Contract report for the Horticultural Development Council

Celery: Evaluation of alternative Seed treatments for the control of *Septoria apiicola* (celery leaf spot)

FV 237a

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Project title:	Celery: Evaluation of alternative seed treatments for the control of *Septoria apiicola* (celery leaf spot).
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The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and accuracy. However because of the biological nature of this work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

Authentication

I declare that this work was done under my supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr K R Green
Research Scientist
ADAS Arthur Rickwood

Signature……. Date.…….

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Signature…….. Date……..

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GROWER SUMMARY

FV 237a

Celery: Evaluation of alternative seed treatments for the control of *Septoria apiicola* (celery leaf spot)

Headlines

- A knowledge review has identified a range of alternative seed treatments to evaluate for control of celery Septoria
- Initial results have confirmed the efficacy of hot water treatment for reducing Septoria infection levels in celery seed.

Background and expected deliverables

Celery leaf spot, also known as 'late blight', is the most destructive disease of field grown crops. Initially seen as small brown spots on leaves and stems, the disease can progress rapidly and render the whole crop unmarketable if left unchecked. Seedborne inoculum is thought to be the major cause of outbreaks of celery late blight. Seed treatment remains an important component of disease management for celery *Septoria*. Thiram is currently used as the standard seed treatment for celery *Septoria* as a warm water soak. This fungicide will not, however, be permitted for organic production beyond December 2003. Alternatives to thiram seed treatment are also highly relevant to conventional producers, given continued consumer and retailer pressure for rationalisation of fungicide usage.

The aim of the project is to determine the efficacy of a range of seed treatments for celery *Septoria* that could provide alternatives to thiram, for both conventional and organic celery production. Seed treatments are to be assessed according to their effects on, i) incidence of seed-borne inoculum, ii) seed germination, iii) seedling infection, and iv) seed viability after storage.

The expected deliverables from this work are:

- A knowledge review on alternative treatments for the control of seed-borne diseases, particularly in relation to *Septoria* spp.

- Information on the efficacy of a range of seed treatments for celery Septoria, enabling the industry to make an informed decision on viable alternatives for use in celery production.

Summary of the project and main conclusions

Completed work for the period April – December 2002:

- A knowledge review on alternative treatments for the control of seed-borne diseases, particularly in relation to *Septori*a spp.
- Selection of seed treatments for evaluation based on the knowledge review
- Preliminary studies to determine appropriate techniques for estimating infection levels in seed batches naturally infected with *S. apiicola.*

Based on the knowledge review, seed treatments under the following categories have been selected for evaluation. It is intended that some of the selected treatments will be appropriate for organic seed production while others will be more relevant for conventional production. Experiments have been initiated on microwave, hot water and UV treatments and have been partially completed. Further experiments are planned for January to March 2003.

- Fungicides: Beret Gold (fludioxonil) and Wakil XL (cymoxanil + fludioxonil + Metalaxyl-M)
- Hot water treatments
- Microwave treatments
- UV treatments
- Disinfectant treatment (hydrogen peroxide)
- Plant oils and extracts
- Biological control (*Pythium oligandrum*)

A batch of celery seed naturally infected with *S. apiicola* has been sourced for use in seed treatment evaluations (hybrid TZ9783). The percentage of seeds in the batch with viable *Septoria* infection was estimated at 10 % based on observation of release and germination of spores from seeds plated on agar plates in individual water droplets.

A rapid method to determine spore germination from treated seed samples has been used successfully to date to evaluate the efficacy of hot water, microwave and UV treatments in eliminating *Septoria* from celery seed. For example, for seed exposed to a hot water treatment

of 48ºC for 30 min following a 16 hour pre-soak, *Septoria* spore germination was 0.4 % compared with 39.1 % in the untreated control.

Financial benefits

None to date

Action points for growers

None to date

SCIENCE SECTION

1. Introduction

Within the vegetable industry, there is increasing interest in alternative seed treatments for the control of seed-borne diseases, both i) in conventional production, due to retailers' preference for minimal pesticide usage, and ii) in organic systems, where the use of conventional seed will not be permitted after December 2003 (EU regulation 2092/91).

There is particular concern regarding diseases for which seed represents the primary source of inoculum such as celery leaf spot, caused by *Septoria apiicola*. Celery leaf spot, also known as 'late blight', is the most destructive disease of field grown crops. Initially seen as small brown spots on leaves and stems, the disease can progress rapidly and render the whole crop unmarketable if left unchecked.

Industry efforts are being made to improve the health of celery seed produced overseas and an ongoing project (HDC FV 237) is addressing the optimisation of fungicide timing as a component of IPM. Seed treatment, however, remains an important component of disease management for celery *Septoria*, since a seed infection rate as low as 1:7000 can lead to economic loss (Maude, 1996) and resistant cultivars are unlikely to be available in the near future. Thiram is currently used as the standard seed treatment for celery *Septoria* as a warm water soak. This fungicide will not, however, be permitted for organic production beyond December 2003. Alternatives to thiram seed treatment are also highly relevant to conventional producers, given continued consumer and retailer pressure for rationalisation of fungicide usage.

