SESSION 3B

SEED TREATMENT - TO TREAT OR NOT TO TREAT?

Chairman:	Professor Graham Jellis *HGCA, London, UK*
Session Organiser:	Dr Anthony Biddle *PGRO, Peterborough, UK*
Platform Papers:	3B-1 to 3B-4
Poster Paper:	P3B-5

A model for setting seed treatment thresholds for common bunt, *Tilletia tritici*

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ABSTRACT

Winter wheat is the largest cereal crop in the UK, requiring over 300,000 tonnes of seed each year. Rapid diagnostic tests for bunt (*Tilletia tritici*) have been developed that allow seed treatment decisions to be based on need. Survey evidence shows that it may be possible to sow over half of the winter wheat grown for ware untreated. However, it is important that the treatment threshold is set at a level that protects both individual and national seed stocks. This paper describes a model that simulates the year-on-year multiplication potential of the disease, at field and landscape scale, describing the whole life cycle including the spatial re-distribution of certified and home saved seed. The model has been calibrated and proven at field scale, by analyses of inoculated wheat experiments. This work has identified a potentially significant soil-borne phase that may be responsible for a residual level of infection despite a historical downward treatment pressure.

INTRODUCTION

One of the most important seed-borne pathogens of wheat is 'Stinking' or 'Common' bunt, *Tilletia tritici* (syn. *T. caries*). Ears of infected wheat develop 'bunt balls' which replace seed. These bunt balls, containing millions of spores, are prone to breakage during harvesting and will contaminate the seed lot (Wilcoxson & Saari, 1996). Bunt can be easily controlled, since spores contaminating the surface of the seed are relatively easy to disinfect with chemical seed treatments and this has become routine practice for commercial seed lots. In the UK, a review of cereal seed health and seed treatment suggested that a move towards targeted seed treatment 'according to need' would improve both seed health and seed production efficiency (Paveley *et al.*, 1997). This has been made possible by developments in rapid molecular diagnostic assays that reduce the time constraints on seed testing to allow treatment (or rejection) of infected seed prior to planting (Cockerell *et al.*, 2004). However, the setting of an appropriate threshold for treatment is complicated by the potential interactions between neighbouring fields (spore dispersal), users of certified and home-saved seed, and a potential soil-borne infection pathway (Yarham, 1993; Yarham & McKeown, 1989). A treatment threshold should place the national bunt population under a constant downward pressure. The objective of this work was therefore to develop a model, describing the life cycle of the bunt disease and built upon field

experimental data, to characterise disease multiplication potential and aid identification of an appropriate threshold for seed treatment according to need.

METHODOLOGY

Field scale model

A mathematical model of plant infection and spore release was developed, drawing upon published data. Central to the model is the assumption that a Poisson function describes the distribution of spores per seed, and that any successful infection by a single spore will result in the whole of the plant being infected and all of the grain on the tillers being replaced by bunt balls (Benada *et al.*, 1995). There is no reduction in tillering or shoot survival with increasing disease incidence. The proportion of plants infected is calculated as a function of the mean number of spores per seed ***m*** and the probability ***b*** of a single spore infecting the seed (Equation 1; Gregory, 1948). The numbers of spores released at harvest, returned adhered to the harvested grain and dispersed are calculated by a simple mass balance (Equations 2 and 3). This requires estimation of the proportion ***d*** of bunt balls that release their contents during threshing, the number ***k*** of spores per bunt ball, and the proportion of spores ***c*** that adhere to the healthy grain. The model parameters were calculated from the results of field experimentation described by Cockerell *et al.* (2004).

Equation 1. Proportion of plants infected (*p*)

$$p = 1 - e^{-b \cdot m}$$

b	Probability of a single spore infecting a plant
m	Mean number of viable spores adhered to seed

Equation 2. Mean number of spores adhered to harvest grain (*s*)

$$s = \frac{p \cdot c \cdot k \cdot d}{1 - p \cdot d}$$

d	Proportion of bunt balls that release their spore contents
k	Mean number of spores per bunt ball
c	Proportion of spores that adhere to healthy grain

Equation 3. Mean number of spores dispersed per hectare (*a*)

$$a = p \cdot (1 - c) \cdot k \cdot d \cdot g \cdot t$$

t	Number of surviving shoots per hectare
g	Number of grains per shoot

Landscape scale model

Iterative, year on year, application of the field scale model allows calculation of the levels of disease in a harvested crop and the number of spores on grain that may be used to seed the following crop. For simulation at the landscape or regional scale, a simple stochastic

framework was built that describes the exchange of potentially contaminated seed between a large number of fields, growing wheat for ware and seed, from certified and home saved bulks. The framework represents the central collection of grain as certified seed (always treated) and ware bulks, and allows for a proportion of home savers (for full details, see Cockerell *et al.*, 2004). At each annual planting a decision whether to treat is made separately for each field, based on the number of spores per seed. Treatments are assumed to have an efficacy of 99.9% against spores on the seed only. The framework tracks the levels of infection in a population of fields and seed bulks, reporting the proportion exceeding quality thresholds.

A critical facet of the model is the dispersal and survival of spores that fall to the soil, rather than adhere to grain, and may infect the following crop. Field experimentation had indicated that the dispersal distance is short with the majority (>80%) of spores observed to fall to the ground adhered to chaff within 100m. Therefore, this model version assumed that spores contaminate only the source field. To represent the likely death of the soil-borne spores between harvest and sowing of the following crop, the proportion remaining viable was reduced exponentially with the time delay. There are currently no data available to set the exponential rate parameter, but it is suggested that it be correlated with rainfall frequency. No field would be sown for seed or ware *ad infinitum* so the use of each field is randomised at each planting.

RESULTS

Field experimentation

By analysis of wheat crops that were sown with known counts of spores per seed, guide values for each of the model parameters have been established. These experiments were carried out at SASA Gogarbank and Bush Estates, and ADAS farms at Rosemaund, Manor Farm and Boxworth, in the period 1996 to 2002. Figure 1 illustrates the sample range of calculated infection probabilities for *cv.* Consort. The calculated value of $\boldsymbol{b}$ was *c.* 5×10^{-5}, and was found to vary by an order of magnitude with site and variety.

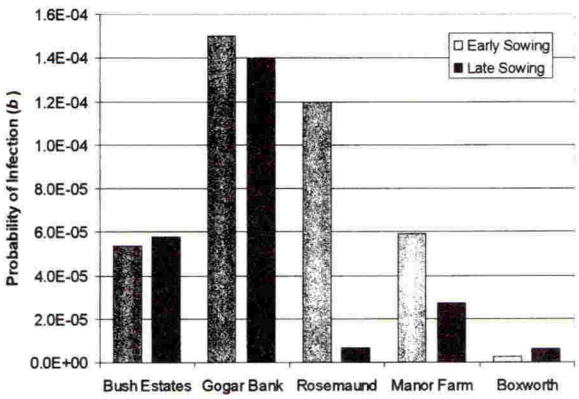

Figure 1. Calculated spore infection probabilties for *cv.* Consort at a spore loading of 1,000 spores per seed at study research sites (1994 and 2001).

The number of spores per bunt ball ***k*** was estimated as 10^7 based on a sample of 20 in a haemocytometer counting chamber. The proportion ***d*** of bunt balls that release their contents during threshing was *c.* 0.80 and the proportion of spores retained adhered to grain ***c*** was *c.* 0.005, indicating that 99.5% of spores are released to the air or fall to the ground with wheat chaff. By using these parameters in the field scale model, we have demonstrated our ability to reproduce observed levels of infection for independent experimental sites at a wide range of spores per seed (Figure 2).

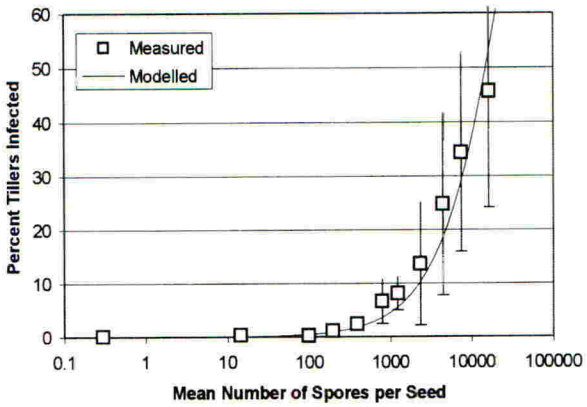

Figure 2. Measured and modelled percentage of tillers infected as a function of increasing spores per seed, for a winter wheat sown on 3rd November 1996 at Cammo Field, Scotland. The bars show the 95% C.I. of the mean of 4 replicates.

Critically, the field experimentation included an investigation into the infection resulting from soil-borne spores (Cockerell *et al.*, 2004). This demonstrated that 1g m^{-2} of soil-borne spores gave rise the same infection as 20,000 spores per seed when spread to the soil on the day of drilling. This effect was included in the model framework by adding the weighted soil spore load (due to spore release from the previous harvest in the same field) to the mean number of spores per seed ***m*** when calculating the likely infection level.

Landscape simulation

The landscape scale model was used to explore the sensitivity of the system to the model parameters and assumptions about the proportion of seed that is home saved and tested for treatment. The output from the model was extremely sensitive to the survival of the soil-borne spores. For a treatment threshold of 1 spore per seed, and in the absence of home savers, the disease was driven to extinction if the average soil-borne spore survival was less than 2.5%. Whereas for a higher survival of 5% the disease persists and the model predicts that 3% of certified seed bulks would return an unacceptable 100 spores per seed. A population of home savers was found to allow the disease to persist, even at low levels of soil-borne spore survival. For any parameterisation, the average infection level in harvested grain across all fields was approximately proportional to the treatment threshold. Without the soil-borne load, certified seed treatment inevitably drove the disease to extinction.

