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Assessment of the potential of dehydrated garlic products to assist with the integrated control of onion white rot.

Dr Jason Dennis
Field Fresh Tasmania
Australia

Project Number: VX99046

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ASSESSMENT OF THE POTENTIAL OF DEHYDRATED GARLIC PRODUCTS TO ASSIST WITH THE INTEGRATED CONTROL OF ONION WHITE ROT.

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FINAL REPORT
TO
HORTICULTURE AUSTRALIA LIMITED

Project No. VX99046

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Purpose:

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June 2002

Contents:

MEDIA SUMMARY:

Onion white rot is a soil borne disease of onions that can cause considerable crop loss. It attacks the roots of onions plants and causes young plants to die or of infection occurs later during the crop, causes bulbs to rot. It permanently infests fields and intensifies over time to cause field-wide plant death. In recent research trials, application of germination stimulants specific for white rot substantially reduced soil populations of the disease and, under ideal field conditions, provided acceptable commercial control on subsequent crops.

The mode of action of this process is unique in plant pathology - the germination stimulants mimic root exudates from onion crops, and trigger the soil borne spore-like structures to germinate. In the absence of a host (onions, garlic and leeks) the germinated spore-like structures starve and quickly die. This effectively reduces the disease levels in the soil, and once reduced below economically important thresholds will allow onion crops to be grown relatively free from the risk of infection by white rot.

More recent research suggests that integrating this method together with fungicides, and possibly biocontrol agents, will provide more reliable and sustained white rot disease control.

In this project, a range of dehydrated garlic samples from several USA companies were imported into Australia and analysed for concentration of germination stimulants.

The use of dehydrated garlic powder from the US, or potentially elsewhere, may be an economically feasible way to reduce disease levels. The main difficulty will be establishing the rate needed which will depend upon analysis of the product. It was observed that the levels of stimulants can change in storage, which could be a problem for any carry over stocks as the shelf life could potentially be limited to only one or two seasons, after which time the volumes needed may be too costly. In the absence of a dehydrated garlic industry in Australia it is unlikely that dehydrated garlic products will be used for application to commercial fields as a means of disease control. The cost and risk of importing product with low analysis is likely to impede industry adoption of this approach.

It is was also determined in this project that post-harvest treatment of new disease patches should not be treated with germination stimulants, as this is unlikely to effectively reduce disease levels owing to a natural dormancy period that occurs in the newly formed spore-like structures.

TECHNICAL SUMMARY:

The pathogenic fungus Sclerotium cepivorum is the cause of the disease white rot of Allium crops. It permanently infests fields and intensifies over time to cause field-wide plant death. In recent research trials, application of germination stimulants specific for S. cepivorum substantially reduced soil populations of the fungus and, under ideal field conditions, provided acceptable commercial control on subsequent crops.

The mode of action of this process is unique in plant pathology - the germination stimulants mimic root exudates from Allium crops, and trigger the sclerotia (resting bodies) to germinate. In the absence of a host (Alliums only) the germinated sclerotia starve and quickly die. This effectively reduces the inoculum in the soil, and once reduced below economically important thresholds will allow Allium crops to be grown relatively free from the risk of infection by white rot.

More recent research suggests that integrating this method together with fungicides, and possibly biocontrol agents, will provide more reliable and sustained white rot disease control.

In this project, a range of dehydrated garlic samples from several USA companies were imported into Australia and analysed for concentration of three germination stimulants; DAS (Diallyl sulphide), DADS (Diallyl disulphide), DATS (Diallyl trisulphide).

The use of dehydrated garlic powder from the US, or potentially elsewhere, may be an economically feasible way to reduce inoculum levels. The main difficulty will be establishing the rate needed which will depend upon analysis of the product. It was observed that the levels of stimulants can change in storage, which could be a problem for any carry over stocks as the shelf life could potentially be limited to only one or two seasons, after which time the volumes needed may be too costly. In the absence of a dehydrated garlic industry in Australia it is unlikely that dehydrated garlic products will be used for application to commercial fields as a means of disease control. The cost and risk of importing product with low analysis is likely to impede industry adoption of this approach. A synthetic liquid product, although costly, provides reliable and repeatable levels of stimulants, which will be essential for industry adoption of this unique disease control strategy.

