SESSION 3
APPLICATION TECHNOLOGY

Chairman and Session Organiser: Dr P B Clayton
PBC Enterprises Ltd, Market Rasen

The development of an image analysis technique for the quantitative analysis of seed treatment coverage on seed

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ABSTRACT

An image analysis system has been developed which can determine objectively the extent of treatment coverage on seed. Using this technique the effects of varying application parameters on coverage have been investigated and a clear relationship between coverage and efficacy of a fungicidal seed treatment has been established.

This technique provides a useful tool for the optimisation of formulation and application parameters for seed treatment products.

INTRODUCTION

Seed treatments are used globally on a range of different crops for many different purposes. Application of a seed treatment containing pesticide is an efficient method of providing protection for the germinating seed, and the emerging seedling, against fungal pathogens and / or insect attack. An important aspect for efficacy of many seed treatments is the extent to which the seed surface is covered by the treatment. The cosmetic appearance of treated seed is perceived to be a reflection of treatment quality (Halmer, 1994). However, the importance of coverage in terms of biological efficacy is less well defined.

Image analysis has been used in many different applications (Glasbey & Horgan, 1995) for quantitative analysis. Measurement and the study of fibre diameters, algal cells, fungal hyphae, electrophoretograms, muscle fibres and soil aggregate pore size are but a few examples. Applications specific to seed analysis include use of image analysis to detect quality factors such as seed dimensions and fungal damage in maize and soybeans (Paulsen *et al.*, 1989). Separation of different seed species, detection of stress cracks and external breakage of maize kernels (Gunasekaran, 1987) and prediction of sprout damage in wheat (Sapirstein, 1999) have also been investigated. The availability of image analysis software (Regent Instruments, 1998) designed specifically for seed morphology and disease analysis highlights the applicability of the technique to the agricultural industry.

The objective of this research was to establish a technique for evaluating surface coverage of treated seed to investigate the effects of application parameters and formulation component change on seed coverage. This paper describes the image analysis technique and methods developed for the analysis of treated seed and indicates a relationship between seed coverage and efficacy, for the fungicidal suspension concentrates evaluated.

MATERIALS AND METHODS

Image Analysis System

The system, illustrated in Figure 1, consists of:

i) a Pentium computer with Trinitron multiscan 17sfII monitor linked to a JVC camera model TK1085 fitted with an 18 to 108 mm zoom lens. The camera is fitted onto a Kaiser 5510 copy stand with Kaiser 5350 lighting set comprised of two light fittings used with 100 or 250 watt bulbs.

ii) an IDPCIH2 Flashpoint peripheral component interconnect (PCI) 2MB combined frame grabber and SVGA card used with the Red, Green, Blue (RGB) colour model in phase alternation line (PAL) monochrome or colour. An advanced 24-bits-per-pixel, true colour, frame grabbing videographics card featuring 16.8 million colour accelerated display with live video capture. The Flashpoint VGA image capture (TWAIN) driver allows use of the Flashpoint board as an image capture source which was used for this research.

iii) The system is used with Media Cybernetics Image-Pro® Plus version 3.1 for windows software.

Figure 1 Image Analysis System

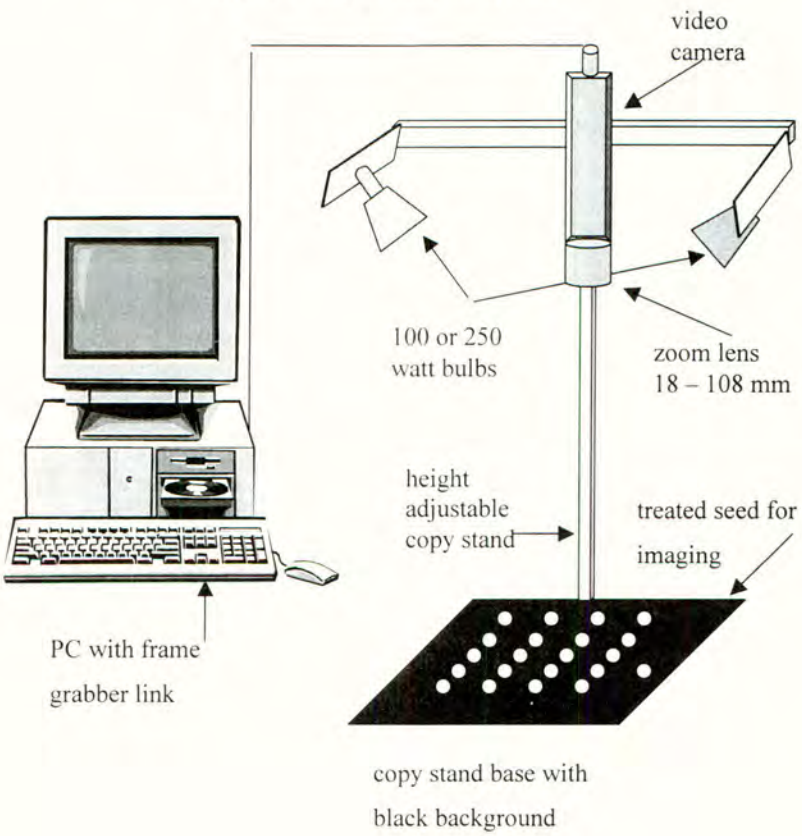


Image analysis operation and calibration

The system was used with the following Flashpoint Twain and camera setup:

i) brightness 27, contrast 51, saturation 26 and hue 32
ii) video standard – phase alternation line (PAL) used with s-video input type
iii) true colour class with 24 bits-per-pixel
iv) a camera aperture setting of F8 was used with 18 mm zoom and column height of 41 cm.

The image analysis system was calibrated to measure pixels as millimetres. To verify the calibration five samples of 21 maize seed (cv Silverio) were image analysed and the area, length and width measurements were compared to results obtained by manual measurement. The mean sum area of the imaged seed was 1778 mm^2 compared to the manual mean sum area of 1772 mm^2.

Image capture

Images are captured by activating a scan command which links to the Flashpoint TWAIN driver. The image becomes live allowing for camera height adjustment and focussing. When the desired image is viewed it is captured and saved as a tagged image file (TIFF).

Measuring

Measurements were made automatically using the tools on the "Measure" menu which allow measurement of single or multiple objects, this is particularly useful for measuring roundness, areas and perimeters of objects. This function was used for measuring seed area and the amount of blue colour treatment on the seed. The Count / Size tool within this function was used to threshold the blue colour against the untreated background colour of the seed. The colour range was selected by identifying an Area Of Interest on the seed which encompassed the blue treatment colour. Then, by using the count feature the coloured area was counted and identified with an outline. The Add / Subtract commands were used to refine the count as necessary to incorporate any areas originally not counted.

