The effect of arbuscular mycorrhizal colonisation on the C economy, growth and nutrition of young grapevines

By

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Declaration

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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Summary

Arbuscular mycorrhizal (AM) C-costs in grapevines were investigated. Since both dormant vines and AM colonisation rely on stored C for initial growth, AM colonisation costs would therefore compete with plant growth for available C reserves. The aims of this study were to assess the host C economy during AM development and the subsequent C-costs of N and P uptake, as well as the effects of C costs on host growth. This was evaluated in two separate experiments; one assessing the symbiotic influence on the C costs of fungal establishment and nutritional benefits, whilst the other one evaluated the effects of the symbiosis on host growth and nutrient productivities.

This study has shown that AM acts as a C sink, competing with the host for available C. Past work on the AM sink effect has focused mainly on the movement of photosynthetic C below ground to support the AM fungus. This however, does not take into account the effect that stored C will have on the C economy of the plant and symbiosis. The role of stored C becomes even more crucial when working with deciduous plants that rely on stored C for new growth at start of a growing season. It has been reported that stored C in AM plants is remobilized at the start of a growing season and then the C reserves are refilled towards the end of the season, when the plants enter dormancy.

The initial costs of AM fungal colonisation were borne by the above-ground C reserves, at the expense of new growth in host plants. These costs were offset once the plateau phase was reached, and the depleted reserves started to refill. Once established, the active symbiosis imposed a considerable below ground C sink on host reserves. In spite of these

costs, the improved P nutrition of AM roots was achieved with a more efficient C-use. This concurs with other findings, that of the belowground C allocated to AM roots, a greater part is used by AM respiration and a smaller part for P uptake. The C costs of the AM fungal phase of rapid development can be seen as negative to root growth and shoot development. These negative effects may continue for a period of time, even during the plateau phase of fungal development. Once the AM symbiosis is fully established, the host growth and development is then improved to a greater extent than in non-AM plants. From this study it can be concluded that AM growth directly competes with host development, but the symbionts revert to a beneficial partnership once it is fully established.

Opsomming

Die C koste van arbuskulêre mikorisa (AM) in wingerdstokke is ondersoek. Beide rustende wingerdstokke en AM koloniseering is afhanklik van gestoorde C vir aanvanklike groei. AM kolonisering sou dus met plantgroei kompeteer vir beskikbare C reserwes. Die doelstellings van hierdie ondersoek was eerstens om die C-ekonomie van die gasheer tydens AM ontwikkeling en die gevolglike C-kostes van N en P opname te bepaal en tweedens sowel as die invloed van C veranderings op gasheergroei vas te stel. Hierdie is in twee afsonderlike eksperimente ondersoek: een om die simbiotiese invloed op die C-kostes van swam-vestiging en voedingsvoordele te bepaal, terwyl die ander die uitwerking van simbiose op gasheergroei en voedings doeltreffenheid evalueer het.

Die ondersoek het bewys dat AM, as ‘n C-sink, kompeteer met die gasheer vir beskikbare C. Vorige werk oor die AM sink-effek het hoofsaaklik gefokus op die afwaartse beweging van fotosintetiese C om die AM-swam ondergronds te ondersteun. Die werk neem egter nie in ag wat die effek van gestoorde C op die C-ekonomie van die plant en simbiose sou wees nie. Die rol van gestoorde C is selfs nog meer belangrik wanneer met bladwisselende plante gewerk word, omdat sulke plante op gestoorde-C vir nuwe groei aan die begin van die groeiseisoen staatmaak. Dit is op rekord dat gestoorde C in bladwisselende plante by aanvang van die groeiseisoen gemobiliseer word en dat die C-reserwes teen die einde van die seisoen wanneer die plante rustyd nader, weer hervul word.

Die aanvanklike kostes van AM kolonisering is deur die bogronds C-reserwes, ten koste van nuwe groei van die gasheerplante, gedra. Hierdie kostes herstel sodra die plato-fase bereik is, waar die uitgeputte reserwes begin hervul het. As die aktiewe simbiose eers gevestig is, sal dit as ‘n onderg P-voeding van AM wortels verkry wordrondse C-sink vir gasheer optree.Hierdie C verbruik word egter as doeltreffend beskou aangesien verbeterde. Dit is bekend dat ‘n groter deel van die ondergrondse C geallokeer word aan AM-wortels, deur middel van AM respirasie en P-opname. Die C-kostes van die AM-fungus tydens die fase van vinnige ontwikkeling, kan ‘n negatiewe effek op wortel- en lootontwikkeling hê. Hierdie negatiewe uitwerking kan vir ‘n tydperk voortdeur, selfs gedurende die plato-fase van fungi-ontwikkeling. Sodra die AM-simbiose volledig gevestig is, word gasheergroei en ontwikkeling tot ‘n groter mate verbeter as in plante sonder AM-fungi. Hierdie ondersoek het bewys dat AM groei direk met gasheerontwikkeling kompeteer, maar dat die simbiose ‘n voordelige vennootskap vorm sodra dit volledig gevestig is.

