Organic seed treatment to control common bunt *(Tilletia tritici)* in wheat

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Abstract

Common bunt caused by the fungus *Tilletia tritici* (syn. *T.caries*) is one of the most devastating plant diseases in wheat. In conventional agriculture the disease is controlled exclusively by fungicide seed treatment, but in organic farming these fungicides are not accepted. Previous studies in India have shown that seed treatment with plant extracts of *Canabis sativa*, *Eucalyptus globulus*, *Thuja sinensis* and *Datura stramonium* was fully effective against the disease under field conditions. Later, in vitro studies have shown that also germination of spores of the Karnal bunt pathogen (*Neovossia indica*) could be prevented by these plant extracts. The experiment was repeated in Denmark with extracts from the same species grown in Denmark, which has climate conditions very different from India. In this experiment, the same seed treatments had no or very limited effect on the frequency of the disease. The treatments were compared with indigenous methods from Europe including salty brine, *Thuja* leaves and lime. These methods had a significant, but insufficient effect on disease suppression.

Introduction

Common bunt (*Tilletia tritici* syn. *T. caries*) is also called stinking smut and in India it is called hill bunt. In conventional agriculture in Europe, common bunt is one of the diseases most intensively treated with pesticides, and about 80-90% of all seed lots of winter wheat in industrialized agriculture are treated with synthetic fungicides (Nielsen *et al*. 1998). In the arid zones of less industialized agriculture, common bunt is still one of the diseases causing most devastating yield losses of up to 30% in some areas (Mamluk 1998). In organic agriculture common bunt is a difficult disease to control in the absence of fungicides (Borgen 2000).

Singh *et al.* (1979) were able to control the infection of common bunt by 100% by soaking wheat seed in plant juices of *Canabis sativa*, *Eucalyptus globulus, Thuja sinensis* and *Datura stramonium.* In order to develop a strategy to control the disease in organic agriculture, the most promising treatments found by Singh *et al.* (1979) were included in a series of treatments in control of common bunt under Danish cropping conditions.

Two thousand years ago, Pliny the Elder (Caius Plinius Secundus) wrote in his Historia naturalis that by mixing bruised cypress leafs into the seed lots, a significant plant disease could be controlled. It is likely that this plant disease was common bunt (Buttress and Dennis 1959). The recommendation was repeated in the Almanac during 16th and 17th century in Denmark (Olsen 1791), but at this time conifers were rare trees in Denmark, and in stead Olsen (1791) recommended seed treatment with lime to control common bunt.

During the 16th century a seed treatment against common bunt was developed by soaking seed into salty water (Woolmann and Humphrey 1924, Buttress and Dennis 1959). Soaking seeds into water or plant juices will increase the water content of the seeds to an extent, where re-drying is required for storage and for sowing with a conventional sowing machine. The drying process is expensive and energy consuming especially in temperate climatic zones, and the proposed designs, where seeds are soaked into a liquid, are therefore not optimal for modern organic agricultural practice in Denmark. The aim of the present study is to investigate the potential of different classic seed treatments to control common bunt in a design applicable to the practice in organic wheat production in Denmark.

MATERIALS AND METHODS

The seeds of the variety Kosack were contaminated with 5 g spores of *Tilletia tritici* per kg seeds, which resulted in a contamination of 1.7 x 106 spores per gram seeds when tested by the ISTA haemocytometer method (Kietreiber, 1984).

Singh *et al.* (1979) treated seed by soaking the seed for 15 minutes into juices of different plants. By this treatment spores of the pathogen will be washed off the seeds. It is therefore not possible to conclude to which extent the effect of the treatment is a washing effect or a chemical effect. To investigate this, a different design was chosen. The species *Thuja sinensis* described by Singh *et al.* (1979) is unknown to the taxonomy used in Denmark, but the common name Chinese Thuja is used for *Thuja occidentalis* in some countries and this species was chosen for the experiment in the hope that they are closely related or even synonyms for the same species. *Thuja occidentalis, Canabis sativa* and *Datura stramonium* were grown in open air in Denmark, while *Eucalyptus globulus* were grown in a green house. Leafs of the plants were put into a cylinder (ø=10 cm, h= 10 cm) with 1 mm holes and pressed under 20 tons. The collected juices were filtered in a sieve with 0.2 mm holes. Seeds were treated in a spinning wheel seed dresser (Hege no. 11) in a dose of 30 ml/kg of the concentrated juices.

Leafs/needles of *Thuja occidentalis* and *Picea glauca* were dried in an oven for 2 hour at 80 C° and grinded into meal. Seeds were then by turn added water and meal until 42 g meal adhered per kg. However, a part of the meal may have fallen off later during seed handling and sowing, since no other adhesive were used. To further investigate the mode of action, treatments were included, where pure oils of *Eucalyptus* and *Pinus* were added in the seed dresser in a dose of 18 ml/kg.

Olsen (1791) recommended a lime treatment where a pile of seed was sprinkled with slaked lime. In the present study a design was chosen, where powder of quick lime (Calcium hydroxide) were mixed with water (2:3) (quick lime turns into slaked lime when mixed with water). 100 ml of this liquid was added per kg, and on top of it 30 g per kg of quick lime powder.

