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Perennial survival of common bunt *(Tilletia tritici)* in soil under modern farming practice

Mehrjähriges Überdauern von Steinbrand *(Tilletia tritici)* im Boden unter modernen landwirtschaftlichen Bedingungen

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Summary

Three years of field trials in Denmark have shown that common bunt *(Tilletia tritici)* is able to infect wheat in crop rotations with several intercrops between susceptible crops. This contradicts most previous literature which concluded that common bunt is a seed-borne pathogen only able to infect through the soil in cases where wheat is grown in two consecutive years. It is argued that the reason for this change in pathogenicity can be explained by a change in farming practice.

Key words: Winter wheat; *Tilletia tritici;* soil-borne disease; *T. caries*

Zusammenfassung

Feldversuche in Dänemark über drei Jahre hinweg zeigen, daß Steinbrand *(Tilletia tritici)* durch bodenbürtige Erreger Winterweizen auch dann infizieren kann, wenn in der Fruchtfolge mehrere andere Kulturen zwischen den anfälligen Pflanzen im Feld liegen. Dieses widerspricht der Mehrheit der Literatur, die Steinbrand als eine ausschließlich samenübertragbare Pflanzenkrankheit auffaßt, die nur dann durch Erreger im Boden infiziertwerden kann, wenn Winterweizen unmittelbar nach einer infizierten Weizenfrucht angebaut wird. Die veränderte Pathogenität wird im Rahmen dieser Arbeit durch veränderte landwirtschaftliche Methoden erklärt.

Stichwörter: Winterweizen; *Tilletia tritici;* Steinbrand; Überdauern im Boden; *T. caries*

1 Introduction

Common bunt (*Tilletia tritici* (Bjerk.) Wint. syn. *T. caries* (DC) Tul.) is normally considered to be an almost strictly seed-borne disease except under very dry conditions, when spores can remain viable in the soil from the harvest of a preceeding infected crop to the sowing of the next (Williams 1987; Yarham and Mckeown 1989) or in cases of intercross between *T. tritici* and the soil-borne pathogen *T. contraversa* (Kühn syn. *T. controversa*) causing dwarf bunt (Kendrick et al. 1964; Yarham 1993).

When common bunt is normally not considered as a soil-borne disease, it is based on trials showing that, when spores are introduced to the seed bed before the sowing of winter wheat, the incidence of bunt is low, and under normal humid conditions decreases during a period of 30–60 days after which the crop will no longer be infected (Bonne 1931; Hungerford 1922; Kühnel 1960; Parlak 1986; Vanderwale and Detroux 1954; Weltzien 1957; Weston 1932; Woolman and Humphrey

1924) and neither will wheat sown in the following spring (APPEL and RIEHM 1914; FOSTER and HENRY 1937; TUBEUF 1902; WOOLMAN and HUMPHREY 1924). The most likely explanation is that spores introduced into a humid soil will germinate and, in the absence of a host, they die.

JOHNSSON (1990) investigated the survival capacity of spores kept in open plastic containers 20 cm under an undisturbed and an uncovered moist soil and showed that the spores were viable after, at least, 10 years in the soil. After this time, they could still infect wheat plants under experimental conditions. However, the practical implications of perennial survival of spores in the soil has never been investigated.

There seems to be a contradiction between the conclusions from previous studies about the ability to infect a crop under farm-like conditions and JOHNSSON's studies showing the extended survival capacity in soil. JOHNSSON explains the contradiction via the experimental design. The test seeds in JOHNSSON's study were placed very close to the spores which may not have been the case in the previous studies under more farm-like conditions.

New observations from farming have indicated that common bunt sometimes occurs in cases where perennial soil transmission seems to be the only possible source of inoculum (BORGEN and KRISTENSEN 1997; NIELSEN and NIELSEN 1994). Also in 1998, I have observed a case of severe bunt in a wheat field where uncontaminated seeds had been used with oat as a pre crop, but where common bunt had been observed in previous years. These examples may indicate that JOHNSSON's discovery (1990) may have implications for practical farming.