To obtain the percentage area of coloured treatment on the seed surface, 21 seeds were placed on a black background, to minimise shadowing. The area of interest, i.e. the complete seeds, were then thresholded, outlined and measured. The coloured treated areas on the seed were then separated via thresholding and measured. The colour measurement was then expressed as a percentage of the complete seed area. An untreated seed was included to aid the thresholding process.

Formulations

Formulations containing a total of 20 % w/w dispersed solids (including the blue pigment) were used. The formulation colour was optimised with a selection of different seed types to identify a system with good contrast for image analysis.

Seeds

Maize seed (cv Silverio) supplied by Maisadour, France was selected as a suitable seed substrate for use with this image analysis system. The seed is flat and of a uniform pale yellow / white colour.

Seed Treatment – "Glass Jar" Method

Seeds (typically 100 g) were transferred to an 800 ml squat form beaker. The treatment, at the required application rate, was injected onto the inside of the beaker close to, but not touching the seed surface. The beaker was then immediately swirled vigorously for 30 seconds to coat the seed and to encourage distribution of the treatment between seeds. The treated seeds were then transferred to a plastic weighing boat for drying at room temperature.

Pathogen Selection for *In-vitro* Efficacy Method

Fusarium moniliforme Sheldon was selected as a pathogen known to infect maize.

Efficacy Method

A method was developed for the *in-vitro* inoculation, treatment and assessment of maize seed to determine seed treatment formulation efficacy. Spore suspensions were prepared by diluting culture sub-samples in distilled water to give a spore concentration of approximately 0.25×10^6 spores / ml. Maize seeds (100 g) were immersed in the inoculum for 1 minute before the inoculum was poured off and the seed transferred to Petri dishes lined with filter paper for drying. The seeds were dried for about 19 hours in a laminar flow booth.

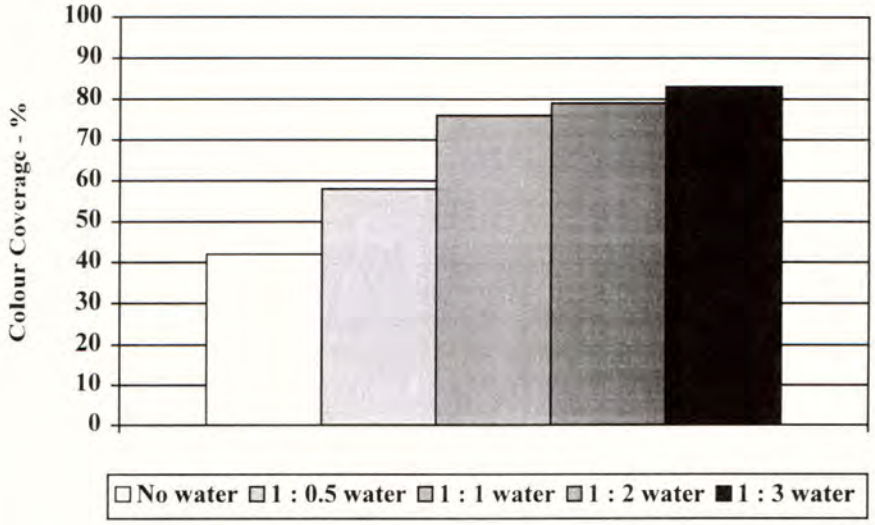
The seeds, in batches of 100 g, were treated using the Glass Jar treating method. One hundred seeds from each treatment were then plated onto potato dextrose agar and incubated at 20 to 25°C for 8 days. The *F. moniliforme* growth was then measured either manually or using image analysis. The percent total infection and infection severity were calculated.

RESULTS

Image Analysis Method

An initial test of the image analysis system was to investigate a technique where the product was applied undiluted to the seed and also co-applied with water. A formulation known to give poor coverage on seed was selected for this study. The formulation was applied at 2 g product / kg seed i.e. constant dose. Water was co-applied at 0, 1, 2, 4 and 6 g / kg seed using the Glass Jar treating technique. Image analysis was carried out, with accuracy estimated to be ± 5%. Results are shown in Figure 2.

Figure 2. Variation in Colour Coverage with Increase in Applied Water Volume

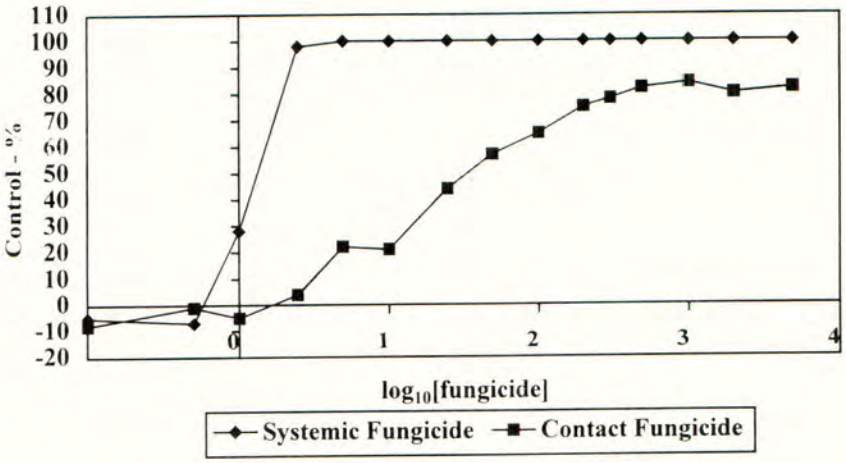


The data in Figure 2 demonstrate that coverage is affected by the co-application of water. Coverage was almost doubled by co-applying the product with an equivalent amount of water. As the volume of water was increased and coverage approached 80 % the magnitude of improvement levelled off.

Dose Response for Fungicides against *Fusarium moniliforme*

Four fungicides were tested for their activity against *F. moniliforme*. The dose response curves of two of these fungicides are shown in Figure 3. The data in Figure 3 show a steep dose response for the systemic fungicide and a more shallow dose response for the contact fungicide. As a result, the systemic fungicide with high levels of activity was selected for further study.

