



# **Increasing the export potential -- opportunities for Australian garlic**

**A report for the  
Rural Industries Research and  
Development Corporation**

by Gisele Irvine and Samantha Sterling

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# Foreword

Two previous projects, BIO-1A, "Garlic, high yielding and virus free for profits", and DAV-124A, "A cholesterol-lowering extract from garlic", were conducted by Biofresh Australia P/L and the Department of Natural Resources and Environment (DNRE) at the Institute for Horticultural Development (IHD), Knoxfield. Project BIO-1A provided a selection of virus-tested garlic cultivars as seed stock for commercial production for both the fresh market and for garlic powder production, used in the pharmaceutical industry and complimentary medicine market. The initial trials of these garlic cultivars found them to be high yielding in the Sunraysia region of Victoria and in the north of South Australia.

Project DAV-124A, carried out by IHD investigated the levels of alliin reported to be a cholesterol lowering extract from garlic, developed protocols for optimising yields of alliin and identified opportunities for Australia as a source of alliin for national and international pharmaceutical companies.

Based on the results obtained from these two projects, the Australian Garlic Industry Association requested that a trial be undertaken to assess the performance of the virus-tested garlic cultivars alongside those usually grown by industry over a two-year period. The study would also measure the alliin content of all the cultivars in the trial, comparing the virus-tested garlic to the industry standards.

This project was co-funded by RIRDC, HRDC, a commercial partner Biofresh Australia P/L, the Australian Garlic Industry Association (AGIA) and the Department of Natural Resources and Environment (DNRE).

This report, a new addition to RIRDC's diverse range of over 800 research publications, forms part of the New Plant Products R&D program, which aims to facilitate the development of new industries based on plants or plant products that have commercial potential for Australia.

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We would particularly like to acknowledge the invaluable assistance of the Sunraysia Horticultural Centre (DNRE), in particular Mr Paul McClure and Mr Colin Bennett.

The authors also acknowledge the State Chemistry Laboratories for conducting the alliin testing for the project and Dr Graeme Hepworth for the statistical analysis.

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# Executive Summary

The principal aims of this investigation were to identify the yield benefits of planting virus-tested garlic, to evaluate the effect of virus-tested garlic on alliin levels and to establish the rate of re-infection by yield reducing viruses

Previous work has shown that Australian garlic is infected by three viruses:

Leek Yellow Stripe Virus (LYSV)  
Onion Yellow Dwarf Virus (OYDV)  
Shallot Latent Virus (SLV)

This investigation confirmed the presence of these viruses in commercial lines in two years trials. High levels of LYSV and OYDV were recorded in commercial lines in the first year but incidence was considerably lower in the second year of trials

Comparison of yields from virus-tested and non virus-tested commercial lines were made over two years in insect proof screenhouses and open field production areas. The results showed;

- Overall results demonstrated variability in yields between cultivars, including virus-tested material, and seasons.
- Results indicated substantial influences of seasonal conditions on yields. In 2000, Biofresh 1 and Biofresh 3 yielded up to 55% better than in 1999 in the field trial.
- Complications over the screenhouse experimental design prevented comparisons between virus-tested cultivars and commercial cultivars. Nevertheless data showed that virus-tested Biofresh 1 yielded 3.65 kg per plot in 1999 and 7.96 kg per plot in 2000 and the industry standard, Aussie White yielded 2.88 kg per plot and 5.76 kg per plot in 1999 and 2000 respectively.

Investigations on alliin production between virus-tested and non virus-tested commercial lines were also made over two years in screenhouse and open bed trials. Plants were grown to maturity and chemically analysed for levels of alliin. In year one climatic conditions were unfavourable for garlic production and no significant differences were found between virus and non virus-tested plants. In year two, which was considered to be more favourable higher levels of alliin were recorded in plants derived from virus-tested cloves.

In one experiment in insect proof screenhouses 19.0 and 19.6 mg/g fresh weight of alliin were recorded from virus-tested and virus-infected Biofresh, compared with 12.8 mg/g fresh weight in the non virus-tested Aussie White, which was used as the industry standard. These results were not repeated with other virus-tested lines in screenhouses nor in a similar experiment in open beds where alliin production in virus and non virus-tested lines was compared.

Studies on virus incidence showed that OYDV and LYSV were more prevalent than SLV in the two years of investigation. Overall results showed that the incidence of virus infection in garlic originating from the virus-tested lines was lower than in the commercial non tested lines. Observations on rates of re-infection indicate that non-persistent aphid transmitted viruses spread slowly in spite of the fact there were two flushes of aphid populations which invaded the trial areas. Consequently incidence of virus in virus-tested plants remained lower than in the non tested commercial lines.

# 1. The Project Objectives

## 1.1 A brief history of garlic production in Australia

Garlic was introduced to Australia through early European immigration. As demand for local garlic increased through the 1970's, the area sown to garlic increased, primarily in Queensland and New South Wales. By 1992 the industry had grown to 350 tonnes per annum with growth remaining static until 1995.

Currently, the garlic industry in Australia is small and targeted primarily at the domestic (fresh) market. Imports are significant, totalling 2,500 tonnes in 1996 and often cheaper than the local product (coming from China, the USA and Argentina). The cheaper imports flood the market, reducing prices. In the 1999/2000 season, garlic production was expected to be in the vicinity of 1000 tonnes of garlic, with fresh garlic available from mid-October through to early June (the latter having been in storage for up to 3 months after harvest). The majority is sold on the fresh market, as there is only limited commercial processing capacity in Australia.

Throughout the world there has been extensive research into the effects of the removal of yield reducing viruses on commercial garlic yield. Overseas studies have shown that virus-tested garlic, where pathogenic viruses are removed and the material multiplied in tissue culture, can lead to yield increases of 20-50% when grown under commercial conditions, compared to the garlic varieties that are virus infected. Anecdotal evidence suggests that the virus-tested material is also cheaper to produce, with a reduced requirement for sprays and disease management. Most large garlic producing countries now use virus-tested seed stock as part of the normal production system. In Argentina in the late 1980's, yields dropped considerably and acreages shrank until growers began using virus-tested seed stock. Currently the garlic industry in Australia is small and targets the domestic (fresh) market. In 1998, 1500 tonnes of garlic were produced in Australia, with a further 3,000 tonnes imported. Australian grown garlic has difficulty competing on the domestic market, as the imported material is cheap, of reasonable quality and available in large volume. The garlic currently grown in Australia has been found to contain all the yield reducing viruses.

In 1989 a joint venture project between Daratech (formerly the commercial arm of the Victorian Department of Agriculture) and Kamvale (a group of private investors) was established to find a replacement crop for the tobacco growers of Victoria. Biofresh Australia P/L was formed, with a focus on research into virus elimination in garlic and the establishment of a tissue culture multiplication scheme to produce virus-tested garlic with a view to increasing bulb weight for a higher yielding crop. The Institute for Horticultural Development was retained as the research arm of the project and the project was started in 1989. The project provided a limited selection of higher yielding virus-tested garlic cultivars able to be grown as quality seed stock to be grown on by commercial growers with a potential for drying and processing for pharmaceutical quality garlic powder.