The aim of the project is to determine the efficacy of a range of seed treatments for celery *Septoria* that could provide alternatives to thiram, for both conventional and organic celery production. Seed treatments are to be assessed according to their effects on, i) incidence of seed-borne inoculum, ii) seed germination, iii) seedling infection, and iv) seed viability after storage.

This interim report includes:

- A knowledge review on alternative treatments for the control of seed-borne diseases, particularly in relation to *Septoria* spp.
- Details of protocols to determine appropriate techniques for estimating infection levels in seed batches naturally infected with *S. apiicola.*
- Description of the seed treatments selected for evaluation.

2. Knowledge review

2.1 Current practice

Celery seed for both conventional and organic production may currently be treated with thiram (Agrichem Flowable thiram). The derogation on use of thiram-treated seeds for organic production is due to expire at the end of 2003.

The maximum thiram concentration for seed treatment is 1 litre product in 300 litres of water. Various techniques can be used to enhance the efficacy of the seed treatment, such as a 12 h pre-soak to activate the pathogen prior to fungicide treatment and the use of warm water for the thiram soak (28-30ºC for 8 h). Treated seed should not be stored for the next season.

2.2 Fungicides

Thiram is currently the only fungicide approved for treatment of celery seeds against *Septoria*. While there are several fungicides used for cereal seed treatment that show activity against *Septoria* on wheat, few are available for treatment of seeds of vegetable or salad crops. This is largely due to i) cost of fungicide registration for minor uses, ii) unwanted growth regulatory effects of some seed treatments, and iii) possible fungicide residue issues.

The following list shows comparative activity of seed treatments against *Septoria* on wheat (W. Clark, ADAS Boxworth, pers. comm.):

<u>Good control</u>

Jockey (fluquinconazole + prochloraz)

Ravine (guazatine)

Premis (triticonazole + guazatine)

Moderate control

Anchor (carboxin + thiram)

Baytan (triadimenol + fuberidazole)

Beret Gold (fludioxonil)

Sibutol (bitertanol + fuberidazole)

Beret Gold (fludioxonil) is reported to give moderate control of wheat *Septoria*. The active ingredient fludioxonil has been used in other European countries and the USA (marketed as Celest and Maxim) as a seed treatment for sweet corn, sorghum and potatoes. Fludioxonil in combination with cymoxanil and metalaxyl-M (Wakil XL) is used in the UK as a fungicide seed dressing for peas, against *Ascochyta*, damping-off and downy mildew. There is also an off-label approval for use of Wakil XL as a seed treatment for carrots in the UK. Given the reported activity of fludioxonil against *Septoria* and the use of formulations for food crops other than cereals, there is merit in evaluating both Beret Gold and Wakil XL for their effects on celery *Septoria*.

2.3 Storage time

Some reports suggest that seed storage may be the simplest method to eliminate *S. apiicola* from celery seeds. For example, Krout (1921) showed that conidia and mycelium from pycnidia in the peduncles and pericarp of celery seed gave only 2-3 % germination after 2 years and were of low vitality. The pathogen was dead after 3 years storage. Seed germination was still acceptable after 4 years suggesting that seed could be stored for 3 years before use, to eliminate seed-borne inoculum of *Septoria*. Other reports, however, suggest that infected plants can still be obtained from 5-year-old celery seeds. Maude (1996) argues that differing results may be due to varying storage conditions, which are a major factor in determining the length of survival of seedborne pathogens. Under storage conditions required to ensure adequate seed germination levels (7.3 % moisture content, 5-15^{o}C), spore viability remains at a high level for 3 years in infected seeds (Maude, 1996). This is in agreement with observations from Tozers Seed Ltd (J. Claxton, B. Lincoln, pers. comm.) and suggests that seed storage is not a reliable method for elimination of *S. apiicola* from celery seed.

2.4 Thermotherapy

The use of hot water, aerated steam and dry heat for seed treatment has been reviewed extensively by Maude (1996). The advantages and disadvantages of these different types of

thermotherapy are well known. In summary, hot water treatment has been used since the 1920s and, before the advent of systemic fungicides in the 1960s, was the only treatment available to eradicate deep-seated infections of seed. Water is twice as effective for heat transfer as steam and five times as effective as dry heat. Therefore, temperatures and/or exposure times have to be increased when steam or dry heat is used to achieve equivalent levels of disease control. However, steam-treated seeds require less drying, seed coats are less damaged and germination is not reduced, compared with hot water treatment (Maude, 1996). Use of hot air at high relative humidity (similar to steam treatment) has reported to be successful for control of seed-borne pathogens on cereals in Sweden (Forsberg 2001) with the advantages over hot water treatment of less seed damage and no need for drying. Sanitation effects were equivalent to those obtained with chemicals for all diseases tested (*Tilletia caries* and *Microdochium nivale* in wheat, *Drechslera teres* and *Ustilago nuda* in barley, and *D. avenae* and *U. avenae* in oats). The author commented that the same method was also effective for treatment of parsley seed for *Septoria petroselini.*

Dry heat is again less damaging to seed (compared with hot water or steam) but the high temperatures and long exposures required, mean that there are practical hazards involved. For example, temperatures of 71-84ºC for up to 11 days were needed to eliminate *Xanthomonas campestris* pv *translucens* from barley seeds (Fourest *et al*., 1990).