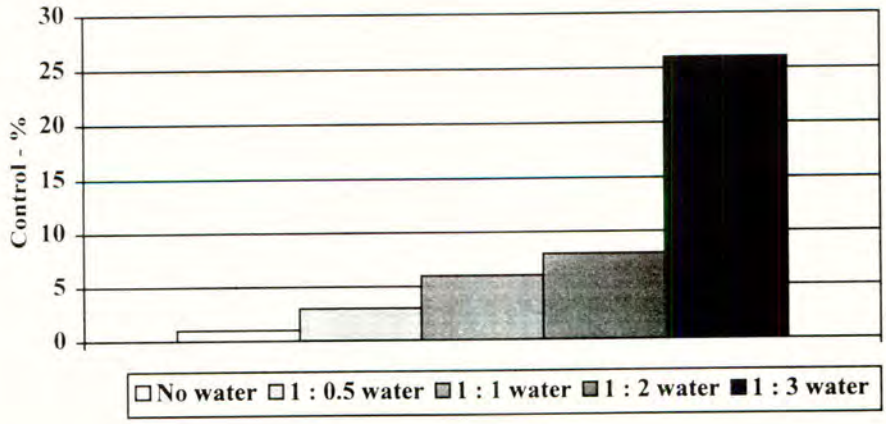
Figure 3. **Dose Response Curves for a Systemic and a Contact Fungicide against *Fusarium moniliforme*.**



In-vitro Efficacy of a Systemic Fungicide Formulation Co-applied with Water

The data in Figure 2 demonstrate that co-application of water with the product improves coverage. To investigate if co-application of water improves efficacy, a formulation designed to give poor coverage when applied undiluted and containing the systemic fungicide, was applied at 2 g product / kg seed i.e. constant dose. Water was co-applied at 0, 1, 2, 4 and 6 g / kg seed using the Glass Jar treating technique. Image analysis was carried out in the manner described above alongside an efficacy test using the method described previously. The results are shown in Figure 4.

Figure 4. **Formulation Efficacy with Co-application of Water**

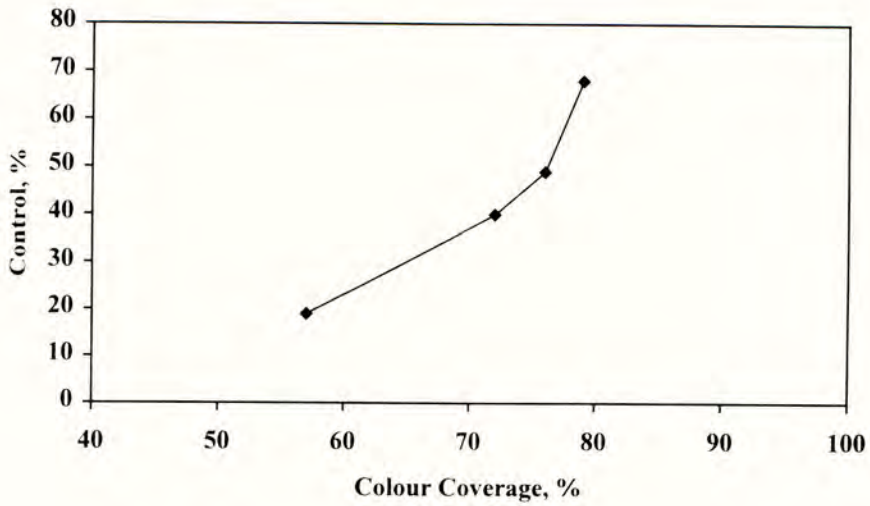


The results demonstrate that as the volume of water applied is increased the efficacy of the chemical improves. Comparison with the data in Figure 2, where coverage increases with co-application of water, implies that coverage affected control.

Determination of the Relationship between Coverage and Efficacy

A further experiment was carried out using the same techniques, where the application volume was held constant. Specially designed formulations of the systemic fungicide were used to give increased coverage at constant volume and constant dose of active ingredient. These results are shown in Figure 5 and indicate a direct relationship between coverage and control of infection.

Figure 5. Variation in Control with Coverage of a Systemic Fungicide against *Fusarium moniliforme* on/in Inoculated Maize Seeds



CONCLUSION

An image analysis technique has been developed for the quantitative analysis of seed treatment coverage on maize seed. The data generated using this technique demonstrate that a direct relationship exists between coverage and efficacy and that coverage can be improved by :

a) co-application of water and
b) use of specific formulation ingredients.

In this way, the quantities of pesticide needed to give the required levels of efficacy can be minimised.

ACKNOWLEDGEMENTS

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Quantitative and qualitative detection of *Pyrenophora* species on barley seed using PCR in advisory seed health testing

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ABSTRACT

A PCR test has been developed to provide a rapid, sensitive test for *Pyrenophora* spp. in barley seed. Using a LightCycler instrument, pathogen DNA was detected and *P. teres* quantified to the picogram level. The amount of pathogen DNA found in artificially produced seed-lots correlated (r=0.931) well with the level of infection found using a conventional agar plate test. Using the standard curve produced from these results the level of infection in a further 60 samples was predicted correctly.

The new PCR method has been adopted as the standard test for the detection of *Pyrenophora* spp. in barley seed at the Official Seed Testing Station for England and Wales. This has led to a more rapid service and reduced the cost of testing to the agricultural sector.

INTRODUCTION

Pyrenophora graminea, the cause of leaf stripe in barley, is a solely seed-borne pathogen capable of causing severe crop losses in both spring and winter crops (Richardson *et al.*, 1976). *Pyrenophora teres* (net blotch) is also seed-borne and although other sources of inoculum contribute to epidemic development, high levels of seed infection are likely to be significant. Traditionally both these pathogens have been detected using an agar plate test (Rennie & Tomlin, 1984) where the pathogens are identified using both colony and spore morphology. Distinguishing between these two pathogens can be difficult due to their morphological similarity and despite their differing importance as seed-borne pathogens, laboratories in some countries do not distinguish between them.

Although the majority of certified barley seed receives a chemical treatment that controls a range of diseases including both *Pyrenophora* spp. many seed merchants test for the presence of *P. graminea* to ensure that they comply with the voluntary standard of not more than 2% seeds infected. A PCR test has been developed to detect both these pathogens in a seed extract (Thomas *et al.*, 1998; Stevens *et al.*, 1998). Recent surveys have shown *P. graminea* to be rare in seed-lots and therefore a qualitative PCR test might be appropriate with the recommendation that any infected seed-lots should be treated regardless of level in order to minimise the risk of this disease increasing in seed stocks. With *P. teres* a quantitative test is required as low levels of this disease are found in the majority of samples but are insignificant in terms of effect in the subsequent crop.