In the present study, I investigated if common bunt can be transmitted through the soil under normal farming conditions.

2 Materials and methods

2.1 Field experiments

The experiments were performed over three consecutive years in eastern Denmark. The soil type in the experimental areas is moreanic sandy loam with conventional cropping in a cereal-dominated rotation under normal farm practice except for the experimental areas.

In five fields, areas of between 850 and 2000 m^2 were grown with bunt-infected winter wheat in different years caused by seed infection. The cultivars were the very tall cv. 'Kosack' except in Field 5 where 'Husar' were grown. The infection rate varied between 65 to 156 infected heads per m^2 as presented in Table 1. This infection rate is estimated to leave between $5–10 * 10^{10}$ spores per m^2 in the field. These areas were chosen as the inoculum source areas. Plants in the source areas were harvested with a combine harvester at the beginning of August leaving chopped straw and spores on the ground. All areas were ploughed at the end of September in the year of inoculation.

In the years following inoculation, assessment areas were chosen within the source areas. Where practically possible, the assessment areas were extended outside the source area and into the rest of the fields, where common bunt had not been observed previously. The crop rotation and the disease incidence in the source areas and the assessments areas are presented in Table 1.

The assessment areas were grown with wheat using untreated seed. Each year the same seed lot was used in all assessment areas and the seeds were found free from bunt-spore contamination tested by the haemocytometer method (KIETREIBER 1984). In 1995 and 1996 the cultivar was 'Pepital' and in 1997 it was 'Husar'. Seed beds were prepared on the same day with seeds sown the day after in all fields. The sowing dates were 27 September 1995, 3 October 1996 and 6 October 1997, which is about 1 to 2 weeks later than recommended for the region.

In all fields, except for Field 3 in 1997, all plants within the assessment areas were diagnosed for bunt infection by visual inspection. In 1996 in Field 1 and 2, the number of plants were only 300 and 1,379, respectively. In the rest of the fields and years, the number of plants diagnosed within the assessment areas varied between 2,500 and 13,500 plants. In 1997 in Field 3, the infection level was very high and instead of assessing all plants, a reduced inspection was made: All plants outside the source area and 2.5 m into the area were assessed. In addition two areas, each of 3.1 m^2 inside the source area, were selected randomly and assessed for disease frequency.

Table 1. Experimental conditions and results in soil-infection trials with common bunt *(Tilletia tritici)* showing the amount of spores from diseased plants in the source areas, the crops and number of years between source and assessment areas, and the frequency of bunt in the assessment area (± half confidence interval at 95 %).

Tab. 1. Experimentelle Bedingungen und Ergebnisse eines Bodeninfektionsversuchs mit Steinbrand *(T. tritici)*. Dargestellt sind Sporenmengen von erkrankten Pflanzen auf den verseuchten Flächen, die Ernten und die Zahl der Jahre zwischen den verseuchten und den Bewertungsflächen sowie die Häufigkeit von Steinbrand in den Bewertungsflächen (± halbe SD bei 95 %)

	Source areas			Assessment areas		
	Crop 1993 (diseased heads per m²)	Crop 1994 (diseased heads per m²)	Crop 1995 (diseased heads per m²)	Crop 1996 (percent bunted plants)	Crop 1997 (percent bunted plants)	Crop 1998 (percent bunted plants)
Field 1	Winter wheat (65)	Winter barley (0)	Kentucky Bluegrass (0)	Winter wheat (0 % ± 0)		
				Kentucky Bluegrass (0)	Winter wheat (1.0 % ± 0.38)	
					Kentucky Bluegrass (0)	Winter wheat (0.04 % ± 0.07)
Field 2	non wheat crop (0)	Winter wheat (66)	Spring Barley (0)	Winter wheat (1.9 % ± 0.72)		
Field 3	non wheat crop (0)	non wheat crop (0)	Winter wheat (75)	Winter wheat (1.5 % ± 0.27)		
				Bunt resistant winter wheat (0 % ± 0)		
				Spring barley (0)	Winter wheat (10.0 % ± 1.3)	
				Spring barley (0)	Spring oat (0)	Winter wheat (0.60 % ± 0.30)
Field 4	non wheat crop (0)	non wheat crop (0)	non wheat crop (0)	Winter wheat (156 diseased heads pr m²)	Winter wheat (0.74 % ± 0.28)	
Field 5	non wheat crop (0)	non wheat crop (0)	non wheat crop (0)	Winter wheat (152 diseased heads pr m²)	Spring barley (0)	Winter wheat (0.75 % ± 0.15)
	Source areas				Assessment areas	