It is was also determined in this project that post-harvest treatment of new disease patches should not be treated with germination stimulants, as this is unlikely to effectively reduce disease levels owing to constitutive dormancy of newly formed sclerotia.

INTRODUCTION:

The pathogenic fungus Sclerotium cepivorum is the cause of the disease white rot of Allium crops. For all practical purposes, S. cepivorum permanently infests soil once it is introduced into previously noninfested fields. Varietal resistance, traditional fungicidal controls and quarantines have been insufficient to control or contain this disease in most circumstances. The common progression seen worldwide is that once infested, the disease intensifies over subsequent cropping cycles, eventually approaching 100% plant loss. One to three cropping cycles may be sufficient in this respect; however, fields usually are abandoned from future onion and garlic production prior to losses reaching high proportion. As a result, crop loss statistics in most countries do not reflect high crop losses or abandonment.

White rot disease already has severely limited onion and garlic production in a number of sub-regions of the U.S. and in sub-regions of all other world continents. Once an onion exporter, Egypt became an onion importer after white rot spread widely (Georgy, 1983). The Victoria area of Australia largely was ruined for onion production several decades ago (Merriman et al, 1980), and the disease is spreading rapidly in Tasmania and New Zealand at this time (J. Dennis, personal observation). Introduced into the western U.S. in the 1930's, white rot forced the relocation of most garlic from the Santa Clara Valley (Gilroy area) by the 1960's, from the Salinas Valley by the 1970's and currently is spreading in garlic and onion fields in the San Joaquin Valley. The Tulelake Basin of California and Oregon is widely infested, as are the Walla Walla onion production region and central and western Oregon (Crowe et al, 1990). Without effective controls, production of most overwintered or cool season onions and garlic in the Western U.S. may cease within a generation. Other major U.S. over-winter onion production regions, e.g. Texas and Georgia, are at risk of becoming infested. The situation is similar elsewhere in the world.

S. cepivorum infects and grows only on Allium species, including onions, garlic, leeks, chives and numerous lesser-known edible species. Alliums produce several unusual amino acids that are precursors of the notable flavor and odor compounds associated with this genus. The small, 0.5-mm diameter survival structures (sclerotia) of S. cepivorum lie dormant in soil until stimulated to germinate by one or more 3-carbon-organic sulfur compounds, typically allyl and propyl forms, which volatilize away from onion and garlic roots (Coley-Smith & King, 1969; King & Coley-Smith, 1968). Sclerotia germinate only once and hyphae may grow up to 2 cm from a sclerotium (Crowe et al, 1980). The fungus radiating from each sclerotium dies if an Allium root is not contacted and infected within a week or so (Crowe et al, 1980a). A few, small secondary sclerotia may form, but are not thought to play a role in the epidemiology of the disease (Entwistle & Munasinghe, 1981; Somerville & Hall, 1987). While S. cepivorum grows saprophytically on many food sources in pure culture, it seems unable to compete with other soil organisms to reproduce in soil, and it is unable to infect and reproduce on non-Allium species (Scott, 1956; Crowe et al, 1980a).

After infection, S. cepivorum grows upward inside and along the outside of roots, rapidly decaying the root in the process. Hyphae radiating from infected roots may infect additional roots of the same or adjacent plants in the planted row. Depending upon the depth of original infection, soil temperature and the length of the growing season, a single sclerotium ultimately may infect 30-40 or more plants in a planted row (Crowe & Hall, 1980a).

Very few sclerotia form in infected roots (Crowe et al, 1980). Once S. cepivorum contacts the stem plate and bulb, bulbs are rapidly decayed, and abundant reproduction occurs. With high soil populations, many shallow infections occur and widespread crop losses result early

in the season. However, under such conditions, the net sclerotial population may decrease slightly, remain stable or increase only slightly, because small plants sustain only limited reproduction. When soil populations are low, fewer and deeper root infections tend to predominate. Nevertheless, substantial crop losses still may result because of secondary spread of infections from plant to plant along the row. In this case, soil populations rapidly increase because many plants are decayed later in the season when larger, providing a larger food base for reproduction (Crowe et al, 1980). Sclerotia were found to decay bulbs from root infections arising as deep as 30 cm (Crowe & Hall, 1980a).