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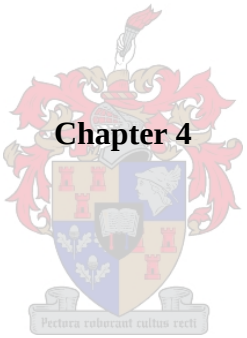

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Chapter 1

Literature review

1.1 Grapevines

1.1.1 Growth and developmental stages

The vines used in the wine industry are produced from cuttings to ensure a true genetic line (Crossen 1997). These cuttings are grafted, rooted and grown in a nursery before being supplied to the industry. The growth and development of vines follow an annual lifecycle. In spring the buds burst and new shoots and leaves are produced followed by the onset of flowering (Crossen 1997). Towards the end of spring and the beginning of summer the shoots continue to grow and the berry set occurs and towards the middle of summer the berries start to ripen (veraison) (Crossen 1997). As autumn progresses and the grapes ripen harvesting will commence and in late autumn the vines lose their leaves and enter a period of dormancy during winter, when pruning takes place (Crossen 1997).

1.1.2 Carbohydrate reserves

Once the young vines have been planted in the vineyard the only source of carbon (C) for the initial plant growth is from C reserves within the plant (Buttrose 1966; McArtney and Ferree 1998). For young vines these reserves are found primarily in the woody stem and to a lesser degree in the roots, but as the vine matures the roots become the major carbohydrate reserve (McArtney and Ferree 1998). The vine is reliant on these reserves until it is has enough photosynthetic tissue to sustain growth. Therefore the different growing tissues of the vine, mainly roots and shoots, act as competing C-sinks (Miller *et al,*. 1996). Later in the season, when the vine begins to build up reserves for the following

season's growth the berries act as a sink and compete with the reserve tissues for carbohydrates (Candolfi-Vasconcelos and Koblet 1990; Schreiner 2003).

1.1.3 Reliance of vines on mycorrhizae

The extent to which plants rely on mycorrhizae varies widely with plant type and soil P availability. It is thought that about 80% of all plants develop an AM relationship, vines being one of the plants that rely on AM for increased nutrient acquisition, especially P (Smith and Read 1997; Possingham and Obbink 1971). The percentage colonisation, degree of growth response and nutritional benefits of AM colonisation of vine roots will vary according to the AM fungal species and the rootstock cultivar involved (Linderman and Davis 2001; Schreiner 2003). In spite of these variations grapevines appear to be reliant on AM fungal colonisation for normal growth and development (Menge *et al,.* 1983; Karagiannidis *et al,.* 1995; Biricolti *et al,.* 1997; Linderman and Davis 2001). It has been reported that coarse rooted species, such as vines, are more reliant on AM colonisation than fine rooted species (Bolan 1991; Eissenstat 1992; Motosugi *et al,.* 2002).

A further reason that vines in the Western Cape might be reliant on AM fungi is acidic, nutrient poor soils that occur in this region. Many of these plants can survive without the AM fungus, but may show decreased growth, especially under nutrient limited conditions. Conversely, if the plants are growing in conditions where nutrients are freely available, the fungus may have a negative effect on the growth of the host plant, due to the C drain caused by the fungus (Johnson *et al,.* 1997).

1.1.4 Inoculation

The fungal species and the rootstock cultivar will determine many of the benefits attributed to the symbiosis (Menge *et al.,*1983; Schubert *et al,.*1988; Karagiannidis *et al.,* 1997). Schubert *et al,.* (1988) inoculated different rootstock cultivars with different AM fungi and found that certain fungal species combined with specific rootstocks increased plant growth to a greater extent than other combinations did. Thus rootstock inoculation can ensure that the vines are colonised by a desirable fungus.