The soil-borne spore survival was calibrated to reproduce the levels of infection reported in recent surveys (Table 1), assuming 25 fields producing certified seed and 320 fields used for home saving in every 1,000 wheat fields. For an average soil-borne spore survival of 2.3%, the model predicts that 10% of fields return seed with more than 1 spore per seed, and 3% with more than 5 spores per seed, with a geometric mean of 0.28 spores per seed. Increasing the soil-borne survival rate to 3.5%, the model predicts that 16% of fields return seed with more than 1 spore per seed, and the geometric mean rises to 0.56 spores per seed. Removal of the home savers causes the disease to go extinct. These analyses did not take account of treatment effects against the soil-borne spores. In practice, the use of seed treatments that are effective against the soil-borne phase will reduce disease further.

Table 1. Percentage of seed samples contaminated with *Tilletia tritici* spores, tested at the Official Seed Testing Station (OSTS) Scotland (2000 to 2004; Cockerell, *pers. comm.*), at NIAB (1991 to 2004; Kenyon *pers comm.*) and tested during the HGCA seed pathogen survey (1992 to 1994; Cockerell & Rennie, 1996).

	Spores per Seed	Year 91	92	'93	94	95	96	97	98	99	00	01	02	03	04
OSTS	>0	-	-	-	-	-	-	-	-	-	28	28	15	22	26
	>1	-	-	-	-	-	-	-	-	-	8	11	6	9	8
HGCA	>0	-	37	43	42	-	-	-	-	-	-	-	-	-	-
	>1	-	16	12	15	-	-	-	-	-	-	-	-	-	-
NIAB	>0	64	69	63	67	53	91	82	37	22	25	22	37	48	43
	>1	5	11	1	2	4	0	17	4	4	3	3	8	10	11

DISCUSSION

A treatment threshold is required that results in a continuous downward pressure on national infection levels. If seed-borne spores were the only source of infection, then application of the field scale model predicts an annual multiplication potential of *c.* 4, giving rise to a 100-fold increase within 4 years if untreated. If seed-borne spores were the only source of infection, then a threshold for treatment could be defined simply in terms of the acceptable level of infection for quality and price. The mean interval between treatments would be dictated by the ratio of the treatment efficacy and the annual multiplication potential; a mean interval of 2 years giving a 66% saving of treatment costs.

However, application of the model without the effect of soil-borne spores inevitably drove the disease to extinction given the current treatment threshold of one spore per seed, in contradiction to recent survey results that established a residual but low level of infection (Table 1). Soil-borne spores from the dispersal and deposition of spores released at harvest can result in high levels of infection if the delay between harvest and drilling is short. The model predicts an annual multiplication potential of up to 450 in worse case situations. Historical seed testing data show that organomercury seed treatments that were not effective against soil-borne spores still resulted in a downward pressure on the disease, with bunt ball detection declining from 30% to less than 1% between 1920 and 1957 (Marshall, 1960), that has been maintained (Cockerell & Rennie, 1996). There is therefore evidence for an effective soil-borne spore multiplication potential of less than one when averaged across a landscape or region. However,

the model suggests that local scale infection incidents, such as described by Yarham (1993), can be responsible for maintaining the residual level of infection. This requires further investigation to establish, experimentally, the decline rate for spore survival in the soil.

The significance of the soil-borne spores may be reduced by the increasing use of systemic fungicide treatments. It is estimated that only 25% of seed dressings were effective against soil-borne spores in 1995, compared to 75% in 2001 (Defra and CSL Cereal Disease Survey). Systemic treatments reduce the likelihood of local infection incidents.

Data from seed testing stations at NIAB and SASA suggest an increase in the targeting of seed treatment using results from seed health tests, and a decrease in bunt (Cockerell, *pers. comm.*; Kenyon, *pers. comm.*).

ACKNOWLEDGEMENTS

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Development and application of a quantitative PCR test for detection of *Microdochium nivale* on wheat seed

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ABSTRACT

Seedling blight of wheat caused by *Microdochium nivale* can occur at high levels in UK wheat seed stocks depending on seasonal conditions. Current advice is that seed with levels of infection above 10% should be treated, though the majority of seed in advisory health tests over a five year period did not exceed this level. A rapid test to detect *M. nivale* in wheat seed stocks was developed to increase time available for decisions on seed treatment in the short period between harvest and sowing. Quantitative PCR enabled the prediction of infection in excess of 10% and reduced testing time from five to seven days to 48 h.

INTRODUCTION

Microdochium nivale is frequently found on wheat seed in the UK. Infection occurs during anthesis, in damp conditions. The fungus colonises the outer layers of the developing grain. If seed is saved from infected grain, *M. nivale* can cause a seedling blight which reduces plant populations in the autumn, and can occasionally lead to crop failure. Seedling blight is controlled effectively by a range of seed treatments. However, tests of winter wheat samples in surveys and commercial tests over a number of seasons have shown that incidence and severity of *M. nivale* in seed is very variable (Kenyon and Thomas, 2001) Despite this, seed treatments have been used routinely for many years regardless of the health status of the seed.

The reasons for prophylactic uses are varied. Certified seed is nearly always sold with a treatment unless untreated seed is requested by buyers. Though a small proportion of farm-saved seed has been sown untreated for many years, the majority has been treated for *M. nivale* and bunt (*Tilletia caries*). Tests for *M. nivale* take between five and seven days on agar plates and though tests for bunt are more rapid, the relatively short period between harvest and sowing for winter wheat has precluded large scale testing. Furthermore, though the threshold for treatment for *M. nivale* was based on field experimentation, the level was precautionary since experiments did not cover worst case situations. This tended to deter growers from testing, as many samples might still require treatment, and cost savings would be limited.

Recent work has demonstrated that a higher threshold for treatment is possible, creating more potential for savings on treatment costs. This paper reviews the incidence of *M. nivale* above the new threshold over the last five years, and describes the development of a rapid PCR test for quantitative detection of *M.nivale* in wheat seed. The potential for improved targeting of seed treatments based on these developments will be discussed.

MATERIALS AND METHODS

Seed tests for *M. nivale*

Two hundred seeds were surface sterilised by immersing them for 10 minutes in a solution of sodium hypochlorite (1% available chlorine) and plated onto potato dextrose agar with 10 seeds per 9 cm diameter plate. Plates were incubated for five to seven days at 22°C under a 12 hour NUV, 12 hour dark cycling regime and then examined for *M. nivale* colonies. This method was used for advisory tests on samples submitted to the Official Seed Testing Station, Cambridge and for all tests used in the calibration of the PCR method.

Development of PCR test

Primers MnivA and Fun28B were used throughout. Primer specificity was checked using a total of 85 isolates of *M. nivale* and a range of other seed-borne pathogens of wheat, other pathogens of wheat and common saprophytes. Seed samples were prepared by milling whole seeds for 10 s using a laboratory blender to produce a coarse powder, or crushing with a hammer to produce a similar powder. DNA was then extracted using CTAB buffer according to the method used by Edwards *et al.*, (2001). Quantitative PCR was carried out in a LightCycler™ using a total reaction volume of 20 µl consisting of 2.4 µl 4mM $MgCl_2$, 2.0 µl master-mix (Biogene Ltd, UK) including SYBR Green 1 (Biogene Ltd, UK); 2.0 µl of each primer; 2.0 µl of DNA extracted from seed; and 11.6 µl of water. Amplification was carried out with an initial denaturing step at 95°C for 30 s followed by 35 cycles of 95°C for 0 s, 55°C for 5 s, 72°C for 17 s and 84°C for 0 s. The temperature transition rate was set at 20°C/s. Acquisitions of fluorescence signal were carried out at the end of every extension step for 50 ms. A melting curve was obtained immediately after amplification to distinguish specific products from non-specific products and primer-dimers. This was done by holding the temperature at 60°C for 5 s and gradually increasing the temperature to 98°C at a rate of 0.2°C/s.

Relationship between PCR result and agar plate infection levels

Quantification of *M. nivale* DNA from seed was examined initially in a series of 25 samples, with a range of *M. nivale* infection levels from 0 to 21% in plate tests. Eight replicate sub-samples of 200 seeds were produced of which four were tested using the agar method and four by the quantitative PCR assay. A second series of samples with infection ranging from 21 to 60% was tested in the same way.

DNA was extracted from between 17 and 26 single seeds crushed individually from each of four lots of one cultivar of wheat having 2, 5.5, 13.5 and 50.5% infection with *M. nivale* assessed by agar plate tests.

A set of 91 samples, with *M. nivale* infection levels ranging from 0 to 70% submitted to the OSTS, Cambridge during autumn 2002 was used to calculate a predictive relationship between PCR result and % infection. A further set of 33 samples was used to test the ability of the calibration to predict % infection in unknown samples.

RESULTS

Seed tests for *M. nivale*

Over a five year period, relatively few samples in advisory seed health tests had greater than 10% infection with *M. nivale*, and samples with more than 30% infection were rare (Table 1).

Table 1. Incidence (% of total samples) of varying infection severities with *M. nivale* in seed samples from winter wheat, 2000-2004

Year	Severity		
	10-20 %	21-30 %	>30%
2000	4.9	1.1	0.0
2001	0.0	0.0	0.0
2002	17.3	8.5	3.8
2003	16.3	3.8	0.7
2004	3.7	1.0	0.0

Development of a PCR test

DNA from all isolates of *M. nivale* tested was amplified by the primers. None of the other pathogens or saprophytes tested gave any amplification product, including seed-borne fungi such as *Fusarium culmorum*, other *Fusaria* species, and *Septoria nodorum*, all of which can be found in agar plate tests. The specific product for *M. nivale* melted at 88.2°C.