MATERIALS AND METHODS

A sample of barley seed (cv. Regina) that had previously been tested positive for *P. teres* (32.5%) using the agar plate method was used to construct 12 different artificial seed-lots with target infection levels between 0 and 10% by mixing with un-infected seeds. 25 samples of 200 seeds were then produced from each of the artificial bulks of which ten were tested using the agar method and ten by the quantitative PCR assay. The DNA was extracted by soaking the seeds for 5 min in 20 ml of TE buffer and the mixture then macerated in a stomacher for 1 min. 15 ml of the liquor was then removed and centrifuged for 10 min at 9000 rpm, the supernatant removed and the pellet freeze-dried. The DNA was extracted from the pellet using the Nucleospin Plant Kit (Machery-Nagel GmbH & Co, Germany) as previously described by Taylor *et al.* 2000. The PCR reaction and quantification were carried out using the LightCycler system, using universal *Pyrenophora* spp. primers. The accumulation of PCR product was measured at the end of each amplification cycle by fluorescence of SYBR green I dye (Figure 1). Any products produced from the test sample were checked using melting curve analysis at the end of each reaction (Figure 2). Quantification of *P. teres* was carried out by plotting the log DNA concentration of known standards against fluorescence from which unknown sample concentrations could be derived. The results obtained as pg of DNA were then correlated with the plate test result.

A further sixty barley seed samples received by the Official Seed Testing Station (OSTS) for advisory testing were divided down to provide two samples each of 200 seeds to be tested for *Pyrenophora* spp. one using the agar plate method and the other by PCR.

During the 2000 harvest all barley samples received by the OSTS to be tested for *Pyrenophora* spp. were analysed using the PCR method. An additional stage to check for the presence *of P. graminea* was included. The success of the DNA extraction was confirmed by using universal ITS primers for barley DNA, since some seed material is always released during stomaching (Figure 3). The level of *P. teres*, expressed as pgDNA was converted to percentage of seeds infected for reporting to the customer.

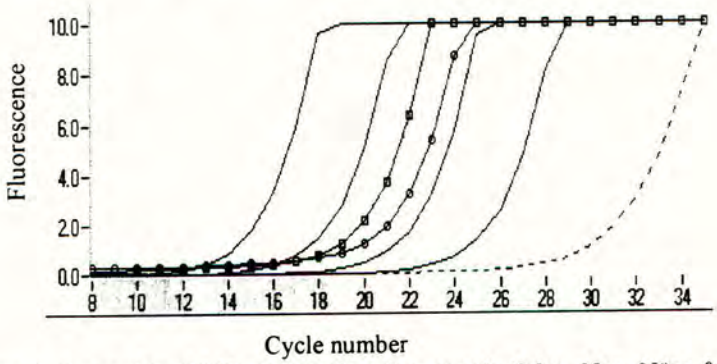


Figure 1. Run profile of PCR products from four controls (2.5pg, 25pg, 250pg & 2.5ng), a water blank (dashed line) and two barley samples with 3% (○) and 15% (□) seeds infected.

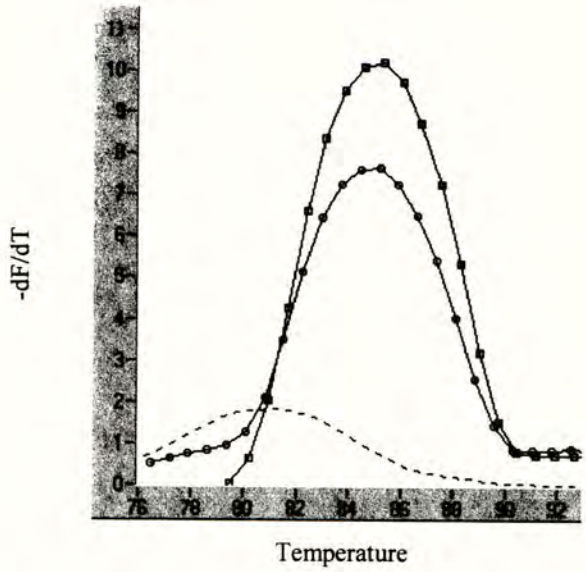


Figure 2. Run melting curves of PCR products from a water blank (dashed line) and two barley samples with 5% (○) and 70% (□) seeds infected.

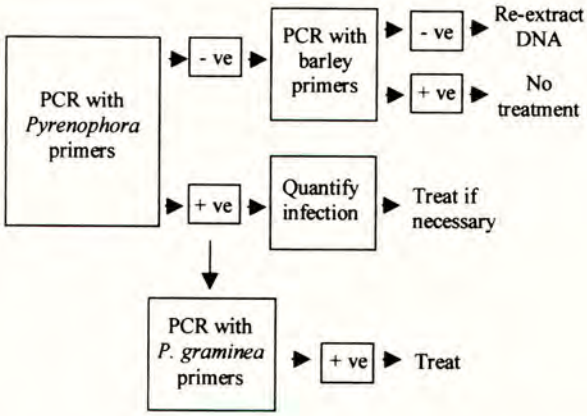


Figure 3. Flow diagram illustrating the various stages in the *Pyrenophora* seed health test.

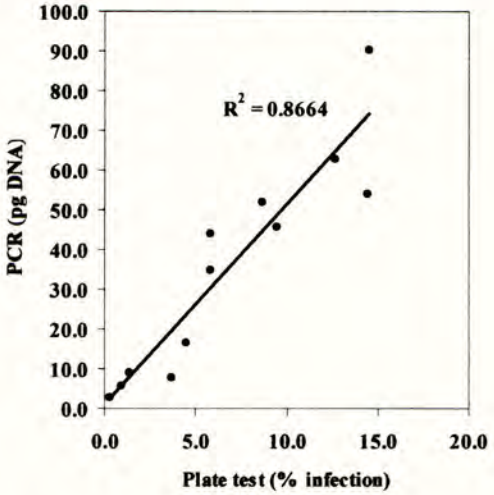
DISCUSSION

Quantification of *P. teres* DNA extracted from both naturally infected seed and samples blended to give target infection levels gave good correlation with the agar plate test. The artificially blended seed gave a gradient of between 0% - 14% infection. A correlation coefficient of r = 0.931 (Figure 4) was obtained between the mean of the plate test results and the mean of the PCR results at each infection level. These data sets were used to produce a linear conversion factor for converting pg of DNA to percentage of seeds infected.

The 60 advisory samples tested ranged in infection from 0% to 89% seeds infected. Good correlation (r = 0.88) was again found between the two techniques (Figure 5). It is worth noting that above 40% infection the variability increases considerably. Other work on *Microdochium nivale* (Unpublished) has shown that the amount of pathogen DNA extracted from individual seeds can vary by as much as 25-fold. Seed loading could then be contributing to this variability seen on the more highly infected seed lots where the effect would be greatest.

