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A cholesterol-lowering extract from

GARLIC

A report for the Rural Industries Research and Development Corporation

by David Eagling & Sam Sterling

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Foreword

Clinical trials have shown that garlic has important health benefits. The most encouraging results have occurred in the area of cholesterol reduction. The compound responsible for these benefits is allicin, one of a number of compounds responsible for garlic's characteristic flavour and odour.

In Australia, attempts to produce allicin extracts aimed at the multi-million dollar international market for cholesterol lowering drugs, initially proved uneconomical due to low yields of allicin and fluctuations between batches.

This publication presents results pertaining to the issue of minimising product variability. The report details investigations on the varietal, field and storage conditions that affect the level of allicin extracted from garlic.

Australia continues to import about two thirds of the total garlic required for consumption. A large proportion of this is from China which, over the last 6 years, has dominated imports into Australia with a low cost product during mid-winter to late spring. This project identifies the potential for market diversification, with Australian garlic proving to be optimal for international health/pharmaceutical markets. Furthermore, the value to consumers of vegetables high in health-giving compounds provides options for the domestic marketing of Australian garlic.

This project was funded from RIRDC Core Funds which are provided by the Federal Government.

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Peter Core
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Contents

List of Tables

List of Figures

Executive Summary

There is currently a multi-million dollar international business in the production and sale of hypolipidaemic (cholesterol lowering) drugs and other treatments. Treatment generally consists of dietary and lifestyle intervention (for up to six months) prior to courses of drugs being prescribed. There is a significant opportunity for garlic supplements to become part of this first line treatment program. Garlic has long been cited as having a range of health benefits and scientists have been investigating the biochemical pathway linked to the production of the specific cholesterol lowering compound, allicin. Garlic has the added benefit of having few known side effects and hence it should be possible to initiate prophylactic treatment earlier than other first line hypolipidaemic therapies.

Previous commercial research undertaken by an Australian company in conjunction with the Monash Medical Centre indicated that Australian grown garlic had a role in the reduction of elevated cholesterol levels, but they found that the yield of allicin from the plant was often low and fluctuated from batch to batch causing their extraction process to become uneconomical.

In 1995 a collaboration between RIRDC, Agriculture Victoria and The Australian Garlic Industry Association, set out to investigate the varietal, production and storage conditions that affect the level of allicin extracted from garlic.

In the first phase of the study, a survey of the allicin levels in garlic grown across Eastern Australia (Queensland, New South Wales, Victoria, South Australia and Tasmania) was conducted. It determined that almost 50% of the samples (95 out of 200) contained allicin levels above the level required to allow economic extraction (> 4.5 mg/g of fresh bulb weight) as determined by commercial interests. This result contrasted with previous studies conducted in other parts of the world, in that allicin levels above 4.5 mg/g were recorded in a relatively large proportion of the samples. Allicin levels peaked at 9.0 mg/g whereas the previous highest result recorded in the scientific literature was 7.3 mg/g.

Later work with a range of garlic varieties at sites in Queensland, Victoria and Tasmania showed that allicin levels above 4.5 mg/g were reproducible across a range of Australian garlic growing environments. Furthermore, a range of factors were identified as being important in optimising allicin levels, in particular the matching of each garlic variety to its preferred growing region on the basis of both allicin production and crop yield.

Management and nutritional factors were identified that enhanced the level of allicin produced by the plants. Allicin increases of up to 35% were recorded with the use of nutrient supplements during crop growth. However, in some varieties this was accompanied by a concomitant decrease in bulb size and further studies are currently underway (in association with the Australian Garlic Industry Association) to determine the best time/s during the growth cycle to apply the nutrient supplements.

Storage trials were conducted to determine the optimum storage conditions for maximum retention of allicin levels over time. Protocols have been developed with the assistance of garlic growers and in particular, Mr. Roger Schmitke.

The high level of allicin present in Australian grown garlic presents two opportunities to the Australian garlic industry. Firstly, the opportunity to market fresh produce to consumers based on its premium quality (health benefits), and secondly, the opportunity to export for the international health and/or pharmaceutical markets. This second opportunity has been investigated in some detail, in association with Agriculture Victoria's agribusiness unit, Austrade and the Australian Garlic Industry Association.

Market research has indicated the potential to value add for the export market with a dried (processed or semi-processed) rather than fresh product. Further research is required to determine the optimal drying/powdering process based on the listed specifications of potential pharmaceutical customers.

Whilst the opportunity for the Australian garlic industry is potentially lucrative, the industry is currently extremely small by world standards. Hence significant industry development is required before the opportunity can become reality.

1. Setting the Scene

1.1 Introduction

Clinical trials have shown that garlic has important cardioprotective benefits including the ability to decrease platelet aggregation (1), lower total plasma cholesterol (2) and reduce blood pressure (3). The most encouraging results have occurred in the area of cholesterol reduction (2). The compound responsible for these benefits is allicin, one of a number of compounds responsible for garlic's characteristic flavour and odour. Allicin is present in garlic bulbs in the form of alliin which is converted to allicin when the bulb is cut or crushed.

There is currently a multi-million dollar international business in the production and sale of hypolipidaemic (cholesterol lowering) drugs and other treatments. An Australian pharmaceutical company has investigated the biochemical pathway linked to the production of allicin in garlic. The company initially produced pilot batches of this compound using garlic supplied by a local distributor. However low allicin yields and fluctuations from batch to batch resulted in the extraction process being uneconomical.

The project aimed to minimise product variability by investigating the varietal, field and storage conditions that affect the level of allicin extracted from garlic. The main objective was to identify garlic varieties and production protocols that optimise allicin yields.

1.2 A brief history of garlic production in Australia

Garlic was introduced to Australia through early European migration. As demand for local garlic increased through the 1970’s the area sown to garlic increased, primarily in Queensland and New South Wales. During the 1980’s, Australian producers adopted the 10 kg carton for small opportune exports within the Pacific rim.

This season (1999/2000) the industry predicts a harvest of 1,000 tonnes of garlic, with fresh garlic available from October through to early June (the latter may have been in storage for up to three months after harvest). The majority will be sold on the fresh market as there is only a limited commercial processing capacity.

Australia continues to import about two thirds of the total garlic required. A large proportion of this is from China which, over the last 6 years, has dominated imports into Australia with a low cost product during mid-winter to late spring (4).