Relationship between PCR result and agar plate infection levels

Quantification of *M. nivale* DNA extracted from the samples with infection levels below 21% gave a linear relationship with a correlation coefficient of 0.87 (Figure 1). When samples with infection greater than 21% were included the relationship was less defined with a correspondingly lower correlation coefficient (0.72) (Figure 2).

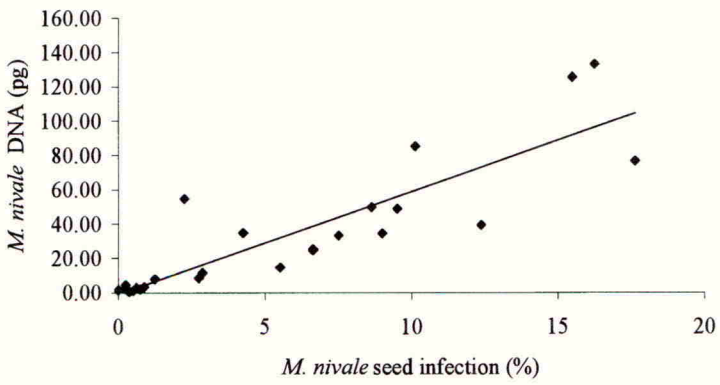

Figure 1. The concentration of *M. nivale* DNA (pg) compared to *M. nivale* infection levels in an agar plate test for samples with low infection levels (0 – 21%).

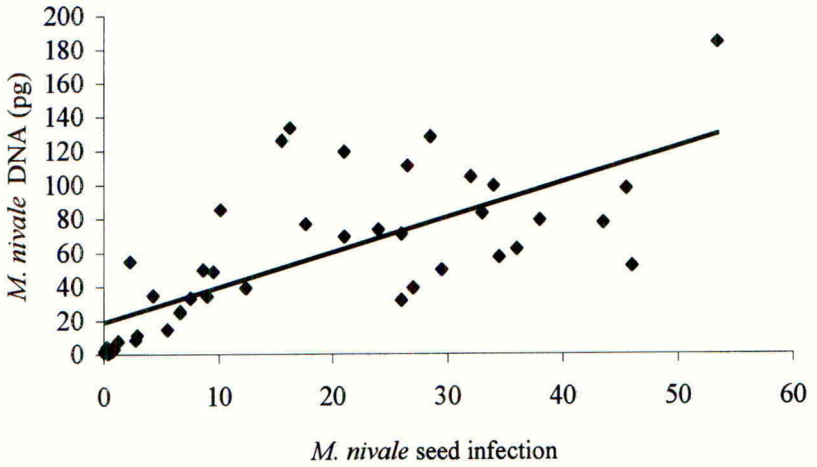

Figure 2. The concentration of *M. nivale* DNA (pg) compared to *M. nivale* infection in an agar plate test in a range of infected samples (0-53.5%).

There was considerable variation in the amount of fungal DNA derived from single crushed seeds (Table 2). In the sample with 13.5% infection, one seed had higher levels of fungal DNA than any of the seeds in the 50.5% sample.

Table 2. Range of *M.nivale* DNA levels (pg per seed) in individual seeds taken from samples with different levels of infection

Infection level (%) in sample	Range of DNA in seeds (pg)	Number of seeds tested
2	1.6 to 9.0	17
5.5	3.5 to 18.6	17
13.5	6.9 to 4156.0	26
50.5	6.4 to 1074.0	20

A correlation of r = 0.78 between pg DNA and plate test % infection was found with the set of 91 samples used to calibrate the PCR result. Log_{10} transformation of both variables improved the correlation, but observed variation was still large, and the 95% confidence intervals of prediction were thus also large (Figure 3).

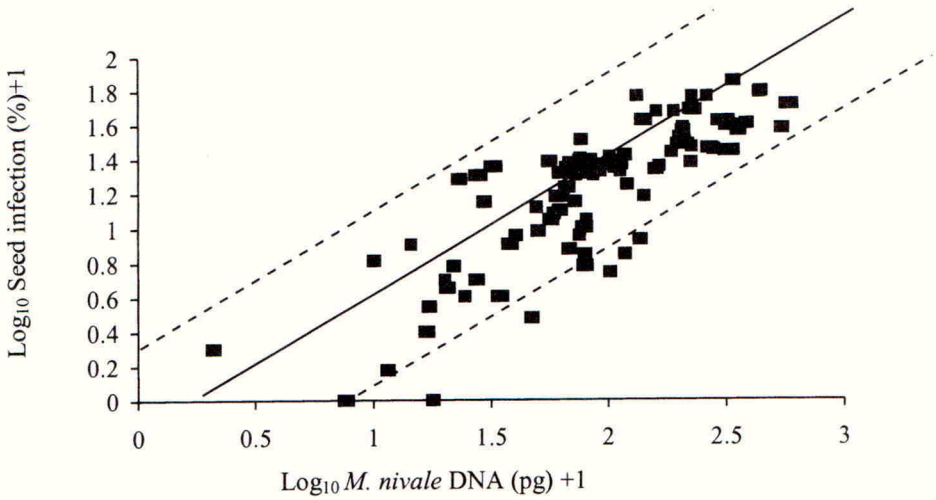

Figure 3. Log transformation of *M. nivale* DNA (pg) compared to the log % of seeds showing infection in an agar plate test with 95% confidence interval prediction (two replicate PCR results per extract are shown separately for each sample).

Regression analysis of data from the 91 sample set was used to derive a prediction of % infection from the PCR result of the 33 sample set, with infections ranging from 0 to 76%. In these samples, PCR DNA values above 100 pg produced agar plate infection levels of 20.6% or greater. Values above 150 pg corresponded to plate levels of at least 28.2%. When the predictions were plotted against the observed plate % data, there was better agreement at levels of infection between 0 and 20% than for infection levels exceeding 20% (Figure 4).

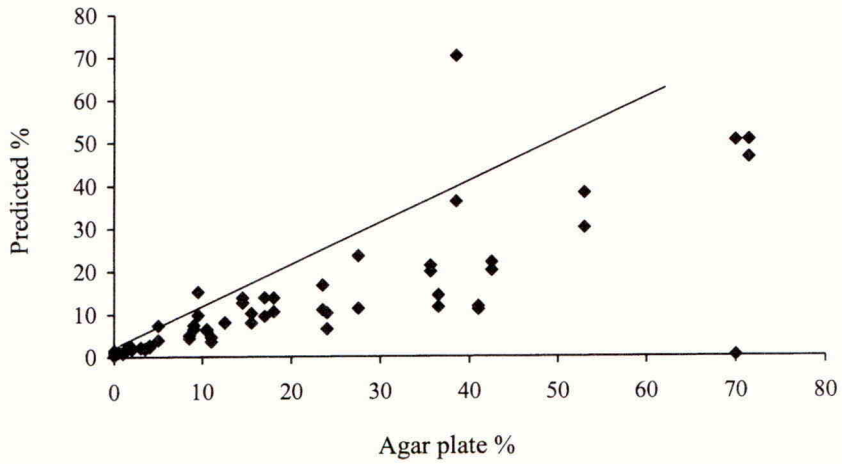

Figure 4. The % seeds infected predicted from *M. nivale* DNA (pg) and % of seeds showing infection in an agar plate test (predictions from two replicate PCR results plotted for each plate test; line shows 1:1 relationship).

DISCUSSION

Results from advisory seed health tests over the last five years indicated that the majority of wheat seed was below the treatment threshold of 10% infection, and that there would be significant potential for savings on seed treatment costs. Advisory seed health tests do not represent comprehensive surveys, and are predominantly carried out for growers intending to farm-save seed. Samples received by the OSTS in Cambridge are mainly from England and Wales and in Scotland, severity tends to be much higher. In England and Wales, only two years, 1997 and 1998, in the last decade have had a high incidence of samples over 10% infection, with 92% and 90% of samples respectively exceeding the threshold. Such sporadic occurrence requires that any strategy for a managed approach to seed treatment must be supported by a seed health test capable of predicting whether a sample is above or below the treatment threshold.

Real-time PCR using the LightCycler™ system was capable of quantifying *M.nivale* DNA from an extract of whole seeds. There was a linear relationship between agar plate result and fungal DNA level, but variability was high, with the greatest variation occurring for samples with more than 20% infection. Variability may be due to experimental method, effects of total DNA (wheat and fungus) extracted, sample weight, or seed loading with *M. nivale* mycelium. Corrections made for seed weight and total DNA extracted had no effect on reducing variability (data not shown). However, the amount of *M. nivale* DNA on individual seeds within a sample showed considerable variation and this may preclude the establishment of a stable relationship between % infection and DNA level. Seed to seed variation was greatest in samples with higher infection levels above the treatment threshold and the PCR assay can provide more accurate quantification at lower infection levels. A predictive relationship with 95% confidence intervals was developed which allowed the conversion of *M. nivale* DNA level to a result which indicates whether the pathogen is above or below the treatment threshold of 10%. The major advantage of the real-time PCR test is speed, reducing the current test length from seven days to 48 hours. The test 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.

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Seed treatments with herbicides for *Striga* control in Africa

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ABSTRACT

Parasitic *Striga* spp. are a major constraint to grain production in sub-Sahara Africa . They cannot be controlled by selective herbicides, except on crops with target-site resistance to systemic herbicides. Spraying herbicides is uneconomic in African conditions, and instead, targeted herbicide use via seed dressing of maize varieties bred with mutant ALS genes has been recently commercialized in Kenya. This technology has tripled yields in heavily infested areas and provided season long control in short season maize. High rainfall can leach the imidazolinone herbicide in short season maize, and normal rainfall leaches it in longer season maize. Controlled release formulants (high capacity ion exchangers) were developed for this seed treatment application to limit herbicide leaching.

