CFPA	DFPT	DFTS	Winetech
Canning Fruit Producers' Assoc. Submit to: Wiehahn Victor PO Box **426** **Paarl, 7620** **Tel: +27 (0)21 872 1501** **inmaak@mweb.co.za**	Deciduous Fruit Producers' Trust Submit to: Louise Liebenberg Suite 275, Postnet X5061 Stellenbosch, 7599 Tel: +27 (0)21 882 8470/1 **louise@dfptresearch.co.za**	Dried Fruit Technical Services Submit to: Dappie Smit PO Box 426 Paarl, 7620 Tel: +27 (0)21 872 1501 **dappies@dtd.co.za**	 Submit to: Jan Booysen PO Box 825 Paarl, 7624 Tel: +27 (0)21 807 3324 **booysenj@kwv.co.za**

			X

Indicate (X) client(s) to whom this final report is submitted.
Replace any of these with other relevant clients if required.

FINAL REPORT
FOR 2009

PROGRAMME & PROJECT LEADER INFORMATION

	Programme leader	Project leader
Title, initials, surname	Prof. M.J. Samways	Dr Pia Addison
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PROJECT INFORMATION

Project number	US/E/A3

Project title	Life table studies on *Anagyrus pseudococci,* an important parasitoid of the key wine and table grape pest, vine mealybug

Industry programme	**CFPA**	
	DFPT	
	DFTS	
	Winetech	
	Other	

Fruit kind(s)	Grapes

Start date (dd/mm/yyyy)	1 October 2006	**End date** (dd/mm/yyyy)	31 Sept 2008

FINAL SUMMARY OF RESEARCH PROJECT

PROGRAMME & PROJECT LEADER INFORMATION

	Programme leader	Project leader
Title, initials, surname	Prof. M.J. Samways	Dr. P. Addison
Institution	University of Stellenbosch	University of Stellenbosch
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PROJECT INFORMATION

Project number	US/ENT-06-A6

Project title	Life table studies on *Anagyrus* sp (near) *pseudococci,* an important parasitoid of the key wine and table grape pest, vine mealybug

Fruit kind(s)	Grapes

Start date (dd/mm/yyyy)	1 October 2006	**End date** (dd/mm/yyyy)	31 Sept 2008

(Give a summary of the *total* project in no more than 250 words).

Anagyrus sp (near) *pseudococci* colonies were established in the laboratory from collected infected *Planococcus ficus* (vine mealybug) to conduct a life table study. The colonies were maintained on *P. ficus*, reared on butternut squash (*Cucurbita mochata*) in a laboratory at approximately 26°C and relative humidity below 60%.

The *A.* sp (near) *pseudococci* colonies were maintained, despite having the necessary rearing facilities, but suffered various setbacks due to amongst others continuous shortage in rearing space with correct rearing conditions. Due to continuous *Coccidoxenoides perminutus* contamination both the *P. ficus* and *A.* sp (near) *pseudococci* colonies could therefore unfortunately never be utilized at full potential. Life tables were conducted for the following constant temperatures 14˚C, 22˚C, 27˚C, 32˚C & 34˚C at a constant humidity of 53 to 55% and a daylight regime of 16L:8D. The lower temperature (T_b) threshold at which no development can be expected is 11°C, upper temperature threshold (T_L) 34°C and optimum temperature threshold (T_{opt}) ~ 25°C. It seems that the South African strain of *A.* sp. (near) *pseudococci* has a narrower temperature tolerance than its American counterpart which shows higher development at both 14˚C and 34˚C. It was further found that *A. sp* (near) *pseudococci* manages to infect *P. ficus* at higher temperatures than those optimal for *C. perminutus.* The incorporation of both parasitoids together could therefore provide much more complete control. This project was initially taken over from Dr Vaughn Walton and formed part of a Masters thesis, which is due to be complete by April 2010.

FINAL REPORT

(Relevant publications may replace the final report)

1. Problem identification and objectives

State the problem being addressed and the ultimate aim of the project.