Once a field becomes infested, soil populations rapidly increase to high levels, then stabilize (Crowe & Hall, 1980a; Crowe et al, 1980). Fields remain infested for many years (Coley-Smith & Cooke, 1971; Crowe et al, 1980; Crowe et al, 1990) during rotations away from Allium crops. During this time populations may slowly decline as sclerotia are lost via undetermined processes (Legget & Rahe, 1985; Crowe et al, 1994), probably including decay and predation. Unfortunately, fields may remain infested at levels commercially damaging to Allium crops after rotations as long as 25-40 years, or perhaps even longer (Crowe et al, 1990).

The flavor and odor compounds may stimulate sclerotia up to 10 cm away from roots (Coley-Smith & Cooke, 1971), so essentially all sclerotia may germinate during the season by natural root leakage of stimulants (Crowe et al, 1980; Coley-Smith, 1987; Coley-Smith & Parfitt, 1986). In this sense, then, nearly the entire population of S. cepivorum is fully committed to successfully completing the current disease cycle and, at the end of the season, essentially all residual inoculum is newly produced. This non-conservative commitment is unusual for soil pathogens, and has suggested to various workers that soil populations of S. cepivorum might be eradicated by efficient artificial application of germination stimulants to infested soil in the absence of Allium crops. Early efforts to achieve such eradication, however, met with only partial reductions in soil populations (40-60%) and insufficient disease control (Elnaghy et al, 1971; Merriman et al, 1980; Merriman et al, 1981; Entwistle et al, 1982). This presumably was because of less-than-optimal soil temperature and moisture, rapid loss from soil of volatile stimulants, incomplete placement of stimulants through the infested soil profile, or re-planting before economic thresholds were achieved.

More recently, Crowe et al (1994) achieved 98-99% reduction in sclerotial population in a series of field trials between 1988-1992. These studies took advantage of improved dosage-response data from Coley-Smith & Parfitt (1986). More importantly, care was taken to
a. apply germination stimulants precisely at times of the year when soil temperature would remain near to the optimum for germination response for as long as possible (Crowe & Hall, 1980b),
b. provide supplementary irrigation to keep soil moisture within the optimum for germination response (Crowe & Hall, 1980b),
c. apply as deeply as possible through the infested soil profile (Crowe & Hall, 1980a),
d. carefully monitor residual sclerotial populations (Crowe et al, 1980),
e. apply germination stimulants only when sclerotia were matured past their period of constitutive dormancy (Coley-Smith, 1960)
f. ensure that no volunteer onions or garlic were present during the treatment period,
g. roll or irrigate the soil surface to contain volatile stimulants as long as possible.

Dennis (2001) in Tasmania recently demonstrated that when the S. cepivorum population was reduced substantially with stimulated germination treatments, full control was then achieved

with Folicur seed treatments on onions. The consensus of numerous white rot pathologists at the 6th Int. Workshop on Allium White Rot in Mexico, 1998, was that such integrated approaches might soon be used to control white rot on full season Allium crops. A number of fungicides with chemistry similar to Folicur are being tested against white rot, along with newer generation products with different mode of actions.

Some early investigations of germination stimulants utilized natural products, but most have relied on petroleum-derived diallyl compounds, including Crowe et al, 1994. Diallyl disulphide is highly stimulatory to S. cepivorum, and seems to persist in soil to elicit germination response for up to 2 months or more (Coley-Smith, 1986; Crowe et al, 1994; Crowe, 1998), the time necessary for full response from the soil sclerotial population (Crowe, 1998).

Commercial development of diallyl disulphide into a formulation suitable for agricultural application is in advanced testing. The product developer recommends 10 liters/ha (1.1 gpa) applying to about 20 cm depth or more into soil under pressure using specialized equipment. Injection shanks are spaced at about 20 cm apart, soil would be irrigated and or rolled to assist sealing in the volatile material to extend soil residence time, as per guidelines provided to United Ag Products by F. Crowe, Oregon State University, 1994. The product cost would be US$65/liter, or US$650/ha (US$263/ac) at 10 liters/ha (R. Ostrowski, United Ag Products, personal communication, 1998). Based on conversations with growers and industry, we believe this cost may be considered prohibitive for many growers, and for repeated applications, which the supplier recommends.

