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Seed Treatment

Challenges & Opportunities

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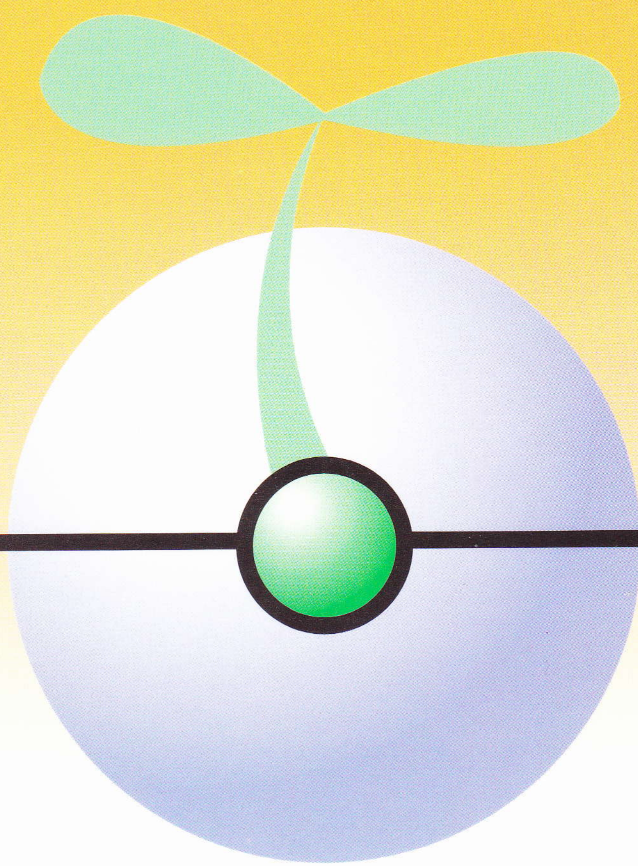

BRITISH CROP PROTECTION COUNCIL

Effect of seed treatment with acetic acid for control of seed borne diseases

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ABSTRACT

In field trials, seed treatment with acetic acid reduced common bunt (*Tilletia tritici*) by 92-96% in winter wheat, and by 83% in spring wheat, without negative effects on germination vigour of the seeds. Leaf stripe (*Pyrenophora graminea*) in spring barley was reduced by 93%. Acetic acid is a cheap and environmental friendly fungicide with a potentially wide scope of application especially in organic agriculture, where conventional pesticides are prohibited.

INTRODUCTION

Lime has been used as a seed treatment against common bunt, *Tilletia tritici* (syn. *T. caries*) since the 18th century (Olsen, 1791), probably acting through a pH effect since lime is a strong base. However, the control of common bunt using lime is not complete, and has found only minor use since the development of more effective seed treatments like copper (Kühn, 1866) and hot water treatment (Jensen 1888a, 1888b). With the development of organic mercury seed treatment (Riehm, 1913) common bunt has been controlled almost exclusively by synthetic pesticides in the industrialised world.

Nevertheless, recently, increasing focus has been placed on the environmental side-effects of synthetic pesticides and there is now a requirement in public opinion and in legislation in some countries to reduce the amount of pesticides used in general. One way to do this is to replace the conventional pesticides with naturally occurring substances (Nielsen *et al.*, 1998). In organic agriculture normal pesticides are not used, and here, seed borne diseases have become a severe problem. Consequently, the use of pH extremes to control seed borne diseases deserves to be reassessed. The treatment of seed with acid to create a very low pH has never been studied for pathogen control, but Hahne (1925) showed that acetic acid has a strong inhibitory effect on spore germination of common bunt *in vitro*. Consequently, the aim of this study is to investigate whether seed borne diseases can be controlled by seed treatment with acetic acid without adverse side effects on seed germination and vigour.

MATERIALS AND METHODS

Field trials were conducted at three sites on Zealand, Denmark: Common bunt at Højbakkegård in 1997 and 1998, leaf stripe (*Pyrenophora graminea* syn. *Dreschlera graminea*) of barley at Flakkebjerg in 2000 and both leaf stripe and common bunt at Mørdrupgård in 2000.

The effect of dose rates of acetic acid was tested by applying increasing amounts of increasingly concentrated acid to seeds of wheat and barley. For experiments with common bunt the winter wheat variety Pepital was used at Højbakkegård in 1997 and 1998 and the spring wheat variety Dragon was used at Mørdrupgård in 2000. The spring wheat seed used had a very low germination ability in order to increase the possibility of achieving a high infection in the field and to detect possible side effects on germination from the seed treatment. The seeds were contaminated with 5 g spores per kg seeds, which resulted in a contamination between 1.7 - 2.0 x 10^6 spores per g seed when tested by the ISDA haemocytometer method (Kietreiber, 1984).

The tests for the effect on leaf stripe were carried out in both years using the variety Alexis which by blotter test was shown to be heavily infected.

In 1997 and 1998, normal fermented 5% vinegar for household use was used (*FDB Lagereddike*). In 2000, different concentrations were made by adding increasing volumes of inert water into concentrated acetic acid (99.9%). After treatment the seeds were stored at 5 °C. Samples were removed for field tests 2-6 days after seed treatment. Germination tests were conducted 1-3 months later at Højbakkegård and Mørdrupgård. At Flakkebjerg, effects on germination were tested by counting the number of emerging plants in the field.