INTRODUCTION

Striga spp. are a major reason that maize yields in the 1.2 million ha sown in sub-Sahara Africa have not increased over the last two decades, but remain around 1.5 tons/ha, which is well below the world average of 4.2 t/ha (FAO, 2005). Although crop rotation, especially with introduced intercrops or rotational crops (Carsky *et al.*, 2000; Khan *et al.*, 2000; Oswald & Ransom, 2001; Oswald *et al.*, 2002), transplanting (Oswald & Ransom, 2002), organic and inorganic fertilizers (Gacheru & Rao, 2001) can partially allay the problem, no control measure has been developed that most subsistence farmers find within their financial means, or that fit well into their traditional cropping systems. Moreover, many of these measures require several seasons of repeated use before they begin to produce yield benefits (Ransom, 2000). Thus, despite widespread extension efforts, they have not been, and are unlikely to be, widely adopted, as they are not what the farmers consider "appropriate" for their needs of providing sufficient food for their families on small, intensively cultivated holdings.

Striga does most of its damage to its host through phytotoxins before the weed emerges from the soil (Gurney *et al.*, 1995), while depleting the crop of photosynthate, minerals, and water (Press & Graves, 1995). *Striga* can be controlled by foliar applications of existing phenoxy herbicides after the *Striga* flower stalk has emerged, requiring spray equipment and high doses

of herbicide. These treatments are too late to be effective for the current season and, if there is a large seed-bank, ineffective for coming seasons. Spray applications of most herbicides would kill intercropped legumes, which are planted by many subsistence farmers in an effort to reduce risk and increase the dietary intake of protein that would otherwise come from maize alone.

Subsistence farmers in Kenya and elsewhere cultivate maize with judiciously used, small inputs of fungicide and insecticide seed dressings, and weeks later, apply a few granules of insecticide into the whorl of maize leaves to control stem borers. We thought that small amounts of herbicide could control the parasitic *Striga* while it is still underground, before the weed debilitates the crop (Abayo *et al.*, 1996; Abayo *et al.*, 1998; Kanampiu *et al.*, 2001). Although economically feasible, such a strategy requires the adoption of new varieties and techniques and thus posed both technical and extension challenges. Maize is an important food throughout sub-Sahara Africa. In Kenya, over 200,000 ha of land is severely infected with *Striga hermonthica*. Doubling yields from 1 ton would produce enough maize to provide 1,400,000 people with their current average annual maize consumption. The areas severely affected by *Striga* in Africa are typically those of the poorest population with the highest percentage of maize in their diet. Despite the apparent focus of the *Striga* problem in poorer areas, acceptance of a solution seemed likely because African farmers adopt new maize varieties and technologies having perceived value, and have adopted hybrid maize in subsistence agriculture over the last two decades in places where *Striga* is not a major problem.

The agroeconomic situation in the problem areas is, therefore, one that can respond to a new variety and implement in parallel micro-application of agrochemicals. Some transgenic (Joel *et al.*, 1995) and mutant (Abayo *et al.*, 1996; Abayo *et al.*, 1998; Berner *et al.*, 1997) herbicide-resistant crops with altered target enzymes (Newhouse *et al.*, 1991) enable the early control of parasitic weeds before or during attachment to the host. The herbicides are exuded from crop roots and kill attached *Striga* as well as its nearby seeds in the soil, before germination (Kanampiu *et al.*, 2002). These herbicides cannot, however, be used conventionally because of cost and effect on intercropped legumes. We thus demonstrated that seed dressings of IR (imidazolinone resistant) maize with small amounts of imazapyr or pyrithiobac could provide season long control of *Striga*, (Kanampiu *et. al.*, 2002). The seed dressings allowed intercropping with legumes (Kanampiu *et al.*, 2002). Results with the material approved by the regulatory authorities and commercialized are described below.

Although this strategy is effective, it has limits. When the soil is very dry during germination, a high local level of herbicide can cause a 2-3 day delay in germination of the IR-maize. Conversely, very high rainfall can wash the herbicide beyond the root zone, allowing establishment of late germinating *Striga*. It was clear that while the treatments were appropriate for Kenya with its 12-14 week maize, there might not be sufficient herbicide available in the longer (20-22 week) season maize, grown where there is only one rainy season per year. We are, therefore, developing the next generation of seed treatments based on high capacity ion exchangers, and report some results below showing that they facilitate *Striga* control under simulated high rainfall, as also described below.

MATERIALS AND METHODS

The breeding of maize varieties that have been released is described in Kanampiu *et al.* (2003). The development of the seed dressing protocols being commercially used are described in

Kanampiu *et al.* (2001; 2002; 2003). Micronized technical grade imazapyr acid is added to commercial fungicide/insecticide seed dressings and applied to the seeds. The slow release formulations have imazapyr bound to anion exchangers such as Dowex 1 or DEAE cellulose, and then were mixed with the fungicide/insecticide seed dressings, or in some cases bound to the seed with polyvinylpyrollidone, as described in Burnet *et al.* (2004).

RESULTS AND DISCUSSION

The research and development described above began utilizing the initial IR-maize developed for the USA market. This material was very susceptible to turcicum leaf blight, leaf rust, gray leaf spot, and maize streak virus disease, as well as having low yield potential. A breeding program was therefore initiated in Kenya to incorporate adaptations to the local environment. High yielding and disease resistant IR-maize inbred lines, hybrids and open pollinated varieties with increased yields were gradually achieved, while the above experiments were being performed with synthetic open-pollinated varieties with increasing levels of adaptation. This material was subjected to extensive multi-site testing in western Kenya (Figure 1).

Multi-site field tests in seven countries demonstrated that herbicide seed-coating of herbicide-resistance maize controls both *Striga hermonthica* and *S. asiatica* (Kanampiu *et al.*, 2003). Varieties adapted for western Kenya did not always outperform local varieties in yield, despite the *Striga* control, indicating a need to back-cross the IR material into locally adapted material to control both *Striga* and improve yields (Kanampiu *et al.*, 2003).

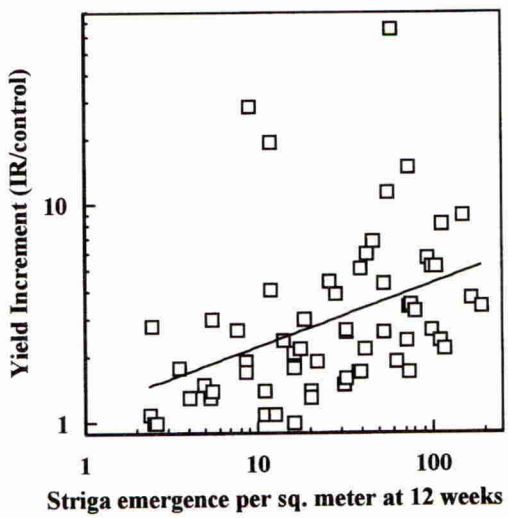

Figure 1. Increment of yield increase of imazapyr seed treated IR maize compared to untreated as a function of *Striga* attachment, in multi-site testing

Following proof of concept in the field, imazapyr was registered as a seed treatment by BASF and the technology and open-pollinated as well as hybrid varieties were tested and approved in Kenya by the regulators after finding excellent *Striga* control and high maize yields (Table 1). Three seed companies have produced 10 tons of seed (enough to plant 550 hectares) for September/October 2005 planting and about 120 tons of certified seed will be ready (6,600 hectares) for March 2006. The first commercial launch in western Kenya was July 2005 after extensive pre-release demonstrations of the technology throughout Western Kenya. The first new maize hybrid will be marketed under the common commercial name *Ua Kayongo H1* (*Striga* killer) with the assistance in dissemination by a collection of NGO's and other international organizations that had previously conducted extensive on-farm demonstrations.

Table 1. Grain yield and *Striga* count of IR-hybrids compared to local hybrids in 10 farmers' fields in western Kenya, 2004

Germplasm	Yield (t/ha)	*Striga* emergence (m^2)	Status
Local susceptible hybrid			
H513	3.2	3.6	(Check)
CIMMYT IR hybrids			
CKT036071-IR	7.2	0.45[a]	Released
CKT036069-IR	6.3	0.81[a]	Released
CKT026065-IR	6.1	0.83[a]	Released
CKT036067-IR	5.9	0.75[a]	Released
CKT026061-IR	5.6	0.76[a]	Not released

[a] The emerged *Striga* did not set seed

It was clear from all the field tests (Figure 1, Table 1 and others) that the new hybrids and inbreds performed far better in Kenya than the local varieties, especially under heavy infestations. The few *Striga* stalks that emerged did so late in the season, such that they failed to set seed, and thus did not replenish the seed bank. The higher yields at low infestation may be due to controlling *Striga* that attached and did not emerge, and/or due to the superior disease resistance of the hybrids.

Where late emergence was observed, it was clear that the duration of *Striga* control was shortened by herbicide leaching due to a longer season or higher raninfall. To reduce leaching and maintain control, the herbicide was combined with novel slow release seed dressings that were generated by binding imazapyr to high capacity ion exchangers (>1meq imazapyr bound/g exchanger). Previously generated formulations using similar technologies had a more than ten fold lower exchange capacity, and would be far too bulky for seed dressings (e.g. Mishael *et al.* (2002). The effectiveness in preventing leaching was demonstrated in a simulation experiment using large pails for cultivating maize with *Striga* (Figure 2). The data show that circa 2-3-fold less herbicide was needed under all rainfall regimes for equivalent *Striga* control. Seed dressings with slow release herbicide were also needed to prevent crop phytotoxicity, early in dry seasons. It was seen in the field that the formulations also abolished the transient phytotoxicity observed with low rainfall. These results were obtained with prototype materials and improved derivatives are in field testing. It appears likely that this approach to targeted pesticide application should be applicable with other pesticides, especially seed or soil applied compounds.