Additional developments lead us to reconsider the utility of natural products in this proposal, as either alternatives or improvements upon the use of petroleum-derived dially disulphide. First, in 1991-1992, following the same procedures to optimize sclerotial responses as for petroleum-derived diallyl disulphide, F. Crowe and T. Darnell (unpublished) also applied commercial food-grade dehydrated garlic powder to the surface of infested soil in Walla Walla, Washington, and tilled the soil to 30 cm. Within 3 months of conducive soil temperatures and soil moisture, 0, 80, 98 and 99% reduction in sclerotial populations were measured for 0, 50, 500 and 5000 lb/ac applications of the garlic powder -- results equivalent to those achieved with various rates of diallyl disulphide in the same field. Since 1992, no further research to refine the minimum effective rate of garlic powder has been conducted.

Regulatory agencies continue to delay approval of petroleum-derived diallyl disulphide products in some countries, as the formulation does contain slight impurities (e.g. pentane) not found in natural products (K. Inkrott, Phillip 66 Company, personal communication, 1994; R. Ostrowski, UAP, personal communication, 1997). However, recently the U.S. Environmental Protection Agency deregulated all natural garlic products from requiring pesticide registration (U.S. Federal Register, March 6, 1996, Volume 61, Number 45). Prior to this ruling, garlic dehydration companies were intrigued by preliminary data, but were reluctant to consider registering their food-grade products as pesticides (Ed Kurtz, American Dehydrated Onion and Garlic Association, personal communications, 1994 and 1996). Since 1996, garlic products may be used without regulation for control of white rot.

Garlic powder and various granulated forms of these dehydrated crops, are normally produced for food usages following slicing, dehydration and milling of bulbs and cloves (Fenwick & Hantley, 1990). Food-grade product recently has been available for as little as $US 0.30/pound, but price clearly will fluctuate with availability. Out-of-grade product is

available at even less cost than food-grade product. Out-of-grade product may be off color (Joslyn & Sano, 1956; Lukes, 1986; Shannon et al, 1967) and sizes or with too high of a bacterial count for normal food marketing channels (Pruthi, 1980; Pruthi et al, 1959). Sale of out-of-grade product for control of white rot, at even lower prices, would be preferable to disposal. Disposal results in financial losses, and can even be problematical because of the odors involved (W. Randle, University of Georgia, personal communication, 1998).

Based on preliminary data from the US, less than 500 pounds of garlic powder per acre would be suitable for commercially acceptable reduction in sclerotial populations of S. cepivorum. If so, the cost of product per acre would be less than 500 x US$0.30 = US$150, and hopefully as low as US$50-100 if effective rates of application are substantially lower. Product costs per acre of US$50-100 would be readily acceptable to commercial onion and garlic growers if there was some confidence that white rot disease control would be achieved. Garlic powder can be sprayed onto and tilled into soil with conventional equipment. Thus, we anticipate that application technology for natural products would be already available to most farmers, and that the cost of application would be modest. Repeated applications would more likely be made, if needed.

Without governmental regulation of natural products as pesticides, and without product labeling to verify product potency, growers would not have any assurances of the concentration of stimulants contained within products such as dehydrated garlic and onion powders and granules, especially following storage and international shipment. The range of concentration of fresh and stored product is unclear, but key components of dehydrated products (such as diallyl disulphide) likely can be determined by chemical analyses. An objective of this investigation is to determine whether routine testing is necessary, and to provide guidelines for those seeking assurance of product potency. Flavor intensity of fresh product may be indirectly measured by pyruvate concentration (Schwimmer & Weston, 1961). However, the intensity flavor and odor compounds in dehydrated materials probably would require direct measurement of either allicin or other allyl products, likely with either liquid or gas chromatography (W. Randle, University of Georgia, personal communication, 1998).

METHODS:

1. ANALYSIS OF DEHYDRATED GARLIC PRODUCTS AND DADS OIL
(Text prepared by Professor R Menary, University of Tasmania)

A range of dehydrated garlic samples from several USA companies were imported into Australia and analysed for concentration of three germination stimulants; DAS (Diallyl sulphide), DADS (Diallyl disulphide), DATS (Diallyl trisulphide). The methodology of extraction and analysis was determined by Professor RC Menary at the University of Tasmania.