For the treatment of celery *Septoria*, hot water treatment has traditionally been the preferred method of thermotherapy. The key to successful treatment involves identifying the band of temperatures/exposure times that achieve pathogen kill without reducing seed germination.

Early studies by Krout (1921) showed that spores of *S. apiicola* were not viable after 30 min exposure at 40ºC and mycelium was non-viable at 45ºC. Temperatures of 40-45ºC increased percentage germination compared with an untreated control, 46-49ºC slightly reduced germination and at 50ºC, germination was significantly reduced. The conclusion was that heating celery seeds in water at 48ºC or 49ºC for 30 min eliminates *Septoria* without appreciably affecting percentage germination. A MAFF advisory leaflet (1963) suggests that 25 min at 50ºC is also effective.

Bant & Storey (1952) showed that it was possible to obtain clean celery crops using hot water treatment alone to treat severely infected seed, provided care was taken to minimise infection

from other inoculum sources. It was noted that seed should be hot-water treated within a year of harvest since the treatment may cause considerable reduction in germination in old seed. However the hot water treatment does not affect the longevity of the seed so it is preferable to treat the seed in the harvest year even if it is not to be sown until later (MAFF, 1963).

Pre-soaking (e.g. 16 h) prior to hot water treatment is considered a useful way of 'activating' the pathogen prior to hot water treatment (J. Claxton, B. Lincoln, Tozer Seeds Ltd, pers. comm.). For seeds with surrounding dead tissue (e.g. glumes, fruit coats etc), pre-soaking may also eliminate air from between dead tissue and the actual seed, facilitating the conduction of heat.

For the actual treatment, it is recommended that muslin bags (or similar) half-filled with seed to allow for seed swelling and free circulation of water are used. Other precautionary measures include heating the water to one degree celsius higher than required, since immersion of seed in the water will lead to a temperature reduction. Once immersed in water, agitate the bags from time to time to ensure that the seeds in the middle are heated to the required temperature. The bags should not be in contact with the sides or base of bath. After the appropriate exposure time, seeds can be immersed immediately into cold water, to stop the heating process.

For drying, spread seeds thinly on absorbent paper and leave to dry in a well ventilated room at ambient temperature for 4-7 days. Drying at higher temperatures may affect seed germination.

Given previous successful commercial use of hot water treatment for elimination of celery *Septoria*, this technique will be included in this project.

2.5 Microwave treatment

There are examples of microwave use to eradicate both surface-borne and more deep-seated infection on seeds and planting material of a diverse range of crops.

Microwave treatment was used to eradicate seed-borne pathogens (including *Fusarium* spp., *Cladosporium, Colletotrichum, Diplodia* and *Xanthomonas campestris* pv *manihotis*) and common saprophytic species in cassava true seed (Lozano *et al*., 1986). Microwave exposure

for 120 s at 1400 W heating power and 2450 MHz eliminated fungi and bacteria. The effectiveness of the treatment depended on reaching an optimum temperature of 77ºC. The time taken to reach the required temperature was affected by container capacity, water volume and seed number. Percentage cassava seed germination after this treatment (95 %) was higher than for non-treated seed (65 %).

On soybean, microwave treatment for 30 s (60 kW at 50 Hz) had no deleterious effects on the viability, vigour, moisture content or ultrastructure of seeds and virtually eliminated all internal fungi. The vigour and viability of seeds irradiated for 45 s or longer were substantially affected (Reddy *et al*., 2000). Seeds microwaved for even 15 s exhibited symptoms of severe heat stress and desiccation, with the testa rupturing and seeds sometimes bursting. This was alleviated by placing a 500 ml beaker containing 100 ml water into the microwave oven.

The effect of microwave treatment on seed germination can vary according to seed moisture content. For example, microwave heating reduced transmission of soybean mosaic virus, with little reduction in germination of seeds treated at 8.5% moisture content, but germination was considerably reduced in seed treated at 16% (Jolicoeur, 1982).

Hankin & Sands (1977) showed that there is a relationship between seed size and its ability to withstand microwave exposure and remain viable. For example, treatment of tobacco seeds with 625 W microwave radiation for 20 min eliminated viable *E. carotovora* var. *carotovora* from the seed surface without reducing seed germination. In contrast, the germinability of cabbage and bean seeds was completely destroyed by a 2 min treatment because they were unable to radiate heat away from the seed during treatment. Tobacco seeds were placed one layer deep on a paper plate for treatment, since glass containers became heated and plastic containers softened during long exposures.