Vine mealybug (VMB) continues to be a key pest in South African vineyards and other crops such as figs (*Ficus carica*). Consumer, but more importantly retailer sentiment is continuously putting more and more pressure on producers to replace conventional management practices by biological control measures. Producers on the other hand were faced with drastic price surges to the extent that biological control is a true competitive option now strongly integrated to holistic approaches. *Coccidoxenoides perminutus* is a known effective control agent of early mealybug instars whereas the beetle *Cryptoleamus montrouzie* predominantly feeds on ovisacks, both locally and abroad. Third to adult mealybug instars are however not targeted by biological control means in South Africa, whereas abroad (USA & Israel) *Anagyrus* sp. (near) *pseudococci*, previously known as *Anagyrus pseudococci*, has proven an ideal addition to integrated biological control for VMB management. During previous work by Walton *A.* sp. (near) *pseudococci* was identified as a prevalent VMB parasitoid present in Western Cape vineyards. Basic information on the developmental biology was lacking and needed in order to understand the importance of this parasitoid, in order to optimize possible future rearing and release methodology.

2. Workplan (materials & methods)

List trial sites, treatments, experimental layout and statistical detail, sampling detail, cold storage and examination stages and parameters.

Insect and plant materials

First and foremost the prevalent *Anagyrus* species in Western Cape vineyards needed to be determined, as recent literature suggested the existence of closely related species that could not be distinguished by morphometrics, but only genetically (Triapitsyn *et. al.,* 2007).

As no co-existence of both *A. pseudococci* and *A.* sp (near) *pseudococci* was found and only the latter was found in Western Cape vineyards a laboratory cultures was established on collected vine mealybug. All VMB were reared on butternut squash, *Cucurbita moschata* L., which was cleaned with a 0.5% bleach solution, temperature regulated at approximately 26℃ and relative humidity kept below 60% to reduce mold growth, whilst providing ideal growing conditions for the mealybugs.

Field collected infected third to adult instar mealybugs were reared separately to “harvest” parasitoids. Reared parasitoids were identified as *A.* sp (near) pseudococci and removed to a further rearing cage where they could mate and reproduce on clean hosts. Once these colonies where sustainable day-old *A.* sp (near) *pseudococci* where removed and sexed and into pairs of 20 males and females.

A single butternut infested with ~1000 mealybugs of predominantly third to adult stages was placed inside a ventilated 5ℓ PET container and assigned to its relevant temperature. Vents were covered with a fine mesh that prohibited exit or entry by any host, predator or parasitoid. The 20-pair *A.* sp (near) *pseudococci* were added to this ventilated container and left to mate and oviposit for 24h. Hereafter all life parasitoids were removed and placed in a further identical container until no life parasitoids were left. A 50% honey/water dilution was available as food source to the parasitoids.

The containers were checked daily for emergence of progeny, which were subsequently removed, sexed, counted and longevity determined.

Parameter determination

Lx, the proportion of individuals alive on day *x,* and *Mx*, the mean number of progeny produced on day *x,* will be determined for the duration of the life span of *A. pseudococci* at each temperature. The net reproduction rate (*R*o) will be determined using $\sum_{x=1}^{t} L_x M_x$,

where *t*=time in days. The mean generation time (*T*) was calculated using (Watson 1969; Price 1984),

$$T = \frac{\sum L_x M_x X}{\sum L_x M_x}$$

These values will subsequently be used to obtain an initial estimate of the intrinsic rate of natural increase (r_m) using (Price 1984),

$r_m=[\ln(R_o)]/T$.

The estimate of r_m will then be used in the first iteration to solve the equation (Watson 1964),

$$\sum_{x=1}^{t} (e)^{-r_m x} L_x M_x = 1, \text{ X = 1, 2, 3, . . ., t days.}$$

The iterations will be continued until the left hand side of the equation is within 0.0001 of the right hand side.

Minimum, maximum and optimum temperatures for development.

The minimum threshold temperature for development will be determined by using linear regression. The regression equation *y = ax+b* describes the rate of development regressing 1/*t* on temperature for *P. ficus* and then solving the regression equation for 1/*t* = 0, where *t* = time in days. The thermal constant (*k*) from birth to adult, in required degree-days (DD), is calculated as *k=1/a* (Liu and Meng, 1999).