Before development of a current standard curve for Sulphur detection, samples of the synthetically produced DADS oil were made up with octanethiol internal standard (C8-S) and run on the GC using the FPD (flame photometric detector) to establish the peak identification (e.g. retention times etc.) and optimum GC settings for the column currently being used.

An initial extraction of 2 garlic powder samples (1 coarse and 1 fine ground) was then made to check the effectiveness of the procedure and to confirm the appropriate concentration range for the standard curve was to be obtained.

The measurement of sulphur over a wide range of concentrations using a FPD is known to involve a non-linear response curve. This is due to the flame composition being highly reducing, with light emission thought to occur because of dimerisation of two sulphur atoms giving an excited state S2* species that then drops to ground state with a resultant release of energy (light). The kinetics of this process dictate a non-linear response described by a quadratic equation (polynomial).

As the initial preparative extractions gave quite variable levels of response for the 3 components measured with much greater response for DATS than DADS or DAS, an extended range of duplicate standards was made up by making 2 dilution series from an initial hexane solution of oil and C8-S std. The measurement of the quantities of these 3 compounds was to be achieved by detection of the amounts of sulphur for each of the peaks (using the standard curve), before conversion to a weight (or concentration) of the actual compounds present by using conversion factors obtained thus:

$$\text{C.F.} = \frac{\text{mass of sulphur in compound}}{\text{molecular weight of compound}}$$

The conversion factors for the 3 compounds are:

Diallyl (mono) sulphide (DAS) ($C_6H_{10}S_1$) $\quad CF = \frac{(32) \times 1}{114} = 0.2807$

Diallyl disulphide (DADS) ($C_6H_{10}S_2$) $\quad CF = \frac{(32) \times 2}{146} = 0.4384$

Diallyl trisulphide (DATS) ($C_6H_{10}S_3$) $\quad CF = \frac{(32) \times 3}{178} = 0.5169$

The response factor for n-octanethiol (C8) Std. $= \frac{32 \times 1}{146} = 0.2192$

The concentrations of DADS oil/C8-S std. were made up in hexane and ranged from 25 - 1000 ug/ml, giving a Sulphur range of approx. 5-220 ug/ml . These samples were run on a silicone HP1 column (30m, 0.32 mm ID, 0.52 mm film thickness) at a split ratio of 50:1. Detection was by a flame photometric detector fitted with an appropriate filter for Sulphur detection.

From the concentrations of C8 standard, the conversion factor was used to calculate the concentration of C8 sulphur in each sample. These concentrations were plotted against the corresponding FPD response results to determine the relationship, which was polynomial with an R^2 of 0.98 to 1.00.

This equation was then used to calculate the concentration of sulphur for the DAS, DADS and DATS in the unknown extract samples (see results table). This was done by using the areas of these peaks measured and deriving for x in the equation using the solver tools function in the Microsoft Excel 98 software.

To determine the concentration of the identified components in the Garlic samples, they were extracted by weighing each out into 2 duplicate samples of approx. 10g and extracted overnight in hexane (10 x v/w) after a known amount of C8 standard (equivalent to 101.49 ug of Sulphur) was added as both a retention time reference for peak identification and as a means of measuring the % recovery of extract/std. through the filtration/drydown process. The extractions were initially sonicated for 10 min. before shaking for the remainder of the extraction period. The samples were then filtered with 2 washes of a further 50 ml of hexane before dry-down in an RVE at 25^{0}C, allowing a weight of extract to be obtained. The extracts were then redissolved in 2.00 ml DCM and 1.00 ml subsamples of this taken for GC analysis.

2. INVESTIGATION OF DENSITY COLUMN TO ASSIST SOIL TEST EFFICIENCY

Inoculum levels in soil are currently assessed by wet sieving soil samples through a 600µm and then a 300µm sieve, and then examining the contents of the 300µm sieve under a dissecting microscope. The sclerotia range in size between 350µm and 450µm and so are collected on the 300µm sieve. Larger and smaller particles are removed during the process. This procedure takes in the order of one hour to fully process 200g of wet soil. The main limitations of this method are that only relatively small amounts of soil can be processed and the time taken makes cost prohibitive. When attempting to map the level of disease within a paddock a large number of samples is needed and pooling samples may result in intense localised patches of disease being missed. The threshold for severe disease has been demonstrated in Tasmania to be as low as 40 sclerotia per kilogram of soil, but may be even lower, making accurate detection in small samples difficult.