1.3 Project objectives

1. Document the levels of allicin in Australian grown garlic.
2. Identify garlic varieties and production protocols that optimise yields of allicin.
3. Document the opportunity for Australian grown garlic as a source of allicin for national and international pharmaceutical companies.

2. The Opportunity for Pharmaceutical Production

The garlic industry in Australia is small by world standards averaging 350 tonnes per annum in the three years prior to 1995. The industry is primarily focussed on the domestic market, with limited opportune exports within the Pacific rim. Imports are significant, totalling 2,500 tonnes in 1996. A large proportion of this is from China which, over the last 6 years, has dominated imports into Australia with a low cost product during mid-winter to late spring (4).

The peak industry body, the Australian Garlic Industry Association, has identified the need for new markets and marketing tools to allow Australian garlic to compete with lower-priced imports. Investigation of allicin levels in Australian garlic offers industry the opportunity to increase markets through:

1. the supply of fresh or semi-processed product to global pharmaceutical companies and / or
2. reposition Australian garlic on both domestic and international fresh markets based on the level of allicin.

3. Methods and Results

Some of the information presented in this report has also been published in the scientific literature as Sterling, S.J. and Eagling, D.R. 1998. Agronomics and allicin yield of Australian grown garlic. *Acta Horticulturae* (in press). See Appendix 1.

3.1 The evaluation of allicin levels in Australian grown garlic

3.1.1 Allicin Level Comparison

A total of 200 garlic samples were collected from 43 producers in the Australian States of Victoria, Tasmania, Queensland, New South Wales and South Australia.

As garlic is vegetatively propagated, it is difficult to determine the genetic heritage of this material. It is estimated that within these 200 samples, some 80+ varieties were represented. This includes a number of the 132 varieties imported from the worldwide USDA collection in 1994 (under Horticultural Research and Development Corporation project VG505), as well as the varieties that currently make up the majority of Australian production.

It is important to note that due to the difficulty in determining genetic lineage of garlic, the varieties in this report are listed by the names provided by growers- they are not necessarily the same as similarly named varieties grown elsewhere.

Each of the 200 samples comprised 3-10 bulbs of an individual variety and these were obtained at least 30 days post harvest (ie. fully cured) to avoid rots or disease problems during transport and storage. Once received, samples were stored at 25°C and 65% relative humidity, until analysed for allicin content. Growers were also asked to fill out a questionnaire detailing information on growing region, soil type and rainfall for each sample. This information was received for a proportion of the samples only, and was used to identify potential factors in the production of crops with high allicin levels (see pages 6-9 of this report). The interaction of growing environment and variety in producing high allicin levels was further investigated in later years of the project.

The analysis of allicin was conducted in all instances by the commercial partner, Pharmaction Pty. Ltd., using High Performance Liquid Chromatography (HPLC).

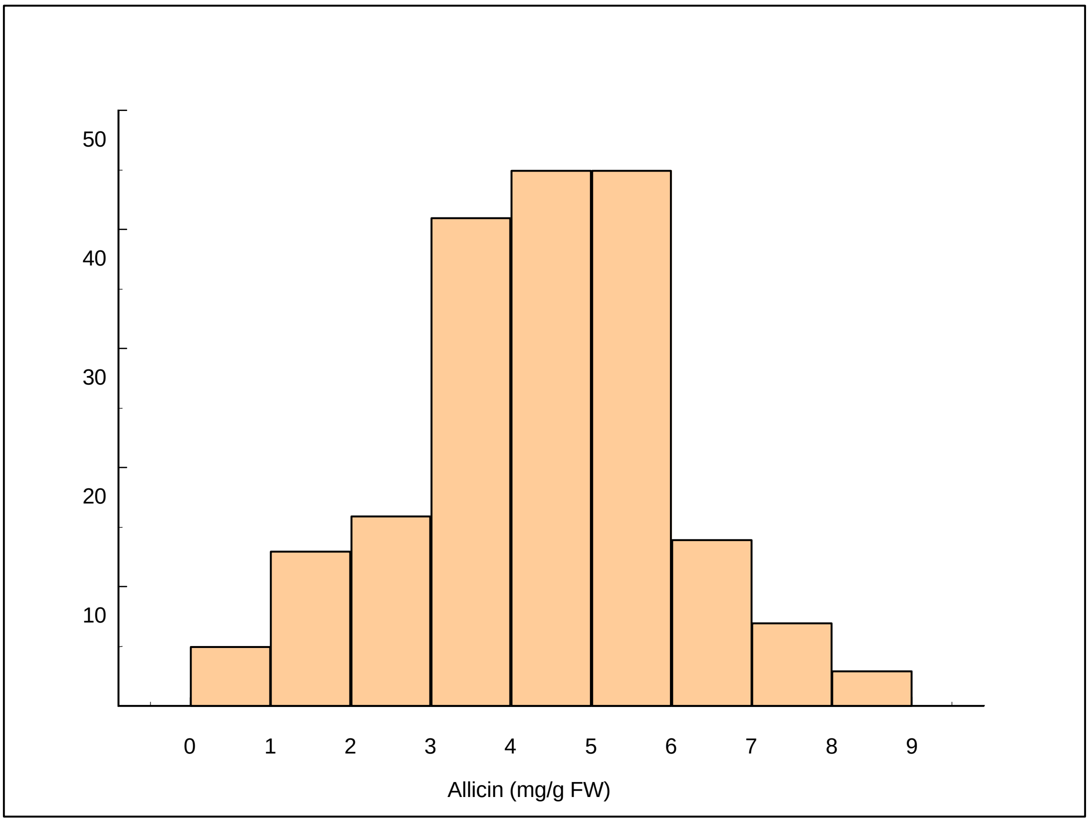
HPLC analyses were performed using a Hewlett Packard 1100, with ODS Hypersil column fitted with C18 pre-column.

Samples for analysis were prepared from each bulb by removing and peeling 2-3 of the cloves. A sample of 5 +/- 1 g was weighed out and blended with 25 ml of the internal standard using a Barmix coffee grinder. The mixture was then incubated for 20 min at 30°C before being centrifuged for 5 min at 4000 rpm. Two ml of the supernatant solution was added to 8 ml of a 50/50 v/v mixture of Milli-Q water and HPLC grade methanol. The supernatant liquid was then filtered through a 0.45μm HPLC filter into amber HPLC vials for assaying. The autosampler was cooled as the allicin content of a methanol/water mixture has previously been found to be unstable at room temperature (6). Results were recorded as mg/g allicin (by fresh weight), and then graphed against regional and climatic factors.