Nonlinear regression will be used to estimate life history parameters for VMB using Briere *et al.* (1999) to determine the upper, optimum and lower threshold temperatures for development. The reciprocal of developmental tempo, *T* will be fitted on temperature (℃) using:

$$r(T) = nT(T - T_b)(T_L - T)^{1/m}$$ (Briere *et al.* 1999), (1)

where *r(T)* is the rate of development at temperature *T*; T_L the upper developmental threshold; T_b the lower developmental threshold temperature, and m and n are empirical constants. The optimum temperature T_{opt} will be calculated using

$$T_{opt} = \frac{(2mT_L + (m+1)T_O + \sqrt{4m^2T_L^2 + (m+1)^2T_O^2 - 4m^2T_OT_L}}{4m+2}$$ (Briere *et al.* 1999), (2) using T_L, T_O, and *m*, obtained from (1).

3. Results and discussion

State results obtained and list any benefits to the industry. Include a short discussion if applicable to your results.
This final discussion must cover ALL accumulated results from the start of the project, but please limit it to *essential* information.

Milestone	**Achievement**
1. Parameter determination	Determined for 14, 22, 27 and 32 and 34°C
2. Min, max and optimum temperatures for development	T_b = 11°C, T_L, = 34°C, T_{opt} ~ 25°C

Insect and plant materials

During the past two seasons, VMB showing signs of parasitoid infection (parasitoidism) where collected from vineyards surrounding the Stellenbosch and Simondium areas (Fig. 1). These mealybugs where kept in a laboratory environment (approx. 26˚C, RH <60%) until parasitoids emerged. The latter where subsequently removed and added to a laboratory *A.* sp. (near) *pseudococci* colony. More field collected *A.* sp. (near) *pseudococci* are regularly added to this colony to keep up genetic diversity. Colony insects were used for the life table studies.

The necessary rearing facilities unfortunately never realized during the course of this project and are still under construction (one year post completion of all laboratory work). The *A.* sp (near) *pseudococci* colonies were however maintained, but suffered various setbacks due to amongst others continuous shortage in rearing space. The facilities used provided no protection against secondary parasitism of the host by *C. perminutus*, often resulting in a lack of adequate hosts, severely affecting the continuity of the colony. The *A.* sp (near) *pseudococci* colonies could therefore unfortunately never be utilized at full potential.

Life table parameters

The following important life table parameters were determined as shown in fig 1 and table 1. The lower temperature (T_b) threshold at which no development can be expected is 11°C, upper temperature threshold (T_L) 34°C and optimum temperature threshold (T_{opt}) ~ 25°C. The net reproduction rate (*Ro*), intrinsic rate of reproduction (*Rm*) and mean generation time *T* are presented in fig 1 and table 1.

Minimum, maximum and optimum temperatures for development.

Due to malfunctioning environmental chambers the planned 17˚C could not be conducted whereas 27˚C had been repeated and initial results discarded. At 14˚C development was lower than expected, with adult *A.* sp. (near) *pseudococci* being very passive. 22˚C & 27˚C provided the highest intrinsic rate of natural increase and net reproduction rate respectively (Table 1). No development was observed from the 34˚C life table, even though the parasitoids were able to survive a few days. It seems that the South African strain of *A.* sp. (near) *pseudococci* has a narrower temperature tolerance than its American counterpart which shows higher development at both 14˚C and 34˚C (Daane et al., 2004).

Further analysis

This work forms the base for further studies on *A. sp* (near) *pseudococci* and its importance as a bio control agent to be used in an integrated approach, together with *C. perminutus* and others against *P. ficus*. Fig. 3 confirms this hypothesis as it can be deducted that *A. sp* (near) *pseudococci* manages to infect *P. ficus* at higher temperatures than those optimal for