In order to allow larger soil samples to be processed without adding significantly to the cost the use of a sucrose density gradient was evaluated. This method is already in use in the US and for soils encountered in the US has provided a more rapid way to process large volumes of soil, which in turn enables the level of detection of inoculum to be more sensitive. In 50% w/v sucrose sclerotia remain suspended in a column of sucrose, with lighter organic matter

rising to the top and heavier soil particles sinking to the bottom. Tasmanian soil samples containing sclerotia were added to 50% w/v sucrose columns and then all levels in the column were assessed for the presence of sclerotia.

3. VERIFICATION OF THE NEED FOR SCLEROTIA PRE-CONDITIONING

Treating new patches of disease after harvest is a targeted disease control measure that is currently not used to reduce new inoculum levels. If successful it would provide an alternative to treating entire paddocks. Previous work has established that newly formed sclerotia have a constitutive dormancy period, which may prevent post-harvest application of germination stimulants from being effective. To test for constitutive dormancy in Tasmania, sclerotia were sampled from infected onions at harvest and then exposed to moist conditions with the presence of germination stimulants at 1, 2 and 3 months post-harvest. Sclerotia collected from the previous season were also incubated to provide a control set of sclerotia where constitutive dormancy was less likely to be a germination-limiting factor.

RESULTS & DISCUSSION:

1. ANALYSIS OF DEHYDRATED GARLIC PRODUCTS AND DADS OIL

Results of FPD analysis of dehydrated garlic products and oil.

COMPANY	PRODUCT	Year of Analysis	DAS (%)	DADS (%)	DATS (%)
Philips Petroleum	DADS Oil (1995 batch)	1996	13.37	62.35	12.70
			DAS (ppm)	DADS (ppm)	DATS (ppm)
Gilroy	Powder (1996 batch)	1996	-	62.53	-
		2001	4.88	5.31	18.59
DeFrancesco	BB Powdered (1270)	2000	2.17	1.19	20.45
		2001	3.10	2.94	17.17
DeFrancesco	Granulated (1271)	2000	6.47	3.21	43.17
		2001	4.52	4.23	20.32
DeFrancesco	Ground (1272)	2000	4.19	3.78	48.03
		2001	6.29	6.20	36.98
DeFrancesco	MR DE Powdered (1269)	2000	3.69	1.05	13.17
DeFrancesco	Minced (1273)	2000	2.36	0.83	21.39
Basic	Granulated (50810)	2000	2.92	1.16	12.44
Basic	Ground	2000	2.11	1.05	13.54
Basic	Powder – Standard (50801)	2000	3.28	2.27	16.93
Basic	Powder – Premium (50801)	2000	4.25	0.97	22.98
Basic	Powder – Export (508018)	2000	1.49	2.08	9.89
Empire	Powder 1090000 (Lot 9212M 2)	2000	0.94	1.44	2.65
Empire	Minced 1050000 (Lot 9196M 1)	2000	1.24	1.15	7.39
Empire	Chopped 1030000 (Lot 9189M 1)	2000	3.48	2.60	27.11
Empire	Granulated 1070000 (Lot 9233M 2)	2000	4.90	1.02	17.82
DeFrancesco	Granulated (1529)	2001	4.38	4.97	21.84
DeFrancesco	Chef's Choice Granulated (1530)	2001	5.58	5.47	23.45
DeFrancesco	Blender's Best Powdered (1528)	2001	3.91	4.50	18.99
DeFrancesco	Powdered (1525)	2001	5.20	6.81	26.36
Empire	Ground (0298)	2001	6.13	7.06	59.74
Empire	Granulate (0298)	2001	6.65	7.56	53.07
Empire	Powder (0298 W)	2001	6.93	6.91	38.14
Empire	Chopped (0298)	2001	8.17	6.15	43.99
Empire	Minced (0298)	2001	4.44	4.12	28.11
Rogers Foods	Powder Export 40*%	2001	4.12	4.40	21.26
Rogers Foods	Powder Fancy 10%	2001	3.07	3.06	15.08