Each seed treatment was repeated 5 times (true replicates), one for each field replicate and one for germination test. After treatment the seeds were stored at 5 C°. Samples were removed for sowing of field tests 4 days after seed treatment. Germination tests were conducted 1 month later.

Field trials were conducted at Højbakkegård, an experimental farm of the Royal Veterinary and Agricultural University, on Zealand, Denmark. In the field trial seeds of each treatment were sown in 4 replicates in 6 m^2 plots of a rate of 400 seeds per m^2. After heading the number of infected ears were counted based on visible macro-symptoms. In average, 2000 plants in each plot were assessed for common bunt infection in each treatment.

Germination tests were conducted as cold sand-tests, testing the germination speed of the treatments (Borgen 2000, Borgen and Kristensen 2001, Borgen and Nielsen 2001). Results were analysed by a Generalised Mixed Model (GENMOD, software SAS ver. 8.01).

Results and discussion

The effect of the different treatments is listed in Table 1.

Table 1. Effect of seed treatments to control of common bunt (Tilletia tritici).

Treatment	Dose ml/kg	Diseased heads	Days for 50% germination
Control		53.3%	9.6
Water	30	46.4%	9.5 n.s.
Salty water	30	30.9% n.s.	10.5 (p=0.0051)
Quick lime *(see text)*		10.9%***	9.0 n.s.
Canabis sativa, juice	30	52.2% n.s.	9.4 n.s.
Eucalyptus globulus, juice	30	52.3% n.s.	9.5 n.s.
Thuja sinensis, juice	30	53.0% n.s.	9.2 n.s.
Datura stramonium, juice	30	57.3% n.s.	9.0 n.s.
Eucalyptus, oil	18	48.9% n.s.	10.1 n.s.
Pinus, oil	18	46.0%*	11.1***
Thuja sinensis, meal	42g/kg	28.7%***	9.7 n.s.
Picea glauca, meal	42g/kg	30.2%***	9.5 n.s.

None of the plant juices used by Singh *et al.* (1979) could significantly reduce the frequency of common bunt in this study, even though they had been 100% effective in their trials. Gupta and Singh (1983) found *in vitro* that plants extracts of the same species inhibited spore germination of the related species *Neovossia (Tilletia) indica*, when spores were soaked in the extracts for 5 days, except for the *Eucalyptus*-treatment, which did not reduce, but instead enhanced the germination of the spores. Sharma and Basandrai 1998 found some reducing effect *in vitro* of *Canabis sativa* and *Eucalyptus tereticornis* when germinating spores of *N. indica* in extracts from boiled leafs of the plants.

There may be different explanations for the contra-dictionary effects of the plant extracts on the development of the bunt disease. The plants used in the trials were different varieties grown under extremely different conditions. The content of primary and secondary metabolites is therefore likely to be very different, and most likely the content of most metabolites are higher in wild plants grown in the Simla hills in India than cultivated and fertilized varieties grown in Denmark, especially in a green house. The design in the treatment procedure was very different, since the seed were not soaked into the liquid in the experiment presented here. The effect of the soaking treatment presented by Singh *et al.* (1979) can not be a washing effect alone, since other plant juices and deluded plant juices had less or no effect on the bunt frequency. However, the soaking treatment may have other effects on the plant-pathogen interaction, which may explain the difference in effect.

Cypressus sempervirens was the only *cypress* species grown in the Roman Empire, and it is therefore likely that this is the species recommended by Pliny to be mixed into the seed lots. *Thuja sinensis* and *Cypressus sempervirens* are closely related species, and applying leaf meal to the seeds significantly reduced bunt frequency, as was the case with meal of *Picea glauca*. It is therefore likely that the recommendations by Pliny and in the Danish Almanacs from the 16th and 17th century actually had an effect on the bunt frequency, even though the effect is not complete in the current study.

Lime used as a seed treatment reduced the bunt frequency by 80%, and the treatment was frequently used in the 17th and 18th century in Europe (Woolmann and Humphrey 1924, Buttress and Dennis 1959). The results indicate that lime can be used to reduce bunt frequency, but in the current design the treatment did not offer a complete control.

Common bunt is a very serious disease, reducing not only yield, but also grain quality. Only a single infected plant per m^2 will make the whole harvested crop smell like rotten fish, and unacceptable to commercial wheat production. It is therefore crucial for the seed propagation that common bunt is under complete control. Some of the classic methods examined in this study reduced the bunt frequency significantly, but could not control the disease sufficiently. These treatments can therefore not be used alone in cases of very high spore load of the seeds.

To control common bunt in organic agriculture it is recommended to used a combination of different measures. This includes discarding the most infested seed lots, use of resistant varieties and removal of spores from the seed e.g. by brushing. On top of this strategy, seed treatments can be used (Borgen 2000). As seed treatment for organic agriculture it is recommended to use mustard or milk powder (Borgen and Kristensen 2001), milk-powder in combination with bio-agents (Borgen and Davanlou 2000), acetic acid (Borgen 2001) or hot water treatment (Nielsen *et al.* 2000), which are more efficient than the ones tested in this experiment. With a combination of these tools, common bunt can be controlled in organic agriculture in the future.

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