C. perminutus. The incorporation of both parasitoids together could therefore provide much more complete control.

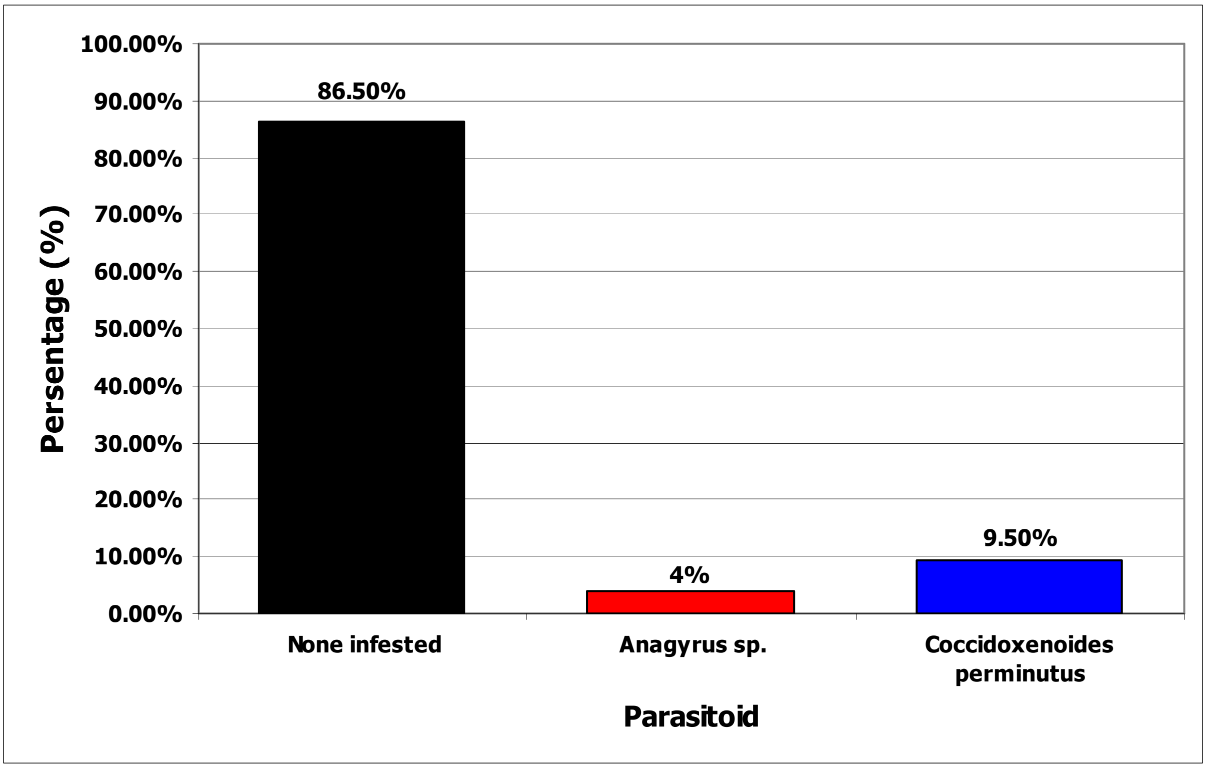

Fig. 1. Parasitism of 3rd instar to adult, field-collected vine mealybug *Planococcus ficus* where no augmentative releases have taken place.