Microwave treatment was tested to eliminate *Ralstonia solancearum* biovar III (bacterial wilt) from ginger rhizomes (http://ibws.nexenservices.com/Talks/talk_15kumar.htm). Rhizomes exposed to 30 s microwave treatments showed no reduction in germination and emerging plants were free from bacterial wilt. Pulse microwaving (4-5 cycles of 10 s with 5 s between cycles) was also effective. Pulsing treatments were also used to eliminate *Ustilago nuda* (loose smut) from barley seed (Stephenson *et al*., 1996).

Other examples include the use of microwaving for the control of seedborne fungi of Southern Pines and Douglas-fir, and also elimination of the yeast *Nematospora coryli* in heavily contaminated dry-milled flour from mustard seed.

2.6 UV radiation

UV radiation is that part of the electromagnetic spectrum between 200 and 400 nm. UV is conventionally divided in to three components.

- UV-C (200-280 nm) is emitted by the sun but is filtered out by the atmosphere, and does not reach the biosphere (Paul & Gwynn Jones, 2002). Thus, UV-C has no relevance in nature, but can be readily produced by artificial lamps, and such lamps can be used to induce a range of biological effects. UV-C effects include direct kill of many micro-organisms and at high doses cause acute damage to higher plants and animals, while lower doses induce changes in plant chemistry, including the synthesis of antimicrobial phytoalexins. Such responses are exploited in the food industry and have potential for disease control (see below).
- UV-B (280-320 nm) does penetrate the atmosphere, but this depends on the concentration of ozone, and it is the UV-B component of sunlight that is affected by stratospheric ozone depletion. UV-B irradiances within the environmental range are lethal to many microbes, including plant pathogens, and induce resistance mechanisms (Paul, 2000). Higher doses, as achieved by a range of artificial "sun lamps" can cause acute damage to many higher plants.
- UV-A (320-400 nm) represents more than 90% of the total UV present in field sunlight. At field doses it is less damaging to plants and microbes than UV-B, but can induce a range of morphological changes in both plants and fungi, and is important in controlling the sporulation of some plant pathogens. High dose UV-A produced from lamps can induce the production of free-radicals in plants and microbes, and this process can be enhanced by the addition of photosensitisers, such as ozone or psoralens. Combined UV-A plus photosentiser treatments are exploited in the food industry and have potential for disease control (see below).

The potential use of UV radiation to control crop disease is founded on two areas of research. The first are studies using relatively low-dose UV treatments to understand the consequences of changes in environmental UV (e.g. Paul, 2000). Such studies confirm that environmental

UV-B may limit the severity of some crop diseases by killing pathogen spores and exposed mycelium, and/or by inducing changes that confer resistance (Rasanayagam *et al*., 1995; Gunasekera *et al*., 1997). On this basis, the use of higher UV-B doses provided by artificial lamps might have potential for disease control, but this requires careful assessment since there may be a threshold beyond which treatment causes plant damage. It is likely that such thresholds will vary between plants and tissues, largely depending on UV-B penetration in to tissues. The effects of UV-B treatments on seed have very rarely been considered in an environmental context but it is considered that UV-B penetration in to seeds is limited by the opaque seed-coat.

The second area of research informing the use of UV for crop disease control is that seeking to utilise UV treatments as a means of food preservation. Irradiation with UV light is increasingly used to reduce post-harvest disease in a range of fruit and vegetable crops (e.g. Mercier *et al*., 2000; Nigro *et al*., 1998; Stevens *et al.,* 1997; Stevens *et al*. 1996; Stevens *et al*. 1999; Stevens *et al*., 1998). In direct contrast to environment UV research, food treatments typically use short, intense UV exposures (seconds-minutes), often using UV-C.

UV-C has a direct germicidal effect on surface micro-organisms and this is clearly an important component for control in some post-harvest diseases (Mercier *et al,* 2000). However, UV-C also induces a systemic increase in host defence processes, conferring resistance to internal tissues not directly exposed to radiation (Brown *et al*., 2001; Mercier *et al*., 2000). This systemic effect, known as “hormesis”, offers more effective and long-lasting disease control in some systems, and is consistent with the wider literature which shows that UV-B also induces host resistance to disease (see above). Thus, UV-B might offer an alternative to UV-C treatments, with the possible benefit of a greater margin of safety between effective disease control and crop damage.

UV-A has already been used to control storage rots and further possible approaches are evident by analogy with other technologies. Psoralens are aromatic compounds found in certain plant tissues that act synergistically with UV-A to inactivate micro-organisms by binding permanently with microbial DNA and RNA, preventing cell replication. This effect is the basis of a method of disinfecting stored blood (Hei *et al* 1999; Margolis-Nunno, 1997). In horticulture, the effects of UV-A treatments might be maximised by the natural psoralen content of some plant families, such as Umbellifers, such as celery, which contain

furanocoumarins , a natural form of psoralen, which are both induced and activated by UV (Finkelstein *et al*., 1994; Zobel & Brown, 1993).