In 1995 a project investigating the levels of alliin in Australian garlic found that Australian garlic and in particular the virus-tested cultivars, returned economically sustainable levels of alliin compared to overseas cultivars. The high levels of alliin found in Australian garlic presents two opportunities for the garlic industry; one the opportunity to supply fresh garlic to consumers marketed on the health benefits and secondly the opportunity to supply national and international pharmaceutical industries with a dried (processed or semi-processed) product with high alliin levels. It is hoped that increased productivity from premium seed stock will assist growers in achieving the volume and consistency of quality required to access the export market for pharmaceutical garlic, and to be more competitive on the domestic market.

The Australian Garlic Industry Association (AGIA) has collaborated with IHD, Knoxfield on two prior RIRDC-funded programs: BIO-1A, "Garlic, high yielding and virus free for profits", aimed at increasing productivity by reducing yield losses from virus (development of virus-tested seed stock), and DAV-124A, "A cholesterol-lowering extract from garlic", aimed at identifying new market opportunities for Australian garlic as a pharmaceutical product based on the level of bioactive compounds (alliin) within the bulb. The success of these programs has led to a rapid expansion in the Australian garlic industry (1200 tonnes expected harvest in 1998, from 130 hectares). The AGIA, in conjunction with commercial interests, sought evaluation of the virus-tested seed stock, both with respect to alliin levels (pharmaceutical quality) and yield increases.

Further expansion of the industry is unlikely but may maintain at the current level given some divergence into organic production and back into fresh market areas. Clear definition of the potential markets will be essential to enable industry to access the new international markets based on pharmaceutical interests.

## 1.2 Why produce virus-tested garlic?

Australian garlic has been shown to be infected by the following yield reducing viruses (Sward, 1990);

Leek Yellow Stripe Virus (LYSV)

Onion Yellow Dwarf Virus (OYDV) and

Shallot Latent Virus (SLV), which causes yield losses when in combination with either OYDV or LYSV (Lot *et al*, 1998).

Onion Yellow Dwarf Virus can be very damaging to garlic crops causing stunting and deterioration during storage with premature sprouting of bulbs being reported. OYDV is transmitted by over 50 species of aphids in a non-persistent manner and by mechanical inoculation (Bos, 1976). According to recent data, LYSV may cause a 15-50% reduction in garlic bulb yield, depending on cultivar and aggravation of the symptoms can occur when plants are co-infected with SLV (Lot *et al*, 1998). Aphids transmit LYSV in a non-persistent manner and by mechanical inoculation. SLV as previously indicated can cause crop losses in combination with other viruses, but is basically symptomless when singularly infected (Diekmann, 1997 FAO/IPGRI Technical Guidelines).

Several distinct viruses commonly infect a majority of vegetatively propagated *Allium* spp. Attempts to characterise viruses infecting garlic often led to confusing results and inappropriate naming of the viruses, eg. Garlic Yellow Stripe Virus, Garlic Yellow Streak Virus and Garlic Mosaic Virus. Studies showed that these viruses were in fact mixtures of now formally described viruses (Diekmann, 1997 FAO/IPGRI Technical Guidelines).

Technology exists that enables the production of garlic from which the viruses have been eliminated. Virus-tested garlic forms the basis of commercial crops in France (Messiaen *et al*, 1994) and Argentina and is under evaluation in the UK. Increased crop yields of 25-50% (Walkey and Antill, 1989) and increased bulb weights of 40-60% (Lot *et al* 1998) have been reported from virus-tested seed stock. Whilst the technology for producing virus-tested garlic is not new, the material evaluated in this project is unique in that this is the first time that the technology has been utilised in Australia, with commercial quantities of virus-tested varieties from Australian seed stock. Biofresh Australia P/L have, over the last ten years, developed commercial quantities of virus-tested cultivars from Australian garlic material.

The introduction of virus-tested garlic onto the Australian market provided the chance to evaluate the two following factors in concert. Does the virus-tested seed stock have similar/higher/lower levels of alliin to the material previously tested in DAV-124-A (the material currently grown commercially throughout Australia)? Does virus re-infection affect these alliin levels?



### **1.3 The principal objectives of this project were;**

1. To identify the yield benefits of planting virus-tested garlic.
2. To evaluate the effect of virus-tested garlic on alliin levels and compare alliin levels found in virus-tested cultivars with the levels found in the cultivars currently grown by the Australian growers.
3. To establish rate of reinfection by yield reducing viruses.

## 2. Materials and Methods

Screenhouse and field trials were conducted in 1999 and 2000 at the Sunraysia Horticultural Centre in North West Victoria. The soil in the Sunraysia region is red sand with no requirement for pH adjustment. The garlic cloves were planted in April of each year and harvested in November of the same year respectively.

### 2.1 Screenhouse Trials

The trials were housed in four aphid-proof screenhouses each with an airlock, two contained the virus-tested material and two contained the commercial material (Figs. 1 and 2). Each plot comprised 120 cloves of each cultivar of garlic and was replicated four times, two in each screenhouse. As far as was practicable the trial was managed using normal cultural practices.

Six cultivars were assessed each year:

#### 1999

Virus tested: Biofresh 1, 2 and 3

Commercial cultivars: Aussie White, Italian and D&S

#### 2000

Virus tested: Biofresh 1, 2 and 3,

Commercial cultivars: Aussie White (different set from 1999) 1VI (field grown virus-infected Biofresh 1) and 3VI (field grown virus-infected Biofresh 3)

At the end of each growing season a leaf from every plant in each plot was sampled, the leaves from each plot were mixed and randomly grouped into six batches of 20 leaves and tested in batches for virus using ELISA. All bulbs in each plot were harvested and weighed at the end of the season.



Figure 1: Aphid proof screenhouse.

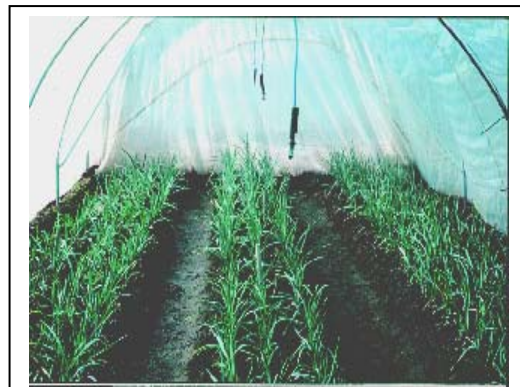


Figure 2: Trial 1 1999. Garlic growing inside aphid proof screenhouse.

### 2.2 Field Trials

The trial was a randomised block design with each block comprising three plots each planted with a different cultivar. Plots were planted with 60 cloves of garlic and blocks were replicated 10 times in 1999 and five times in 2000.