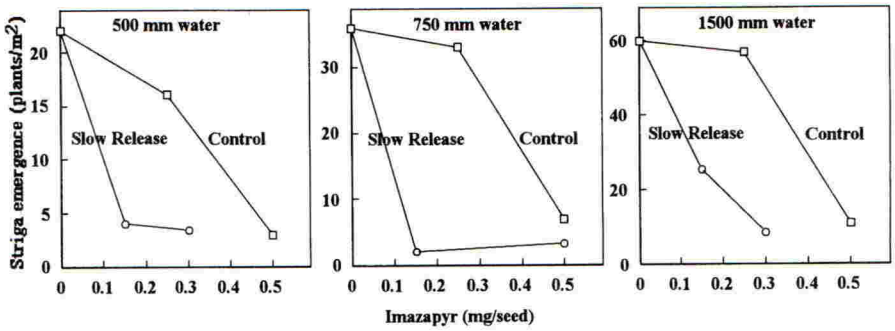

Figure 2. Enhanced control of *Striga hermonthica* on IR-maize with slow release formulations of imazapyr (averaged) under different rainfall regimes. *Striga* emergence was measured 12 weeks after planting using either unformulated imazapyr or the formulated form. Natural rainfall was supplemented by sprinkler irrigation to achieve the desired regimes.

The slow release formulants used are GRAS (generally regarded as safe by the US FDA), generally biodegradable, and the pesticides are always in the parent form so that no "novel" pesticides are formed that would require registration of new molecules.

In summary, herbicide seed treatments provide affordable season long *Striga* control suitable for subsistence farmers. In many cases, a general weed free zone was observed by the farmers around the maize plants, allowing later and easier hand weeding and less weed competition with the crop. Although already very successful, the approach is being improved through the combination of improved locally adapted varieties and optimised herbicide formulations to enable its broader adoption in diverse agro-ecological zones in sub-Sahara Africa.

ACKNOWLEDGEMENTS

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Improved plant protection by a unique seed delivered nematicide

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ABSTRACT

Abamectin applied as a coating to cotton and various vegetable seed at rates of 0.1 to 0.3 mg a.i./seed resulted in early season seedling protection against plant-parasitic nematodes that was comparable to current standards of soil-applied non-fumigant nematicides. In a cucumber field trial, abamectin treatments resulted in faster fruit setting and development as well as higher yield compared to the non-treated control. In 55 US cotton field trials with predominantly root-knot and/or reniform nematode infestations, seed coating with abamectin at 0.15 mg/seed resulted in an average yield increase that was at least equivalent to the soil-applied standard aldicarb at 841.4 g/ha. This results in a significant reduction of nematicide application and in combination with insecticide and fungicide seed coatings is a novel tool for plant protection.

INTRODUCTION

Management strategies for soil borne plant-parasitic nematodes generally are designed to reduce population levels below damage thresholds since eradication is rarely feasible in the field. Such practices include crop rotation, resistant cultivars, cultural practices, and nematicides.

Successful cropping in fields infested with plant-parasitic nematodes should allow the crop sufficient time to establish a healthy root system. Typically, this is accomplished within four to six weeks of planting. However, this situation can change when plants are subjected to additional biotic or abiotic stress.

Current organophosphate or carbamate nematicides are limited by the efficacy and environmental toxicological constraints. Most of these contact materials have a nemastatic rather than nematicidal mode of action. Repeated applications lead in some cases to accelerated soil biodegradation that further reduced their activity (Smelt *et al.*, 1987). Enhanced biodegradation has been reported with all currently registered carbamates and organophosphate nematicides (Moens *et al.*, 2004). Although some of these compounds showed potential as seed treatments, the loading rate was a delicate balance act between sufficient efficacy and phytotoxicity (Truelove et al., 1977, Brown, 1984, Rodriguez-Kabána

and Weaver, 1987, Townshend, 1990). Consequently, seed treatments with nematicides did not gain acceptance (Rodriguez-Kabána and Kokalis-Burelle, 1997).

Abamectin is a mixture of macrocyclic lactones (> 80% avermectin B1a and < 20% avermectin B1b) that are metabolites of *Streptomyces avermitilis* and possess exceptional insecticidal and anthelmintic activity (Putter *et al.*, 1981). The activity on *Meloidogyne incognita* in greenhouse tests at rates of 0.16-0.24 kg abamectin/ha was found to be about 10-30 times more potent than current contract nematicides. Use of abamectin as a soil-applied nematicide seemed initially promising (Garabedian and Van Gundy, 1983) but further tests showed the product often lacked activity in the field (Nordmeyer and Dickson, 1985), possibly due to abamectin's low water solubility (~8 ppb) and tight binding to soil and organic matter. Currently, abamectin is widely registered and extensively used as an insecticide, miticide, and anthelmintic, but not as a nematicide against plant-parasitic nematodes. However, recent reports have demonstrated the effectiveness of abamectin as a seed coating for early season protection against nematode pests (Becker *et al.*, 2003, 2004).

This project focused initially on cucumber and cotton. Cucurbits are extremely sensitive to root-knot nematodes and no useful resistance has been found in commercially available cultivars. The relatively large seeds allowed evaluating various loading rates prior to optimizing the formulation. Cotton presented the opportunity to evaluate the nematicide seed coating in large-scale field trials over a wide geographical area. In US cotton, estimated yield losses due to plant-parasitic nematodes exceed 4% (Koenning *et al.*, 2003). Aldicarb is applied to ~90% of the cotton fields that receive a nematicide. Typically, it is applied in the furrow to reduce both early season insects and plant-parasitic nematodes. However, increasing cost and potential regulatory issues drive the search for economically reasonable and ecologically sensible alternatives. The currently available seed treatments against plant-pathogenic fungi and insect pests have made significant improvements with the introduction of phenylpyrrole, triazole, strobilurin, and neonictinoid chemistries. This paper will demonstrate the usefulness of abamectin as a unique new nematicide seed coating.

MATERIALS AND METHODS

Laboratory tests

All seeds were treated and provided by Syngenta Crop Protection. Initially, abamectin was evaluated in pouch and greenhouse trials using seed coating loading rates of 0.03, 0.1, and 0.3 mg a.i./seed. These trials were conducted on cucumber seed (cv. Straight Eight, Burpee Seed Co.) against root-knot nematode (*Meloidogyne incognita* race 1) using phenamiphos as a standard treatment. The pouch tests were conducted according to a modification of a previously published method (Preiser *et al*, 1981). Two seeds were inserted into each pouch and inoculated with 300 root-knot nematode eggs in 9 ml of water. A second set of pouches was inoculated with 600 root-knot eggs 7 days after seeding. After 5 days, for each treatment, 6 replicates were selected to ensure sets of seedlings with uniform growth. The pouches were arranged in a randomized complete block design and placed in an upright position in a growth chamber at 26°C and 14,700 lx illumination with a 12-hour day-night cycle. The pouch moisture level was monitored daily and water was added as needed. The numbers of root-knot galls were counted after 2 weeks.

Greenhouse experiments

Pulp pots were filled with 500 cc steam-pasteurized river bottom sand. Two identical sets of 5 treatments with 6 replicates were prepared. The first set was infested at seeding; the second

set 7 days later. In the first set, each pot was infested with ca. 5,000 eggs of *M. incognita* race 1. This level of inoculum resulted in a medium disease pressure (expected gall rating of ~5). The second set was inoculated with ca. 10,000 eggs, which results in very severe disease pressure and even death of seedlings in the non-treated controls. Root-knot inoculum was raised during the previous three months on tomato plants in the greenhouse. Nematode eggs were harvested by a bleach/sieving extraction (Hussey and Barker, 1973). Each pot received slow release fertilizer at the recommended rate for tomato production. The plants were incubated in the greenhouse at ca. 24°C +/- 3°C and ambient lighting. Irrigation was applied daily as needed. Five weeks after inoculation, the plants tops were cut off, dried in a drying oven overnight, and their weight recorded. The roots were placed in eryoglaucin solution overnight and the stained egg masses of root-knot nematodes were counted (Omwega et al., 1988). Root galling was rated on a scale of 0-10 (0 = no galling) (Zeck, 1971). The eggs were released by shaking the egg masses in 10% bleach solution for 1 minute. Sub-samples were counted under a dissecting scope. Soil sub-samples were collected from the pots and second-stage juveniles (J2) were extracted with modified Baerman funnel at 26°C for 5 days. The J2 were enumerated with the aid on a dissecting scope.

Field trials

A cucumber trial was conducted at the University of California South Coast Research and Extension Center, Irvine, CA. The soil at this site was a San Emigdio sandy loam (12.5% sand, 75.4% silt, 12% clay, 0.45% organic matter, and pH 7.2), previously cropped to root-knot nematode-susceptible tomatoes. The site was heavily and fairly uniformly infested with *M. incognita* race 1. The trial was designed as a randomized complete block with 5 replications on 10 m x 0.68 m beds. Fertilizer, weed and insect control, and irrigation were applied using drip tubing with 2 l/hr emitters spaced at 0.3 m. All agricultural operations were conducted according to local standards. Oxamyl was applied as Vydate L at 2.24 kg a.i./ha via the drip irrigation system during 2 hour runs. This application was at 2 and 6 weeks after planting, leaving the young seedlings intentionally unprotected for the first two weeks. All data were subject to ANOVA and, if appropriate, means separation with Fisher's LSD (Super ANOVA, Abacus, Berkeley, CA).