Germination tests in lab were done in the form of a cold sand test in plastic plates containing 1.5 kg sand with water (65ml H_2O/kg quartz sand). Sowing depth was 1.5 cm and temperature was 10 °C. The emergent number of seedlings was counted every day for 5 consecutive days after first emergence. There were 3-4 replicates.

In the field trials at Højbakkegård and Mørdrupgård, treatments were sown in 1.25 m rows with 8 or 10 replicates. The total number of plants assessed in these trials was 1-2000 on average in each treatment. The seeds in the trial at Flakkebjerg were sown in 9 m rows with 200 seeds with 4 replicates. After heading, the number of infected ears (common bunt) or plants (leaf stripe) were counted based on visible macro-symptoms.

Data of diseased plants and germination rate was analysed by a generalised linear model (GENMOD in SAS ver. 6.12).

RESULTS

Common bunt

In winter wheat, common bunt was controlled by 96% and 92%, respectively, in the years 1997 and 1998 at the dose of 20 ml of 5% acetic acid per kg seed (Figure 1). No negative effect on seed vigour was recorded at this dose. However, at the higher dose of 30 ml/kg in 1997 and 40 ml/kg in 1998, germination vigour was significantly reduced in terms of germination speed.

Even when low vitality seeds of the very susceptible spring wheat variety Dragon were used at Mørdrupgård in year 2000, the bunt frequency was still very low in all plots. The use of low vigour seeds resulted in low field germination in both treated and untreated plots with an average of only 37 ears per plot. Because of a low number of plants, the effect of many treatments were not statistically significant, especially infected plants (Table 1). However, the optimal dose was still found to be about 20 ml/kg in a concentration between 5% and 20%.

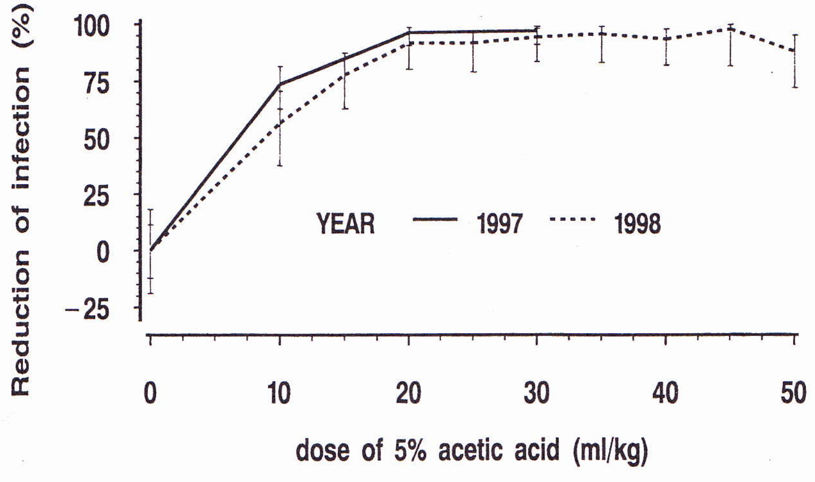

Figure 1. Control of common bunt in winter wheat in two years of field trials. Effect of increasing seed application rate of 5% acetic acid on germination vigour. Højbakkegård 1998. Bars indicate 95% confidence interval.

Table 1. Effect of different combinations of dose and concentration of acetic acid seed treatments on percent reduction in frequency of common bunt in spring wheat. Grey cells indicate treatments with a significant reduction in germination vigour. Experimental year 2000 at Mørdrupgård. Average infection in control plots was 8.0%. (n.s. = not significant).

	Dose of acetic acid (ml/kg)					
Concentration	5	10	20	30	40	50
5 %	20 n.s.	49	79	62 n.s.	54 n.s.	63
10 %	25	59 n.s.	83	70	63	70
20 %	4	39	75	54	45	55 n.s.
30 %	30	55	30 n.s.	67	60 n.s.	67 n.s.
99.9%	46	66	86 n.s.	74	69 n.s.	75 n.s.

Barley leaf stripe

In spring barley at Mørdrupgård the infection of leaf stripe was reduced by 93% at the dose of 20 ml/kg of concentrated acetic acid (99.9%) with no significant effect on field emergence (Table 2).

