Effect of Seed Treatments with EM in Control of Common Bunt (*Tilletia tritia*) in Wheat

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Abstract.

Common bunt is one of the most important diseases in winter wheat, and one of the most intensively pesticide treated diseases in regions where this pathogen occurs. When pesticides are not used in seed propagation, i. e. in organic agriculture, the disease is normally a serious problem. EMI is a product used in Kyusei Nature Farming, containing a bunch of different microorganisms against various fungal plant diseases. In order to develop methods to diminish pesticide use, and to improve organic agriculture, the potential of EM to regulate this pathogen was investigated. In 1 996 seeds contaminated with teleutospores of Tilletia tritici were treated with two levels of EMI, two levels of acetic acid and two levels of milk powder. A combination of EMI and milk powder were tested as well.

EMI had a significant effect on disease rate (87. 6% reduction) when applied in high dosages (150ml/kg seed), but in this dose germination vigour and field emergence were also negatively affected. The use of the same dose of autoclaved EMI had almost the same effect on disease rate and germination vigour. This might indicate, that the causative agent in the EMI product is not the effective microorganisms themselves, but the metabolites of the organisms, most probably lactic acid. Using acetic acid (1.22M, 30mllkg) it was possible to get a better control of the pathogen with no significant harm to germination. Based on these trials it can be concluded, that EMI is not an optimal control agent against common bunt. However the use of EMI did reduce the disease rate, and this can- therefore be a positive side-effect If EMI is used as a seed treatment for other reasons.

Introduction

Common bunt is one of the most damaging plant diseases known in agriculture. The fungus (*Tilletia tritici syn. T.caries*) grows systemically in wheat plants (*Triticum aestivum*). Instead of normal kernels in the spikes, they develop into bunt balls filled with fungus spores. During harvest, the bunt balls break, and the spores attach to the healthy seeds, The spores stink due to the release of simple nitrogen components like tri-methyl-amin, which gives the disease the synonym stinking smut. When contaminated seeds are sown, the spores will germinate in synchrony with the kernels and infect the germinating plants systemically and thereby close their life cycles.

Common bunt is an old disease. Spores were found on seeds from ancient Mesopotamia 4000 years ago (Johnsson 1990), and it is likely, that the disease has been a problem to wheat production ever since the domestication of this plant species. Since then, the disease has been one of the most intensively treated diseases in the history of plant protection (Woolman and

Humphrey 1924, Buttress and Dennis 1959, Sharvelle 1979). Since seed treatments with organic mercury started in the 1920s, research on the disease has been limited, as this treatment was not cheap and effective. Mercury is now banned in most industrialized countries due to environmental reasons, but other synthetic fungicides have taken its place in the control of bunt.

In organic farming, seed treatments with agro-chemicals are not used. Common bunt is therefore, in this cropping system, a significant threat to wheat production. Research in ecological regulation of this disease are going on in Europe, mainly focussing on different seed treatments like plant extracts, organic compounds, hot water treatment and antagonistic bacteria (Heyden 1997, Spiess and Dutscnke 1992, Becker and Weltzien l993, Bergman, 1996, Borgen and Kristensen 1996, Gerhardson et al 1996).

Kyusei Nature Farming is an organic cropping system mainly differing from other ecological systems by the intensive use of EM. Among other purposes like plant nutrition, EM is used in plant protection against various fungal diseases. In Kyusei Nature Farming the question is therefore straightforward: Can EM be used in the control of common bunt ?

Materials and Methods

Seeds of wheat, variety Pepital, were abundantly contaminated with teleutospores of *Tilletia tritici* at a rate of 1,975,000 spores per gram seed. Pepital is a winter wheat variety known to be very susceptible to common bunt with no known resistance genes. Samples of 100 g of contaminated seed were treated with different applications in a laboratory spinning wheel seed dresser (Hege no. 11). Among the applications were two levels of EM, 40ml/kg and 150ml/kg. The formulation EM1 was used in this trial. These treatments were compared with two levels of 1.22 M acetic acid, 20ml/kg and 30ml/kg. In order to develop the method and to determine the working mechanism, EM1 was autoclaved for 20 min at 120 ºC, and a seed lot was treated with 150ml/kg. One sample was treated with a combination of 40ml EM1/kg and 20g skimmed milk powder per kg. This treatment was compared with two levels of milk powder, l0g/kg and 30g/kg.

The treated seed lots were sown on 11th of October 1996 in a randomized field block trial in small plots of approximately 125 seed per plot in 10 replications. After tillering all heads were diagnosed for bunt infection, and the frequency of bunted heads was calculated.

The different treatments were tested for side-effects on germination vigour. This was done by germinating 100 seeds in quartz sand and water (65ml/kg), and the number of sprouts in each sample was counted on three consecutive days. This germination test was replicated three times at low temperatures (10-12 ºC). Because of the differences in temperature in the different replications in the germination test, it was necessary to express the germination vigour as an applied germination index. This is done by the formula:

$$(n_1 / N_1 + 0.9*n_2 / N_2 + 0.8*n_3 / N_3)*100/C$$

were n is the number of sprouts on the day l, 2 or 3, N is the average of number of sprouts on that day in the test. This equation is in each replication divided by the average of the replication, C and multiplied by 100. This transformation given a good description of the relative germination vigour of the seed sample, and thereby the eventual phytotoxic side effect of a seed treatment.

The statistical calculation in Figure 1 is done by a Ryan test on the transformed data (1n(x)+ 1) using the GLM option REGWQ in the software SAS ver 6.12.