In the first year of field trials, two virus-tested varieties, Biofresh 1 and Biofresh 3 (supplied by Biofresh P/L) were grown in randomised blocks with a virus-infected variety (Aussie White) which is commonly grown commercially, particularly in the Sunraysia region. All three varieties were

planted both in aphid-proof screenhouses (to maintain the virus-tested status of some of the material for future trials) and in the field, to allow re-infection of some of the material (both to gauge yield and alliin differences, and to provide infected supplies of B1 and B3 for future trials).

At the end of each growing season a leaf from every plant was sampled, the leaves from each plot were mixed and randomly grouped into six batches of 10 leaves and tested in batches for virus using ELISA. All bulbs in each plot were harvested and weighed at the end of the season.



Figure3: Trial 2 1999. Garlic growing in outside field trial area.

## 2.3 ELISA

Batches each comprising 20 leaves from the screenhouse trials and 10 leaves from the field trials were tested for OYDV, LYSV and SLV using commercially available ELISA kits (BIOREBA AG ®) used in accordance with the manufacturer's directions. Each well was duplicated, and positive and negative controls were included on each plate, a sample was considered positive if the OD reading was twice the average of the negative controls. Samples were tested in April and November of 1999 and in November in 2000.

## 2.4 Statistical Analysis

### Virus infection levels

Virus infection levels were estimated from the proportions of batches of samples that contained infected leaves (Moran *et al*, 1983) and (Hepworth, 1996).

### Yield analysis

Yields were measured as weight of garlic bulbs in each plot in the screenhouses and in the field trials. Comparisons between treatments were carried out using ANOVA.

## 2.5 Alliin Analysis

Alliin was measured by the State Chemistry Laboratories using HPLC and protocols developed by Pharmaction P/L. These protocols are outlined in the report to RIRDC, "A cholesterol lowering extract from garlic", DAV-124A. HPLC analyses were performed using a Hewlett Packard 1100, with ODS Hypersil column fitted with C18 pre-column.

From each garlic bulb, one sub-sample was prepared as follows: each clove was removed and peeled. The cloves were then homogenised (in solvent, in a blender) and a sub-sample taken from the

homogenate for further extraction and analysis. The number of bulbs (and therefore the number of sub-samples taken to produce each data point) varies between the cage and outdoor trials, due to the increased variability encountered with the material grown outdoors. In the cage trials, 4 replicates of each treatment were planted, and 3 bulbs from each replicate plot being sampled (providing 12 data points for each treatment). In the outdoor trials, 10 replicate plots were planted, and 2 bulbs sampled from each replicate plot (20 data points for each treatment).

### **Screenhouse Trials**

Three bulbs from each replicate were randomly selected for alliin analysis, with each bulb being individually sampled (cloves peeled and homogenised and a sub-sample taken for each bulb).

### **Outdoor Trials**

Two bulbs from each plot were randomly selected for alliin analysis, and individually sampled as for the screenhouse trials.

## **2.6 Aphid Monitoring**

During the duration of both growing seasons aphid numbers were monitored using yellow pan traps. The traps were filled with water containing a small amount of detergent to break the surface tension and a small amount of copper sulphate to reduce the growth of algae. One trap was placed inside the crop and one placed outside the crop. Traps were collected and drained every two weeks and the aphids counted.

## 3. Results

### 3.1 Virus status and yield analysis

#### 3.1.1 Virus Status

In the 1999 screenhouse trials, infection levels in the commercial cultivars were 100% for LYSV and from 0.8% to 100% for OYDV both yield reducing viruses. Virus infection levels in the VT Biofresh cultivars ranged from 0% to 4.7% for SLV, which is considered to be latent but were below 1% for the yield reducing LYSV and OYDV (Table 1). Virus infection levels in the VT Biofresh cultivars were less than the commercial standard of 5%.

In 2000 virus infection levels in the VT Biofresh cultivars ranged from 0% to 3.4% for SLV, which is considered to be latent but were below 1% for the yield reducing LYSV and OYDV (Table 1). Virus infection levels in the VT Biofresh cultivars were less than the commercial standard of 5%. The infection levels in the commercial cultivar Aussie White were less than in the previous year for LYSV. This may be due to the fact that a different planting set was used from the previous year.

**Table 1: Virus status/infection percentage, Screenhouse Trial 1999 and 2000  
(VT =virus-tested)**

Cultivar	LYSV (%)	OYDV (%)	SLV (%)
<b>1999</b>			
Biofresh 1 (VT)	0.8	0	0
Biofresh 2 (VT)	0.8	1.8	0
Biofresh 3 (VT)	<0.8	0.8	0
Aussie White	100	0.8	4.7
Italian	100	100	0
D&S	100	1.8	<0.8
<b>2000</b>			
Biofresh 1 (VT)	0	0.8	0.8
Biofresh 2 (VT)	0	0.8	0.8
Biofresh 3 (VT)	<0.8	0	3.4
1VI (B1 virus-infected)	0.8	<0.8	1.8
Aussie White	7.13	0	4.7
3VI (B3 virus-infected)	1.8	1.8	7.13

**Table 2: Virus status/infection percentage, Field Trial 1999 and 2000.  
(VT =virus-tested)**

Cultivar	LYSV (%)	OYDV (%)	SLV (%)
<b>1999 (April)</b>			
Biofresh 1 (VT)	<1.66	3.63	Not done
Aussie White	1.66	1.66	Not done
Biofresh 3 (VT)	<1.66	3.63	Not done
<b>1999 (November)</b>			
Biofresh 1 (VT)	3.63	9.17	1.66
Aussie White	100	13.75	1.66
Biofresh 3 (VT)	3.63	13.75	0
<b>2000 (November)</b>			
Biofresh.1 (VT)	<1.66	1.66	1.66
Biofresh 2 (VT)	<1.66	3.63	1.66
Biofresh 3 (VT)	1.66	<1.66	13.75

In the April ELISA testing in 1999, virus infection levels in the commercial cultivar Aussie White were 1.66% for LYSV and OYDV. Virus infection levels in the VT Biofresh cultivars ranged from <1.66% for LYSV to 3.63% for OYDV. In the November ELISA testing in 1999, field trial infection levels in the commercial cultivar Aussie White were 100% for LYSV and 13.75% for OYDV both yield reducing viruses. Virus infection levels in the VT Biofresh cultivars ranged from 3.63% for LYSV to 13.75% for OYDV and 1.66% for SLV, which is considered to be latent (Table 2).

In 2000, virus infection levels in the VT Biofresh cultivars ranged from 1.66% to 13.75% for SLV, which is considered to be latent. All the VT Biofresh cultivars contained virus levels below the commercial standard of 5% (Table 2).

Infection in the field with the yield reducing viruses LYSV and OYDV and SLV in combination with either LYSV or OYDV was low when testing was carried out at the beginning of the growing season. This suggests that either the virus titre had not yet built up or high aphid numbers had not yet invaded the crop. The levels did increase for all viruses by the end of the growing season but to a relatively low level in the VT cultivars.