Table 2. Effect of different combinations of dose and concentration of acetic acid as seed treatments on percent reduction in disease frequency of barley leaf stripe (*Pyrenophora graminea*). Grey cells indicate treatments with a significant reduction in germination vigour. Experimental year 2000 at Mørdrupgård. Average infection in control plots was 17.4%. (n.s. = not significant)

Concentration	Dose of acetic acid, ml/kg: 5	10	20	30	40	50
0 %	28,6 n.s.	4,2 n.s.	6,4 n.s.	8,3 n.s.	-1,6 n.s.	7,2 n.s.
5 %	-7,8 n.s.	10,4 n.s.	26,9 n.s.	32,9 n.s.	54,5	84,8
10 %	-6,2 n.s.	12,7 n.s.	67,8	82,4	95,8	84,5
20 %	8,1 n.s.	26,7 n.s.	84,3	96,1	93,3	50,6 n.s.
30 %	29,2 n.s.	36.0 n.s.	90,3	91,0	99,5 n.s.	48,8 n.s.
99.9%	12,7 n.s.	68,5	93,4	99,7 n.s.	99,7 n.s.	94,4

At Flakkebjerg 2000, fewer combinations of concentration and doses was tested, and an optimal dose was not found and none of the treatments reduced germination vigour significantly (Table 3). The results from Flakkebjerg are consistent with the results from Mørdrupgård, and also with previous published results with increasing doses of 5% acetic acid (Nielsen *et al.*, 2000).

DISCUSSION AND CONCLUSION

Acetic acid is a naturally occurring substance with a high biodegradability and a very low oral toxicity to humans, game birds and others that may come into contact with seeds treated with fungicides. However, acetic acid is a corrosive substance that will evaporate from the seeds during seed treatment and so precautions should be taken to ensure human health and safety at work. We believe that substituting conventional fungicides with acetic acid will reduce the general environmental impact of seed treatments. Seed treatment with acetic acid would be cost effective, since it is a cheap substance and treated seeds remaining unsold could be used for animal feed, while seeds treated with more ecologically-toxic fungicides must be incinerated under controlled conditions.

In the winter wheat experiments, the infection of common bunt was high but a reduction of infection by 92-96% was still achieved when treated with 20 ml/kg of 5% acetic acid, without

Table 3. Effect of different combinations of dose and concentration of acetic acid on the percent reduction of frequency of barley leaf stripe (*Pyrenophora graminea*). Field trial at Flakkebjerg 2000.

Dose ml/kg	Concen-tration	% infected plants (95% confidence intervals)	% reduction
Control		14,0 (10,9 - 17,9)	-
Imazalil		0,2 (0,0 - 2,0)	98,3
5	30 %	6,5 (4,4 - 9,7)	53,4
5	40 %	5,8 (3,8 - 8,7)	58,9
5	50 %	2,9 (1,4 - 5,8)	79,3
10	15 %	13,0 (9,6 - 17,4)	7,5
10	20 %	9,3 (6,6 - 13,0)	34,0
10	25 %	6,4 (6,1 - 6,6)	54,6
20	7,5%	11,2 (10,6 - 11,9)	19,9
20	10 %	10,4 (10,2 - 10,6)	26,0
20	12,5%	5,7 (5,4 - 6,1)	59,4

affecting the germination vigour of the seeds. In spring wheat the infection was lower and a reduction of only 75-83% was recorded. The reasons for the differences in effect between the experiments in winter wheat and spring wheat are unclear. It may be caused by the differences in crop, in differences in infection level or the fact that the spring wheat was grown from very low vitality seed that may have made the seeds more sensitive to the acetic acid treatment.

The experiments with spring barley showed that barley leaf stripe can be effectively controlled by a high concentration of acetic acid (99.9%) at a dose rate of 20 ml/kg. In previous experiments only a low concentration of 5% have been used (Nielsen *et al.*, 2000), but the general pattern of control was similar to these experiments. The ratio surface area to volume is higher in barley than in wheat and *Pyrenophora graminea* is present within the seed coat while *Tilletia tritici* occurs as loosely attached spores on the seed surface. These facts may be the reason that the concentration of acid needed to control the pathogen is higher in barley than in wheat and why barley seeds are less sensitive to acetic acid, exhibiting a relatively lower reduction in seed vigour.

Common bunt is a very devastating plant disease since presence of only a few infected plants can give the whole crop an odour of rotten fish, and consequent crop loss. The disease control treatments must therefore be very effective against this disease (Borgen, 2000a). Even the control level of 92-96% found in these experiments is not adequate for seed lots of susceptible varieties with a high spore load. Therefore, seed treatments with acetic acid cannot stand alone, but must be combined with other measures in an integrated strategy. This could involve physical removal of spores or use only according to specific contamination thresholds dependent on the susceptibility of the varieties to be sown (Borgen, 2000a).

Although barley leaf stripe is an important seed borne disease, reducing yield when disease levels are high, the need for complete control is less critical than for common bunt in wheat. A control effect of 93% as achieved in these experiments is therefore believed to be acceptable in some cases e.g. the last generation of organic seed production.

In organic agriculture, conventional fungicides are prohibited and the current practice of discarding all infected seed lots is a major constraint on organic cereal propagation. Consequently, acetic acid could be an interesting new weapon to use against seed borne pathogens in organic agriculture particularly if combined with other treatments approved by organic growers (Spieβ, 2000).

In recent years, soil borne infection of common bunt has been of increasing importance in wheat production (Borgen, 2000b). Some systemic pesticides effective against common bunt are also effective against soil borne pathogens, and provide near 100% control of all seed borne infections (Nielsen, 2001). However, organic products or biological products have only a low effect on the soil borne infection of common bunt, which could give be a problem if common bunt was introduced in an organic field

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