Ideally seed health tests should be specific for the pathogen of interest, sensitive enough to detect appropriate threshold levels and repeatable both within and between samples. The technology should also be transferable between laboratories and allow tests to be completed in a time scale and at a cost that satisfies the customer. The use of the traditional agar method for the detection of *Pyrenophora* spp. whilst providing the sensitivity required does not satisfy the other criteria, with the skill level of the analyst conducting the test being critical to the overall accuracy. Results from a recent International Seed Testing Association (ISTA) workshop showed that laboratories working with sub-samples from one initial bulk recorded results over a very wide range (Thomas personal communication). The PCR test described here used a LightCycler, which allows quantification of unknown samples by comparison to standards amplified in parallel reactions (Morrison *et al.*, 1998). Several workers (Hu *et al.*, 1993; Mahuku *et al.*, 1995; Nicholson *et al.*, 1996; Nicholson *et al.*, 1997) have reported quantification of fungal plant pathogens. However these involved lengthy post PCR steps which are absent when using a LightCycler system where PCR and detection occur in one tube. The complete test from receipt of sample to final result can be completed in several hours rather than the seven days required for the conventional plate test. The system could also analyse with a much higher number of samples, with a single LightCycler handling up to 300 samples per day. It thus offers a greater opportunity for farmers and seed merchants in the UK to complete seed health testing within a time scale that allows a treatment decision based on knowledge of disease risks. Comparative testing of the primer sequences and methodology using ISTA evaluation protocols will be sought in order to establish the technique as a future standard.

Figure 4. Correlation between conventional agar plate test results and quantitative PCR



of *P. teres* DNA from a constructed infection gradient. Each point represents the mean of ten replicate samples for each test.

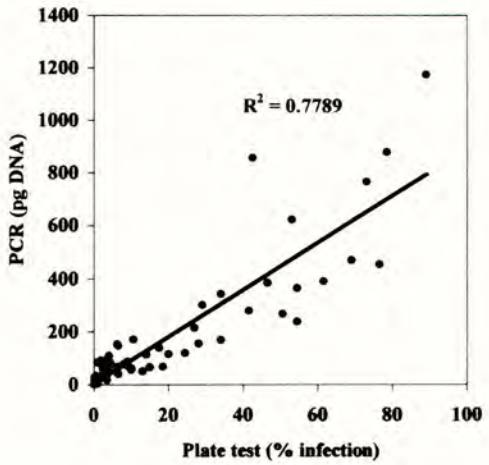


Figure 5. Correlation between conventional agar plate test results and quantitative PCR of *P. teres* DNA from 60 seed samples received by OSTS for advisory testing.

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Heat sanitation of cereal seeds with a new, efficient, cheap and environmentally friendly method

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ABSTRACT

A new high precision method for sanitation of seed from seed-borne pathogens has been developed. The method uses warm air which is in moisture equilibrium with the grain. Sanitation results comparable to those obtained with chemical seed treatment have been obtained in field results against many important seed-borne pathogens, without affecting plant development. The method is suitable for treatments of thick seed layers at short durations, which makes it promising for large scale treatments at a low cost.

INTRODUCTION

Healthy crops require healthy seeds, which makes most kinds of cropping dependent on seed treatment. Environmental and human health concerns have made it desirable to reduce the use of chemical fungicides in conventional farming, and organic farming, devoid of chemical pest control, has severe problems with disease-infected seed. Heat treatment of seed has, in this connection, again become interesting, but it has to be more convenient than the earlier method of hot water treatment. A cheap, environmentally friendly and efficient method using steam, intended for both conventional and organic farming, has been developed. The method is under test on a European level: FAIR project FAIR5-CT97-3664 - Demonstration of a biologically sustainable and environmentally friendly high precision thermal seed treatment method (DEST).

The principle behind seed sanitation using heat is the knowledge that many seed-borne pathogens do not survive as high temperatures in water as the host seed (Jensen, 1888). An interval of treatment temperatures can be found where the seed gives healthy plants without reducing or delaying germination or other kinds of damage on plant development (Figure 1).

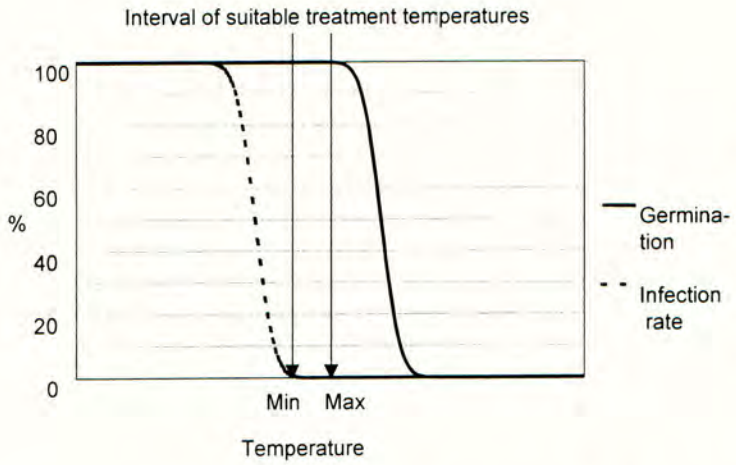

Figure 1. A temperature interval can be found where the seed is sanitised from the pathogen without affecting plant development.

MATERIALS AND METHODS

The idea behind the new treatment method is that by using hot air at a high relative humidity, the treatment conditions should be close to those in warm water, but without raising moisture content in the seeds. This could be achieved by thoroughly controlling the air humidity according to the modified Henderson equation (Henderson, 1952; Thompson, 1967) for calculation of parameters for moisture content equilibrium between grain and air. Heat treatment using air instead of water eliminates the need for expensive drying, it facilitates high precision control of treatment temperature and time, cooling of seed is uncomplicated and the method is also more suitable for large scale treatment, which reduces treatment costs. A laboratory scale treatment equipment has been constructed for evaluation and research (Figure 2).

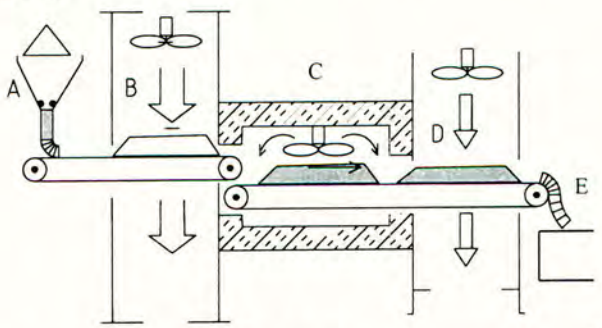

Figure 2. Schematic view of the heat treatment equipment: A. Feeding, B. Heating phase, C. Constant phase, D. Cooling phase, E. Discharging.

