech.vurv.cz/gringlobal/search.aspx, Accessed: 30.03.22

[4]Buerstmayr H. & Ehn M.(IFA Tulln), personal communication (2022)

[5]Borgen A. (Agrologica), personal communication (2022)

[6]Oberforster M. (AGES), personal communication (2022)

## Additional Results

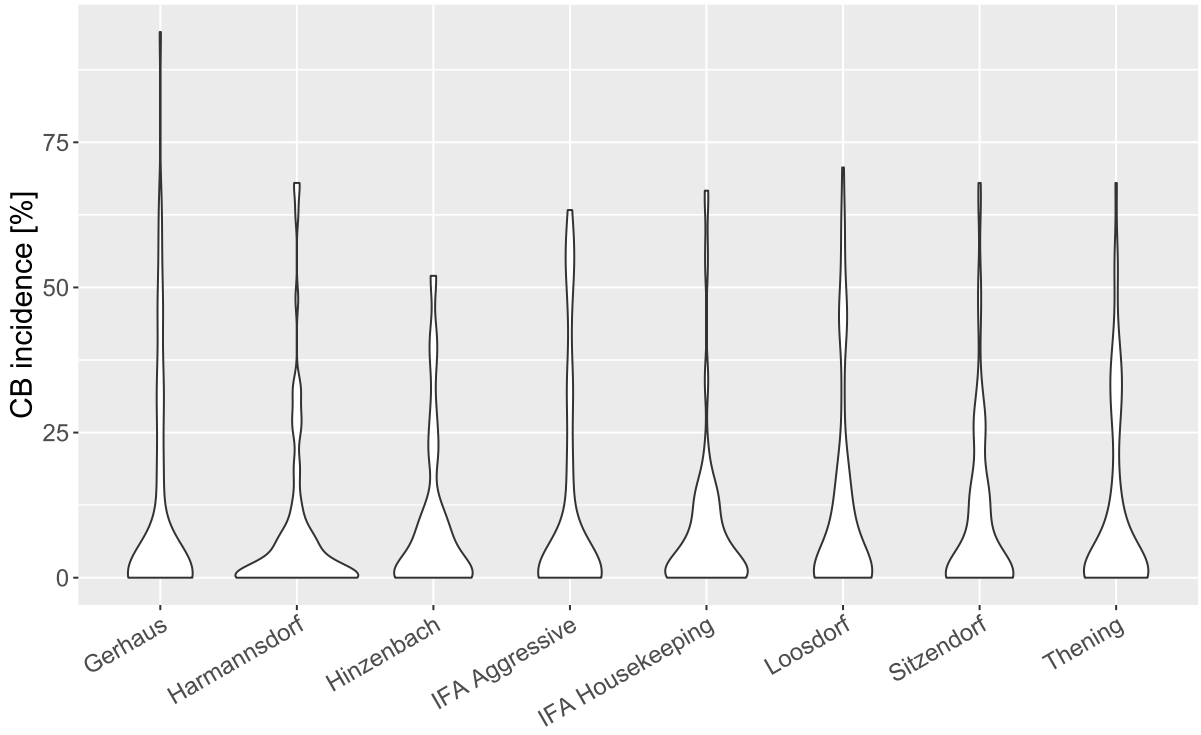


*Figure 21: Violin plot showing* common bunt *incidence [%] of different isolates. Shapes of the violins represent the distribution of the data.*

*Table 13: ANOVA table of AMMI stability analysis: ISO represents the effect of the respective isolate, REP the replication effect and GEN the genotypic effect of the wheat accessions, Df = Degrees of freedom*

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| **ISO** | 7 | 1485.44 | 212.21 | 11.13 | 1.46E-03 |
| **REP(ISO)** | 8 | 152.60 | 19.07 | 1.56 | 0.14 |
| **GEN** | 39 | 154527.43 | 3962.24 | 323.58 | $<2*10^{-16}$ |
| **ISO:GEN** | 273 | 17828.55 | 65.31 | 5.33 | $<2*10^{-16}$ |
| **Residuals** | 312 | 3820.47 | 12.25 | | |

