REVIEW OF POTENTIAL SEED TREATMENTS FOR USE IN ORGANIC CEREALS

INTRODUCTION

Historical background

For centuries, naturally occurring or derived products, such as animal urine, wine and bruised cypress leaf concoctions, have been applied to seed to improve the health of the growing crop, albeit without knowledge of the target pathogens. In the late 17th Century, wheat seed salvaged from a ship-wreck in the Bristol Channel subsequently produced a bunt-free crop, leading to adoption of the practice of "brining" seed. Lime was also used as a seed treatment against bunt from the 18th Century, its effectiveness being due to a pH effect (Olsen, 1791). Our knowledge of fungi as causal agents of disease increased markedly thanks to the work of Prevost: he found that very low concentrations of copper sulphate prevented germination of wheat smut spores. In the 19th Century, hot water treatment of seed was found to be efficacious against seed-borne disease (Jensen, 1888). Arsenic and formalin have also been used in the past to control bunt and smuts respectively. However, it was not until the development of organomercury and subsequent fungicidal seed treatments in the 20th Century that seed-borne diseases were adequately controlled. The decline of historically devastating diseases, such as bunt and the smuts, of cereals in conventional farming is thus due primarily to routine use of fungicidal seed treatments but also to seed certification. Even so, occasional scares of a revival of these "forgotten diseases" (Yarham & Jones, 1992) have occurred, due to problems of fungicide resistance in the pathogens and withdrawal of key active ingredients, e.g. organomercury. Such instances have given a timely reminder to the Organic sector, where seed health may be compromised and conventional seed treatments unacceptable, of the danger of a major resurgence of seed-borne disease problems.

Current situation

The main seed source (c. 60%) of organic cereal seed is "bought-in non-organic" (recleaned only) (Taylor *et al*, 2001). Approximately 20% is "bought-in organic" and 20% is "home-saved". The percentage of the latter could rise after 31 December 2003, as use of non-organic seed will not then be permitted. This could lead to a rise in seed-borne disease problems. At present in the UK, organic cereal seed is not treated with any substance (Dr DM Kenyon, NIAB; Dr J Welsh, EFRC: pers. comms).

The main target diseases which could pose a real threat are **bunt** (*Tilletia tritici*) and **glume blotch** (*Stagonospora nodorum*) in **wheat**; **leaf stripe** (*Drechslera graminea*), **net blotch** (*D teres*) and **loose smut** (*Ustilago nuda* f sp *hordei*) in **barley**; **loose smut** (*Ustilago avenae*) and **leaf spot** (*D avenae*) in **oats**. In addition **ergot**, *Fusarium* spp and *Microdochium nivale* must also be considered potential problems for most or all of the cereal species. Dornbusch *et al* (1992) found bunt, *Septoria* spp and *Fusarium spp* to be present in organic wheat seed lots. Speiss (1999) reported the rejection of 25-30% of organic wheat seed in Germany in the 1980s, due to bunt contamination. In Denmark, *c.* 50% of all seed lots destined for organic use are

discarded on the basis of seed-borne disease levels assessed in laboratory tests (Borgen, 2002).

This Review attempts to collate contemporary research and information on cereal seed treatments which could potentially be adopted in organic systems.

THE TREATMENTS

Nielsen *et al* (1998) considered that there was no useful alternative to chemical (conventional) seed treatments in the short-term for organic growing and that use of healthy seed, allied with seed testing, was paramount for control of seed-borne diseases. It is essential that seed treatments in organic systems form only a part of an <u>integrated</u> control strategy, which also includes healthy seed, resistant varieties and agronomic practices *e.g.* crop rotation. The requirement for seed for organic crops to be produced organically from 2004 will put more pressure on both seed producers and organic farmers to ensure that seed-borne diseases are well controlled.

This Review will consider seed treatments under three headings: substances applied to seed, physical treatment of seed and biological control agents applied to seed.

1. <u>Substances applied to seed</u>

a) Acetic acid

Increasing the pH on the seed surface with alkaline treatments, *e.g.* with chalk or wood-ash, has long been known to give control of bunt (Buttress & Dennis, 1947). Spiess & Dutschke (1991) also found that different alkalis gave good bunt control.