The internal standard used was ethyl p-hydroxybenzoate as this allowed a shorter run time thus reducing the effect of allicin instability, and prepared by adding approximately 500 mg of ethyl p-hydroxybenzoate to 20 ml of methanol AR and shaking until dissolved. Nine hundred ml of 80°C Milli-Q water was then added and the standard mixture cooled to room temperature, before being diluted to a final volume of 1L with Milli-Q water.

Results of 3.1.1
The 200 samples tested recorded allicin yields in the range 0.5 to 9.0 mg/g (by fresh weight) with the majority of samples in the 3.0 to 6.0 mg/g range (Figure 1). The distribution of yields appears similar to a normal distribution.

Figure 1: Frequency distribution of allicin levels in 200 garlic samples



Commercial interests determined that an allicin level of 4.5 mg/g (by fresh weight) was required for extraction to be economically viable. Almost 50% (95 out of 200) of the samples assayed had allicin yields over 4.5 mg/g, (ie "pharmaceutical grade") and a further 10% of the samples were in the 4.0- 4.5 mg/g range.

As a comparison to allicin levels previously cited in the scientific literature, the highest previously recorded allicin level was 7.7 mg/g (by fresh weight) for Romanian Red garlic grown in New York, USA (Table 1). Six samples of the Australian grown garlic tested in the current study recorded allicin yields higher than 7.7 mg/g. The next highest allicin yield recorded prior to this study was 6.7 mg/g for Spanish Roja grown in the USA and 6.6 mg/g for Laotouxu garlic obtained from China (5,6). Fifteen Australian grown samples from the current study exceeded this level and a number of these samples are listed in Table 1.

It is important to note however, that the HPLC analyses were conducted by different laboratories and therefore methods may vary between these studies. Our method was independently verified by a German laboratory, so to the best of our knowledge the results are comparable.

Table 1: Allicin levels (from fresh garlic samples) recorded in Australia and other countries

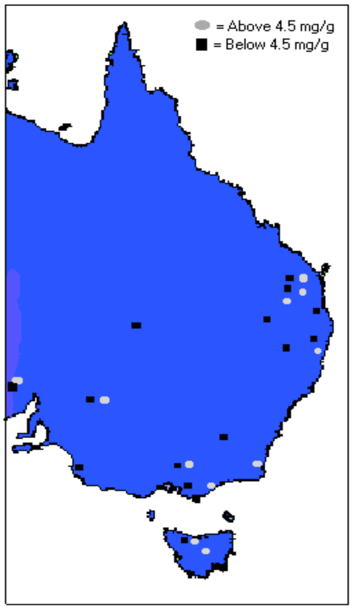
Country of origin	Allicin mg/g FW	Country of origin	Allicin mg/g FW
Australia	9.0	Switzerland#	3.6
Australia	8.4	Switzerland#	2.4
Australia	8.3	USA*	7.7
Australia	7.8	USA*	6.7
Australia	7.8	USA*	6.1
Australia	7.7	China^	6.6
Australia	7.6	China^	6.4
Australia	7.4	China^	5.1
Australia	7.3	Japan@	5.3
Australia	7.3	Japan@	5.1
Australia	7.0	Japan@	5.0

#From (7) Ziegler and Sticher, 1989
*From (5) Koch and Lawson, 1996
^From (6) Lawson, Wood and Hughes, 1991
@From (8)Ueda et al, 1991

Influence of growing region

Production of pharmaceutical grade garlic was not restricted to any specific growing region across Eastern Australia. All of the regions that supplied garlic samples recorded allicin levels both above and below 4.5 mg/g (Figure 2).

Figure 2: Locations of garlic crops according to allicin levels in Eastern Australia.



Influence of soil type and rainfall

Based on the information provided by growers (see attached questionnaire, Appendix 2), samples that reached or exceeded 4.5 mg/g were sourced from across a wide range of soil types and rainfall levels. Soil types were categorised as: sand, sandy loam, loam, clay loam or clay and there were samples grown in all soil types that recorded allicin levels above 4.5 mg/g FW.

Samples with allicin levels above 4.5 mg/g FW were sourced from areas with annual rainfall of 300mm-1000mm, which is not surprising as the crop is irrigated, hence actual water availability would have been similar across these crops. It is interesting to note however, that allicin yields above 4.5 mg/g were not recorded in areas where rainfall was greater than 1000 mm annually.

The questionnaire results obtained from growers indicated a tendency for high allicin levels to be recorded in soils with high pH levels (pH 8), and in regions where light quality (number of cloud free days) could best be described as "high" (from a choice of very high, high, average, low). Whilst it is not possible to draw strong conclusions on the basis of this sort of information, it would seem that the factors positively affecting allicin levels may include low rainfall, high soil pH and high levels of sunlight. These conditions are known to lead to increased sulfur compound concentration in related vegetables such as onions (9,10). Further investigation of these issues was carried out in the second year of field trials.

Influence of variety

Varietal influence on allicin yield was not detected in this initial research. Where the same variety was grown in different locations, different allicin levels were recorded (Table 2). The variation between sites was such that it was impossible to detect varieties, or groups of varieties, that stood out as preferential producers of allicin.

Table 2: Variation in allicin levels for the same variety grown at different sites

Variety	Allicin Content (mg/g FW)	Crop Location
Italian White 1	6.6	Purga, Qld.
Italian White 1	5.3	Glen Innes, NSW
Italian White 1	4.6	Quirindi, NSW
Purple	5.0	Purga, Qld.
Purple	4.5	Quirindi, NSW
Purple	3.7	North Dorrigo, NSW
Purple	3.1	Tenterfield, NSW
Purple	1.8	Bevendale, NSW
Purple	1.7	Walcha, NSW

3.1.2 Comparison of varieties grown at several different locations

The following year, a number of garlic varieties were selected for comparison at four field sites across Eastern Australia. The varieties were chosen to represent a range of garlic genotypes- California Early and Australian White are softneck or non-bolting varieties, whilst Italian and Glenlarge are hardneck or bolting varieties. Three of these varieties (California Early, Australian White and Italian) constitute a large proportion of current Australian production. Glenlarge was included as it is a heat tolerant variety known to grow well under the conditions at site 1.