*Table 14: Tukey posthoc results: The second column shows differences in average common bunt incidence between the two isolates indicated in the respective row of the first column*

| | differences [%] | p -value |
|---|---|---|
| **Loosdorf-Harmannsdorf** | 4.24 | $9.63*10^{-12}$ |
| **Loosdorf-IFA Housekeeping** | 4.17 | $1.98*10^{-11}$ |
| **IFA Aggressive-Harmannsdorf** | 3.82 | $9.67*10^{-12}$ |
| **IFA Housekeeping-IFA Aggressive** | -3.76 | $2.00*10^{-9}$ |
| **Harmannsdorf-Gerhaus** | -3.32 | $1.77*10^{-7}$ |
| **IFA Housekeeping-Gerhaus** | -3.26 | $3.42*10^{-7}$ |
| **Thening-Harmannsdorf** | 2.81 | $2.13*10^{-5}$ |
| **Loosdorf-Hinzenbach** | 2.79 | $2.46*10^{-5}$ |
| **Thening-IFA Housekeeping** | 2.74 | $3.77*10^{-5}$ |
| **Sitzendorf-Loosdorf** | -2.46 | $3.71*10^{-4}$ |
| **IFA Aggressive-Hinzenbach** | 2.38 | $6.92*10^{-4}$ |
| **Sitzendorf-IFA Aggressive** | -2.04 | $6.95*10^{-3}$ |
| **Hinzenbach-Gerhaus** | -1.88 | $1.91*10^{-2}$ |
| **Sitzendorf-Harmannsdorf** | 1.78 | $3.21*10^{-2}$ |
| **Sitzendorf-IFA Housekeeping** | 1.72 | $4.59*10^{-2}$ |
| **Sitzendorf-Gerhaus** | -1.54 | 0.107 |
| **Hinzenbach-Harmannsdorf** | 1.45 | 0.159 |
| **Thening-Loosdorf** | -1.43 | 0.170 |
| **IFA Housekeeping-Hinzenbach** | -1.38 | 0.207 |
| **Thening-Hinzenbach** | 1.36 | 0.226 |
| **Thening-Sitzendorf** | 1.03 | 0.593 |
| **Thening-IFA Aggressive** | -1.02 | 0.604 |
| **Loosdorf-Gerhaus** | 0.92 | 0.723 |
| **Thening-Gerhaus** | -0.52 | 0.983 |
| **IFA Aggressive-Gerhaus** | 0.50 | 0.986 |
| **Loosdorf-IFA Aggressive** | 0.42 | 0.995 |
| **Sitzendorf-Hinzenbach** | 0.33 | 0.999 |
| **IFA Housekeeping-Harmannsdorf** | 0.07 | 1.00 |