### 3.1.2 Yield Analysis

Complications over the screenhouse experimental design prevented comparisons between virus-tested and commercial cultivars. In the screenhouse trials yields varied between cultivars in both years. In 1999, when individual cultivars were compared, Biofresh 1 and 2 yielded better than Biofresh 3 ( $p \leq 0.05$ ). There were no significant differences in yield between commercial cultivars ( $p = 0.063$ ).

In 2000, Biofresh 1 and 2 yielded better than Biofresh 3 ( $p \leq 0.05$ ).

Yields varied between cultivars in both years in the field trials. In 1999, Biofresh 1 and Aussie White yielded better than Biofresh 3 ( $p \leq 0.05$ ). The yield of Biofresh 1 was similar to that of Aussie White (Table 6).

In the 2000 field trial, Biofresh 1 yielded better than Biofresh 3 ( $p \leq 0.05$ ). Biofresh 2 yielded better than Biofresh 3 ( $p \leq 0.05$ ). Biofresh 1 and 2 were not significantly different in yields.

Yield data was extrapolated into commercial tons per acre and tonnes per hectare for the purpose of reporting to growers. This extrapolation was carried out with an explanation to the growers that this

should be taken only as an indication of potential yield as the commercial reality may vary (see Tables 5 and 6.

**Table 5: Yields of garlic grown in aphid proof screenhouses, 1999 and 2000. VT=virus-tested**

Cultivar	Yield per plot (kg) (adjusted for 120 plants per plot)	Yield/hectare (tonnes)	Yield/acre (tonnes)
<b>1999</b>			
Biofresh 1 (VT)	3.65	9.12	3.70
Biofresh 2 (VT)	4.41	11.02	4.46
Biofresh 3 (VT)	2.14	5.35	2.16
<b>Ftest 0.002</b>			
<b>l.s.d (p =0.05) 0.872</b>			
Aussie White	2.88	7.20	2.91
Italian	2.47	6.18	2.50
D&S ex SA	4.02	10.05	4.06
<b>Ftest 0.063</b>			
<b>l.s.d (ns)</b>			
<b>2000</b>			
Biofresh 1 (VT)	7.96	19.90	8.05
Biofresh 2 (VT)	7.09	17.70	7.16
Biofresh 3 (VT)	5.50	13.75	5.56
<b>Ftest 0.005</b>			
<b>l.s.d (p =0.005) 9.42</b>			
Aussie White	5.76	14.40	5.83
1IV (Biofresh 1 VI)	4.54	11.35	4.60
3IV (Biofresh 3 VI)	5.01	12.52	5.07
<b>Ftest 0.064</b>			
<b>l.s.d (ns)</b>			

**Table 6: Yields of garlic grown in the field trial area, 1999 and 2000. VT= virus-tested**

Cultivar	Yield per plot (kg) (adjusted for 60 plants per plot)	Yield/hectare (tonnes)	Yield/acre (tonnes)
<b>1999</b>			
Biofresh 1 (VT)	1.98	4.95	2.00
Aussie White	1.74	4.35	1.76
Biofresh 3 (VT)	1.34	3.35	1.36
<b>Ftest 0.001</b>			
<b>l.s.d (p =0.05) 0.41</b>			
<b>2000</b>			
Biofresh 1 (VT)	3.54	17.70	7.16
Biofresh 2 (VT)	3.36	16.80	6.80
Biofresh 3 (VT)	2.66	13.30	5.38
<b>Ftest 0.001</b>			
<b>l.s.d (p =0.05) 0.124</b>			

### 3.2 Aphid Monitoring Results

In the field trials there were two flushes of aphid activity each year occurring in autumn (May) and in late spring (October-November), (Figs 7 8).

In year one of monitoring the aphid flush occurred both inside and outside the crop areas, whereas in year two the aphid flush was largely inside the crop. At this stage the plants are most vulnerable to

virus transmission due to the vector pressure and especially in autumn the availability of fresh plant growth.

In year 2 of aphid monitoring a large population occurred within the crop in autumn (May). These data may be slightly inaccurate due to the fact that the sampling was set up and the samples collected by a different person than in the previous year. Aphid numbers do appear to be less in the spring of 2000.