From the areas obtained for each peak, the concentration of Sulphur in each was calculated using the quadratic equation and the solver function described. Given that the each redissolved GC sample should have contained 50.74 ug/ml of sulphur (i.e. 101.48 ug in the total sample) it is possible to calculate % recovery of the C8-S std. If it is assumed that this also represents the % recovery for all the extract components being measured (which would seem reasonable given that they are all highly soluble in the solvents used), then it is possible to adjust each result for the unknown components to a theoretical 100% recovery figure (labelled converted ug/ml S in table.2), thus eliminating error due to differences in filtration rates, evaporation and spillage etc, as a result of both human error and differences in the

particle size of the Garlic. This would seem to be particularly applicable given the wide variation in the range of % recovery figures obtained, something which is not surprising given the range of particle sizes (and thus wettability and rinsing efficiency) amongst the samples, although no obvious trend could be detected.

From the Sulphur concentration results, actual concentrations of each component were calculated using the conversion factors, before adjustment for % recovery to total ug extracted figures. The means for the pairs of GC duplicates from each extract were then calculated and these were used to calculate the yield of each component expressed as ppm from each garlic sample extracted using the sample extract weights. These results were then used to calculate the mean yield (ppm) from each duplicate set of garlic tissue extracts.

It should be noted that in all cases there were significant unidentified sulphur volatile peaks eluting later than the ones being measured and that the proportion of these to the peaks being measured varied greatly across the sample range. It was also observed that samples that were rerun on the GC after periods of 2 days or more (at room temperature) after the original GC analysis, had a greater proportion of these unidentified peaks with the concentration of the 3 measured peaks appearing to fall. Although this effect was not quantified, it is clear that the 3 volatiles being measured are relatively labile.

Efforts were made in this analysis to standardize the treatment of all the samples and to minimize the temperatures that the samples were subjected to with the temperature of drydown reduced from 30^0C to 25^0C. There was however, a relatively high degree of variability between the measured compositions of both the GC duplicates and the extract duplicates, both for measured compounds and later eluting unknowns. Such variables as duration in the GC autosampler (at room temperature) before injection cannot be ruled out as causes of this error because of the labile nature of the compounds measured. The sample order in the auto sampler was reversed for every 2^{nd} set of GC duplicates in an attempt to cancel out the possible effects of this sort of error. Given that the garlic samples may have been subjected to much severer conditions in drying, preparation, storage and transport it is considered that these results represent reasonably accurately the true relative concentrations of DAS, DADS and DATS in these Garlic samples as they were upon arrival at the laboratory.

From the final results obtained it is apparent that there is a wide range of the three germination stimulants in the sample range tested. It is also clear that in most of the samples, the dominant germination stimulant is DATS rather than DADS, unlike the 1996 garlic powder where the DADS level in the powder was measured at 60ppm. It is possible that some factor, such as temperature, in the extraction process resulted in conversion of DADS to DATS, though everything about the extraction procedure was kept as similar as possible in each year except extraction time, which was increased from 2 hours to 4 hours and the actual laboratory temperature, which fluctuates with outside seasonal conditions. The samples were kept in the dark at 25^0C for the extraction period and the highest temperature involved in the whole process was 30^0C during dry down which was identical to the extraction in 1996. It is unlikely that such a conversion would have resulted from the extraction procedures and therefore the results here represent the true levels in these dehydrated garlic samples.

Commercial development of a petroleum based germination stimulant containing diallyl disulphide into a formulation suitable for agricultural application is in advanced testing. The product cost would be US$65/liter and at 10 liters/ha would cost in the order of $1,300 per

hectare in Australia, however trials in Australia have shown that only half this amount of product is needed to achieve the same level of control.

The petroleum-based product provides 6.25L DADS per hectare. The garlic powder tested in 1996 contained 60ppm DADS, which would require in the order of 1,000kg per hectare to deliver the same level of DADS. At a cost of US$0.30/lb for high food grade, application of this product would result in a total cost in the order of $1,300 per hectare in Australia, however cheaper lower grade product is available and lower rates should be effective. The new samples tested in this report have lower concentrations of DADS than the previously tested powder and so would require even greater amounts of powder per hectare to deliver the same amount of DADS. The unexpected presence of high levels of DATS makes interpretation of the results difficult and further input is being sought to clarify the significance of the presence of high levels of this germination stimulant. The results also show that the level of stimulants does decline during storage and that the balance between DAS, DADS and DATS also changes during storage. Such variances would potentially increase the difficulty of establishing filed rates in the future, unless batches could be routinely screened for stimulants using similar procedures to those reported in this study.