Beltwide cotton trials in 55 fields with various degrees of plant-parasitic nematode infestation (mainly *M. incognita* race 3 and *R. reniformis*) were conducted from 2001 to 2004. In each trial, all seed was coated with the fungicides mefenoxam + fludioxonil + azoxystrobin ('Dynasty CST') at 0.3 mg a.i./seed. The three experimental treatments were: (1) non-treated, (2) aldicarb ('Temik 15G') applied in furrow at 5 lbs of product per acre (841.4 g a.i./ha), and (3) abamectin ('Avicta') at 0.15 mg a.i./seed (24.7 gr. a.i./ha) + thiamethoxam ('Cruiser') at 0.34 mg. a.i./seed. Thiamethoxam was used to control early season insects. Among the ratings taken on these trials were nematode assessment and yields.

RESULTS AND DISCUSSION

Laboratory Tests

All abamectin-treated cucumber seeds germinated readily with no obvious signs of phytotoxity at the three rates tested. Each rate significantly reduced galling caused by root-knot nematodes (*M. incognita*) even if the infestation was delayed for 7 days (Figure 1). The good efficacy in this closed soil-less system was expected as Wright et al (1984) had demonstrated avermectins

can impair invasion and development of *M. incognita* at $3x10^{-4}$ g a.i. cm^{-3}. The fact that invasion and development of J2 were reduced at concentrations much lower than needed to immobilize them lead to speculation that abamectin might be affecting larval behavior prior to invasion (e.g. ability of host finding, choosing penetration sites). In vitro tissue binding studies suggested that effects of avermectins on invertebrates might be experienced at nanamolar or even picomolar concentrations (Fisher & Mrozik, 1992).

Greenhouse experiments

The two highest rates (0.1 and 0.3 mg/seed) significantly reduced root-knot nematode-caused galling (Figure 2, 3), and nematode egg masses (Figure 4, 5). Based on enumeration of J2 in the roots 7 days after seeding (data not shown), the reduction of egg masses is mainly related to fewer females in plants developed from abamectin coated seeds. Even at extremely high infestation levels that killed all non-treated seedlings, the majority of the abamectin-treated plants survived. The population reducing effect of the 0.1 and 0.3 a.i. mg/seed rate was not diminished when nematode infestation was delayed for one week. These trials were conducted under conditions that were close to ideal for the nematode and the efficacy of the seed treatment. The previously steam-pasteurized soil was likely to be less diverse in microbial population that might potentially influence the speed of the biodegradation of the active compound. In addition, by using sand as the growth substrate, the previously reported (Putter *et al*, 1981) inactivation by binding to organic matter or clay minerals was presumably greatly reduced. Finally, watering limited to plant need reduced the change of leaching even though avermectins are not very mobile in soil (Putter *et al*, 1981).

Field trials

The cucumber seed treatment trial was established under a high initial root-knot nematode population density (P*i*). Soon after emergence, all seedlings in the non-treated plots or in the oxamyl-treated plots that received the nematicide treatment about 2 weeks after seeding appeared stunted compared to the seedlings in the plots with abamectin seed treatments. The inability of oxamyl to compensate for the two-weeks of non-protection confirms earlier reports that at least with certain crops the first couple of weeks determine a large part of the yield potential (Seinhorst, 1995, Ploeg and Phillips, 2001). Height measurements (Figure 6) and biomass (Figure 7) taken at mid-season show that there was no difference among the abamectin seed treatments, while the difference to the non-treated control and oxamyl treatment remained significant. Root galling at this time was minor. However, there was an obvious lack of feeder roots in the non-treated control and oxamyl treated plots. At harvest, the feeder roots in the abamectin treated plots were heavily galled while the control plots had plants with a few main roots and very little galling. Consequently, the J2 populations were higher in the plots with abamectin treated seeds although the reproduction index (P*f*/P*i*) did not differ significantly among the abamectin treatments and the non-treated control (Figure 8). It is likely that the initial nematode attack of the feeder roots in the non-treated control caused the abortion of those roots. Similarly, the application in the oxamyl plots came too late to stop this early nematode attack. The loss of feeder roots was likely promoted and accelerated by micro-organisms that followed the nematode into the roots. Often plants from abamectin-treated seed appear lighter in color than the non-treated control. Clearly, benefits of abamectin treatments in the field need to take potential nematode/fungal interactions into consideration. Fruit setting and development were faster in all the abamectin treatments compared to the non-treated control and oxamyl treatment. Furthermore, both fruit weight and number per plot at the first

harvest were significantly increased. The yield differences between the plots with seed treatments and non-treated or oxamyl treated plots were only minor at the second harvest. This was an indication that the non-treated plants either caught up with the treated ones or, more likely, that the larger root systems in the abamectin treatments provided more feeding sites for the nematodes. The increasing root-knot nematode population started to affect the treated plants in diminishing the root size advantage. The highest combined yield was recorded in plots seeded with abamectin (0.3 mg/seed) and treated with oxamyl 2 and 6 weeks after seeding (Figure 9).

The data show the value of seed treatment. The length of the beneficial effect of seed coating will certainly depend on the crop and the environmental conditions. The presented cucumber trial was conducted under high disease pressure with soil temperatures that allow two root-knot nematode generations. In earlier research with furathiocarb, carbofuran, or oxamyl nematicide seed treatments wheat yields increased when used in cereal cyst and lesion nematode infested fields (Brown, 1984, Orion and Shlevin, 1989). Part of that success was likely due to a relative short fall season before the decreasing soil temperatures during the winter months also reduced the parasitic activity of the nematodes.

Beltwide Cotton Trials

Yield assessments from the 55 field trials indicated an average 10.8% yield increase of the nematicide treatments compared to the non-treated control (Fig. 10). In assessing the yields, if the differences were greater than 3%, the assumption was made they were indeed different. Abamectin out-yielded aldicarb in 46% of the trials, aldicarb out-yielded abamectin in 29% of the trials, and abamectin was comparable or better than aldicarb in 73% of the trials. When the treatments were compared to the non-treated control, abamectin was less in 11% and aldicarb was reduced in 22% of the trials. A more precise analysis must take the pre-season nematode-infestation levels and potential insect and disease infestations into consideration, as in some cases there might not have been sufficient disease pressure to warrant nematicide application at all. In others, the systemic insecticidal activity of aldicarb might have provided additional benefits. However, despite the risks of a fairly general evaluation, the abamectin seed treatment proved to be at least equal to aldicarb application at 840 g/ha.

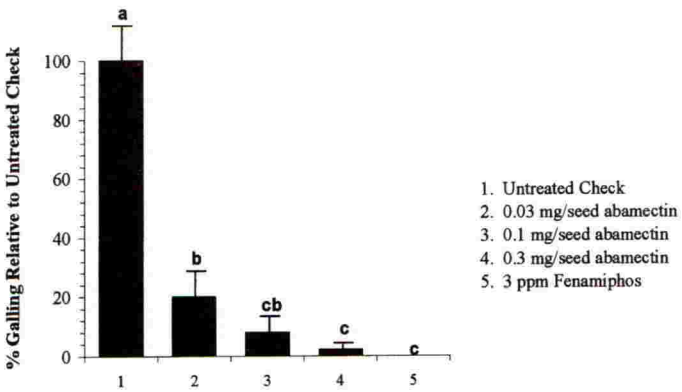

Figure 1. Effect of abamectin on root galling caused by *Meloidogyne incognita* in a soil-less pouch test; concurrent seeding, and nematode inoculation.

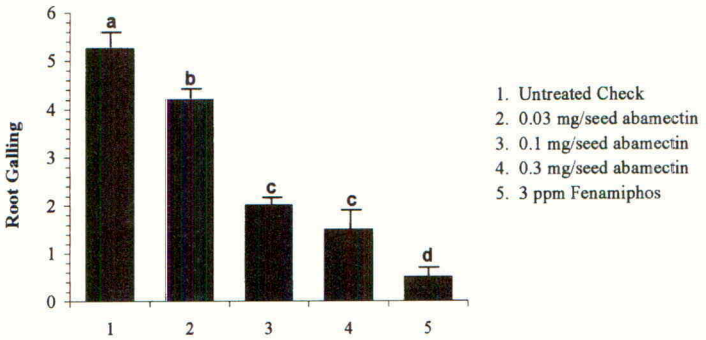

Figure 2. Effect of abamectin on root galling (0-10 scale) using ca 5,000 eggs of *M. incognita* at seeding. Bars indicate standard error and the same letters indicate means are not significant different (P= 0.05).

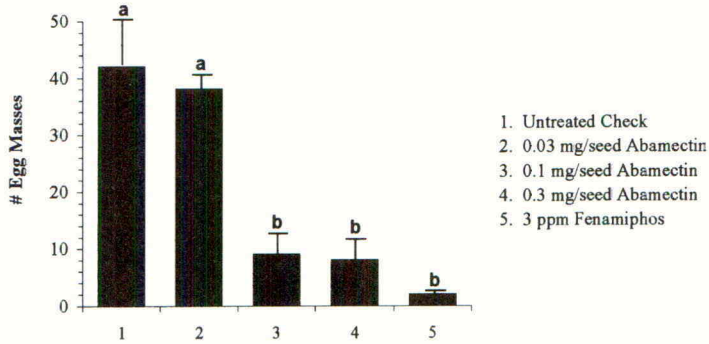

Figure 3. Effect of abamectin on root galling (0-10 scale) using ca 10,000 eggs of *M. incognita* inoculum 7 days after seeding. Bars indicate standard error and the same letters indicate means are not significantly different (P= 0.05).