The clear success of UV treatments for the control of post-harvest diseases offers a basis of the use of this technology as a seed treatment. It seems likely that UV-C and UV-B treatments could control seed-borne infections by a combination of surface sterilisation and hormesis, as in stored fruit and vegetables. UV-A treatments offer an alternative approach, especially in seed where natural concentrations of psolarens may synergise the effects of this waveband on microorganisms. To date, the effectiveness of UV seed treatments has been tested in only one study. This showed that UV-C treatments similar to those used in the food industry offer a chemical-free approach to the control of seed-borne black-rot (*Xanthomas campestris*) infection in Brassicas (Brown *et al.*, 2001). The UV irradiations used in the current project include UV-C treatments, as used by Brown *et al.* (2001) and compares these with the effects of UV-B and UV-A.

2.7 Other radiation methods

A well-known preservation method, aimed at killing all harmful flora on and in the treated material, is irradiation of food. This requires full irradiation using gamma radiation as well as high-energy electron beams. In contrast, a technique using low energy electronic beams penetrates only the outer layers of seed so that resident pathogens are eliminated without damage to the embryo. Promising results were obtained against *Tilletia caries*, *Septoria nodorum*, *Fusarium culmorum* and *Microdochium nivale* on winter wheat, bean infected with *Pseudomonas phaseolicola*, and carrot infected with *Alternaria* spp. and *Xanthomonas campestris* (Linder & Jahn, 1998). Burth *et al.* (1991, cited in McGee, 1995) also found that low energy electonic beams eliminated *Tilletia caries* and *Septoria nodorum* from wheat seeds in the pericarp and testa without adverse effects on germination.

Other radiation methods tested for seed treatment include the use of solar heat (e.g treatment of loose smut in India), laser treatments and radiowaves (see for example, Maude, 1996)

Clearly, there are a number of radiation techniques that could have potential for control of celery *Septoria* and may be worth trialling in the future. Given the scope and limited duration of this project, two methods for which equipment is readily available, microwave and UV treatment, have been selected for evaluation.

2.8 Disinfectants

HDC review CP 4 (1992) gives a detailed review of chemical disinfectants used in commercial horticulture. Chemicals used routinely as disinfectants are also used in certain situations to eliminate fungi from seed surfaces. For example, treatment of douglas fir seed with 3 % hydrogen peroxide (4 h) gave approximately 80 % control of surface-borne *Fusarium* spp without adversely affecting germination. Treatment of shortleaf pine seeds with 70 % ethyl alcohol followed by 1 % sodium hypochlorite for 60 s eliminated *Fusarium subglutinans* f. sp. *pini* (pitch canker fungus) (www.sfws.auburn.edu/sfnmc/class/fy614/peroxide.html). Acetic acid (5 %) was effective for treating leaf stripe (*Pyrenophora graminea*) on barley seed but there were problems with reduced seed germination levels (Nielsen *et al*., 2000).

Disinfectants represent a cheap alternative where elimination of surface-borne fungi from seed is required. However, the usefulness of disinfectants for eradication of more deep-seated seed infection such as *S. apiicola* is not clear, although pre-soaking seeds in water may facilitate fungicidal or fungistatic activity. In addition, the effect of product concentration and soak duration on seed germination would require detailed study. In this project, studies will focus on hydrogen peroxide, which although it has strong oxidising properties has the advantage of breaking down into oxygen and water and so leaves no toxic residues.

2.9 Plant extracts, essential oils etc

Legislation for organic agriculture is based on EU regulation no. 2092/91. While preventative measures against pests and diseases are preferred, in cases of acute threat to the crop, the use of materials listed in Annex II of the regulation is permitted, including plant oils.

The anti-fungal properties of essential oils, compounds fractionated from essential oils and crude extracts from a wide range of medicinal plants, which are natural sources of antimicrobial substances, have been reported. Use of such substances in the liquid or vapour phase may have potential for seed treatment (H. MacTavish, ADAS Phytochemical Unit, pers. comm.). Examples of activity are given below.

The essential oils of *Cymbopogon citratus*, *Eucalyptus citriodora* and *Ageratum conyzoides* provided 100 % inhibition of the mycelial growth and germination of spores of *Didymella bryoniae* (gummy stem blight of melon) (Fiori *et al*., 2000). In a brief review, the same author

reported that crude extract of *E. citriodora* can inhibit the mycelial growth of *R. solani*, *Sclerotium rolfsii*, *Phytophthora* sp., *Alternaria alternata* and *Colletotrichum graminicola*, while extract of *C. citratus* leaves can inbibit mycelial growth of *Fusarium solani* f. sp. *phaseoli*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*. Daferera *et al*. (2002) showed that the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis* was completely inhibited by oregano, thyme, dictammnus and marjoram essential oils at relatively low concentrations (85-300 ug/ml). Clarkson *et al.* (1999) showed that there was some activity of 2 % mint oil against downy mildew (*Peronospora parasitica*) in brassica seedlings. Trials testing natural products versus downy mildew of lettuce showed that rape seed oil significantly reduced disease levels (P. Gladders, ADAS Boxworth, pers. comm). In terms of seed treatment, the essential oil of *Ocimum adscendes* at a concentration of 0.1 % protected the seeds of *Capsicum annuum* against storage fungi and was more effective than treatment with fungicides (Asthana *et al*., 1989).