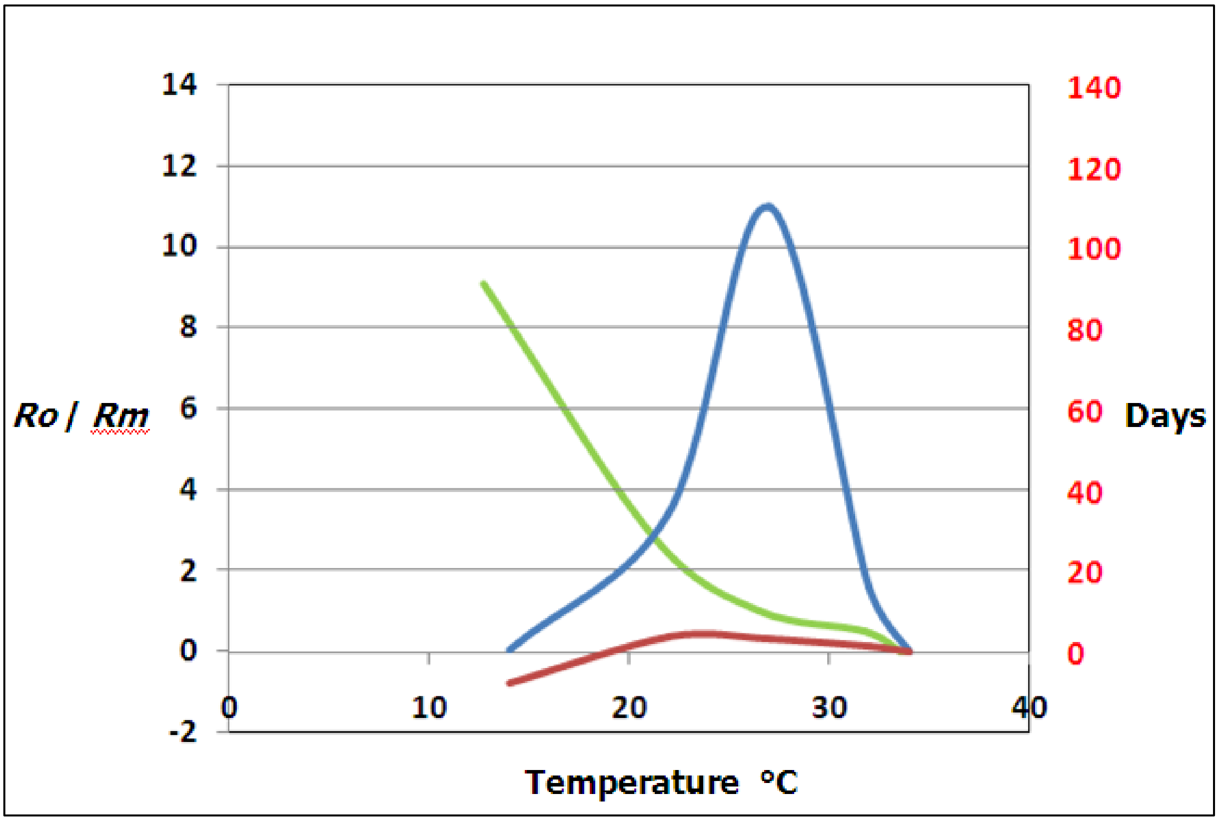

Fig. 2. Life table parameters (blue = net reproduction rate {*Ro*}, green = mean generation time {*T*}, red = Intrinsic rate of reproduction *{Rm}*)

Table 1. Life table parameters of *Anagyrus* sp. (near) *pseudococci* at five constant temperatures.

Life table parameters	14°C	22°C	27°C	32°C	34°C
Ro (Net reproduction rate)	0.05	3.45	11.00	1.60	0.00
Rm (intrinsic rate of natural increase)	-0.75	0.38	0.32	0.14	0.00
T (mean generation time in days)	92	29	11	6	0