RESULTS

Verification

In order to test the theory about the influence of the relative air humidity, glass-house trials were performed with barley seed infected by *Drechslera teres*, treated in thin layers with a laboratory scale heating equipment designed to keep the temperature and air humidity constant during the treatment. Treatment series at a range of temperatures were performed both at 50 % mc. and at equilibrium moisture content at 5 min duration. The seeds were germinated for 11 days in soil at 4°C and the plants were then grown in the glass-house. Net blotch symptoms began to develop and the infected plants were counted. The resluts showed that the higher air humidity created the required temperature interval where seed was viable and resulting fully healthy plants were achieved.

Field trials

Within the DEST project, field trials are being performed in Italy, Austria, Germany, Denmark and Sweden using cereal seed of local cultivars, infected with diseases common in each area, treated with the new method. The seed lots were treated in a fluidized bed version of the laboratory treatment equipment in thick seed layers for higher capacity. The results are very promising, and in most cases comparable to treatment effects obtained by the most common chemicals. In the Swedish field trials, performed at various locations in the country, the sanitation effects have been comparable to those obtained with chemicals against all tested diseases so far - *Tilletia caries* and *Microdochium nivale* in wheat, *Drechslera teres* and *Ustilago nuda* in barley, and *Drechslera avenae* and *Ustilago avenae* in oats. The treatment effects were sufficient for quality seed except for *Ustilago nuda* in barley, where even the chemical treatment had insufficient effect. Table 1 displays the range of sanitation rates typical for the new method for some important seed-borne diseases. In other countries of the project partners, many seed lots infected with other serious diseases have been treated with successful results obtained from field trials.

Table 1. Ranges of sanitation rate typical for the new method.

Crop	Disease	Sanitation rate
Wheat	*T. caries*	> 99 %
Barley	*D. teres*	> 90 %
Oats	*D. avenae*	> 90 %

FURTHER DEVELOPMENT

Optimization of treatment time

In order to further optimize the treatment effects, a study has been performed to find the influence of the treatment time on the effect on sanitation rate and germination. The investigation was performed using barley seed infested with *Drechslera teres* and the effect was studied in glass-house. Short treatment durations appeared to give better sanitation rate maintaining full and undelayed germination. This result would be expected for all diseases situated close to the seed surface, since rapid heating can harm fungal cells near the surface without penetrating to the seed embryo. This is also favourable for treatment capacity.

Large scale treatment

To be able to use the method for practical agriculture a large scale treatment equipment is required to provide large quantities of seed and to reduce seed treatment cost. An equipment with treatment capacity of 1 ton of seed per hour has been constructed within the DEST project for demonstration. The treatment effect obtained in this large scale equipment will be evaluated in field trials during 2001. The equipment is constructed for continous flow of seed, where the heating is performed in a fluidized bed for even temperature and high capacity.

DISCUSSION AND CONCLUSIONS

Heat treatment using air which is in moisture equilibrium with the grain can sanitize seed of cereal crops from many important seed-borne diseases without affecting plant development.

The research has shown that the treatments can be performed in thick layers and with short treatment durations with effects comparable to those obtained with chemical treatments. This indicates that the method could be suitable for large scale use and therefore has potential to provide practical agriculture with healthy and environmentally benign seed.

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Alleviation of seed imbibitional chilling injury using polymer film coating

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ABSTRACT

Polymer seed film coating has been widely used in the seed industry for cosmetic colouration, seed identification, and most importantly, significant reduction of agrochemical dust-off of treated seeds. In addition, there are increasing demands in providing seeds with physiological benefits through polymer seed coating. SB2000, a functional organic-based polymer, was tested on cold susceptible species, such as *shrunken-2* corn, soybean, and dry beans. Beneficial responses were measured in laboratory and field studies. Up to 30% increase in cold test results and field emergence in cold and wet soils was measured on selected crops. The polymer coating retards imbibition during the first few hours of hydration, which is attributed to reduced seed membrane damage and seed leakage resulting in less imbibitional chilling injury.

INTRODUCTION

Film coating is defined as an application of a thin polymeric film over the seed surface. Film coating was initiated to control agrochemical dust-off of treated seeds. Different colourants and cosmetic materials were also added for improvement of seed appearance and seed variety identification (Ni, 1997). During the past 10 years, polymer film coating has made significant contributions to the seed industry by reducing hazards of agrochemicals to people, animals, and the environment, in improving the preciseness of chemical loading onto seeds, and in improving the efficacy of seed treatment chemicals due to both the precise loading and the reduced loss of seed treatment chemicals (Halmer, 1988). Film coating is mainly used on vegetable seeds and other high value crop seeds. Polymer film coating has limited use on field crop seeds due to the low seed cost, and low application rates by conventional seed treatments. For example, the application rate of polymer film coating on tomato seeds could be as high as 7% weight gain, but it is only 0.33 ml per kilogram on soybean seeds. Therefore, the polymer film coating must provide added value to the seeds before it can be broadly used on field crop seeds.

Recently, film-coating polymers with special functions have been developed to provide seeds with physiological benefits. One such material, SB2000 is a functional polymer for seed film coating, and is an organic-based polymer developed by Seedbiotics. Unlike those film-coat polymers which, in their crystalline state, completely block water from entering the seeds, SB2000 slows down seed water uptake during the first few hours of exposure to water. The polymer is mainly used on cold susceptible species, such as, *Phaseolus* spp., *shrunken-2* corn, cotton and soybean seeds.

Seed hydration

Seed water uptake during seed germination is generally divided into three phases. Initially, the water content of dry seeds increases rapidly when seeds are sown on a wet substrate (phase I), then reaches a plateau where seed moisture content changes little or increases slowly (phase II), and finally reaches another increase in water content marked by radicle

emergence (phase III) (Vertucci, 1989; Taylor *et al.*, 1992). Phase I is a physical process governed by the availability of water and the matric forces of the seed constituents, such as cell walls, proteins, and carbohydrates. During the plateau phase, metabolism is activated, proteins and enzymes that are needed to mobilize the stored reserves and to initiate growth are synthesized. Seeds enter phase III when embryo growth begins and radicle emergence occurs. Growing plant tissues absorb large quantities of water to expand their cells, resulting in another increase in the water content.

Imbibitional Chilling Injury (ICI)

ICI is caused by rapid water uptake under cold conditions, and occurs during phase I. The sensitivity of a seed to imbibitional stress is related to three factors: the initial seed moisture content, the temperature of the medium, and the imbibition rate (Pollock, 1969). The degree of the imbibitional injury is mainly dependent on the interaction of these three factors. The seed moisture potential of a dry seed can exceed -100 MPa because of its low matric potential, but the soil water potential must be greater than -1.5 MPa to permit germination. As the seed hydrates in the wet soil, water moves into the seed rapidly due to the large water potential gradient between the dry seed and the soil. In contrast, the cell membranes are still in their dry gel phase and are undergoing a phase transition from the dry gel phase to the liquid crystalline phase and have low elasticity. The rapid increase in seed moisture may result in cell membrane rupture due to rapid cell expansion. ICI is more severe when the susceptible seeds are imbibed rapidly at low temperatures.