Table 3: Location of field sites

Site	Location	Co-ordinates	Average daily temperature (°C)	Annual rainfall (mm)
1	Southern Queensland (Gatton)	27° 34', 152° 17'	20	840
2	Northern Victoria (Irymple)	34° 15', 142° 12'	17	270
3	Southern Victoria (Toora)	38° 40', 146° 20'	14	970
4	Northern Tasmania (Burnie)	41° 04', 145° 54'	13	1000

Sites two and three fall within the latitudes where most garlic is planted in Eastern Australia. Sites one and four are slightly outside the common regions for garlic production and represent areas where specialised garlic varieties (sub-tropical varieties at site one and cold resistant varieties at site four) would normally be planted. In order to examine the effect of climatic differences on allicin production, it was decided that the varieties would be planted at all three mainland sites (sites 1, 2 and 3), even though the common varieties would not perform well under the conditions at site 1. It was not possible to plant these same varieties at site four as the lateness of planting necessary to avoid winter frosts would have rendered the planting material non-viable. The two sites representing major garlic production (sites 2 and 3) had additional locally sourced varieties sown; Marlborough and Peter's Brown at site 2 and Mexican at site 3. Site 4 comprised only varieties M and B due to genotype / environment constraints.

Planting occurred at the normal time for garlic production in each region (earlier plantings in the northern regions getting steadily later with latitude). At each site, all varieties were planted at the same time, but harvested individually when each one reached maturity.

The seed stock for each variety was obtained from a single source in each case. Where the variety was planted at more than one site, the original material was divided evenly between the sites. The varieties were planted in a randomised block design with five replicates at each site. Each plot was 1.5 m long, with 10 cm plant spacings (15 plants per row). Machinery utilised at sites 1, 2 and 4 allowed three rows of garlic to be planted in each plot (20 cm between rows), whilst four rows were planted at site 3. Planting dates, based on optimising garlic initiation, were: Site 1, March 1997; Site 2, April 1997; Site 3, May 1997 and Site 4, August 1997. Management practices utilised were the standard for the region in each case.

Allicin levels were analysed by HPLC, using the method outlined in 3.1.1. Samples comprised two bulbs which were selected randomly from each of the five replicates. Mean allicin levels were calculated for each cultivar at each site and expressed as mg allicin per gram of fresh (cured) bulb weight (Table 4). The standard error was calculated for each mean. Means were compared using analysis of variance, and the associated least significant difference (lsd) at p = 0.05 (Table 4).

Results of 3.1.2

Table 4: Comparison of mean allicin levels (in mg/g fresh weight ± standard error) recorded at each site:

	Variety	Site 1 (Gatton)	Site 2 (Irymple)	Site 3	Site 4
HARD-NECK	Glenlarge	5.0 ± 0.3	6.1 ± 0.2	4.8 ± 0.8	
	Italian	7.8^ ± 1.8	5.3 ± 0.4	2.7 ± 0.4	
	Mexican			3.2 ± 0.8	
	Variety M				5.7 ± 0.7
SOFT-NECK	Cal. Early	5.4^ ± 1.1	5.6 ± 0.1	--	
	Aust. White	6.4^ ± 0.1	5.1 ± 0.4	--	
	Marlborough		6.6 ± 0.2		
	Peter's Brown		5.0 ± 0.5		
	Variety B				5.5 ± 0.7
	lsd (p = 0.05)	**#**	**0.7**	**1.2**	**0.9**

^Bulbs failed to form properly for these varieties at Site 1 and therefore (#) an lsd value was not calculated. Allicin levels are provided as a comparison only.

--These varieties were unavailable for analysis due to a *Fusarium* infestation at harvest.

Varietal influence on allicin levels
At sites 2 and 3 there were significant differences in the level of allicin between varieties (Table 4). However, the status of the material as either hardneck or softneck was not significant, as the pharmaceutical requirement was achieved at each of the sites irrespective of this character, except site 3, where the softneck varieties were unavailable for analysis due to Fusarium (Table 4).

Influence of site location on allicin levels
There was a substantial difference in the level of allicin for variety Italian at sites 1, 2 and 3 and variety Australian White at sites 1 and 2 (higher levels at the more northern sites). Variety Glenlarge had higher levels at site 2, than at either site 1 or site 3 (Table 4).

The results indicate that a key criteria for optimal allicin production is the matching of the production area with appropriate varieties.

Other influences on allicin levels
Varieties planted at sites 1 and 4 which represented areas considered marginal for garlic production, all recorded allicin levels above the pharmaceutical requirement. This indicates that allicin production in Australia is not restricted to the common garlic growing areas of South-East Australia.

Studies of similar sulfur compounds in other vegetables (such as cysteine sulfoxides, which are flavour compounds in onions) have shown a relationship between increased rainfall and reduced flavour levels. It has been shown that increased rainfall leads to a decrease in sulfate uptake from the soil and results in a lower concentration of these compounds in the bulb (9,10). Whilst there are a range of variables that may affect the allicin levels recorded in our study (water, soil pH, light availability etc.), the variety Glenlarge may be seen to uphold this observation, (reduced allicin levels at sites of higher rainfall), while varieties Italian and Australian White do not. There were varieties at every site (including the high rainfall site 4) that recorded high allicin levels. It may well be that this is an adaptation of particular varieties to wetter environments, and indeed we found that the optimisation of varieties to their preferred growing environment led to high allicin levels in all cases (refer to Table 5).

Whilst varieties can be found to suit most environments, it is important to note that some regions are preferable for allicin production, as large numbers of varieties will produce the required allicin levels when grown in these regions. As noted previously, these regions consist of warm, dry climates with high soil pH (ie the conditions under which sulfate uptake is favoured, requiring no varietal adaptation to allow allicin concentration). Much of Australia's garlic production already occurs in regions with these characteristics. The issue of sulfur uptake was further addressed in nutrient trials (refer to section 3.2).

Three varieties at site 1 (Italian, California Early and Australian White) failed to produce proper bulbs due to the lack of an environmental cue. The plants reached maturity and were harvested and allowed to dry as per normal. Analysis was conducted on the base of the stem (ie where the bulb would normally form) and allicin was detected in amounts comparable to other varieties (Table 4). This indicates that the absence of a bulb at maturity does not preclude allicin production.