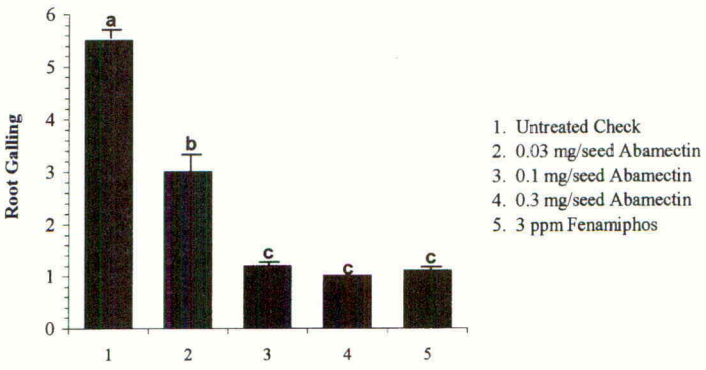

Figure 4. Effect of abamectin on egg mass production of *M. incognita* using ca 5,000 eggs at seeding. Bars indicate standard error and the same letters indicate means are not significant different (P= 0.05).

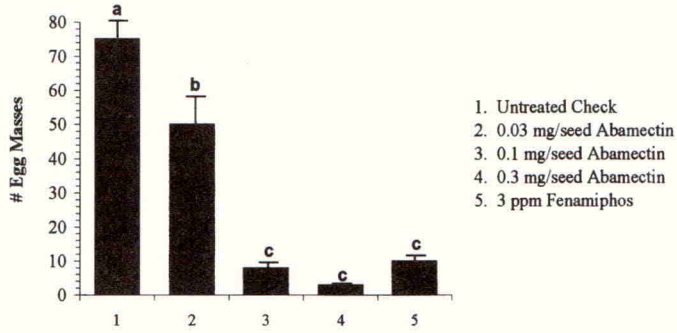

Figure 5. Effect of abamectin on egg mass production using ca 10,000 eggs of *M. incognita* inoculum 7 days after seeding. Bars indicate standard error and the same letters indicate means are not significant different (P= 0.05).

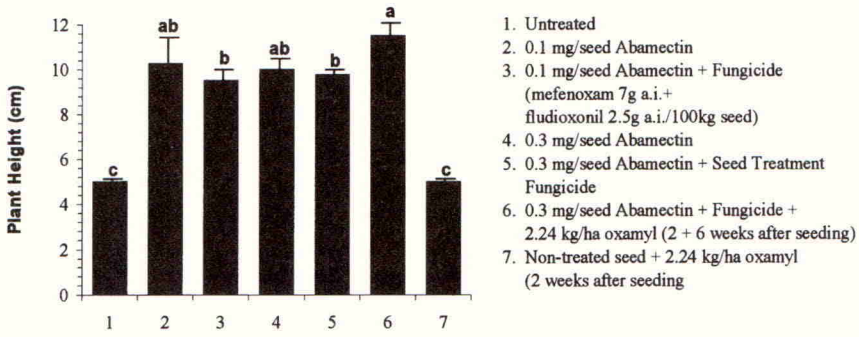

Figure 6. Impact of abamectin on cucumber plant height 30 days after seeding in the field in California. Treatment means are 8 plants per plot, and 5 replicates per treatment. Bars indicate standard error and the same letter indicate means are not significantly different (P= 0.05).

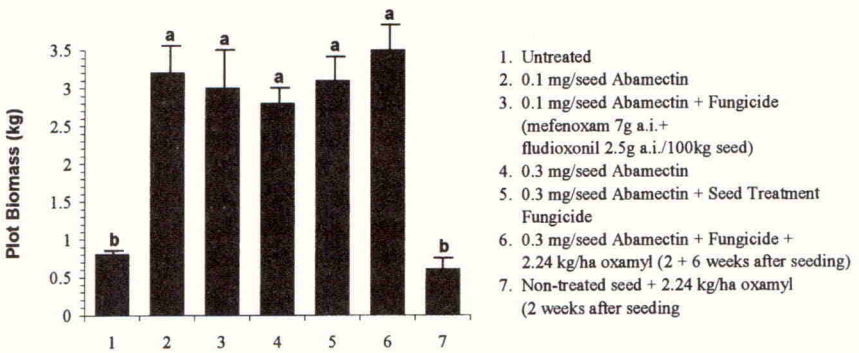

Figure 7. Impact of abamectin on cucumber fresh foliar biomass at 6 weeks after seeding. Evaluations made on 12 plants per plot, 5 replicates per treatment. Bars indicate standard error and the same letters indicate means are not significantly different (P= 0.05).

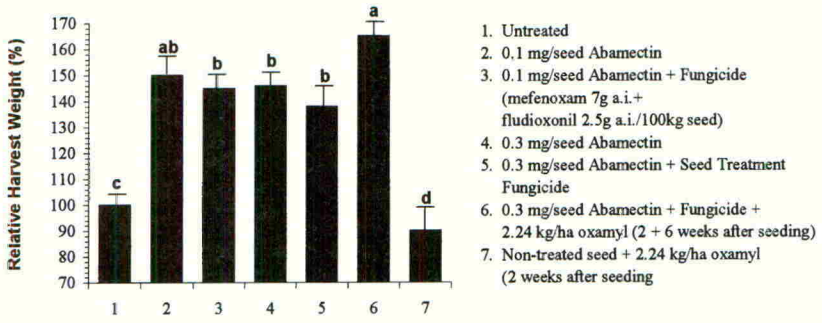

Figure 8. Relative yield of cucumbers from two pickings (non-treated check = 100%). Bars indicate standard error and the same letters indicate means are not significantly different (P= 0.05).

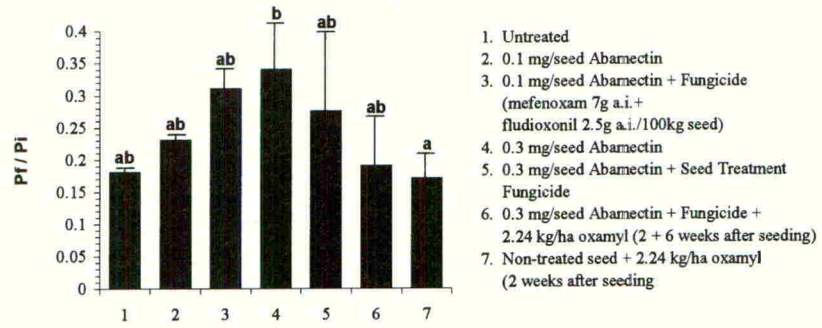

Figure 9. Effect of abamectin on nematode reproduction in a root-knot infested field in California P*f*/P*i* (harvest J2 population/pre-season J2 population) was log (x + 1) transformed for statistical analysis. Non-transformed data shown with bars indicating standard error. The same letters indicate means are not significantly different (P= 0.05).

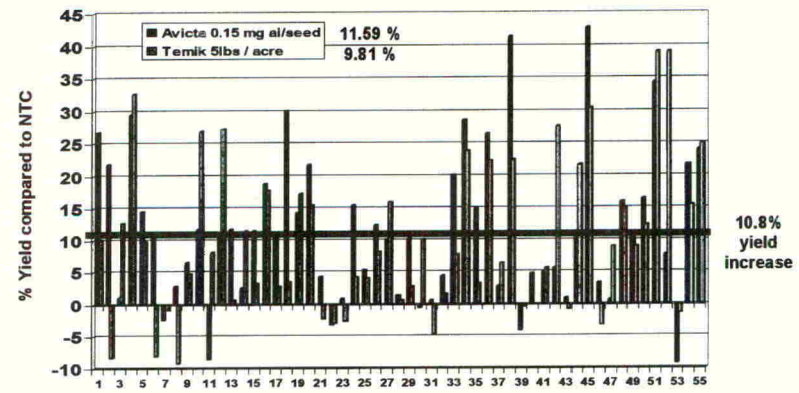

Figure 10. Yield comparison of Avicta and Temik in 55 cotton trials conducted in the US between 200 and 2004 against nematodes.

CONCLUSIONS

Abamectin applied as a seed coating at rates between 0.1 and 0.3 mg a.i./seed provided excellent early season protection against a range of plant-parasitic nematodes. In cucumbers, the significant delay of root-knot nematode attack resulted in more fruits and higher weights compared to the non-treated control. In cotton, this biological activity provided yields equal to, or better than the current standard aldicarb in >70% of the 55 field trials conducted in the US. Abamectin resulted in a mean yield increase of 11.59% over the non-treated control. The dramatic reduction in pesticide application rates attributed to seed coating technology is a major advancement in crop protection against plant-parasitic nematodes. Avicta received in 2005 an EPA label for use on cotton as a reduced risk pesticide.

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Na-dikegulac, a novel chemical for enhancement of seed storage potential and seedling health of two pulse crops

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ABSTRACT

Seed pretreatment of grass pea (*Lathyrus sativus* cv. BR-13) and black gram (*Vigna mungo* cv. WB-17) with sodium-dikegulac (Na-DK, 250 µg/ml) significantly reduced the loss of seed germinability and the time (h) required for 50% germination (T_{50}) under artificially imposed accelerated ageing condition (99.5% r.h., 32±2°C temp.) for 30 d. Seeds pretreated with the chemical for 8 hours before imposition of accelerated ageing treatment ameliorated such deleterious effects on germination. The chemical also significantly arrested the ageing-induced reduction of protein and DNA contents as well as activity of catalase in seed kernels. Potential performance of the seedlings, raised from acceleratedly aged (0 and 30 d) seeds was measured in terms of chlorophyll and protein levels as well as activities of catalase and IAA-oxidase in leaves of 20 d old seedlings. The seedlings raised from the chemical pretreated seeds were superior to control ones as per the biochemical and physiological data recorded in this investigation. Sodium-dikegulac appears to be efficient for maintenance of seed storage potential as well as field performance of seedlings of the two grass pea and black gram cultivars.

INTRODUCTION

Maintenance of seed storage potential and seedling health in tropical and subtropical countries including India is a serious concern to growers due to high temperature and high relative humidity prevailing in major parts of the country almost throughout the year (Basu, 1994).