Nielsen et al (2000) demonstrated a dose-response effect with acetic acid for leaf stripe control in barley. A rate of 50 ml/kg seed of 5% acetic acid was required to fully control the pathogen but gave a reduction in plant emergence; lower dose rates gave some control without affecting emergence. Borgen & Nielsen (2001) described field trials with acetic acid seed treatments in Denmark from 1997 to 2000. 20 ml/kg seed of 5% acetic acid gave a reduction of 92-96% in seed-borne (but not soil-borne) bunt of winter wheat, without adversely affecting germination; higher doses (30 or 40 ml/kg) reduced germination. In spring wheat, only 75-83% control was achieved; the reasons for this were unclear. 93% control of leaf stripe in spring barley was achieved with 20 ml/kg of 99.9% acetic acid, with no adverse effect on germination. The higher concentration is required for leaf stripe control as the pathogen is situated within the seed coat, rather than on the seed surface as is the case with bunt. It was considered that 92-96% bunt control was still insufficient and that treatment should be dependent on a treatment threshold and on variety; thus, an integrated control strategy was essential. The level of leaf stripe control was considered satisfactory for this disease. Work in Norway by Henriksen (2002) showed that application of 20 ml acetic acid per kg seed at 20% and 35% concentrations significantly reduced barley leaf stripe

and oat loose smut, although the higher rate reduced germination slightly in laboratory tests. Further work indicated that 25% and 30% concentrations also reduced oat loose smut.

Acetic acid has also been shown to control some seed-borne diseases of vegetables: *e.g.* in France, a combination of vinegar, cinnamon and micro-nutrients gave 90% control of *Alternaria dauci* in carrots, without associated phytotoxicity problems (Lizot *et al*, 2002).

Using acetic acid has many advantages: it is a cheap, naturally occurring, highly biodegradeable product with very low oral toxicity to humans, game birds *etc.* and unused treated seed could be safely used for animal feed (Borgen & Nielsen, 2001). However, precautions for operators would be necessary, as it is corrosive due to evaporation during seed treatment.

b) Mustard flour

The disease control effect of mustard flour is thought to be due to the action of glucosinolate or mustard oils on the fungal pathogens.

Spiess & Dutscke (1991) showed that mustard flour could control seed-borne bunt of wheat but could cause reduced seed germination at high rates. More recently, Borgen & Kristensen (2001) found that yellow mustard flour applied at up to 10 g/kg wheat seed controlled seed-borne bunt; higher doses (33 or 43 g/kg) gave no additional control but reduced germination vigour. There was no control of soil-borne bunt. Flours derived from other *Brassica* spp. were less effective. Mustard flour applied at 10g/kg seed reduced infection of rye stem smut (*Urocystis occulta*) by 91%, without adversely affecting germination.

Mustard oil is allowed for organic use in Germany and is classed as a "plant protective oil" under Annex II of the EU regulations. A commercial product – "Tille-Kur" – is available. It is not accepted for use in Denmark. In Switzerland, TillecurTM exhibited good efficacy against bunt without negative effects on germination (Schachermayr *et al*, 2002).

c) Milk products

The action of milk powder on some pathogens is probably due to competition from saprotrophic microorganisms using the substance as a nutrient source. Bunt, in particular, is very sensitive to availability of oxygen in competition with other microorganisms using sugar as a nutrient source. Borgen & Kristensen (2001) found that milk powder applied to wheat seed at a rate of 43 or 80 g/kg gave maximum control of seed-borne bunt but there was a reduction in germination vigour at or above these rates, probably due to decreased availability of oxygen. The frequency of plants infected by soil-borne bunt was reduced by 91%. It was considered that the negative effect on germination may be too large to be acceptable and that limited doses of milk powder (*e.g.* 20g/kg), in combination with bio-control agents, may offer effective control without associated germination problems in the future (Borgen & Davanlou, 2000). Schachermayr *et al* (2002) reported that skim milk powder (at 80g/l) controlled bunt well but reduced seed germination by 5%.

One fermented milk product (of a number tested) applied at 20 ml/kg seed reduced the number of plants infected by barley leaf stripe and oat loose smut (Henriksen, 2002). Borgen & Kristensen (2001) also demonstrated control of stem smut of rye with application of milk powder at a rate of 50g/kg seed: 92% control was achieved, with no adverse effect on germination.