### 3.2.1 Year 1, 1999

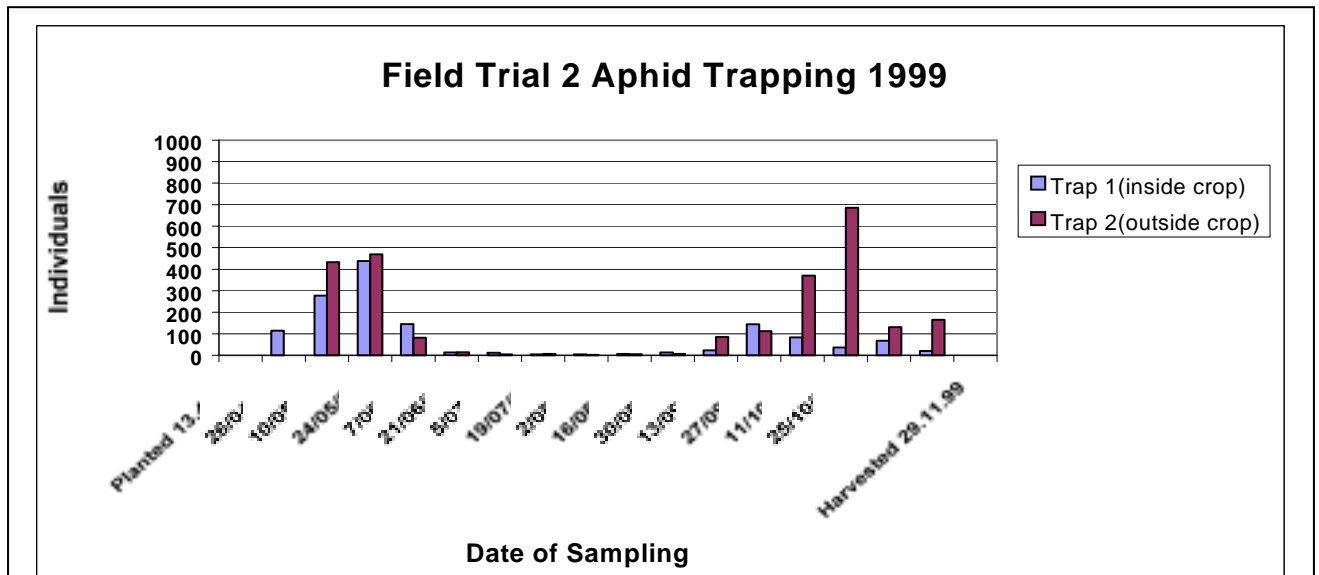


Figure 7: Aphid trapping 1999, indicating aphid numbers

### 3.2.2 Year 2, 2000

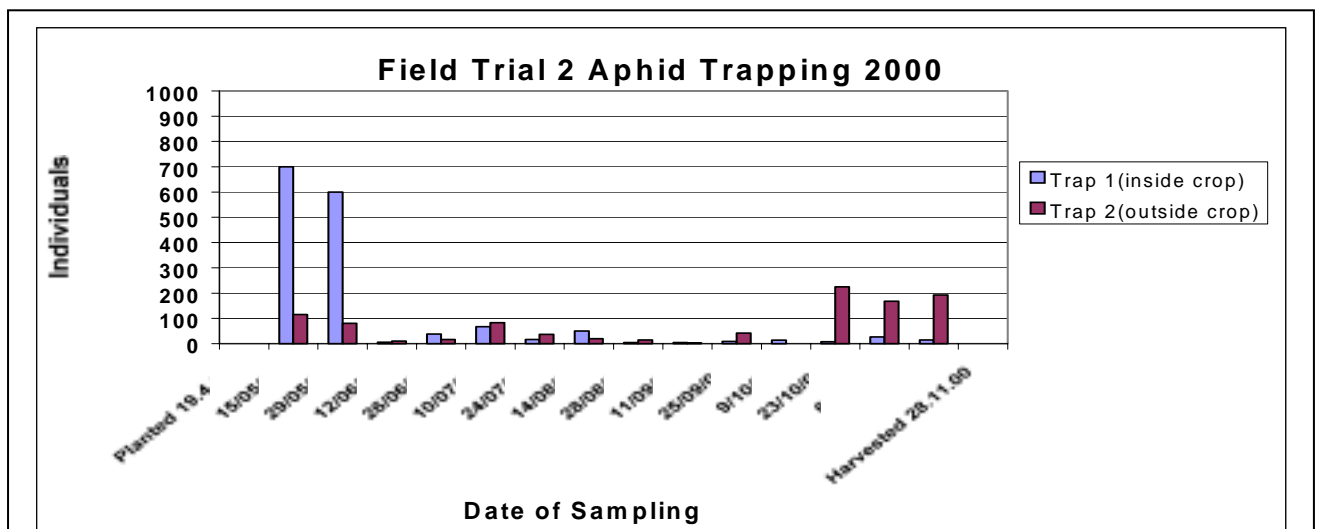


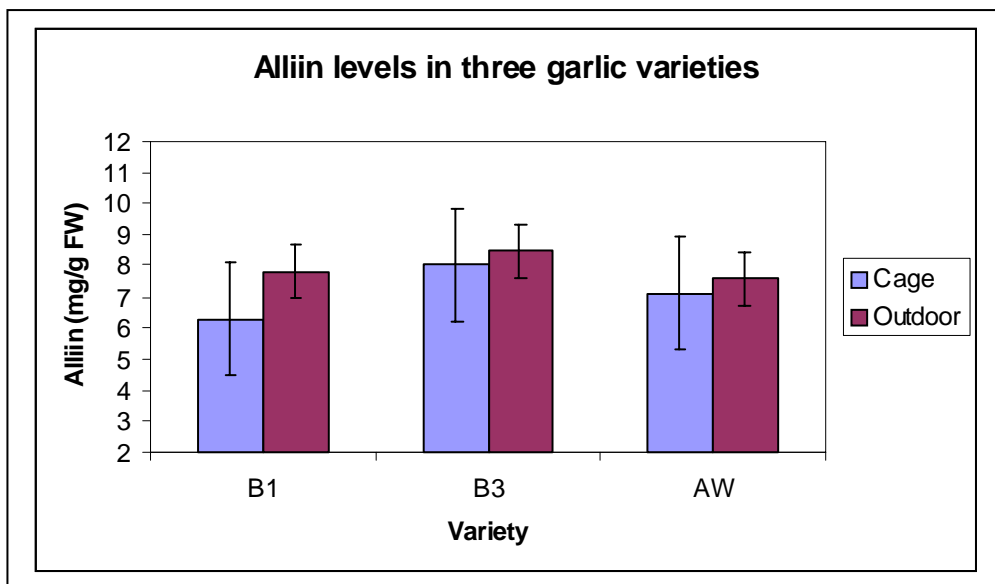
Figure 8: Aphid trapping 2000 indicating aphid numbers.

### 3.3 Alliin Levels

The results of experiments in the screenhouse and field situations are presented below.

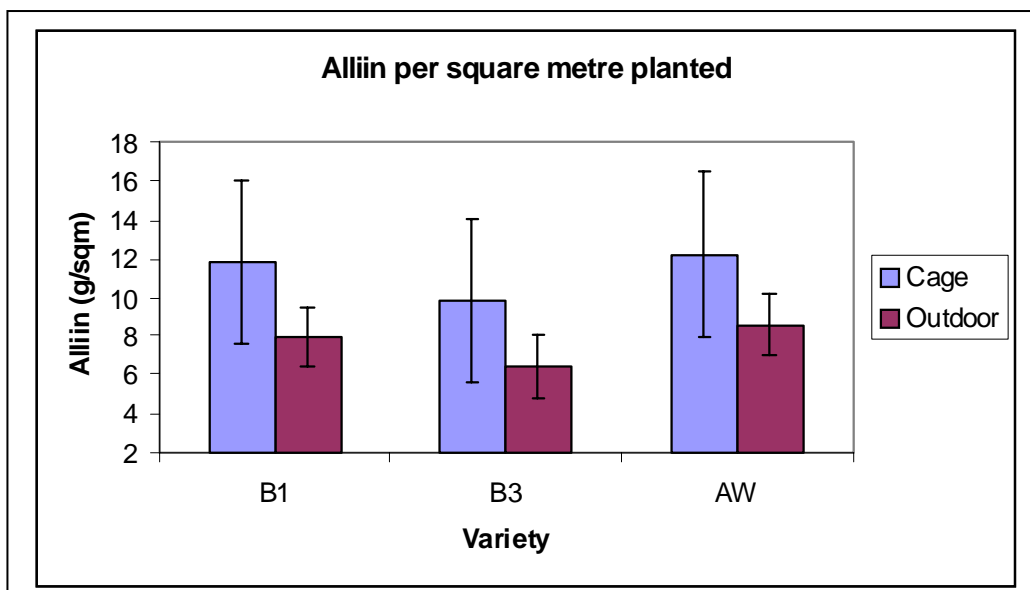


### 3.3.1 Year 1



**Figure 9: Comparison of alliin levels in garlic grown in cages and outdoors, for three different varieties. Error bars indicate L.S.D. values.**

Figure 9 presents alliin levels as mg/g fresh weight of garlic tissue. Comparisons between Biofresh 1, Biofresh 3 and Aussie White show no significant differences between alliin levels in plants grown in screenhouse or field situations.