After inspection, each year all above-ground plant material was removed from the assessment areas in order to prevent contamination of the areas with new bunt spores.

Field 1 was inoculated in the growing season 1992–93. After ploughing, the field was sown with winter barley undersown with Kentucky Bluegrass *(Poa pratensis)*. The grass field was harvested for seed production in 1995, and the soil was consequently undisturbed in the period prior to the experiment starting. In 1995, an assessment area of 2.5 × 11 m was prepared with a rotary cultivator and the day after sown in the middle of the inoculum source area.

In 1996, a new assessment area in the field was ploughed including a part of the source area. A band, 1.25 m wide, was sown in the field through the source area and extended out on both sides. The rest of Field 1 remained with the grass cover. In 1997, a new assessment band was ploughed and sown with the same design as in 1996.

Field 3 was contaminated in the growing season 1994–95. The field was ploughed before establishment of the experiment, the first year with no intercrop in between. The assessment area was made by driving the sowing machine from one side of the field through the source area and out on the other side. This was done both in a East-West and a North-South direction, and the cross-shaped assessment area extended 25–50 m from the source area.

In 1995, where no intercrop was grown between the source area and the assessment area, and special attention was, therefore, put into the risk of disease from volunteer wheat plants. Within the source area, an additional assessment area of 2.5 × 25 m was sown with the bunt-resistant winter wheat cv. 'Stava'.

The field was ploughed after harvest in 1996 and an assessment area was sown as a band through the source area and extending 25–50 m outside the source area. The rest of the field was left uncovered until spring when oats were sown. This was repeated in 1997, except that the oats were replaced by barley.

In Field 2, 4 and 5, assessment areas of 27½, 40 and 80 m^2 were sown in the middle of the source areas not extending outside into the not contaminated area. These fields were only used in each 1 year.

2.2 Statistics

Confidence intervals were set up under the assumption that the infected plants were binomially distributed within the assessment areas.

3 Results

Assessment areas covered both the source area and, in some cases, areas outside the source area. The frequency of bunt within the source areas is presented in Table 1.

In the first year (1996) in Field 3, one infected plant was found 8 m north of the source area. Apart from this, no infected plants were found within a distance of more than 3 m from the source area in the other three directions. No infected plants were found in the plot grown with the bunt resistant cv. 'Stava'. In Field 1 and 2, the assessment areas did not extend outside the source areas.

In the second year (1997) in Field 1, infected plants were evenly distributed within the source area, and no infected plants were found more than 1 m from the source area. In the assessment area in Field 3, infected plants were evenly distributed within the source area including the nearest 3 m outside of it. Three infected plants were found 5–5.5 m east of the source area and four plants were found west of the area at a distance of 6.5 m, 6.6 m, 12.0 m and 23.0 m from this area. The assessment area in Field 4 did not extend outside the source area.

In the third experimental year (1998), no infected plants were found outside the source areas in Field 1 and 3. The assessment area in Field 5 did not extend outside the source area.

4 Discussion

Infections of winter wheat in the assessment areas were directly related to the source areas, where infected wheat had been grown in the previous years. Infected plants were only found sporadically outside the source area, which shows that the actual infection did not come from seed contamination.

The few infected plants found outside the source areas indicate that the spreading of spores with tillage equipment and by the wind are of limited significance compared with their survival in the soil.