Vineyards infested with soil pathogens such as nematodes often require fumigation treatments. However, the fumigant clears the soil of both desirable and undesirable soil microbes, including AM fungi (Menge *et al,.* 1983; Linderman and Davis 2001). Therefore the inoculation of vines before planting in fumigated soils is needed to ensure AM fungal colonisation of the vine roots. Menge *et al,.* (1983) reported that the vines that were planted in fumigated soils and were not inoculated had stunted growth compared to the inoculated vines. Inoculation also increases the vines ability to rapidly establish itself upon replanting (Linderman and Davis 2001), minimizing the effects of transplantation shock, especially under P limiting conditions.

1.2 Arbuscular mycorrhizae

1.2.1 Host benefits

The extent to which a plant will benefit from the AM symbiosis is determined by the fungal species and the plant species involved (Menge *et al,.* 1983 and Karagiannidis *et*

al,. 1997). The species of fungus found in the soil can differ from one location to the next and is usually determined by the soil characteristics (pH etc) and the vegetation cover (Nappi *et al,.* 1985 and Shubert and Cravero 1985). Certain species of fungi will be dominant in a soil that is covered by a specific host plant.

The primary function of AM fungi is the uptake of P from the soil and supplying it to the host plant in exchange for carbohydrates. However, AM fungi also offer a number of other beneficial functions to the host. Nikolaou *et al,.* (2002) reported that AM vines had increased leaf N, P, K and Ca concentrations compared to non-AM vines. Similarly, Marschner and Dell (1994) showed that AM fungi acquired 80% of plant P, 25% of plant N, 10% of plant K, 25% of plant Zn and 60% of plant Cu, indicating the role that AM plays in the overall mineral nutrition of plants and not just P acquisition. It is generally accepted though that the increase in the growth of plants colonised with AM fungi is attributable to increased P nutrition (Sanders and Tinker 1971; Smith 1982; Bolan 1991 and Orcutt and Nilsen 2000).

AM colonisation also has a number of non-nutritional benefits. Karagiannidis and Nikolaou (2000) found that AM colonisation protects plants from the influence of heavy metals such as Pb and Cd. The metals are taken up by the fungus and complexed with polyphosphate, thus preventing their transport to the host (Orcutt and Nilsen 2000). The negative effects of lime-induced chlorosis are also alleviated by the colonisation of roots with an AM fungus. Bavaresco and Fogher (1996) reported that the AM colonisation of

grapevine roots, grown in calcerous soils, resulted in increased shoot growth compared to the non-mycorrhizal plants.

AM colonisation also benefits the host plant by increasing the host's resistance to soil-borne pathogens in a number of different ways. Improved nutrition, primarily P but possibly Zn and Cu, aid in the suppression of root pathogens (Perin 1990; Marschner 1995 and Orcutt and Nilsen 2000). Increased production of phenolics and isoflavonoids is thought to also increase the host's resistance to colonisation (Orcutt and Nilsen 2000). The resulting lignification and suberization of the root due to colonisation lowers the risk of colonisation by root pathogens (Dehne and Schönbeck 1979 and Yedidia *et al,*. 1999). Another hypothesis states that the AM fungi and pathogenic fungi compete for the same colonisation sites on the host's roots. Therefore AM colonisation limits the number of sites available for pathogenic fungi colonisation and lowers the host susceptibility to colonisation by these fungi (Waschkies 1994 and Vigo *et al,*. 2000).

1.2.2 The C-cost of vesicular arbuscular mycorrhizae

AM fungi are dependent on the host plant as a C source and therefore act as a C sink. There is conflicting evidence as to whether or not the percentage colonisation of the root by AM fungi is related to the soluble carbohydrate content of the root. Pearson and Schweiger (1993) found that colonisation was negatively correlated with the soluble carbohydrate content of the root, whilst Thompson *et al,*. (1990) found a positive correlation. This may be because the conflicting experiments were carried out during different developmental stages of colonisation. The three stages of colonisation are the

lag phase, the phase of rapid development and the plateau phase (Smith and Read 1997). Pearson and Schweiger (1993) carried out their experimental work towards the end of the phase of rapid development, when the colonisation period starts to decline and therefore is a subsequent decline in the demand for C by the fungus. Thompson *et al,.* (1990) experimented during the end of the lag phase and the start of the phase of rapid development, when the demand for C is high. It appears that the process of colonisation does depend on carbohydrates from the root during the initial phases and then reaches equilibrium as the process of colonisation comes to an end and a stable symbiotic relationship develops.