Yield - Varietal Analysis
Reporting of allicin levels in terms of allicin per gram of bulb weight indicates that even small or underdeveloped bulbs produce high levels of allicin (Table 5). However, a more useful measure of economic commercial allicin yields is allicin per square metre of production (Table 5).

Table 5: Allicin produced per area of garlic planted

Site	Type	Variety	Allicin (mg/g ± standard error)	Yield (kg of garlic harvested per m^2 planted)	Allicin produced per m^2 planted (g/m^2)
2	HARD	Glenlarge	6.1 ± 0.2	0.8 ± 0.11	4.8 ± 0.02
2	HARD	Italian	5.3 ± 0.4	1.9 ± 0.08	9.9 ± 0.03
2	SOFT	Cal. Early	5.6 ± 0.1	1.8 ± 0.09	9.8 ± 0.01
2	SOFT	Aust. White	5.1 ± 0.4	1.9 ± 0.09	9.7 ± 0.03
2	SOFT	Marlborough	6.6 ± 0.2	0.8 ± 0.05	5.3 ± 0.01
2	SOFT	Peter's Brown	5.0 ± 0.5	1.5 ± 0.04	7.8 ± 0.02
4	HARD	Variety M	5.7 ± 0.7	1.47 ± 0.08	8.4 ± 0.06
4	SOFT	Variety B	5.5 ± 0.7	1.66 ± 0.06	9.0 ± 0.04

The optimal garlic varieties for a particular region are those that produce high yields AND high allicin levels.

3.2 The effect of nutrition practices on allicin production

3.2.1 Low dose sulfur applications

Studies conducted in the USA on cysteine sulfoxides in onions (compounds that are very similar in structure to allicin), have found that the availability of nutrients during growth can influence the production of these compounds (10,11). On this basis an additional trial was conducted at site 2 (Irymple) to investigate the role of nutritional supplements in enhancing allicin levels. As there are a range of sulfur compounds in garlic, the aim was to determine whether increased sulfur availability during growth increases allicin concentration, rather than leading to the production of non-target compounds.

Six replicates of six varieties (Australian White, Glenlarge, Italian, California Early, Marlborough and Peter's Brown) were planted in a randomised block design. Half of the plots (three of each garlic variety) were randomly selected and had additional foliar applications of sulfur applied monthly. The other half were control plots (normal nutritional practices for garlic in the region). The sulfur applications consisted of Lignosulfinate applied at a rate of 12 Lt/Ha.

Results of 3.2.1
There was no significant difference ($p > 0.05$) in either yield (as measured by bulb size and weight) or allicin levels between the foliar treatments at site 2. However, there was a tendency for both yield and allicin levels to increase with foliar applications of sulfur (Figures 3 and 4). This result was developed further in following seasons by investigating the rate of application and including a second nutrient treatment.

As an aside, the levels of allicin recorded at site 2 ranged from 3.7 to 9.2 mg/g allicin by fresh weight which is consistent with our previous finding that Australian grown garlic contains comparatively high levels of allicin.

Figure 3: Mean fresh weight (after curing) of six garlic varieties grown at Irymple, with and without additional foliar applications of sulfur

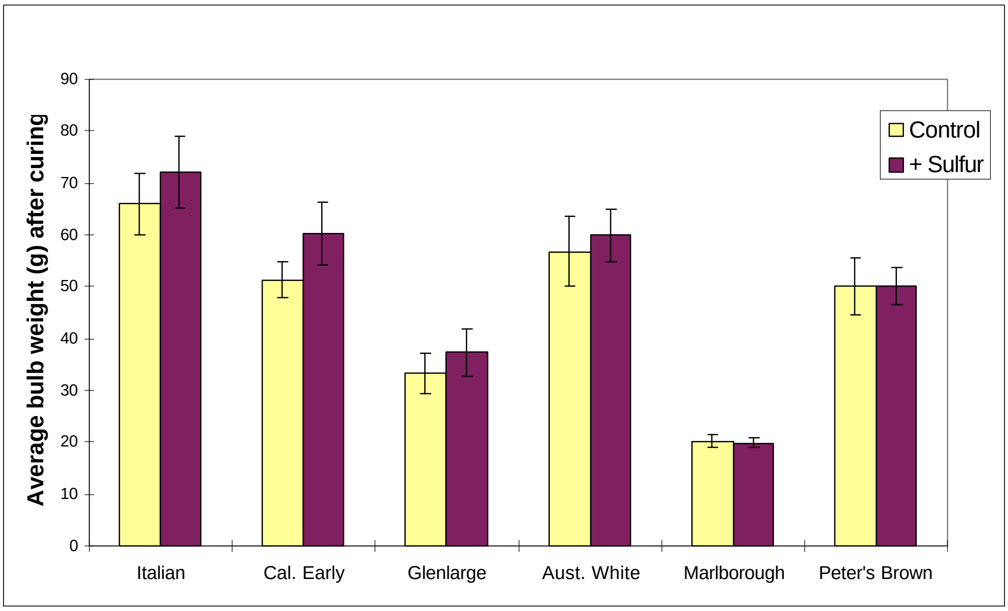
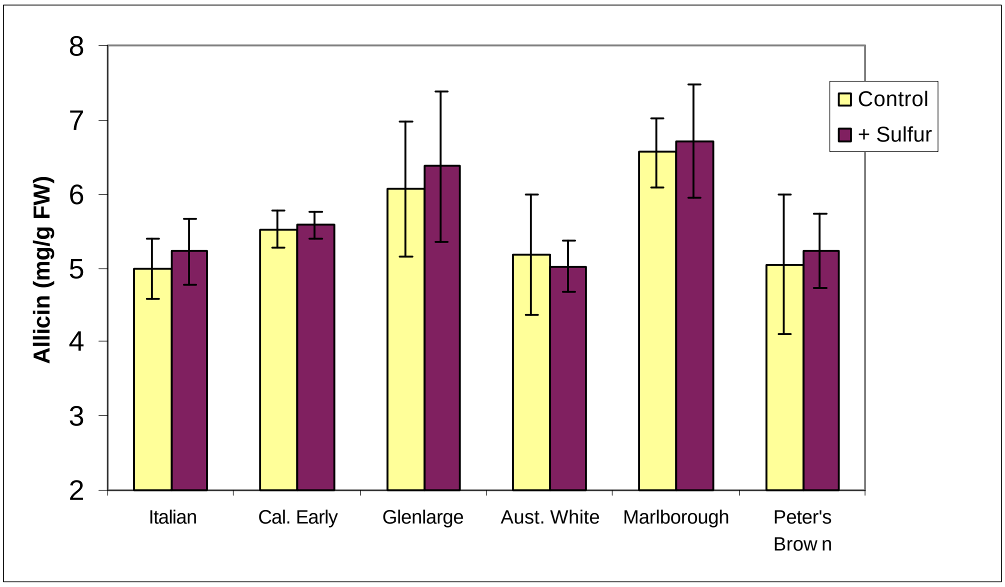