Results

The results of the experiment are presented in Figure 1. The low doses of EM1 and milk powder had no significant effect on either bunt rate or germination vigour. The higher dose of milk powder, the low dose of acetic acid and the combination of milk powder and EM1 reduced the bunt rate significantly with no significant effect on germination vigour. The reduction in germination vigour due to EM1+milk powder had a p-value of 0.14. The high dose of EM1, both regular and the autoclave, reduced bunt rate, but reduced also the germination vigour significantly. The differences between sterile and regular EM1 had a p-value of 0.62. The high dose of acetic acid had the best effect on bunt disease, significantly better than even the highest dose of EM1. The reduction of germination vigour of the seeds was in this trial not significant with a p-value of 0.30.

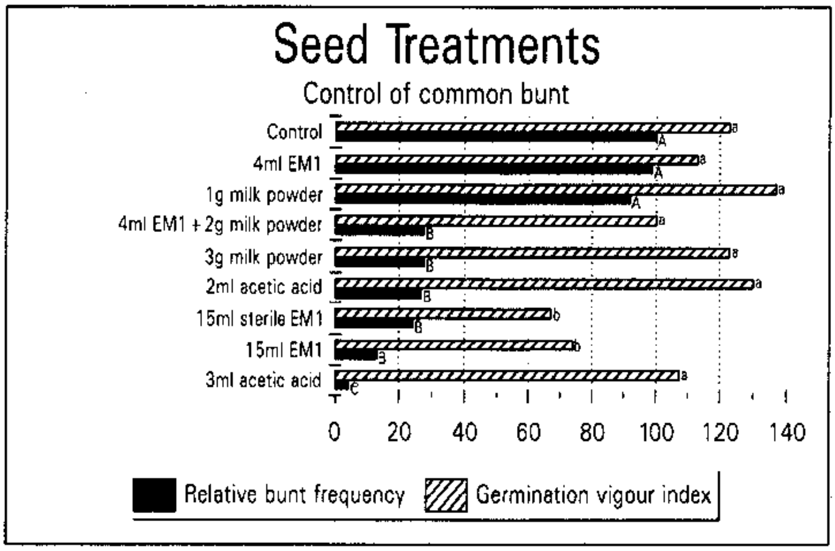

Figure 1. Result of Field with Common Bunt. Bunt Rate in Untreated Control = 27.4%.

Columns with Difficult Letters are Signinficantly Different due to Ryan Test ($p<0.05$)

Discussion and Conclusion

The control of common bunt is crucial for the production of quality wheat. Only a small number of infected heads in the field will reduce of the wheat because of the stench of the bunt spores. 50 per cent of the people in a Swedish experiment could smell the presence of only 1000 spores per gram of seeds (Johnsson 1991). This level can be obtained by a field frequency of less the 0.1 per cent infected heads (Borgen unpublished). The multiplication of bunt frequency from year to year will depend on wheat variety and climate conditions especially during germination, but is often about 100 times under Danish climate conditions in susceptible varieties (Borgen 1992, Borgen unpublished). In order to prevent a multiplication of the bunt rate from year to year, the sum of control measures must therefore have an efficiency of more than 99 percent.

It has been possible to obtain a significant reduction in bunt rate in the field trial by treatment with EM1. The reduction in bunt rate seems to be proportional with the amount of EM1 applied to the seed sample. However, the treatment also causes a significant reduction in germination vigour proportional to the dose applied. A better control of the bunt can therefore not be obtained by an increase of the dose, because it would also increase the phytotoxic effect on germination. An efficiency of 99 per cent can therefore not be obtained by seed treatment alone with EM1 without an unacceptable decrease in germination vigour.

Seed treatment with autoclaved EM1 (150ml/kg) resulted in a control of the bunt frequency almost as good as with the same amount of fresh EM1. This indicates, that the working mechanism of the EM1 on the bunt is not primarily a biological effect, but rather a physical or chemical effect of the metabolites in the product. This might be lactic acid, since EM1 is declared to contain 80 per cent lactic acid bacteria with a pH of about 3.5.

It is confirmed by treatment with acetic acid, which also gives a significant reduction in bunt rate. With acetic acid 30ml/kg it is possible to obtain a significantly better effect on bunt control with no decrease in germination vigour. Acetic acid is as such a better control agent against common bunt than EM1. The acetic effect on bunt spore germination is known (Hahne 1924), but has never been used before as control agent in the field.

The treatment with milk powder gives a significant control of the bunt without reduction in germination vigour. This effect is related to bacterial activity, using the milk powder as a nutrient source (Becker and Weltzien 1993). The combination of milk powder and EM1 gives a better control, than EM1 or milk powder alone. The effect on germination vigour was not significant compared with control (p=0.15) loss. It can not be explained from this experiment whether this effect is caused by the bacteria in the EM1 product or it is the acetic effect, that increases the efficiency of the milk powder on bunt control in the combined formulation. From this study there seems to be no argument for the use of the combined formulation compared with 30 g milk powder or 2 ml acetic acid, since they have the same effect on the disease, but without a tendency to reduce germination vigour.

In this study, only the effect on common bunt and seed vigour has been investigated. The environmental impact of the use, or the effect on yield due to increased release of nutrients from the humus fraction in the soil has not been studied.

On this background it can be concluded that EM1 is not an optimal seed treatment against common bunt, since other treatments like acetic acid or milk powder give the same or better disease control with less harm to germination vigour. If however EM1 is used as a seed treatment in Kyusei Nature Farming for other reasons, i.e. to increase nutrient availability in the soil, the EM1 can in combination with other measures contribute to the regulation of common bunt.

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