A commercial formulation of orange oil plus ethoxylated alcohol (Orosorb) is currently being trialled in the UK against insect pests (www.citrusoilproducts.com). Trials to evaluate whether the product also has fungicidal properties may also be worthwhile.

Apart from plant oils, there are many other 'natural' products which are reported to have fungicidal properties. Their use for organic agriculture depends on the interpretation of EU regulation no. 2092/91 by individual countries. For example, seed treatment with mustard flour can lead to a 91 % reduction of stem smut (*Urocystis occulta*) in rye and common bunt (*Tilletia caries*) in wheat (Borgen & Kristensen, 2001). In Germany, mustard combined with horse-radish extract is marketed as SBM (Borgen *et al.,* 1996) and is permitted for organic agriculture, using the argument that the effect of mustard is from the glucosinolates or mustard oils. Milk powder was similarly effective in reducing infection levels of rye stem smut and wheat common bunt (Borgen & Kristensen, 2001), although seed vigour was affected. In Germany, milk powder is considered as a plant strengthener and is therefore not evaluated as a plant protective agent in Annex II but as a fertiliser listed in Annex I as an animal-derived product. In contrast, neither milk powder nor mustard flour are permitted for their fungicidal use in organic agriculture in Denmark because they are not listed in Annex II.

2.10 Biological control

There are numerous reports of potentially valuable biological control micro-organisms, some of which are supplied as seed treatments. However, the development process to bring these treatments into commercial practice is long due to formulation difficulties, storage stability, shelf life of products after application to the seed and erratic biological efficacy. In addition, development may be hindered by the fact that while chemical seed treatments function across a broad range of environments, the ecological niche in which a biological control agent (BCA) can function may be relatively narrow.

When using a biological control seed treatment, a key issue is correct loading of the appropriate number of cells on each seed. Various carriers and polymers have been used to increase the survival rate of the organism. Some biological control agents are adversely affected by the combination with some traditional chemical seed protection. BCA seed treatments may come in dry formulations as dusts, dry spores and gum/talc powders. Liquid formulations are also available for sprays, dips, fluid drilling gels and solid matrix priming. These may be designed for large-scale application or planter box treatments.

There are a number of modes of action employed by BCAs that lead to seed and seedling protection; these can be broadly categorised as antagonism, antibiosis, competition and mycoparasitism.

Cotton has been the first large-scale agronomic crop treated with biological control agents for the suppression of seedling diseases of the rhizosphere. Much of the cotton planted in the USA is treated with one or more biological control agents (STEC, 2000).

Scroth & Hancock (1981) described increased yield in several crops with *Pseudomonas* seed treatment, probably due to a displacement of root-infecting fungi and bacteria that, in total, reduce plant growth. Interestingly, there may also be future potential for use of *Pseusomonas* spp in control of celery *Septoria*. In Canada, 204 bacterial isolates were collected from celery leaves and celery field soils (Lovering *et al*., 1996). Three isolates of *Pseudomonas* species and an unidentified gram-positive bacteria were found to prevent formation of *Septoria* pycnidia on agar and to inhibit conidial germination on leaf discs. Plants treated with one of the isolates developed only 5 % of the number of *Septoria* lesions recorded on untreated plants.

Pythium oligandrum and *Coniothyrium minitans* are well documented mycoparasites which have shown considerable promise as biocontrol agents of a range of damping-off plant pathogens (e.g. *Pythium ultimum*, *Rhizoctonia solani*) and *Sclerotinia sclerotiorum*, respectively (Whipps, 1997). In the UK, progress has been made in developing commercial seed treatments using *P. oligandrum* and *C. minitans* (Whipps & McQuilken, 2001). A commercial preparation of *Pythium oligandrum* is available in the Czech Republic as Polyversum® (Biopreparaty). Promotional literature suggests that the seed treatment (which can also be applied as a root or stem drench) has a wide range of activity e.g. versus *Pythium*, *Rhizoctonia*, *Phytophthora*, *Phoma*, *Verticillium* and *Sclerotinia*. This product will be trialled in this project.

3. Studies to estimate infection levels in seed batches naturally infected with *S. apiicola*

3.1 Introduction

In this project, experiments are being carried out to determine the most effective conditions for alternative seed treatments (e.g hot water, use of botanical extracts) in terms of reducing seed infection without reducing percentage seed germination. A batch of seed naturally infected with *S. apiicola* has been sourced for this work (celery hybrid TZ9783, stock number 37/1169, 85 % seed germination). Tests are required to determine percentage infection in the original seed batch and then to evaluate the effect of individual seed treatments on infection levels.