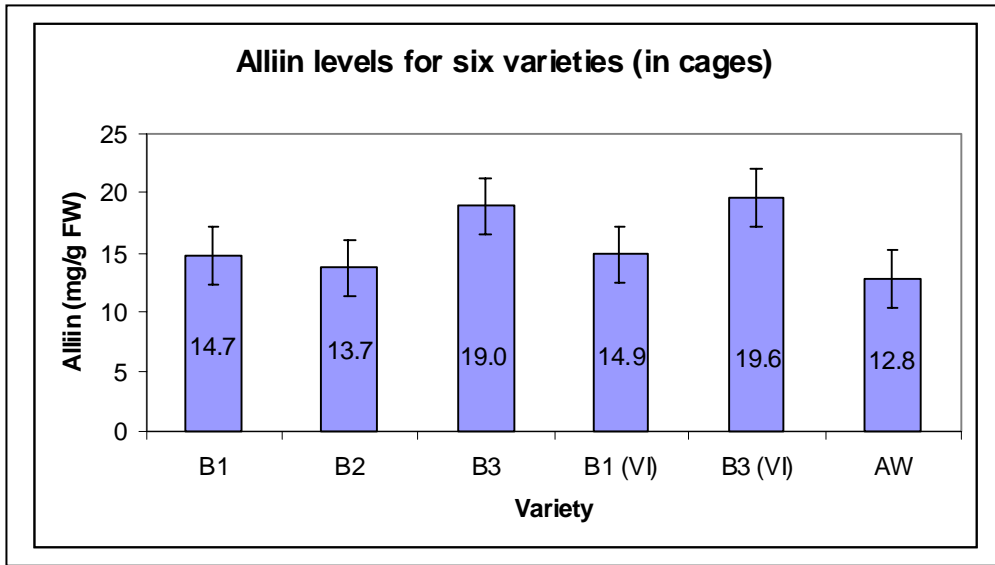


**Figure 10: Alliin produced per area planted. Error bars indicate L.S.D. values.**

Figure 10 presents alliin levels recovered on an area basis, expressed as g/sq m. Comparisons between Biofresh 1, Biofresh 3 and Aussie White show no significant differences between alliin levels in screenhouse or field situations.

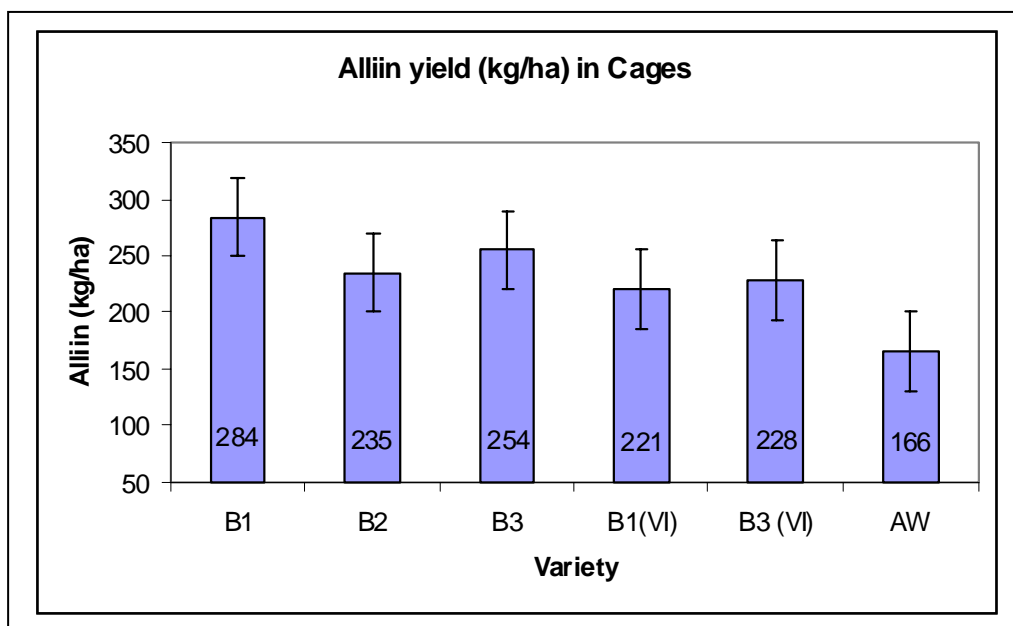
### 3.3.2 Year 2

In year 2, experiments were established in screenhouses and compared the alliin levels in virus-tested plants (Biofresh 1 and Biofresh 3) with virus-infected plants designated Biofresh 1 (VI) and Biofresh3 (VI) and with the industry standard Aussie White.



**Figure 11: average alliin levels (for 3 bulbs from each of 4 plots = 12 replicates) with error bars representing l.s.d. values**

Figure 11 presents alliin levels as mg/g fresh weight of garlic tissue. The results show that levels of alliin in Biofresh 3 and Biofresh 3 (VI) are significantly higher than in Biofresh 2 and Aussie White. There were no significant differences between industry standard Aussie White and virus-tested Biofresh 1 plants.

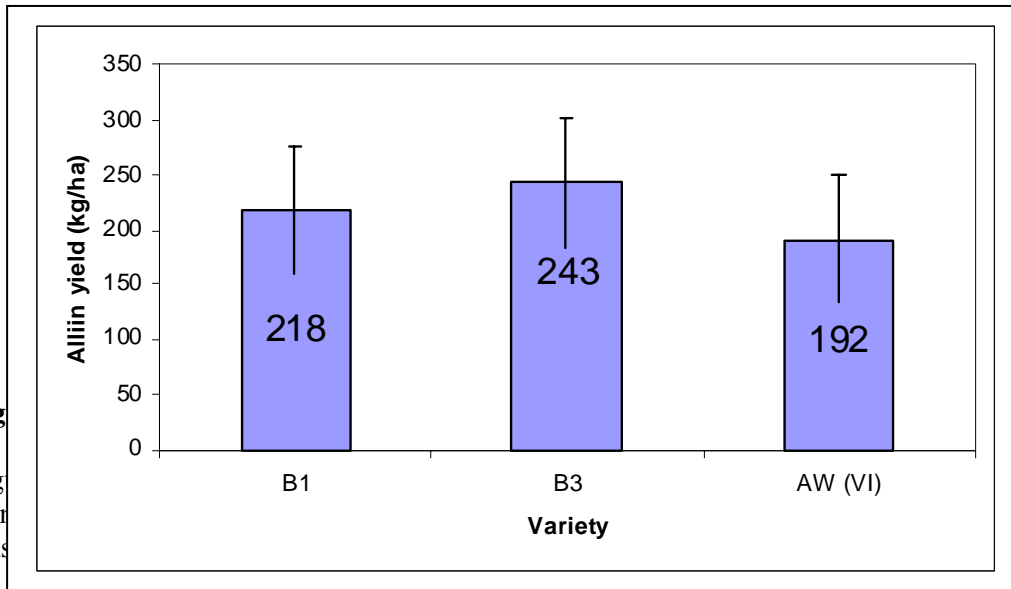


**Figure 12: average alliin levels (for 3 bulbs from each of 4 plots) taking into account the average yield for each variety. Error bars represent l.s.d. values.**

Figure 12 presents alliin levels recovered on an area basis expressed as kg/ha. In this case the results show significantly higher levels in Biofresh 1 and Biofresh 3 than in Aussie White. There were no significant differences between industry standard Aussie White, Biofresh 1 (VI) and Biofresh 3 (VI) and virus-tested Biofresh 2.