In Germany, milk powder is classified as a "fertiliser" in Annex I of the EU Regulations. Its action could also be interpreted as "protecting natural enemies" (saprophytic microorganisms). It is not accepted for organic use in Denmark.

d) Miscellaneous substances

Byron & Hall (2002) reported inhibition of *Fusarium culmorum* and *Alternaria alternata* from cereals *in vitro* with clove and eucalyptus oils. *In vivo* experiments applying these oils to wheat and barley seed are planned in the future.

Henriksen (2002) applied horse-radish (20% suspension) at 40 and 60 ml/kg seed but found little or no effect on barley leaf stripe or oat loose smut. When mixed 50:50 with 35% acetic acid, horse radish had the same effect as 20% acetic acid alone (see above).

2. Physical treatment of seed

a) Hot/warm water treatment

It has been known for many years that heat sanitation of seed provides disease control. Thus, Jensen (1888) stated that "many seed-borne pathogens do not survive as high temperatures in water as the host seed". Treatment can be either "hot water" *i.e.* temperatures $>50^{\circ}$ C and short duration (<10 mins), or "warm water" *i.e.* temperatures $<50^{\circ}$ C and long duration (1-3 hrs). The use of warm-water treatment to control various diseases, including leaf stripe and loose smut, was reported by Lind & Ravn (1918). Winter *et al* (1996, 1998) also found that hot-water treatment would control leaf stripe and that warm-water controlled loose smut.

Nielsen *et al* (2000) described contemporary Danish research with hot- and warmwater treatments of barley seed. Leaf stripe levels were reduced considerably by hot water treatment (HWT) at 50 and 55°C, the effect being enhanced if pre-treatment in warm water for 10, 20 or 30 mins was included. However, treatment at 55°C for 6 mins decreased emergence, especially if pre-treatment was employed. Loose smut was controlled by pre-treatment at 45°C for 2 hrs, followed by HWT at 50 or 55°C, although the higher temperature resulted in reduced germination. Winter *et al* (1996, 1998) also found that HWT at 52°C for 10 mins, or warm water treatment at 45°C for 2 hrs, controlled leaf stripe and that warm water treatment at 45°C for 3 hrs gave control of loose smut. Schachermayr *et al* (2002) reports Swiss work in which warm water treatment (45°C for 2 hrs) reduced wheat seed infection by *M nivale* from 20% to 1% and *by S nodorum* from 55% to 0.5%; in the field there was a significant increase in emergence compared to the untreated control. HWT has also been found to give good control of some seed-borne diseases of vegetables, including *Septoria* and *Alternaria* spp (Jahn *et al*, 2002).

To minimise problems of re-drying and reduced germination, treatments with the lowest possible temperature and shortest duration necessary for control should be employed.

b) Hot air treatment

Forsberg (2001) described a technique and equipment in Sweden which used hot air at high RH to provide conditions similar to those in warm water, but without raising the moisture content of seed. Field trials using treated seed gave a level of control of bunt and *M nivale* in wheat, net blotch and loose smut in barley, and leaf spot and loose smut in oats comparable to that with conventional seed treatments. The benefits of this method were the short treatment duration, elimination of the need for expensive drying, easier control of temperature and seed cooling, and suitability for large scale treatment.

c) Hot steam treatment

In Norway, Henriksen (2002) employed a steam chamber, used for steaming plants and equipment in glasshouses, to treat barley and oat seed. Steam applied at 60° C for 60 or 90 min reduced barley leaf stripe and oat loose smut but there was some effect on seed germination. At 55°C, there was little or no disease control.

d) Other methods

Irradiation of wheat seed to control bunt was mentioned by Nielsen et al, 1998.

Physical removal of bunt spores by brush treatment has also been employed (Nielsen *et al*, 1998).

Schroder *et al* (1998) described the technique of electronic cleaning of seed ("e-dressing"). Here, winter wheat seed is bombarded with low energy electrons, which have a biocidal effect. Bunt, *Septoria nodorum* and *Fusarium culmorum* were controlled to varying degrees by this method, with no adverse effect on subsequent yield. A pilot plant in Germany has treated commercial seed by e-dressing. Lindner *et al* (1990) previously showed that e-dressing was more than 80% effective in controlling leaf stripe in winter barley.