Celery seed infected with *Septoria* usually bear pycnidia of the fungus, which exude spores when wetted. The simplest methods of quantifying seed infection involve recording the percentage of seed with visible pycnidia or determining the percentage of seeds that exude *Septoria* spores when placed in individual water droplets. The main disadvantage of these methods is that they do not provide information on spore viability (ability to germinate and infect the host).

Maude (1963, 1964) and Hewitt (1962) describe a variety of techniques that have been used to test the viability of *Septoria* on celery seed. Based on these techniques, three methods were tested in this study to determine percentage viable infection in celery seed. The most

appropriate method was used to confirm the percentage of seeds infected with viable *Septoria* in the seed batch being used.

Methods that require checking individual seeds for spore release and germination are time-consuming when numerous seed samples are being processed. For seed treatment experiments which will each include several replicated treatments, it was considered that a method was needed to provide a rapid measure of the efficacy of individual treatments in reducing seed infection compared with an untreated control. A protocol to collect spores from treated seed batches and compare spore germination was devised for this purpose and is described below. For selected treatments, a further protocol may be used to confirm whether spores from treated seeds are still pathogenic on host tissue.

3.2 Methods

3.2.1 Experiments to determine percentage celery seeds infected with viable *S. apiicola*

i) 100 seeds were placed in individual droplets of sterile distilled water (SDW) on microscope slides. After 15-30 min, a sterile loop was used to agitate the seed and water droplet. The seed and part of the water droplet were then removed from the slide and streaked on a plate of potato dextrose agar amended with streptomycin sulphate (PDA+S). The plates were incubated at 20ºC for 16 h. Each seed/streak was examined under the microscope for spore release and germination. The percentage of seeds releasing spores that subsequently germinated was recorded.

ii) Four seeds were placed onto each of 25 plates of PDA+S. A water droplet (SDW) was added to each seed. Using a microscope, each droplet/seed was examined and those releasing spores were marked. Plates were incubated at 20ºC for 16 h and the seeds were then re-checked for spore release and spore germination. The percentage of seeds releasing spores that subsequently germinated was recorded.

iii) Fresh healthy celery petioles were wiped with 70 % alcohol. Five small slits were made along the length of each petiole with a sterile scalpel and one seed inserted per slit (20 petioles). 'Control' petioles were also set up with slits but no seeds. The petioles were covered with moist tissue paper towel and incubated at 15-20ºC for 24 h. The petioles were subsequently stored at ambient temperature in the laboratory with their bases in water. Lesion development was assessed after 2 weeks.

3.2.2 Rapid method to compare percentage germination of *Septoria* spores in samples of treated celery seed

Samples used for experimental treatments are processed as follows. Immerse each 2 g seed sample in 15 ml distilled water in a conical flask. Place flasks on an orbital shaker for 2 h. For each flask, pipette 1 ml of liquid into a universal tube and centrifuge at 2000 rpm for 10 min. Discard the supernatant and resuspend the pellet in 0.5 ml distilled water. For each sample, check for the presence of *Septoria* spores using a haemocytometer. For samples in which spores are observed, spread 100 µl spore suspension onto each of 3 plates, incubate at 20°C for 16-20 h and determine percentage spore germination. Also record observations on appearance of spores and germ tube development.

3.2.3 Method to determine the pathogenicity of spores

Excise leaves from a healthy celery plant, disinfect with 1% NaOCl solution and rinse in distilled water. Place leaves in a 45°C water bath for 15 s, then blot leaves dry and place in a plastic box (with lid) lined with damp paper towel. Spray the leaves to run off with a spore suspension obtained from the treated seed sample. Incubate samples at 20°C under light and examine for lesion and pycnidial development in 14 –21 days.

3.3 Results and Discussion

Method i): Four percent of seeds were observed to release *Septoria* spores. Spores released showed 100 % germination. The main disadvantages of the method were that it was laborious and it was also difficult to locate spores on the plate because they were widely distributed due to streaking. In addition, the result did not tally with preliminary observations of seeds in water droplets on microscope slides, which showed 13 % of seeds releasing spores.

Method ii): In total, 15 % of seeds on the plates exuded spores in the 24 h period. 10 % of seeds exuded spores which subsequently germinated. This technique was straightforward, was considered to be more reliable than method i), and will be used where it is necessary to ascertain percentage viable infection of celery seed samples. While it is useful to get a base-line indication of percentage viable infection in the seed stock, infection levels in untreated controls will need to be re-checked for each seed treatment experiment because of the tendency for the viability of *S. apiicola* in celery seed to decrease over time.

Method iii): The celery petiole method could be useful since it can give an estimation of 'total' seed infection, ie the percentage of seeds that can give rise to infection from spores in pycnidia and/or deep-seated mycelium of *Septoria*. However our trial was unsuccessful since the petioles began to rot before any individual *Septoria* lesions were visible. The trial was repeated with different incubation conditions but the outcome was the same.