Also the two virus-tested cultivars Biofresh 1 and Biofresh 3 were grown in field plots and compared with the industry standard Aussie White.

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## 4. Discussion

The experimental demonstration of the benefits of virus-tested garlic over standard commercial cultivars has proved to be challenging. Much more work is required under field conditions to collect data sets which will provide the basis for sound conclusions. In spite of this requirement for more information, the results from this work do provide some indication of the positive benefits from virus-tested material and of areas of future work.

The results from two consecutive years' trials show that some of the virus-tested cultivars yielded higher than commercial cultivars commonly grown by Australian industry. Investigations did not show a consistent pattern of higher alliin levels in garlic grown from virus-tested plants. When considering the alliin yield per fresh weight of garlic tissue, the only significant difference was recorded from one cultivar of plants grown in insect proof screenhouses in the second year of trials. These results were not repeated with other cultivars in the same or other experiments.

It would seem from the results of the trials that environmental factors (in screenhouse or field) and climate may have influenced the amount of alliin produced per unit of garlic tissue. The interdependency, or not, of these factors on alliin production clearly needs more investigation to establish the conditions which are required to maximise alliin. While the factors which influence alliin production are not well understood, there seems to be clear yield benefits from using virus-tested material. This is an important result because it indicates that viruses have consistent adverse effects on productivity which are independent of external factors such as climate.

There are two important challenges ahead before the benefits of virus tested garlic can be realised. One is to confirm, through longer term studies, the benefits of yield and alliin production. The second is to develop an economic analysis of the conventional vs virus-tested production. This is essential so that businesses can understand the costs associated with use of virus-tested plants and whether the agronomic benefits (yield and alliin) are sufficiently significant to translate into long term profitable returns.

## 5. References

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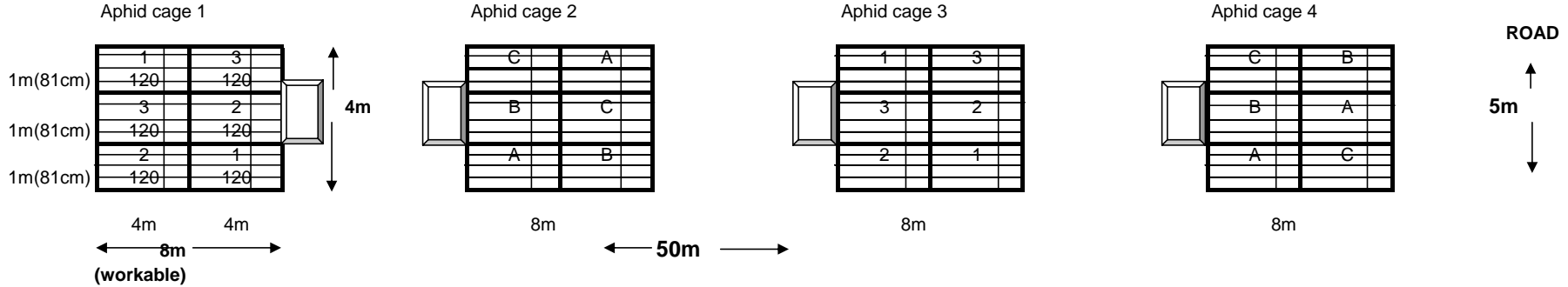
## 6. Appendices

# APPENDIX 1: Trial design 1999 (Excel worksheet)

RIRDC proposal 1999-2003 15.4.99

Field trials and questions to be asked;

Trial 1 to be grown in the Fishers I 1999



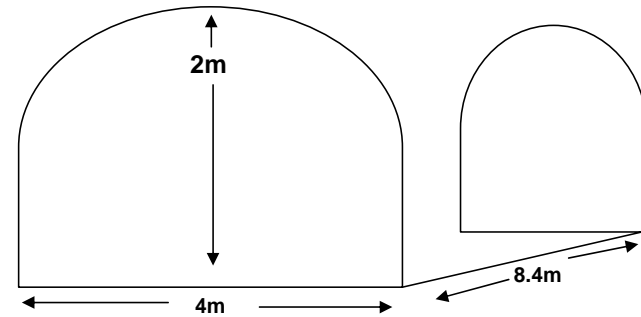
NB monitor for aphids and spray at regular intervals, zero tolerance.

Each tractor width of 81cm would have 3 rows in it therefore we can plant between 100-150 cloves in each plot. =120

Each cage would have plots of garlic cloves sown 10 cm apart in 4 metres of three rows there would be 120 cloves. First 2 m <6g cloves. Second 2 m >4g cloves. =120 total.

- Cage1 Virus tested garlic of three cultivars 1, 2, 3  
~2kg of each cultivar
- Cage2 Virus infected garlic of one-two cultivars A, B, C  
~2kg of each cultivar
- Cage3 Virus tested garlic of three cultivars 1, 2, 3  
~2kg of each cultivar
- Cage4 Virus infected garlic of one-two cultivars A, B, C  
~2kg of each cultivar

<p>1=B1 ex Biofresh 2=B2 ex Biofresh 3=B3 ex Biofresh</p> <p>A=Aussie White ex SA B=Italian ex SA C=D&amp;S ex SA</p>
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Therefore we need ~12kg of Biofresh material @\$21 = \$252.00

1. Yield of virus tested and virus infected industry standard.
2. Alliin levels of virus tested and virus infected industry standard
3. Virus testing using ELISAs on the crop in July and November. (6 batches of 20 leaves = 120 plants per plot x 24 plots = 144 tests twice a year = 4 pla ( use 95% confidence level that <5% infected (test 100 or 95% confidence level that <3% infected)

NB: The whole plot area is approximately 50m in length of all the cages and approximately 5m wide.

NB: Plant after powdering with Sumisclex, Thiram and Benlate.

NB: Planted straight after breaking up on April 13th 1999.

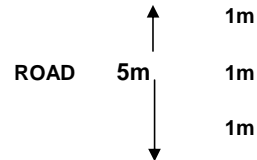
**RIRDC proposal 1999-2003 15.4.99**

Trial 2 grown in the Mid Area

1999

- 1 = virus tested e.g B1
- 2 = virus infected e.g Australian White
- 3 = virus tested e.g B3

20m with 0.1 metre spacings between 2 m plots = 21-22 metres



	J 2m	I 2m	H 2m	G 2m	F 2m	E 2m	D 2m	C 2m	B 2m	A 2m
1m	1	3	2	3	1	2	2	3	1	1
1m	2	1	3	2	3	1	1	2	3	2
1m	3	2	1	1	2	3	3	1	2	3

Plant clove size randomly.  
 10 replicates of 60 plants per plot.  
 Plant cloves 10 cm apart, 60 cloves per plot, all up = 1800 cloves  
 ~ 4-6g per clove = ~10kg of Biofresh and infected material.  
 ~ 7-8 kg of Biofresh material @ \$21 kg = \$150

1. Infection of virus tested material in the first year given natural aphid populations but with a source of virus. Tested using ELISAs.
2. Aphid levels by trapping with yellow pan traps. Aphid species ID?
3. Alliin testing virus tested material.
4. Yields of previously infected industry material and "virus tested" material under pressure.