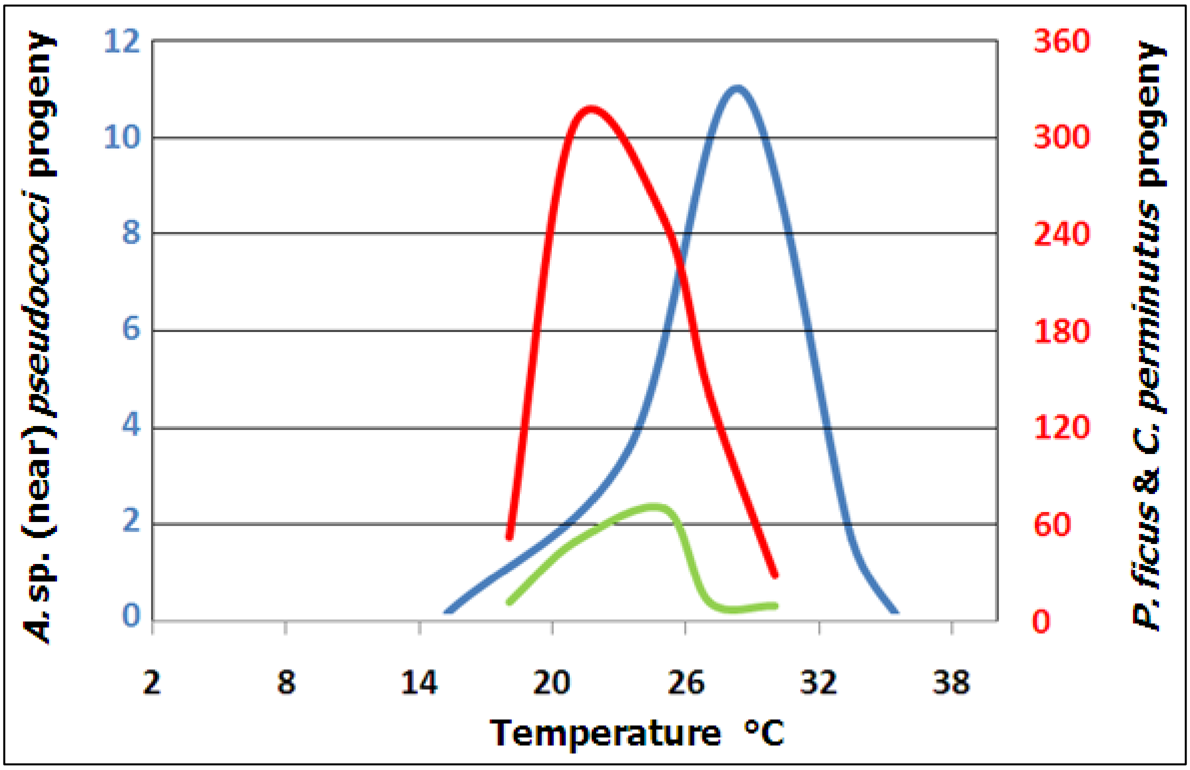

Fig. 3. Comparison of *A.* sp (near) *pseudococci* Ro (blue) to that of *C. perminutus* (red) and *P. ficus* (green).

References

Briere, J. F., Pracros, P., Roux, A. Y. le, Pierre, J. S. 1999. A novel rate model of temperature-dependent development for arthropods. Environmental Entomology 28 (1) : 22-29.

Daane, K.M., Malakar-Kuenen, R., & Walton, V.M., 2004. Temperature-dependent development of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae) as a parasitoid of the vine mealybug, *Planococcus ficus* (Homoptera: Pseudococcidae). *Biological Control* 31, 123-132.

Liu, S.S., Meng, X.D., 1999. Modeling development time of *Myzus persicae* (Hemiptera: Aphididae) at constant and natural temperatures.Bull. Entomol. Res. 89, 53–63.

Triapitsyn S.V, González D, Vickerman D.B, Noyes J.S, White E.B, 2007, Morphological, biological, and molecular comparisons among the different geographical populations of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae), parasitoids of *Planococcus* spp. (Hemiptera: Pseudococcidae), with notes on *Anagyrus dactylopii, Biological Control* 41: 14–24.

Walton, V.M. & Pringle, K.L., 2004. A Survey of Mealybugs and Associated Natural enemies in Vineyards in the Western Cape Province, South Africa. *South African Journal of Enology and Viticulture* 25(1), 23–25.

Walton, V.M., & Pringle, K.L., 2005. Developmental biology of vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae), and its parasitoid *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae). *African Entomology* 13(1):143-147.

Watson, T.F. 1964. Influence of host plant condition on population increase of *Tetranychus telarius* (Linnaeus) (Acarina: Tetranychidae). *Hilgardia* 35: 237–322.

4. Accumulated outputs

List ALL the outputs from the start of the project.

The year of each output must also be indicated.

Technology developed

Human resources developed/trained

Part of a Masters thesis, student (Martin Wohlfarter) due to graduate April 2010.

Patents

Publications (popular, press releases, semi-scientific, scientific)

One scientific article due in 2010.

Presentations/papers delivered

Wohlfarter M, De Waal J.Y, Van Heerden C. & Addison P, 2008, Clearing the clutter; A life table analysis of the strain of *Anagyrus* sp. (near) *pseudococci* (Hymenoptera: Encyrtidae) found in vineyards of the Western Cape province, South African, and subsequent successful taxonomic specimen identification, Proceedings of the XXIII International Conference of Entomology, 6-12 July, Durban, South Africa.

Wohlfarter M, 2007, A life table analysis of *Anagyrus pseudococci*, Extended abstracts 5th International Table Grape Symposium, 14-16 November, Somerset West, South Africa: 119-120.

Mgocheki N. & Wohlfarter M, Development of a biological control programme for vine mealybug, *Planococcus ficus* Signoret (Homoptera: Pseudococcidae) in vineyards, 21st Annual SCB meeting – Vinecology workshop, 27 June 2007, Stellenbosch, South Africa.

4. Total cost summary of project

	Year	CFPA	DFPT	DFTS	Winetech	THRIP	Other	TOTAL
Total cost in real terms for year 1	2005				40 000			**40 000**
Total cost in real terms for year 2	2006				44 480			**44 480**
Total cost in real terms for year 3	2007				49 480	29688		**79 168**
Total cost in real terms for year 4	2008				51 776	21776		**73 552**
Total cost in real terms for year 5								
TOTAL					**185 736**	**51 464**		**237 200**