The rapid method to determine spore germination from treated seed batches (Section 3.2.2) has been used successfully to date in trials testing the efficacy of hot water, microwave and UV treatments for the elimination of *S. apiicola* from celery seed. For example, Table 1 shows differences in spore germination after a range of hot water treatments. Full results from these experiments, including seed germination data, will be presented in the project final report. The pathogenicity test described above will subsequently be used to determine whether spores from seeds exposed to the most effective hot water treatment are able to infect host tissue.

Table 1. Percentage germination of *S. apiicola* spores from celery seed after hot water treatments

Treatment no.	16 h pre-soak in water	Hot water treatment	Mean spore germination After treatment (%)
1	No	No	39.1
2	No	50ºC, 25 min	4.0
3	No	48ºC, 30 min	2.9
4	Yes	No	10.3
5	Yes	50ºC, 25 min	1.3
6	Yes	48ºC, 30 min	0.4

4. Conclusions

Based on the knowledge review, the seed treatments described below have been selected for evaluation. It is intended that some of the selected treatments will be appropriate for organic seed production while others will be more relevant for convential production. Experiments have been initiated on microwave, hot water and UV treatments and have been partially completed. Further experiments are planned for January to March 2003.

Fungicides

The effect of fludioxonil seed treatments (Beret Gold and Wakil XL) on celery seed infection and germination will be determined. In addition, seedlings from treated seeds will be grown under conditions conducive for development of *S. apiicola* to determine whether the number of infected seedlings is reduced in comparison with an untreated control.

Hot water treatment

The following treatments have been tested:

Treatment no.	16 h pre-soak in water	Hot water treatment
1 (control 1)	No	No
2	No	50°C, 25 min
3	No	48°C, 30 min
4 (control 2)	Yes	No
5	Yes	50°C, 25 min
6	Yes	48°C, 30 min

*The purpose of the pre-soak is to activate the pathogen prior to hot water treatment

The effects of treatments on spore germination were recorded earlier in this report. Seed germination tests are ongoing.

Microwave treatment

There will be five microwave treatments of different durations (30, 45, 60, 90 and 120 sec) and an untreated control (0 sec). Seeds will be treated dry but with a beaker of water in the microwave oven to act as a 'heat sink'. Results of this experiment will be used as the basis for further testing, comparing different treatment durations and seed immersion in water.

UV treatment

UV treatments are being carried out by Dr N. Paul, Institute of Environmental and Natural Sciences, Lancaster University. The following experiments have been devised in consultation with Dr Paul.

Experiment 1 – Comparison of UV-C, UV-B and UV-A

Based on previous use of UV-C as a seed treatment and for post-harvest disease control, a dose of 104 J m^{-2} will be used initially. Since the effective disease control using UV-B or UV-A is expected to require higher doses, two doses will be used in initial studies of these wavebands: 104 J m^{-2} to allow direct comparison with UV-C and 105 J m^{-2}. These treatments plus an unirradiated control will initially be applied to seeds that have been pre-soaked.

Experiment 2 – Comparison of the effects of UV treatments on pre-soaked and non pre-soaked seeds

On the basis of experiment 1, one waveband and dose will be selected and applied to pre-soaked or non pre-soaked seed.

Experiment 3 – Dose response

On the basis of experiments 1 and 2, a single waveband will be selected and its effects investigated in more detail through a dose response study. Seed will be exposed to a range of five doses, from zero (unirradiated control) to a maximum based on results from earlier experiments.

Disinfectants

Seed treatment with hydrogen peroxide will be tested using a commercial formulation (e.g. Jet 5) investigating the effects of i) pre-soaking seed in water, ii) duration of soak in hydrogen peroxide.

Plant extracts, essential oils etc

Three essential oil treatments will be selected in consultation with Dr H. MacTavish of ADAS Phytochemicals Unit. The treatments will include oils that are reported to have anti-fungal properties, and that are widely available (e.g. tea tree, eucalyptus and mint). Studies will investigate dose response, in addition to comparison of efficacy of liquid and vapour phases. Trialling of commercial products such as SBM and Orosorb may also be included.

Biological control treatment

The efficacy of *Pythium oligandrum* in the form of the product Polyversum® will be tested.

Conclusions from studies to estimate infection levels in seed batches naturally infected with *S. apiicola:*

- A batch of celery seed naturally infected with *S. apiicola* has been sourced for use in seed treatment evaluations (hybrid TZ9783).

- The percentage of seeds in the batch with viable *Septoria* infection was estimated at 10 % based on observation of release and germination of spores from seeds plated on agar plates in individual water droplets.

- A rapid method to determine spore germination from treated seed samples has been used successfully to evaluate the efficacy of hot water, microwave and UV treatments in eliminating *Septoria* from celery seed. For example, for seed exposed to a hot water treatment of 48^{o}C for 30 min following a 16 hour pre-soak, *Septoria* spore germination was 0.4 % compared with 39.1 % in the untreated control.

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Technology transfer

Meeting

27 June 2002, K. Green met with B. Lincoln and J. Claxton of Tozer Seeds Ltd to discuss seed testing protocols, candidate treatments etc.

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