Figure 4: Mean allicin levels for six garlic varieties grown at Irymple, with and without additional foliar applications of sulfur



3.2.2 The final year of field trials

In 1999, two field trials (3.2.3 and 3.2.4) were conducted to evaluate the role of nutrients (sulfur and nitrogen) in allicin production. The trials were located at the Sunraysia Horticultural Centre, Irymple, (site 2 in the previous results) as the region had shown itself to be a premium growing region for pharmaceutical-grade garlic in previous trials.

3.2.3 The effect of nitrogen and sulfur supplements on allicin production

Three garlic varieties, Australian White, California Early and Shumex, were chosen for their previously determined 'pharmaceutical grade' allicin levels and known ability to grow in the Irymple region.

The varieties were sown in a randomised block design with five replicates of each variety by treatment combination (see below). Each plot was 1m long with three rows of garlic. Cloves were planted at 10cm intervals (30 plants per plot). Management practices were best practice for garlic in the Sunraysia region, and the same as for the previous year's Irymple garlic trial.

The treatments consisted of :

- Normal management practice with no additional nutrient applications (Control)
- Normal management practices plus a monthly foliar application of sulfur (18 Lt/Ha Lignosulfinate) (Treatment 1)
- Normal management practice plus a fortnightly foliar application of sulfur (18 Lt/Ha Lignosulfinate) (Treatment 2)
- Normal management practice plus a fortnightly foliar application of nitrogen (12Lt/Ha Lig-Nit) (Treatment 3)

Results of 3.2.3
Allicin analysis was conducted on fully cured bulbs (approximately six weeks after harvest). Two bulbs were randomly selected from each plot and analysed for allicin. The results were averaged for each variety/treatment combination, and standard errors were calculated.

Sulfur
There was a significant increase ($p < 0.05$) in allicin levels with the application of sulphur (treatments 1 and 2 - see Figure 5). This finding applied to all three varieties, as the variety by treatment interaction was not significant. The average allicin levels for control, treatment 1, treatment 2 and treatment 3 (all varieties combined) were: 4.4, 5.4, 5.7 and 4.6 respectively, with an l.s.d. of 0.4. This indicates that treatments 1 (sulfur monthly) and 2 (sulfur fortnightly) positively influence allicin levels, whilst treatment 3 (nitrogen) has little effect. This is noteworthy in that the control (4.4 mg/g allicin) was close to the pharmaceutical prerequisite of 4.5 mg/g, whilst the sulfur treated bulbs were well above the prerequisite, (5.4 and 5.7 mg/g for treatments 1 and 2 respectively), elevating a borderline situation into a sought after range. From an economic point of view, the benefits gained from doubling the sulfur rate (i.e. fortnightly applications versus monthly applications) were not great enough to justify the extra time and money required to carry them out.

Nitrogen
As previously noted, there was no benefit to allicin production or bulb weight with the additional foliar application of nitrogen.

It is important to note that the nutrient applications also negatively affected bulb weight in varieties Australian White and Californian Early (Figure 6). Thus nutrient supplementation is complex, as the benefits gained through increased allicin levels may be offset by yield reductions (measured as bulb weight) resulting in no net gain when expressed as allicin produced per area planted (Figure 7). For varieties where nutrient supplementation does not negatively affect bulb weight (such as Shumex), significant gains in allicin yield can be obtained (expressed as allicin produced per area planted). Figure 7.

Figure 5: Allicin levels for three garlic varieties treated with nutrient supplements

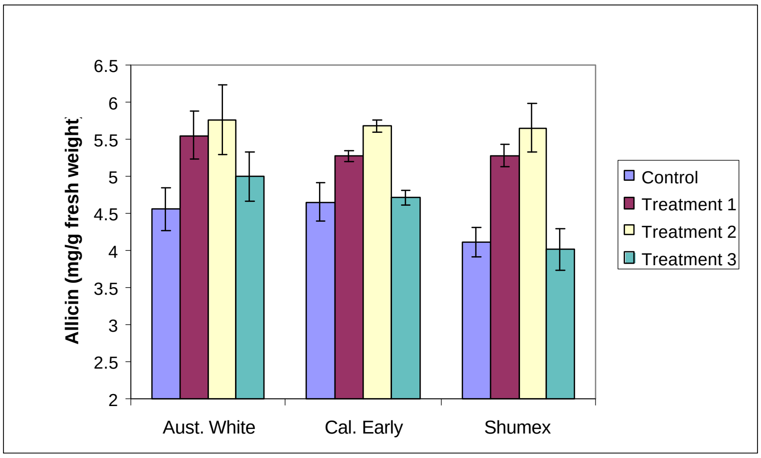


Figure 6: The effect of nutrient supplements on average bulb weight (fresh weight)