**NB Virus infected material should be infected with all three viruses relevant to the industry, OYDV, LYSV and SLV. Worst case scenario.**

**NB Use yellow pan traps and empty every two weeks, count and sort.**

**NB ELISA testing in April and November to assess infection. (6 batches of 10 plants =60 plants x 30 plots = 180 tests = 4 plates twice a year.**

**NB: Planted after powdering with Sumisclex, Thiram and Benlate.**

**NB: Planted straight after breaking up on April 13th 1999.**



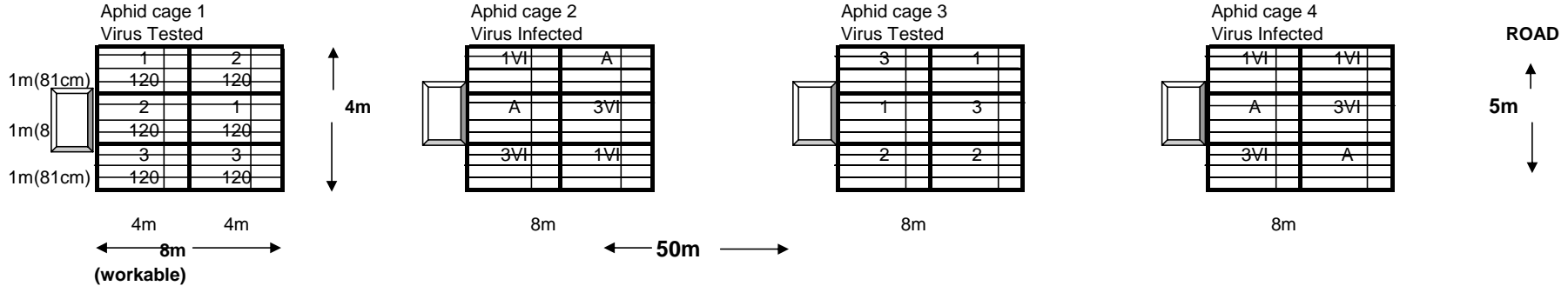
# APPENDIX 2: Trial design 2000 (Excel worksheet)

RIRDC proposal 1999-2001

14.3.00

Field trials and questions to be asked;

Trial 1 to be grown in the Fishers I 2000



NB monitor for aphids and spray at regular intervals, zero tolerance.

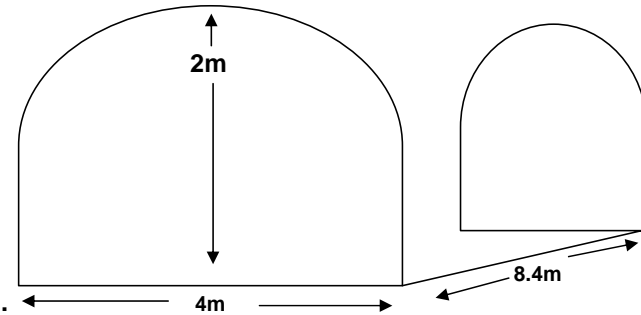
Each tractor width of 81cm would have 3 rows in it therefore we can plant between 100-150 cloves in each plot. =120

Each cage would have plots of garlic cloves sown 10 cm apart in 4 metres of three rows there would be 120 cloves = 120 total.

- Cage1 Virus tested garlic of three cultivars 1, 2, 3  
~2kg of each cultivar = 240 cloves of each cultivar
- Cage2 Virus infected garlic of three cultivars A, 1VI, 3VI  
~2kg of each cultivar = 240 cloves of each cultivar
- Cage3 Virus tested garlic of three cultivars 1, 2, 3  
~2kg of each cultivar = 240 cloves of each cultivar
- Cage4 Virus infected garlic of three cultivars A, 1VI, 3VI  
~2kg of each cultivar = 240 cloves of each cultivar

**1=B1 ex Biofresh (480 cloves)**  
**2=B2 ex Biofresh (480 cloves)**  
**3=B3 ex Biofresh (480 cloves)**

**A=Aussie White ex SA (480 cloves)**  
**1VI = Biofresh virus infected (480 cloves)**  
**3VI = Biofresh virus infected (480 cloves)**



1. Yield of virus tested and virus infected industry standard and Biofresh infected material.
2. Alliin levels of virus tested and virus infected industry standard and Biofresh virus infected material.
3. Virus testing using ELISAs on the crop in November. (6 batches of 20 leaves = 120 plants per plot).  
( use 95% confidence level that <5% infected (test 100 or 95% confidence level that <3% infected)

NB: The whole plot area is approximately 50m in length of all the cages and approximately 5m wide.

NB: Plant after powdering with Sumisclex, Thiram and Benlate.

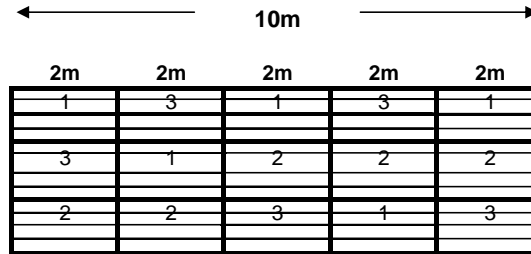
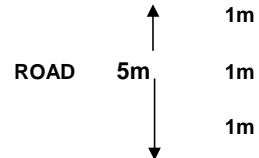
NB: Planted straight after breaking up on 19th April 2000.

**RIRDC proposal 1999-2001 14.3.00**

Trial 2 grown in the Fishers Block 2000

- 1 = virus tested e.g B1
- 2 = virus tested e.g B2
- 3 = virus tested e.g B3

10m with 0.1 metre spacings between 2 m plots = 11 metres



Plant clove size randomly.  
 5 replicates of 60 plants per plot, 15 plots in total.  
 Plant cloves 10 cm apart, 60 cloves per plot, all up = 900 cloves

- B1 = 300 cloves
- B2 = 300 cloves
- B3 = 300 cloves

1. Infection status of virus tested material in the first year given natural aphid populations but with no source of virus grown outside. Test using ELIS/
2. Aphid levels by trapping with yellow pan traps.
3. Alliin testing virus tested material.
4. Yields of "virus tested" material.

**NB: Virus-tested material should not be infected with all three viruses relevant to the industry, OYDV, LYSV and SLV.**

**NB: Use yellow pan traps and empty every two weeks, count and sort.**

**NB: ELISA testing in November to assess infection. (6 batches of 10 plants =60 plants x 15 plots = 90 tests.)**

**NB: Planted after powdering with Sumisclex, Thiram and Benlate.**

**NB: Planted straight after breaking up on 19th April 2000.**