Once established, the fungus acts as a sink for photosynthate from the host plant. It has been estimated that the fungus receives between 10% and 23% of the plant's photosynthetically fixed carbon (Snellgrove *et al,.* 1982; Koch and Johnson 1984; Kucey and Paul 1982; Jakobsen and Rosendahl 1990). Black *et al,.* (2000) showed that mycorrhizal plants have a higher photosynthetic rate than non-mycorrhizal plants. This may be because of either an increased level of phosphate in the leaves due to the mycorrhizae (Azcon *et al,.* 1992; Black *et al,.* 2000) or because the AM fungus acts as a C sink (Snellgrove *et al,.* 1982; Kucey and Paul 1982; Koch and Johnson 1984; Jakobsen and Rosendahl 1990). Both explanations have been found to be true, but under different conditions and for different plants. Therefore it may result from a combination of both, depending on the growing conditions and the developmental stage of both the fungus and the plant.

The carbon taken up by the fungus is incorporated into the growth and development of new fungal structures and spores. More than 90% of the root can be colonised by an AM fungus (Motosugi *et al,*. 2002) and can constitute up to 20% of the root dry mass (Harris and Paul 1987). Respiration of colonised roots was found to be between 6.6% and 16.5% (depending on fungal species) higher than uncolonised roots in cucumber plants (Pearson and Jakobsen 1993). The increased respiration rate contributes to the sink effect of the fungus and indicates that colonised roots have a higher metabolic activity than non-colonised roots.

There are three main ways that organic C is lost from the host via the fungus. Firstly, via the loss of sloughed off fungal material. Secondly through the release of fungal spores into the soil and thirdly via the exudation of organic acids and phosphatase enzymes by the fungus. Fungal mycelia are constantly being replaced because of older material either breaking off as the root pushes through the soil or dying and being released into the soil. Bethlenfalvay *et al,*. (1982) found that as much as 88% of the fungal biomass was external of the root for soybean. Similarly Olsson and Johansen (2000) found that 70% of the fungal biomass was external mycelium on cucumber roots. This will account for a large portion of C lost into the soil since the external hyphae will eventually be released into the soil. The release of spores from the external mycelium accounts for a high percentage of lost organic C. In a study done by Sieverding (1989) it was estimated that 919 $kg.ha^{-1}$ of plant C went into the production of spores, which are subsequently released into the soil by the fungus. Furlan and Fortin (1977) found spore production was affected by the amount of C which is available to the fungus. The third means of organic

C loss is through the exudation of organic acids and enzyme phosphatases by the fungal hyphae in order to aid in the uptake of nutrients such as phosphate. However, the main body of evidence supporting this has been found in ectomycorrhizae (Bolan *et al,*. 1987). Although the release of organic acids is not thought to be the primary means of P uptake (Bolan 1991), it does constitute a loss of organic C from the host.

1.2.3 Soil phosphate

Phosphate occurs in high concentrations in the soil, yet it is immobile and remains largely unavailable to plants (Van Tiehelen and Colpaert 2000). Phosphate is found in two main forms, organic (Po) and inorganic P (Pi). For agricultural soils Po can make up as much as 70% of total soil P, yet it is not readily available for uptake by plants (Lambers *et al,*. 1998). Po occurs in three main forms in the soil: soluble P in the soil solution; insoluble P adsorbed onto the surfaces of soil particles or as organic matter within the soil (Anderson 1980). The primary compounds in which Po is found are inisitol phosphate, glycerophosphate (phospholipids) and nucleic acids (Anderson 1980; Adams and Pate 1992). Po is converted to the inorganic form by soil microbes, which feed off organic substances exuded by the plant (Richardson 1994) or by the action of acid phosphatases, which are secreted by the root (Adams and Pate 1992). These enzymes hydrolyze organic-phosphate containing compounds in the soil, releasing Pi into the soil and consequently making it available for uptake by the plant (Kroehler and Linkins 1991).

Pi makes up a much smaller component of soil P, but is available to plants for uptake. Pi can be found in soil solution, adsorbed onto the surfaces of soil particles or precipitated

as discrete minerals. It is the Pi in the soil solution that constitutes the primary source of P for the plants (Bolan 1991). Pi precipitates out of solution as Fe and Al phosphates in acidic soils and as Ca and Mg phosphates in alkaline soils (Bolan 1991). The form in which these phosphates are found and their availability to the plant is pH dependent (Sample *et al,*. 1980).