Garlic powder and various granulated forms of these dehydrated crops, are normally produced for food usages following slicing, dehydration and milling of bulbs and cloves. Food-grade product recently has been available for as little as $US 0.30/pound, but price clearly will fluctuate with availability. Out-of-grade product is available at even less cost than food-grade product. Out-of-grade product may be off colour and sizes or with too high of a bacterial count for normal food marketing channels. Sale of out-of-grade product for control of white rot, at even lower prices, would be preferable to disposal. Based on preliminary data in the USA, less than 500 pounds of garlic powder per acre would be suitable for commercially acceptable reduction in sclerotial populations of S. cepivorum. If so, the cost of product per acre would be less than 500 x US$0.30 = US$150, and hopefully as low as US$50-100 if effective rates of application are substantially lower. Even with poor Australian dollar exchange rates, this cost would be acceptable and is significantly lower than the experimental petroleum based germination stimulant.

2. INVESTIGATION OF DENSITY COLUMN TO ASSIST SOIL TEST EFFICIENCY

The use of a density gradient to assist the efficiency of assessing soil for sclerotia did not facilitate any time saving for processing Tasmanian soils. It was observed that not all sclerotia settled at the same point in the gradient, with some sinking rapidly and some remaining at the surface whilst the majority settled in the middle of the column. When large amounts of soil are added it is possible for some sclerotia to be dragged down with the soil particles and then buried in the bottom layer, which would be discarded before the sieving operation. Where it is most likely to be beneficial is where the soil has a high sand content in the same size range as the sclerotia, as was the case with some of the US soils. In this instance the sand would be rapidly separated by the gradient and would leave a much smaller sample for inspection with the dissecting microscope. The gradient is unlikely to assist where soils have a high organic matter content, such as Tasmania or where soils are difficult to disperse due to clay content, such as Tasmania. Care needs to be taken when transferring samples from the gradient to the sieves, as there is potential for sclerotia to be lost.

3. VERIFICATION OF THE NEED FOR SCLEROTIA PRE-CONDITIONING

Sclerotia sampled from infected onions at harvest and then exposed to moist conditions with the presence of germination stimulants at 1, 2 and 3 months post-harvest could not be triggered to germinate. Sclerotia collected from the previous season incubated under the same conditions were triggered to germinate. This result is consistent with other studies about constitutive dormancy in sclerotia and confirms that post harvest application of germination stimulants would not provide an effective method to lower inoculum levels of newly formed sclerotia.

RECOMMENDATIONS:

The use of dehydrated garlic powder from the US, or potentially elsewhere, may be an economically feasible way to reduce inoculum levels. The main difficulty will be establishing the rate needed which will depend upon analysis of the product. The observation that the levels of stimulants can change in storage could be a problem for any carry over stocks as the shelf life could potentially be limited to only one or two seasons, after which time the volumes needed may be too costly. In the absence of a dehydrated garlic industry in Australia it is unlikely that dehydrated garlic products will be used for application to commercial fields as a means of disease control. The cost and risk of importing product with low analysis is likely to impede industry adoption of this approach. The synthetic product, although costly, provides reliable and repeatable levels of stimulants, which will be essential for industry adoption of this unique disease control strategy.

The use of a density gradient to assist the efficiency of assessing soil for sclerotia should be evaluated for localised soil conditions. Where it is most likely to be beneficial is where soil has a high sand content in the same size range as the sclerotia, as is the case with some of the US soils. In this instance the sand would be rapidly separated by the gradient and would leave a much smaller sample for inspection with the dissecting microscope. The gradient is unlikely to assist where soils have a high organic matter content, such as Tasmania or where soils are difficult to disperse due to clay content, such as Tasmania. Care needs to be taken when transferring samples from the gradient to the sieves, as there is potential for sclerotia to be lost.

Post-harvest treatment of new disease patches should not be treated with germination stimulants, as this is unlikely to effectively reduce disease levels owing to constitutive dormancy of newly formed sclerotia.