No infected plants were found in the assessment area grown with the resistant 'Stava' wheat in 1996. This indicates that the infections were not related to volunteer plants. This is supported by the fact that the cvs. 'Husar' and 'Pepital' grown in the assessment areas have very short straw, while the cv. 'Kosack', used in the source areas (except Field 5) is a very tall cultivar. Volunteer plants would, therefore, probably be taller than other plants, and this was not observed. This very strongly indicates that the infections shown in Table 1 originated exclusively from soil-borne inoculum.

The highest infection level did not occur when wheat was grown directly after an infected crop, but in all 3 years, the highest level occurred when winter wheat was grown in the second year after an infected crop. The most likely explanation for this is that, after harvest of the infected crop, the fields were ploughed before sowing of the succeeding crops. The majority of the spores will, therefore, have been buried about 25 cm under the seeds. Johnsson (1990) and Woolman and Humphrey (1924) showed that the position of the spores just a few centimetres under the seeds significantly reduces the infection level compared with a situation where spores are placed above or around the seeds. Ploughing was recommended by Woolman and Humphrey (1924) as a measure to prevent transmission of soil-borne inoculum from harvest to sowing, but in the present cases has not succeeded in the full prevention of infection.

When 1 year of barley is grown between two winter wheat crops, spores from the infected crop are ploughed down the first year, and then ploughed up again the following year. Seeds will, therefore, be placed much closer to the majority of the spores in a situation of 1 year with an annual intercrop than when wheat is grown directly after an infected crop.

In Field 1, no infection occurred in the first year, but in the second and third years infection was seen. At least in the second year, the infection level was relatively high considering that wheat had not been grown in the area for 4 years. The main reason for this is probably that the plot was not ploughed in the first year, but only rotary cultivated, which is a much more superficial soil treatment. Infectious spores are, therefore, likely to remain undisturbed in the soil after this cultivation method. In the second year, the soil was ploughed before sowing, possibly resulting in better physical contact between spores and seeds. In the third year, a very low infection rate was observed. The infection level in all fields was lower in this year. Therefore, it is not possible to conclude if the decay of the spores may have started to accelerate in the soil after 5 years or if it is an expression of differences in infection between the years.

The present experiment not only confirms that resting spores of *Tilletia tritici* can survive under the plough layer for at least 5 years, but also shows that their survival can have practical implications under normal farming conditions.

The infection level in the assessment areas were, in all cases, much lower than in the source areas. This may indicate that soil transmission can maintain the disease in an area for a certain period, but the disease incidence is likely to be reduced from year-to-year if new uncontaminated seeds are used sequentially. However, none of the infection levels found in this study, and certainly not the infection of 10 % found in Field 3 in the second year, would be acceptable in commercial wheat production and it would have decisive impact in the case of seed production in the area. However, the experiments have been conducted on only one soil type, a sandy loam quite common in the eastern part of Denmark. The impact of soil type, the impact of ploughing depth, and the impact of the time from harvest to ploughing and from ploughing to sowing for the persistence and disease incidence have not been studied here, but would be interesting subjects for future studies.

Johnsson (1990) explained the contradiction between his results and results from previous studies via the experimental design as described in the introduction. I doubt that this is the only explanation, since my results confirm those of Johnsson under normal farming conditions. A more plausible explanation is, therefore, that the germination and the breakdown of dormancy of the spores is highly influenced by external factors such as the oxygen pressure (Rabien 1928) and the presence of light and ions (Ettel and Halbsguth 1963; Gassner and Niehmann 1954; Hahne 1927; Rabien 1928; Schauz 1972; Zseheile 1965). Ungerminated spores, buried under 20–25 cm soil, may be prevented from germination until light-, oxygen- and ion-conditions are favourable for germination. This may not occur before the reploughing of the field.

New research have shown that several species of collembolans, which are some of the most frequent invertebrates in the upper soil layers but almost absent in the depth of 20 cm, ingest and thereby kill bunt spores (DROMPH and BORGEN, unpublished). The survival of spores in a ploughed field has not been studied previously. All previous studies on persistence of common bunt in soil have been done under unploughed conditions. This I believe is the explanation for the contradictotyconclusions in this and previous studies on the subject of soil transmission of common bunt.

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