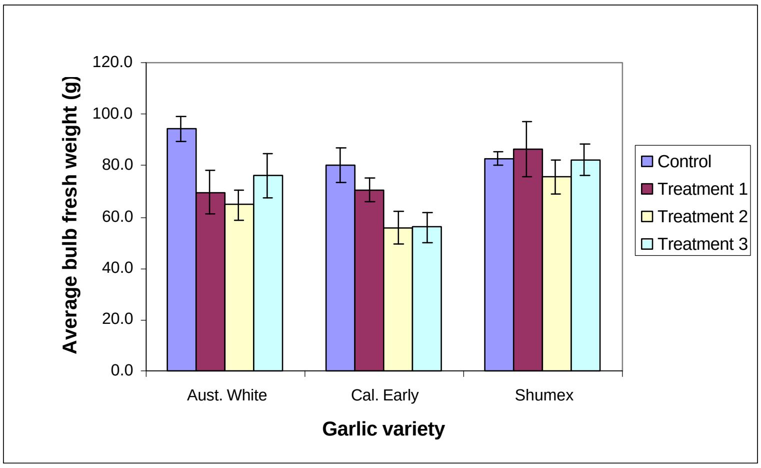
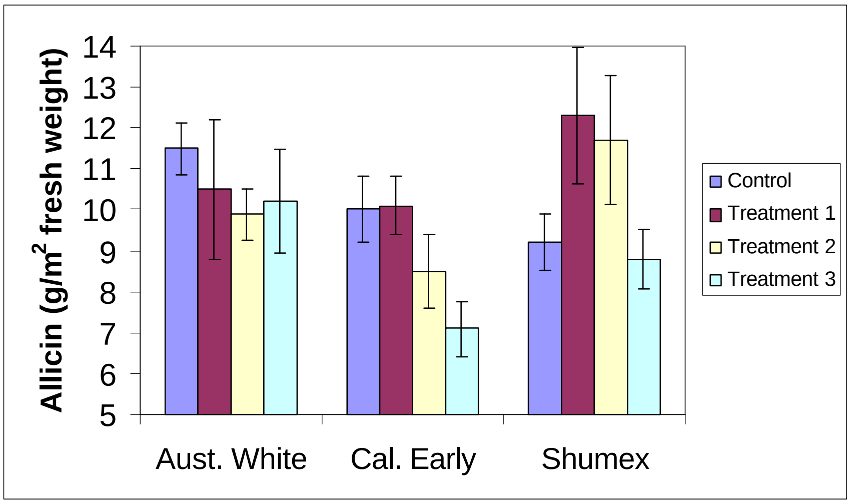


Figure 7: Allicin produced per m² garlic planted, with and without additional foliar applications of sulfur or nitrogen



3.2.4 Allicin production during different growth stages

In 1999 the level of allicin was measured throughout the season. Allicin is produced in the leaves of garlic plants during growth and stored in the bulb at maturity (2), but little is known about the dynamics of allicin accumulation or the site of allicin storage during plant development.

Two varieties, Australian White and Shumex, were grown at site 2 (Irymple) under normal management protocols for the region. Starting at the 4-leaf stage (approximately 12 weeks after sowing), two plants of each variety were dug up and the level of allicin in the leaves, stem and bulb analysed (Figure 8a, 8b). This process was repeated every three weeks until bulbing (early November) and then at two weekly intervals until harvest (early December).

Figure 8a: Allicin levels in the bulb, stem and leaves during growth (Variety Shumex)

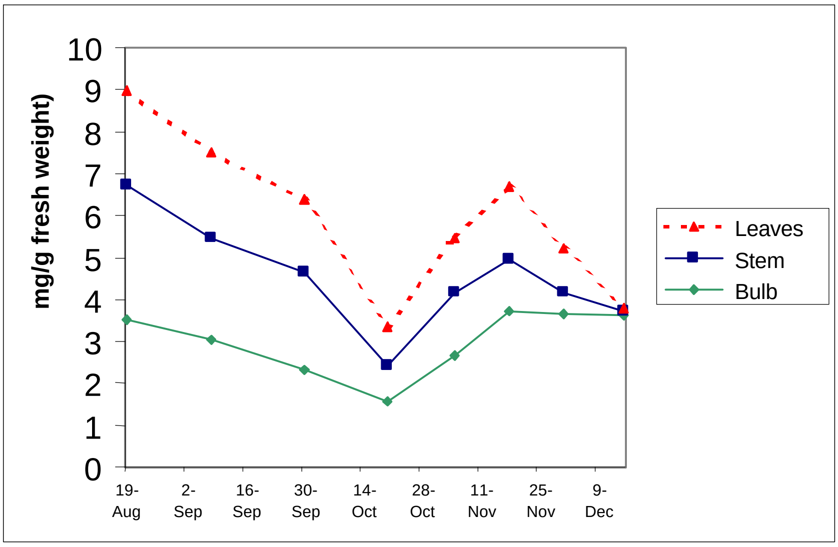
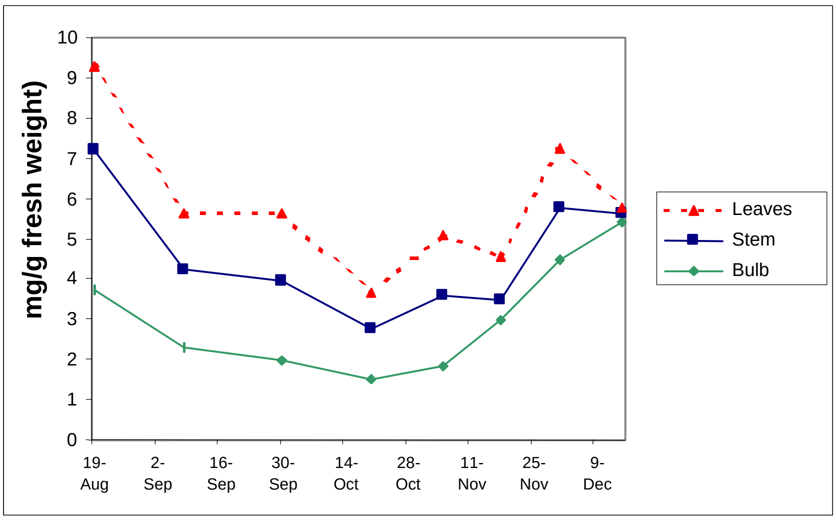


Figure 8b: Allicin levels in the bulb, stem and leaves during growth (Variety Australian White)



Results of 3.2.4
The highest allicin levels recorded in the stem and leaves occurred at the 12 week stage (the first measurement taken in the above experiment). During spring the allicin levels in all areas of the plant dropped steadily until the onset of bulbing. From this time until harvest allicin levels increased steadily in each part of the plant, with levels dropping off slightly just prior to harvest (9th Dec). With allicin peaking during the early and late stages of plant development, it is postulated that the application of foliar nutrients at these times may influence overall allicin production. This theory is currently being investigated in trials at the Sunraysia Horticultural Centre, with the aim of optimising the timing of nutrient applications to allow increased allicin production without the side effect of bulb size/weight reduction as recorded in part 3.2.3.