Because of the problem of seed storing in India due to adverse ambient storage situations, an attempt was made in this investigation to prolong the storage life of black gram and grass pea seeds which undergo rapid deterioration under storage, using sodium-dikegulac.

Since its first report as a potent plant growth regulator (Bocion *et al.*, 1975) sodium-dikegulac (2, 3 : 4, 6-di-O-isopropylidene-α-L-xylo-2 hexalofuranosate) or 'Atrinal' was established as a growth retarding chemical performing a number of physiological functions in plants including regulation of seed vigour and viability (Bhattacharjee *et al.*, 1986; Bhattacharjee, 2001). The effect of the chemical was assessed in the laboratory and in field experiments.

The prime objective of the present investigation was to investigate the efficacy of sodium-dikegulac on enhancement of seed storage potential and seedling health of black gram and grass pea cultivars.

MATERIALS AND METHODS

The experiments were carried out with 100% viable seeds of black gram (*Vigna mungo* cv. WB-17) and grass pea (*Lathyrus sativus* cv. BR-13) under artificially imposed environmental condition i.e. accelerated ageing to obtain expeditious and relatively uniform results.

After surface sterilization (0.1% $HgCl_2$ for 90 s) the seed samples of each cultivar were soaked in the aqueous solution of sodium-dikegulac (250 µg/ml) or distilled water (control set) for 4 h and then dried back to the original dry weight of the seeds. This was repeated twice allowing maximum penetration of the chemical into seeds and avoiding commencement of germination. The total duration of treatment was 8 h in two instalments (4h + 4h in close succession). The pretreated seed lots were then stored in separate cloth bags in a desiccator in which 99.5% r.h. was preimposed by keeping 250 ml 1.57% H_2SO_4 within it. This experimental set-up was kept at 32±2°C for 30 d allowing the seeds to experience forced ageing treatment and H_2SO_4 was changed at 7 d intervals to restore the desired r.h. throughout the 30 d period.

Data on germination behaviour and metabolism of seeds were analysed after 0, 15 and 30 d of accelerated ageing. Analysis of seedling health, in terms of some biochemical parameters, were recorded from 20 d old seedlings grown from acceleratedly aged seeds (0 and 30 d).

Percentage germination of seeds was assessed following the ISTA rules ISTA (1976). The time required for 50% germination of seeds (T_{50}) was determined following the method of Coolbear *et al.* (1984).

Protein and DNA levels as well as activity of catalase were analysed from seed kernels of each sample following the method of Lowry *et al.* (1951), Cherry (1962) modified by Choudhuri & Chatterjee (1970) and Snell & Snell (1971) respectively.

Potential status of 20 d old seedlings raised from 0 and 30 d of accelerated ageing seeds were analysed from leaves of each treatment in terms chlorophyll and protein levels and activities of catalase and IAA-oxidase enzymes.

Extraction and estimation of chlorophyll and protein was done as per the methods of Arnon (1949) and Lowry *et al.* (1951) respectively. Activities of catalase and IAA-oxidase were done as per the methods of Snell & Snell (1971) and Gordon & Weber (1951) respectively.

The data were statistically analysed as per Panse & Sukhatme (1976). In tables LSD values (at 5% level) were incorporated.

RESULTS

Data clearly showed that with the progressive period of accelerated ageing (0 to 30 d), percentage germination (Fig. 1) was decreased and T_{50} values (Fig. 2) were increased both in control and sodium-dikegulac treated seed lots. But the magnitude of decrease or increase was significantly less in sodium-dikegulac pretreated seed lots.

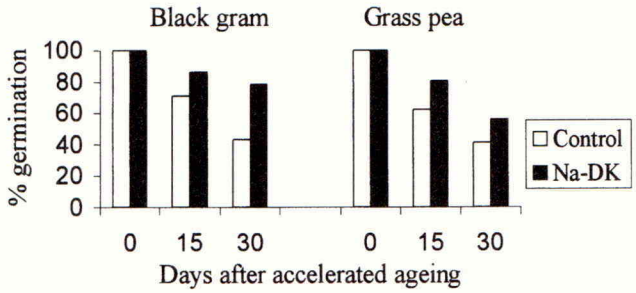
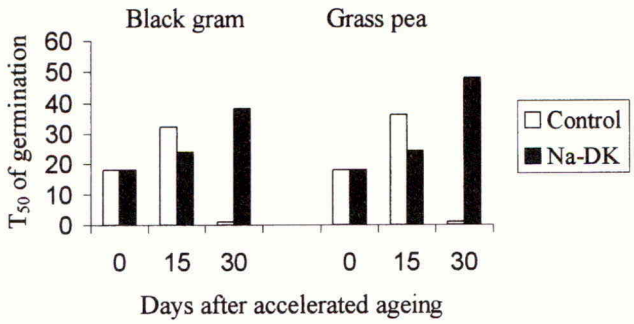


Figure 1. Effect of seed pretreatment with Na-DK on percentage germination of black gram and grass pea seeds stored under accelerated ageing condition for 30 d.



— Nonattainment of 50% germination

Figure 2. Effect of seed pretreatment with Na-DK on T_{50} values (h) of germination of black gram and grass pea seeds stored under accelerated ageing condition for 30 d.

Concomitantly, ageing-induced reduction of catalase activity (Fig. 3) as well as protein and DNA levels (Table1) in seed kernels were diminished by the seed pretreating agent.

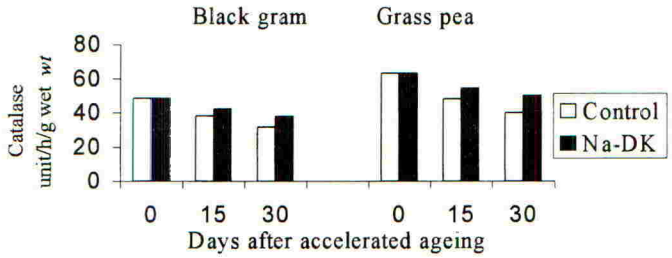


Figure 3. Effect of seed pretreatment with Na-DK on the changes of catalase activity of black gram and grass pea seeds stored under accelerated ageing condition for 30 d.

Table 1. Effect of seed pretreatment with Na-DK on protein and DNA contents in kernels of black gram and grass pea seeds stored under accelerated ageing condition for 30 d.

Seed species	Treatments	Protein (mg/g wet *wt*)			DNA (µg/g wet *wt*)		
		Days after accelerated ageing					
		0	15	30	0	15	30
Black gram	Control	238.46	209.20	156.28	71.25	60.19	46.44
	Na-DK	238.66	232.14	211.54	71.36	68.21	54.17
	LSD (*P*=0.05)	NSD	18.95	15.58	NSD	6.00	4.65
Grass pea	Control	280.62	211.98	181.53	70.20	54.16	39.96
	Na-DK	280.68	239.17	216.72	70.28	62.00	48.15
	LSD (*P*=0.05)	NSD	20.11	17.96	NSD	5.42	4.01

LSD, Least singnificant difference; NSD, No significant difference.

Table 2. Effect of seed pretreatment with Na-DK on chlorophyll and protein contents as well as activities of catalase and IAA-oxidase in leaves of black gram and grass pea seedlings (30 d old)raised from seeds which experienced accelerated ageing for 0 and 30 d.

Seed species	Treatments	Chlorophyll (mg/g fresh *wt*)		Protein (mg/g fresh *wt*)		Catalase (mg/g fresh *wt*)		IAA-oxidase (unit/h/g fresh *wt*)	
		Days after accelerated ageing							
		0	30	0	30	0	30	0	30
Black gram	Control	3.06	1.00	65.08	22.48	28.61	20.01	33.62	56.13
	Na-DK	3.27	3.01	68.17	61.11	29.03	24.18	32.09	42.04
	LSD (*P*=0.05)	NSD	0.11	NSD	2.30	NSD	2.11	NSD	3.25
Grass pea	Control	3.07	0.98	56.62	28.14	32.00	20.11	30.09	56.17
	Na-DK	3.14	2.17	58.18	50.04	33.18	30.07	29.39	39.02
	LSD (*P*=0.05)	NSD	0.10	NSD	2.85	NSD	2.73	NSD	3.21

LSD, Least singnificant difference; NSD, No significant difference.

Reduction of chlorophyll and protein contents as well as activity of catalase was found in leaves of seedlings raised from accelerated aged seeds for 30 d, and such reduction was significantly alleviated in the chemical pretreated samples. Sodium dikegulac also significantly decreased ageing-induced enhancement of IAA-oxidase activity (Table 2).

DISCUSSION

Results of the present study showed that high r.h. treatment accelerated the ageing and deterioration of black gram and grass pea seeds as would be evident from the progressive fall of germination percentage and enhancement of T_{50} hours. However, sodium-dikegulac pretreated seed lots slowed down the ageing-induced loss of germination, reduced T_{50} values, alleviated the loss of protein and DNA (Table 1) contents as well as activity of catalase (Fig. 3). The results thus, indicate that the pretreating chemical rendered the seeds substantially tolerant to withstand the unfavourable storage environment. Efficacy of sodium-dikegulac on maintenance of seedling health can also be supported from the data on the levels of chlorophyll, protein as well as activities of catalase and IAA-oxidase (Table 2) in leaves of 20 d old seedlings. These parameters are often considered as reliable indices for determining the potential status of plants and vigour of seeds (Chattopadhyay, 2003). The experimental chemical showed a considerably promising role on maintenance of seedling health.

Whatever might be the mechanism for the chemical-induced retention of seed vigour and viability under storage and superior health status of seedlings under field condition, results of this investigation can at least indicate that sodium-dikegulac has some efficacy for enhancement of storage potential of seeds and field performance of seedlings raised from such potentiated seeds of the test species.

ACKNOWLEDGEMENTS

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