Soybean seeds with low initial moisture seeds were imbibed at 25 ^{O}C, and seed vigour (expressed as radicle length) only decreased slightly with an increase in the imbibition rate. In contrast, a linear decrease in seed vigour occurred with an increase in imbibition rate when the same seeds were imbibed at 5 ^{O}C. However, there were no deleterious effects of imbibition on soybean seed vigour at low temperature if imbibed slowly (Vertucci, 1989).

Imbibition rate is a major factor causing damage and various attempts have been taken to slow down the imbibition rate and hence to alleviate ICI. Imbibition rate can be decreased by breeding snap bean seeds with semi-hard seed characteristics (Dickson & Boettger, 1982); imbibing seeds in polyethylene glycol solutions (Powell *et al.*, 1986); incorporating polyethylene glycol (Khan & Taylor, 1986) or hydrophobic materials (Sooter & Millier, 1978) in seed pellets; and film coating seeds with polymers, which contain hydrophobic materials (Hwang & Sung, 1991; Priestley & Leopold, 1986; Taylor, 1987). Of all the approaches, regulation of seed water imbibition through polymer film coating should be the most convenient modification of seed coat properties as plant breeding may take years to accomplish, and film coating is faster and less costly than pelleting. In addition, most seeds have some form of chemical treatment applied and polymers with hydrophobic properties can be easily applied together with these seed treatment chemicals either during seed treatment or during seed film coating.

Coating systems have been tested to reduce ICI, and Priestley & Leopold (1986) applied lanolin in an organic solvent on soybean, cotton, and corn seeds at a rate of 20-30g/kg seeds.

The seeds were then imbibed at 2 ^{O}C for 18 hours in flats and later transferred to a greenhouse for 2 weeks. The coating significantly decreased imbibition rate, improved both seedling emergence and fresh weight in soybean, only improved seedling emergence in cotton, and showed no improvement on corn seeds. In a similar approach, Taylor (1987) and Taylor *et al.* (1992) collaborated with the seed industry to evaluate aqueous-based

polymer formulations and coating technology. Percent change in seed moisture content per hour during the first 2 hours of imbibition, warm and chilled laboratory germination percentage, and field emergence were measured on seeds with 8% or 12% moisture content (Taylor *et al.*, 1992). Several polymer coatings showed a significant reduction in the imbibition rate during the first 2 hours and a negative correlation was found between field emergence and the changes in seed moisture content per hour (Taylor *et al.*, 1992). For seeds with an initial moisture content of 8%, the field emergence of polymer film coated seeds was at least 10% greater than the control (in the 1988 field trial). Only one coating formulation improved field emergence with initial seed moisture content of 12%. However, none of these coating formulations was commercialized partially because the seed industry concentrated more on the agrochemical dust-off control and seed cosmetics than physiological benefits of polymer film coating.

RESULTS

Four market classes of dry beans (*Phaseolus* spp.) with different cold susceptibility were tested. For green baby lima and kidney beans, 8 replicates of 25 seeds each were tested, and for black turtle and navy beans, 4 replicates of 50 seeds each were tested. The warm tests were performed following the AOSA rules (AOSA, 1995) by germinating the seeds in rolled towels for 8 days at 25 ^{O}C. The cold tests were conducted by using a combination of the soil and rolled towel method. Both the soil and presoaked paper towels were pre-chilled at 5 ^{O}C.

The seeds were placed on the rolled towel covered with soil obtained from a bean field. The seeds were first incubated at 5 ^{O}C for 5 days, then transferred to 15 ^{O}C for 5 days, and finally incubated at 25 ^{O}C for 8 days. The seeds were coated with SB2000 at 2% weight gain containing seed treatments. While there were no significant difference between coated and the control (treated but without SB2000 coating) in warm germination percentage, significant differences were observed in cold test except for lima bean. Lima beans are very susceptible to the cold conditions and the germination percentage is greatly reduced.

Field emergence test of the same seeds plus two more kidney bean cultivars were also conducted. Three replicates of 100 seeds each were planted on May 15, 1997 in Wisconsin in cold, wet soils. The stands were counted 34 days after planting, and SB2000 showed significant improvement in stand establishment of the cold susceptible varieties. The polymer showed no significant effect on those varieties that were cold tolerant (Table 1). Conductivity tests performed on the same treatments also indicated that SB2000 film coating significantly reduced seed leakage (data not shown). Similar improvements from the polymeric coating were reported for snap beans when tested under laboratory stress conditions (Taylor & Kwiatkowski, in press).

Table 1. Comparison of warm and cold germination test results of 4 dry beans (Phaseolus spp.) polymer film coated with SB2000 at 2% weight gain[1].

Variety	Warm Test[ns2]		Cold Test		Field Emergence	
	Control	2% Coating	Control	2% Coating	Control	2% Coating
Black Turtle	93	96	61	89**	78	75[ns]
Navy	80	73	23	48**	46	76**
Green Baby Lima	79	78	20	20[ns]	4	22**
White Kidney	89	94	77	80[ns]	70	81*
Light Red Kidney	-	-	-	-	95	96[ns]
Dark Red Kidney	-	-	-	-	81	85[ns]

[1] Comparisons are made within the same test.

[2] ns - no significance, *significant at $p<0.5$; **significant at $p<0.01$; ***significant at $p<0.001$.

The polymer was also tested on *sh2* corn 'How Sweet It Is' (HSII) from Crookham Company (Caldwell, Idaho, USA). SB2000 was applied at 2% weight gain with chemical seed treatments. Standard warm germination (Warm), accelerated aging (AA), and cold (Cold) tests were conducted according to AOSA rules (AOSA, 1995) followed by a field emergence (Field) trial. Sixteen rows with 40 seeds per row were planted on April 22, 1997 in Caldwell, Idaho with final stand being counted 36 days after planting. As expected, there were no significant differences between the control and the film-coated seeds in Warm and AA results. However, 2% film coating improved cold test results and field emergence by 18 and 11 percentage points, respectively (Fig. 1).

The effect of the polymer on cold test results of soybean seeds is shown on Figure 2. While the chemical fungicide seed treatment alone Improved germination in the cold test in comparison with the control (untreated, uncoated), SB2000 coating further improved germination in the cold test when the polymer application rate was as low as 1.3 ml per kg seeds. In general, the cold test results were further increased as polymer application rate increased. At an application rate of 5.2 ml per kg seeds, the polymer improved seed cold germination percentage by 24 percentage points in comparison with the chemical seed treatment check (Fig. 2).