## Climate Data

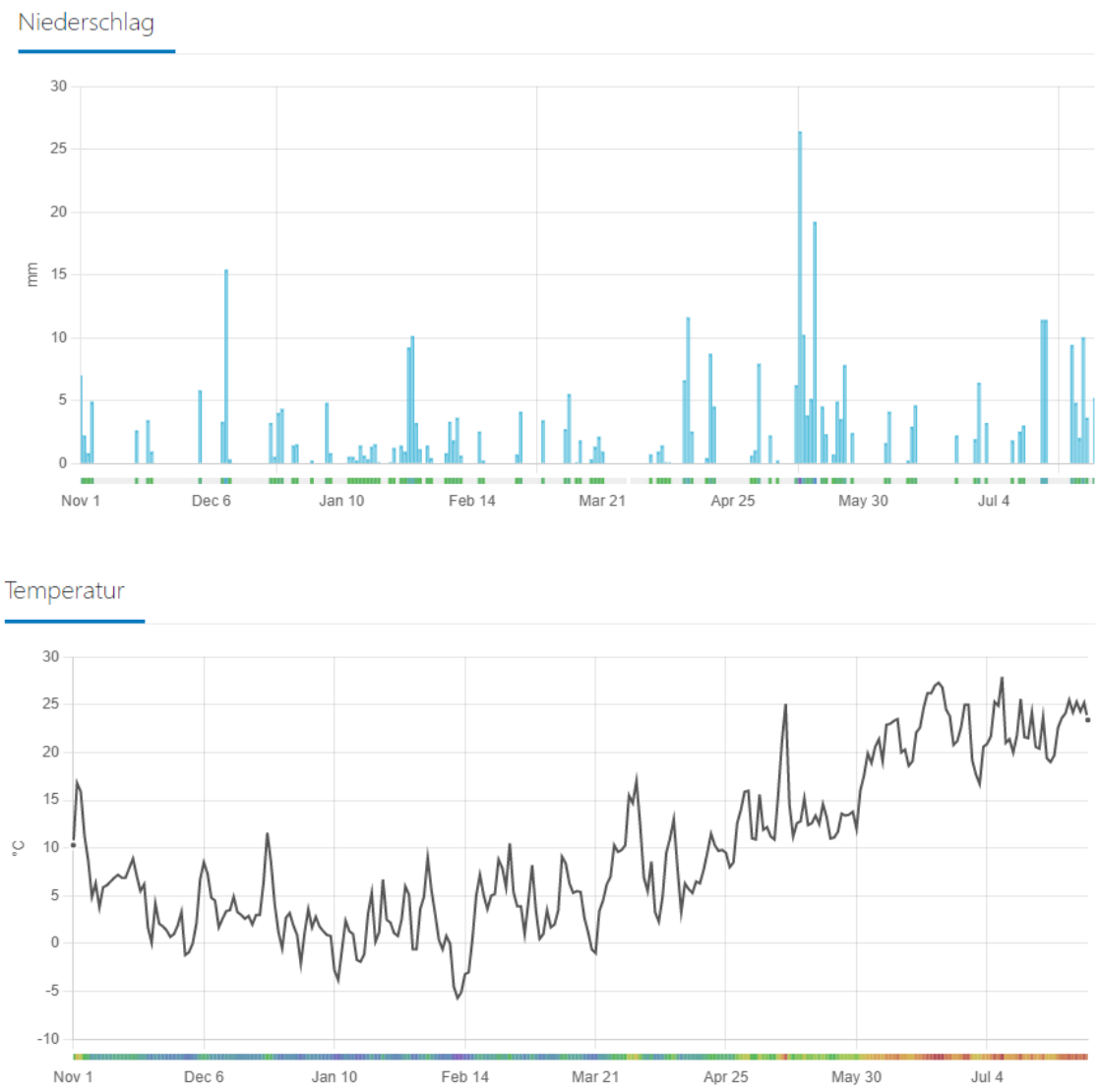


*Figure 22: Precipitation data (upper graph) and daily mean temperature (lower graph) measured in Tulln from 1st of November 2020 to 31st of July 2021. Source: Meteostat*

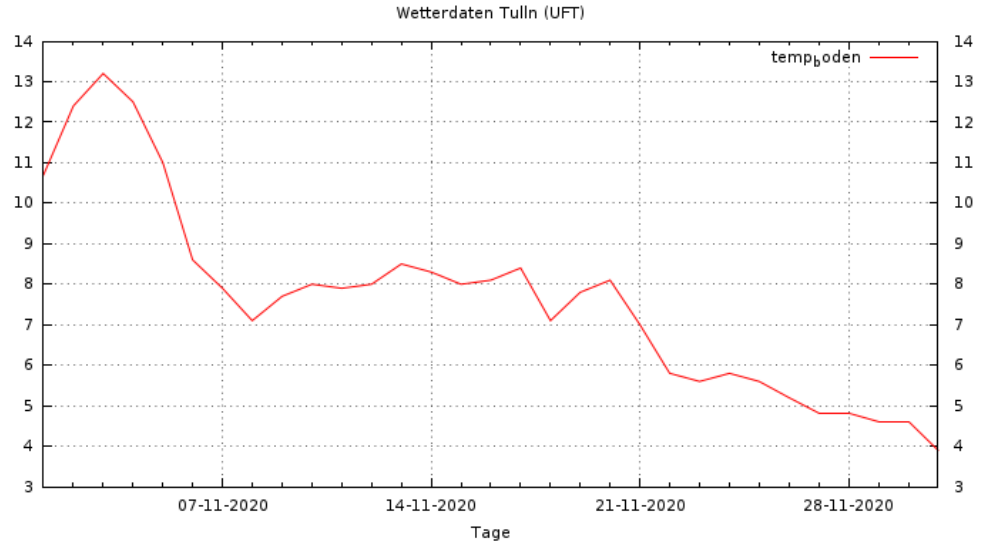


*Figure 23: Daily mean soil temperature from 1st of November to 1st of December 2020 in Tulln (BOKU 2022)*