3.3 Post-harvest considerations for optimal allicin retention

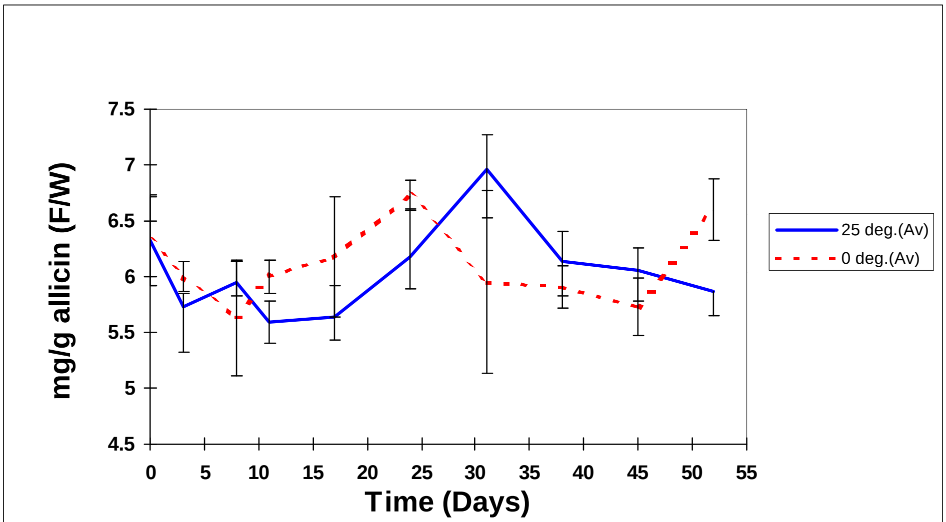
3.3.1 Initial experiments

The effect of storage on allicin levels was investigated using variety Australian White and two storage treatments:
0°C as recommended for garlic storage (12), and
25°C as the majority of Australian garlic is stored under ambient conditions.

A sample of 200 bulbs from an individual crop was obtained and nine bulbs were randomly selected and assayed for allicin content using the HPLC analytical procedure previously described (3.1.1). The remaining bulbs were divided randomly into two groups, with one group put into storage at 0°C and the other at 25°C. Storage was on open wire racks in controlled temperature coolrooms. At each of ten subsequent time intervals over eight weeks, nine bulbs from each temperature treatment were selected at random and assayed for allicin content. Allicin yield was graphed against time for each temperature treatment.

Results of 3.3.1
At most times, no significant differences between the two storage temperatures (0°C and 25°C) were detected. The exceptions were at 12, 24 and 52 days, when allicin producing potential in garlic stored at 0°C was significantly higher than in bulbs stored at 25°C. However, the levels recorded at these times varied at most only +/- 0.41 mg/g from the initial allicin yield of 6.3 mg/g recorded at the commencement of the trial. Allicin producing potential for both storage treatments fluctuated about a mean of 6.1 mg/g allicin, with a maximum of 6.9 mg/g and a minimum of 5.6 mg/g allicin (Figure 9).

Figure 9: Fluctuations in allicin levels with time in storage



In subsequent field experiments the storage of garlic was significantly affected by infection with a pathogen (*Fusarium*) which resulted in losses of up to 40% of the garlic material. Discussions with industry indicated that this was not uncommon. As the above experiment indicated that garlic for pharmaceutical markets could be stored under a range of temperatures, the remaining part of the storage trials were focused on reducing losses associated with pathogen outbreaks.

3.3.2 Storage protocol

The following definition of preferred storage conditions to minimise pathogen infection of garlic bulbs, and to optimise storage in general, is based on a combination of industry knowledge and experimentation.

Results of 3.3.2

Experimentation undertaken by Mr. Roger Schmitke over a number of years has resulted in the following definition of optimal storage of pathogen-free garlic:

1. A storage temperature of 25-27°C, and
2. A relative humidity (RH) of 60-62%.

Temperatures above this range will increase the rate of dehydration of the bulbs, while storage of garlic at temperatures below this range will result in increased rates of sprouting with subsequent losses in quality and marketability.

Note: storage of garlic was not attempted at temperatures below 18°C in the above mentioned study.

Our experiments, with material already infected with a pathogen indicated that further losses and deterioration of bulbs could be significantly limited with storage at either 25 °C or 0°C. Storage at 0°C did not result in any adverse effects on the bulbs as they remained in good condition and were suitable for assaying.
However, the bulbs would have been unsatisfactory for use as planting material as extreme cold retards the bulb's ability to grow. The bulbs stored at 25 °C were plantable, although some treatment of the cloves would be necessary to reduce the likelihood of re-infection.

In summary, the combination of these studies has shown the following with respect to the storage of garlic:

1. The level of allicin fluctuates in storage irrespective of the temperature.
2. The potential for pathogen infection is of greater consideration to the storage of garlic and its subsequent use in pharmaceutical markets than manipulations of temperature and humidity to optimise allicin levels.
3. The suggested storage conditions for garlic are a temperature range of 25-27°C and a relative humidity of 60-62%. Where humidity control is not available, it is vital that bulbs are stored in a well-ventilated area with maximal air flow around each bulb. Where temperature is strictly controlled, some varieties may require a period of cooling prior to planting.
4. In instances where the bulb material is not required for re-planting and is intended for use by pharmaceutical markets only, garlic can be stored at 0°C to limit the losses associated with pathogen infections that may already be present.

4. Discussion of Results - Commercial Opportunities

4.1 The domestic market

Significant opportunities to market Australian garlic as "healthier" than imported material (based on higher levels of allicin) are being realised by the Australian Garlic Industry Association. A substantial media interest in this aspect of the research has resulted in articles in The Age, The Herald-Sun and The Sunday Herald-Sun, The Weekly Times, Good Fruit and Vegetable and many local newspapers (Appendix 2).

4.2 The export market

Opportunities to market Australian garlic on world markets are also significant, with the European health product market a key focus. The ability of Australia to produce garlic of high pharmaceutical quality, in association with contra-seasonal production, represents an important opportunity for export.

Discussions with pharmaceutical companies mediated through Agriculture Victoria's agribusiness unit and Austrade in Germany indicate that there remains a number of constraints to capturing the above opportunity. Specifically, issues regarding

- security of supply
- strategic considerations such as number and size of suppliers
- the provision of QA procedures to guarantee allicin levels, and
- the potential to moderate supply/price cycles

These will need to be addressed by industry to fully realise the potential of the opportunity.

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6. Appendices (not availble in this online version)

6.1 Appendix 1: Agronomics and allicin Yield of Australian grown garlic (*Allium sativum)*

(Paper accepted for publication in *Acta Horticulurae)*

6.2 Appendix 2: Questionnaire circulated to growers