The two main ways in which plants can come into contact with soil P are by root interception and by diffusion. Mass flow plays a role in the movement of other more mobile nutrients, but phosphate is bound too tightly to the surface of soil particles to move by mass flow (Bolan 1991).

Even when P is spatially available to the root, it is not necessarily available for uptake by the plant. Therefore acidifying and chelating compounds (citric acid, malic acid, oxalic acid and piscidic acid) are often excreted from the root (Marschner 1995). The acidification of the rhizosphere increases the solubility of P in alkaline soils. The chelating compounds (organic acids and phenolics) bind to the cations which are bound to the phosphate groups, thus releasing the P for uptake (Marschner 1995).

1.2.4 P-uptake by the AM fungus

Increased growth of AM plants can be associated with an increase in the uptake rate of P (Sanders and Tinker 1971; Smith 1982). This increased uptake of P can be attributed to a

number of factors. Firstly, the fungus provides an increased absorptive area (Sanders and Tinker 1973). The hyphae are much finer structures than roots or root hairs and therefore have a greater surface area to volume ratio, giving them a larger absorptive surface (Bolan 1991). The fine fungal hyphae are also capable of accessing pockets of P that the larger root structures would not be able to access, even within the rhizosphere (Smith *et al,.* 1982, Jakobsen and Rosendahl 1991; Smith and Read 1997). The finer structures of the AM fungus also means that for the same C expenditure, more hyphae can be produced than roots, resulting in a greater number of absorptive structures (Jones *et al,.* 1991). The hyphae are also capable of extending past the depletion zone around the roots, accessing P that would normally be unavailable to the plant. The root depletion zone usually extends no further than about 1 cm from the root, but hyphae have been found to grow as far as 12 cm from the root (Li *et al,.* 1991a; Marschner 1995). Another attribute of the fine structures of the hyphae is that they are produced relatively quickly, which enables the hyphae to proliferate rapidly when they come across a pocket of P in the soil, allowing them to compete more effectively with other soil microbes for the available P (Smith and Read 1997). The extensive hyphal network of the mycorrhizae creates a shorter distance for the P in the soil to diffuse, therefore increasing the amount of P available for uptake (Sanders and Tinker 1973). Additionally, the hyphae have a lower k_m value (higher affinity) for P than the roots, thus making it possible for the hyphae to take up P from lower concentrations in the soil solution. This effectively means that the hyphae have a lower threshold value for P uptake (Bolan *et al,.* 1983; Smith and Read 1997; Van Tiehele and Colpaert 2000).

Increases in the uptake of P can be a result of AM roots accessing sources of P which are not available to non-AM roots. This is made possible by increased rates of solubilization of ordinarily insoluble Pi, or the hydrolysis of Po. The AM roots accomplish this by exuding phosphatases and chelating agents. However, there is conflicting evidence as to what extent chelating agents and phosphatases are used by AM fungi, although it is common in ectomycorrhizal fungi (Bolan 199;1Smith and Read 1997).

The final factor resulting in higher uptake rates is the ability of AM hyphae to incorporate the Pi into polyphosphates and rapidly store the P taken up. P is stored in three forms in the hyphae: soluble orthophosphate (Harley and Loughman 1963); soluble polyphosphate (Martin *et al,.* 1983); and polyphosphate granules (Chilvers and Harley 1980). The conversion of Pi into polyphosphates and the rapid storage of P would circumvent the negative feedback inhibition on P uptake experienced in roots, allowing for more continued uptake.

The costs of the AM symbiosis is considerable to the host plant (Snellgrove *et al,.* 1982; Koch and Johnson 1984; Kucey and Paul 1982; Jakobsen and Rosendahl 1990) and much of the C is sequestered to maintain fungal growth and nutrient uptake benefits. For nutrient benefits, it has been estimated that hyphae provide between 70% and 80% of the P in a mycorrhizal plant (Li *et al,.* 1991b), which would have obvious C cost implications to the host. In dormant hosts such as young grapevines, non-photosynthetic C from stored reserves is the only energetic currency for new growth and AM fungal costs. Therefore it is pertinent that the C costs involved in the establishment and the consequent nutrient

benefits of the AM symbiosis be assessed in order to understand the dynamics of the partitioning of stored C between host plant and fungal components.

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Chapter 2

General Introduction

2.1 Viticultural benefits of symbiosis

The colonisation of grapevines by AM fungi benefits the vines in a number of ways, resulting in improved growth of the colonised vines. Depending on the growing conditions of the vines, different AM benefits will contribute to the growth status of the plant.