## R-code

The following subsection shows excerpts of the R-code used for statistical analysis.

```
#### heatmap
po <- ggplot(blues.sus2, aes(x=variable, y=ï..GEN )) +
  geom_tile(aes(fill = value)) +
  scale_fill_distiller(palette = "YlGn", direction = 1) +
  labs(title = "Heatmap of Common Bunt Infection Levels",
       y = "Genotypes in Test Set", fill = "CB incidence") +
  theme(
    legend.position="right",
    legend.text = element_text(size = 14),
    legend.title = element_text(size = 14),
    axis.title=element_text(size=12,face="bold"),
    axis.text=element_text(size=12),
    plot.title = element_text(size=20, hjust=0.5, face = "bold")
  ) +labs(x=NULL, y= NULL)

po  + guides(x = guide_axis(angle = 45))
po


#### Plots

ggplot(data=mydata1, aes(x = Isolat, y = CB_incidence)) +
  geom_boxplot() +
  stat_summary(fun = mean, geom = "point", col = "red") +  # Add
points to plot
  stat_summary(fun = mean, geom = "text", col = "red",     # Add
text to plot
               vjust = 1.5, aes(label = paste("", round(..y..,
digits = 2))))+
  labs(x = NULL, y = "CB incidence [%]")+
  guides(x = guide_axis(angle = 30))+
  theme(text = element_text(size=17))


ggplot(data=mydata1)+
  geom_violin(mapping =aes(x= Isolat, y = CB_incidence))+
  labs(x = NULL, y = "CB incidence [%]")+
  theme(text = element_text(size=16))+
  guides(x = guide_axis(angle = 30))
```

```
#boxplot of all genotypes ordered by their susceptibility
ggplot(data =mydata1)+
  geom_boxplot(mapping = aes(
    x = CB_incidence,
    y=  reorder(GENOTYPE, CB_incidence, FUN=mean), color= group))+
  labs(x="CB incidence [%]", y=NULL)+
  geom_vline(aes(xintercept = 5))


### boxplot differentials ordered by their susceptibility
ggplot(data =mydata2)+
  geom_boxplot(mapping = aes(
    x = reorder(GENOTYPE, CB_incidence, FUN =mean),
    y=  CB_incidence), color = "3")+
  coord_flip()+
  labs(x=NULL, y= "CB incidence [%]")+
  geom_hline(aes(yintercept = 5))+
  theme(text = element_text(size=17))
```

```
##### Correlations
### Test for normal distribution: if p<0,05 no normal distribution
shapiro.test(mydata3$plantheight)
shapiro.test(mydata3$dateofheading)
shapiro.test(mydata3$CB_incidence)
shapiro.test(mydata3$dateofflowering)

#no normal distribution in the data set

### correlations using Spearman's rho

Ko1 <- tidy(cor.test(mydata3$plantheight, mydata3$CB_incidence
,method="spearman"))
Ko2 <-tidy( cor.test(mydata3$dateofheading,
mydata3$CB_incidence,method="spearman"))
Ko3 <-tidy( cor.test(alle_Daten$ mydata3$dateofflowering,
mydata3$CB_incidence,method="spearman"))


plot((mydata3$dateofheading, mydata3$CB_incidence, xlab= "date of
heading (days after 1st of May)", ylab ="CB incidence [%]",
     sub="n=640, rho = -0.11")
abline(lm(mydata3$CB_incidence ~ mydata3$dateofheading), col =
"red", lwd = 2)

plot(mydata3$dateofflowering, mydata3$CB_incidence, xlab= "date of
flowering (days after 1st of May)", ylab ="CB incidence [%]",