3. Bio-control agents applied to seed.

a) Pseudomonas chlororaphis

Gerhardson *et al* (1998) reported field trials carried out in Sweden and other countries using the naturally occurring bacterial strain MA 342 (*Pseudomonas chlororaphis*) to control several seed-borne diseases. Similar control to conventional seed treatments was achieved for bunt (seed-borne) and glume blotch of wheat; leaf stripe, net blotch and covered smut of barley, and leaf spot and loose smut of oats. Weak activity against *Fusarium* spp and *Bipolaris sorokiniana* was noted. There were no detrimental effects in pathogenicity, toxicity and animal feeding tests. An oil-based commercial formulation – Cedomon – was registered and accepted for organic use in Sweden and this has been used on a considerable acreage of spring barley.

In comparison with conventional seed treatments, Nielsen (2001) found some control of seed-borne, but not soil-borne, bunt using MA 342 applied as a spore suspension at 600 ml per 100 kg of wheat seed.

b) Other potential microorganisms

Pratt *et al* (2002) found that the fungi *Nectria inventa* and a *Pythium oligandrum*-like oomycete inhibited growth of *M nivale* in vitro. The results were sufficiently encouraging to prompt furthur work using these fungi as seed treatments *in vivo* to combat seedling blight caused by *M nivale*.

LEGISLATION

Detailed discussion of the legislation surrounding use of products for seed treatment in organic agriculture is outside the scope of this review. However, certain points have become apparent during compilation:

The EU Regulation 2092/9, revised by EU Reg. 1488/97 (EU, 1997), states the main methods of controlling pests, diseases and weeds solely by non-chemical methods. Only in cases of immediate crop threat can recourse be made to "plant protection products" stated in Annex II(B). The latter lists four categories of product, some of which have specific conditions of use, and is rather limiting in scope:

- I. Substances of crop or animal origin
- II. Micro-organisms used for biological control
- III. Substances in traps and/or dispensers
- IV. Other substances from traditional use in Organic farming

In some EU member states, some listed products are not considered to be plant protection products and are thus not subject to plant protection legislation. The list also excludes any product not registered as a plant protection product in at least one member state. In the UK, the above EU Regulation is implemented under the UK Register of Organic Food Standards (UKROFS, 2001). This includes the list of products given in the EU Regulation, with the added proviso that they may only be used in so far as approval is given for use under the Control of Pesticides Regulations, 1986.

International conformity has been attempted under the IFOAM Basic Standards banner (IFOAM, 2000), Appendix 2 of which gives a list of "products for plant pest

and disease control". The list provides only very general descriptions and gives restrictions for many products. Additional inputs are listed in Appendix 3, but use of these would be outside the EU regulations, as far as EU countries are concerned.

Thus, attempts to harmonise production standards in the EU and internationally have been only partially successful. Stopes *et al* (2000) stated that "there are important inconsistencies between the EU Organic Regulations, National organic standards, as well as EU and National rules concerning pesticide approval". Pinniger (1996) found that only 20 "botanical" substances were approved for organic use in the EU and only two in the UK. Many apparently benign substances with fungicidal and insecticidal activity were not approved for use in the UK. Other anomalies, such as the permission for use of of mustard flour ("Tille-Kur") and milk powder in Germany, but not in Denmark, also exist (Borgen & Kristensen, 2001).

CONCLUSIONS

Recent research has provided considerable evidence that non-conventional seed treatments can give disease control often comparable to that of conventional treatments and thus show great promise for use in organic cereal production. A Summary is shown in the Appendix. The question of which techniques would be acceptable to the bodies regulating organic production (and pesticide use) in the EU and UK is outside the scope of this review.

Substances, such as acetic acid, mustard flour and milk powder, added to the seed can provide control of seed-borne wheat bunt; acetic acid also controls barley leaf stripe. These two diseases probably pose the greatest threat of all the seed-borne diseases to cereals (Yarham & Jones, 1992). The main disadvantage of such treatments is the reduction in seed emergence caused by high dose rates; for milk powder, this effect may preclude treatment unless dose rates are considerably reduced and additional materials *e.g.* a biological control agent added (Borgen & Kristensen, 2001).