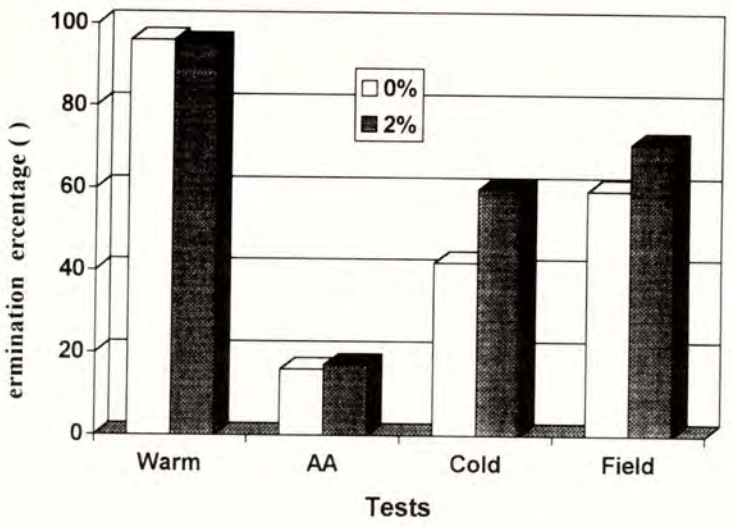

Figure 1. Standard warm germination (Warm), accelerated aging (AA), cold tests (Cold), and field emergence (Field) tests of *sh2* corn seeds film coated with SB2000 at 2% weight gain.

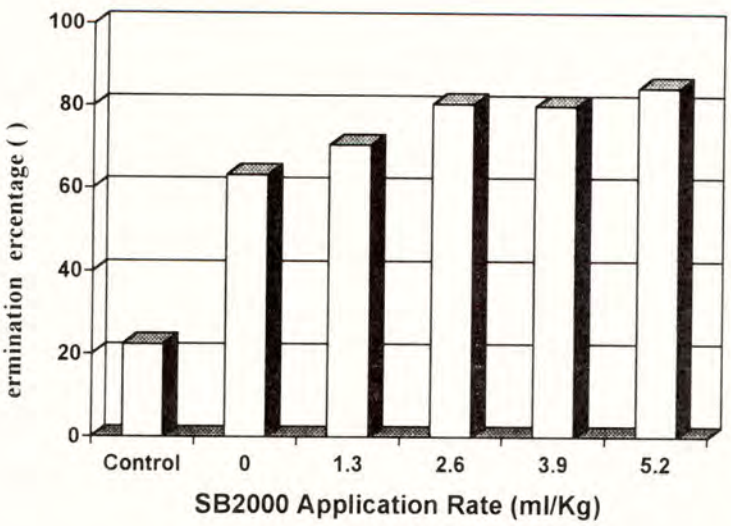

Figure 2. Cold test results of soybean seeds of control (untreated, uncoated) and coated with SB2000 at various application rates with Apron XL (0.21 ml/kg seed) treatment.

To understand the mechanism by which the polymer affects cold test results, seed moisture contents of coated and uncoated soybean seeds were monitored during the first 32 hours of the seed water uptake. The seeds were coated with an application rate of 5.2ml polymer/kg seeds (the water content of SB2000 is 83%). To insure that the control had the same seed moisture as the coated one, 4.3ml water per kilogram seeds was applied. Four replicates each of 25g seeds were measured using the oven method. The initial seed moisture contents were approximately 9% for both treatments. After 2 hours of imbibition, seed moisture content for coated and uncoated seeds were 22 and 24%, respectively. At 4 hours, the difference between the coated and the control was 3.5 percentage points. During the first 8 hours of imbibition, the seed moisture contents of the coated seeds were consistently about 3 percentage points lower than the control (Figure 3). Seed moisture content of both the treatments reached the same level after 24 hours of imbibition.

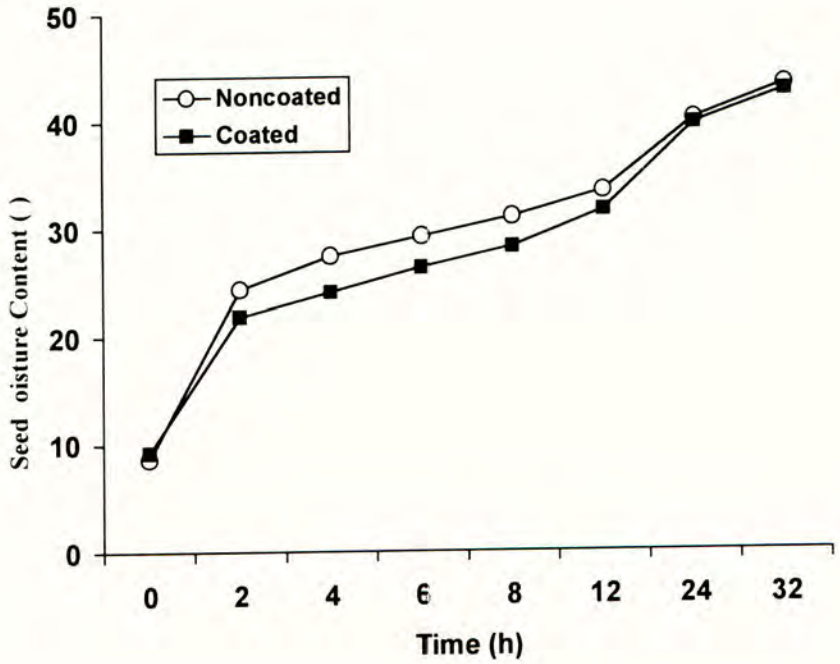

Figure 3. Water imbibition time courses of uncoated and SB2000 polymer coated soybean seeds at 5.2 ml/kg seed at 25 ^{o}C.

CONCLUSION

SB2000 is a seed film-coating polymer particularly designed to retard seed water uptake during the first few hours of imbibition. The slower rate of water uptake may prevent cell membrane damage and hence reduce the leakage of proteins and carbohydrates. As proteins and carbohydrates are food sources of microbes, the reduction in leakage may result in a reduction in microbial attack of the seeds. Applications of SB2000 on various cold-susceptible species have shown improvement in both the cold tests and field emergence. Coating is one of the most convenient methods of alleviating ICI. In addition, the low application rate of the polymer makes the technology feasible for low cost field crop seeds. Effectiveness of SB2000 in improving field emergence and stand establishment is dependent upon cultivar susceptibility to cold conditions and sowing in cold and wet soils.

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