     sub="n=640, rho = -0.09")
abline(lm(mydata3$CB_incidence ~ mydata3$dateofflowering), col =
"red", lwd = 2)

plot(mydata3$plantheight, mydata3$CB_incidence, xlab= "plant height
[cm]", ylab ="CB incidence [%]",
     sub="n=640, rho = -0.27")
abline(lm(mydata3$CB_incidence ~ mydata3$plantheight), col = "red",
lwd = 2)
```

```
##### ANOVA including interactions
interaction2 <- aov(CB_incidence ~ Isolat + GENOTYPE + Isolat *
GENOTYPE + Isolate/Replication, data = mydata1)
summary(interaction2)

##post-hoc:
tukey.int2<-TukeyHSD(interaction2)

post_hoc <-as.data.frame(tukey.int2$Isolat)
post_hoc


##### AMMI (agricole)
TAMMI.mod <- with (mydata1, AMMI(ENV = Isolat, GEN= GENOTYPE,
REP=WH, CB_incidence, console = T))

TAMMI.mod$ANOVA
TAMMI.mod$analysis #IPC und F-Test
#GxE matrix -> deviations from mean
array(TAMMI.mod$genXenv, dim(TAMMI.mod$genXenv),
dimnames(TAMMI.mod$genXenv))


###PC1 vs CB_inc (AMMI1).
plot(TAMMI.mod, 0 ,1, gcol= ”darkblue”, ecol = ”darkgreen”, number
=F, xlab = ”CB incidence [%]”)

AMMI <- FA.AMMI(TAMMI.mod)
```

```
#####GGE-Biplot Analysis
biplot(GGB, main = NULL, sub = NULL, scale=T,
       cex.gen = 0.5,
       cex.env = 0.7,
       col.gen = "darkblue",
       col.env = c("darkgreen", "purple", "red"),
       zoom.gen = 0.98, flip = c(2,1),
       comps= (1:2), origin = 0, hull =T)



###Stability-Analysis in ME (Mega-Environment)
CBi_env1 <- mydata1 %>% dplyr::filter(Isolat %in% c("Thening",
"Hinzenbach", "Loosdorf"))
CBi_env2 <- mydata1 %>% dplyr::filter(Isolat %in% c("Sitzendorf",
"Harmannsdorf", "IFA Housekeeping"))
CBi_env3 <- mydata1 %>% dplyr::filter(Isolat %in% c("Gerhaus", "IFA
Aggressive"))


GGB1 <- gge(CBi_env1, CB_incidence ~ GENOTYPE:Isolat,
            env.group = MEGAENVIRONMENT, center =T, scale =T, ggb
=T)

biplot(GGB1, main = NULL, sub = NULL, scale=T,
       cex.gen = 0.5,
       cex.env = 0.7,
       col.gen = "darkblue",
       col.env = "purple",
       zoom.gen = 0.98, flip = c(2,1),
       comps= (1:2), origin = 0, hull =T)


GGB2 <- gge::gge(CBi_env2, CB_incidence ~ GENOTYPE:Isolat,
            env.group = MEGAENVIRONMENT, center =T, scale =T, ggb
=T)
biplot(GGB2, main = NULL, sub = NULL , scale=T,
       cex.gen = 0.5,
       cex.env = 0.7,
       col.gen = "darkblue",
       col.env = "darkgreen",
       zoom.gen = 0.98, flip = c(2,1),
       comps= (1:2), origin = 0, hull =T)
```

```
GGB3 <-gge:: gge(CBi_env3, CB_incidence ~ GENOTYPE:Isolat,
            env.group = MEGAENVIRONMENT, center =T, scale =T, ggb
=T)

biplot(GGB3, main = NULL , sub = NULL, scale=T,
       cex.gen = 0.5,
       cex.env = 0.7,
       col.gen = "darkblue",
       col.env = c("red"),
       zoom.gen = 0.98, flip = c(2,1),
       comps= (1:2), origin = 0, hull =T)



### ranking environments
library(metan)
gge_model <-metan:: gge(mydata1, Isolat, GENOTYPE, CB_incidence)
k <- plot(gge_model, type = 6, title = F, main=F, col.env =
"darkgreen", cex.env = 2)
```