Hot/warm water treatments have been shown to control barley leaf stripe and loose smut, especially if pre-treatment is included in the process, although high temperatures can reduce seed emergence. Perhaps even more interesting is the hot air method (Forsberg, 2001) which gave good control of a number of seed-borne diseases of wheat, barley and oats. This technique has the advantages of shorter treatment duration, easier temperature control and suitability for large-scale treatment. Hot steam treatment is a potentially useful method, if problems of associated reduced emergence of seed can be overcome. "e-dressing" of seed also looks useful, if the process is acceptable for organic use.

The bio-control agent *Pseudomonas chlororaphis* has given control of a number of seed-borne diseases of wheat, barley and oats (Gerhardson *et al*, 1998) and offers an exciting prospect for the future. As "Cedomon", it is already in commercial use on organic spring barley in Sweden. Some fungal species have also shown promise as bio-control agents in experimental work.

It would be interesting to know whether novel materials such as "plant activators", which trigger the defence mechanisms of plants against fungal infection, have any

activity against seed-borne diseases. The product "Bion" is approved for use as a spray in conventional agriculture in France and Germany but not in the UK; it is not known whether or not this chemical is acceptable in organic systems.

Many authors, *e.g.* Nielsen *et al* (1998), emphasise the need for seed treatment to be just one component of an <u>integrated</u> disease control strategy, which also includes use of resistant varieties and suitable agronomic practices. Muller (2002) stated that cultivars resistant to the seed-borne diseases must be developed in the long-term, due to the problems inherent in organic seed multiplication. Also, decisions on use/non-use of seed treatment should be made on the basis of seed testing, similar to the practice of "treatment according to need" (Thomas *et al*, 2001) employed in conventional agriculture. In Denmark, the problems of certification of organic cereal seed are being addressed (Nielsen, 2002). Currently, seed for organic use is analysed and discarded if seed-borne disease levels are greater than threshold values; however, the latter are being re-defined in the context of organic production.

There is an urgent need for greater clarity concerning definitions of what constitutes a "plant protection product" (as opposed to, say, a "fertiliser") and their approved use under the UK Control of Pesticides Regulations. More European and International harmonisation of production standards is essential, as interpretation of product usage varies considerably between different countries.

The requirement for only organically produced seed to be used for organic crops from 2004 will put pressure on both seed producers and farmers to ensure that seed-borne diseases are kept to an absolute minimum. Use of seed treatments, in combination with resistant varieties, seed certification and agronomic measures, can have a major impact in combatting the potentially damaging seed-borne diseases of organic cereals.

RECOMMENDATIONS FOR RESEARCH

From this literature review, the following seed treatments appear to be worthy of field testing in the UK:-

- Acetic acid: against wheat bunt, barley leaf stripe and, possibly, the smuts.
- Mustard flour: as "Tille-Kur" against bunt and smuts.
- Hot/Warm water treatment: against many diseases of wheat, barley and oats.
- *Pseudomonas chlororaphis*: as "Cedomon" against various diseases.
- (Hot Air/Hot Steam treatment, e-dressing: against many diseases, BUT specialist equipment required?)
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APPENDIX: SUMMARY OF MAIN TREATMENTS

The following Table summarises the information in this Review on activity of the products/techniques used to control seed-borne diseases; levels of control varied and some had detrimental effects on seed germination (see text). The list may not be exhaustive:-

	Acetic acid	Mustard flour	Milk products	Hot/warm water	Hot air	Hot steam	e-dressing	Irradiation	Brush treatment	Pseudomonas chlororaphis
Bunt (W)	+	+	+	+	+		+	+	+	+
(seed-borne)										
Bunt (W)	х	Х	+							Х
(soil-borne)										
Dwarf bunt										Х
(W)										
S nodorum (W)				+			+			+
Fusarium spp				+	+		+			Х
&/or M nivale										
(W)										
Leaf stripe (B)	+		+	+		+	+			+
Loose smut (B)				+	+					
Net Blotch (B)					+					+
Covered smut (B)										+
Spot Blotch (B)										Х
Leaf spot (O)					+					+
Loose smut (O)	+		+	+	+	+				+
Stem smut (R)		+	+							

W = Wheat, B = Barley, O = Oats, R = Rye

+ = Control; x = No/little control.

SID5 OF0330 Annex 9.