The AM fungus takes up minerals from the soil and supplies them to the vine, thus improving the mineral nutrition of the vines. Nikolaou *et al,.* (2002) reported that AM vines had greater concentrations of N, P, K and Ca in their leaves. Similarly, Motosugi *et al,.* (2002) demonstrated that inoculated vines had higher P concentrations in their leaves. The higher leaf P concentrations also enables the vines to maintain a higher photosynthetic rate. Nikolaou *et al,.* (2003b) demonstrated that AM colonised vines had higher CO_2 assimilation rates than uncolonised vines, but no differences in the pruning weight of the plants.

AM fungi also aid in the uptake of water and contributes to an improved water status in vines, enabling the vines to grow under low irrigation or survive water stressed conditions. Nikolaou *et al,.* (2003a) determined that AM vines had an improved water status and drought sensitive rootstocks showed better growth when colonised by an AM

fungus under non-irrigated conditions. Motosugi *et al,*. (2002) reported that AM colonised roots were more efficient in the uptake of water compared to uncolonised roots.

2.2 AM efficiency and C-flux modeling in AM plants

The transfer of fixed C from the host to the fungus has a direct effect on the host plant. As mentioned earlier, the effects will vary according to the light and soil nutrient levels and according to the fungal and host spp. involved. Koide and Elliott (1989) described this relationship mathematically using various models. They developed models describing both the gross benefit of the mycorrhizal colonisation and the net benefit of colonisation.

Gross benefit was defined as the difference between the quantity of gross C assimilation (mole C) in mycorrhizal and non-mycorrhizal plants over a given period of time (Koide and Elliott 1989):

$\Delta A^{g}_{m} - \Delta A^{g}_{nm}$

Where ΔA^{g}_{m} and ΔA^{g}_{nm} is the gross C assimilation of the mycorrhizal and non-mycorrhizal plants during that time interval respectively.

The net benefit of colonisation for the same time period was described as the difference between mycorrhizal and non-mycorrhizal C accumulation (moles C) in the whole plant over the given time period (Koide and Elliott 1989):

$\Delta C^{w}_{m} - \Delta C^{w}_{nm}$

Where ΔC^{w}_{m} and ΔC^{w}_{nm} represent the amount of C accumulated in the mycorrhizal and non-mycorrhizal plants over the given time period.

Koide and Elliott (1989) also described the efficiency of the relationship in terms of P acquisition, P utilization and below ground carbon utilization. The efficiency of the P acquisition was defined as:

$$\frac{\Delta P^{w}}{\Delta C^{b}}$$

Where ΔP^{w} is the total P that has accumulated in the plant during the given time interval and ΔC^{b} is the total below ground C expenditure over the same time period (Koide and Elliott 1989). This describes the efficiency of the relationship in terms of the amount of P taken up compared to the amount of C used for the uptake of P. C^{b} can be calculated as follows (Koide and Elliott 1989):

$$C^{b} = C^{r} + C^{o} + C^{n}$$

Where C^{r} is the C that is allocated to the root tissue, C^{o} is the C lost via root below ground respiration and C^{n} is the non-respiratory, below ground C loss.

The efficiency of P utilization was defined by the following equation (Koide and Elliott 1989):

$$\frac{\Delta C^{w}}{\Delta P^{w}}$$

Where ΔC^{w} is the total amount of C accumulated, in the whole plant, over the same period (Koide and Elliott 1989). This efficiency can be applied to any of the respective plant components.

The final model proposed by Koide and Elliott (1989) was used to define the efficiency of below ground C utilization and was expressed as the ratio ΔC^{w}: ΔC^{b}. This ratio is the product of the previous two models:

$$\frac{\Delta C^w}{\Delta C^b} = \frac{\Delta C^w}{\Delta \mathrm{P}^w} \times \frac{\Delta \mathrm{P}^w}{\Delta C^b}$$

Koide and Elliott (1989) defined C^b (see above) as the total below ground C expenditure, which included all the C in the living tissue of the root system and the C lost from the root, via exudation, leaching, respiration, cell death and direct transport to the fungus. Jones *et al,*. (1991) went one step further and formulated two models, which defined C^b in terms of the factors influencing the changes in C^b. The first model expressed C^b as a function of the C fixed via photosynthesis:

$$C_{b(Pn)} = Pn \frac{\%C_{BG}}{100 - \%C_{SR}} t$$

Where $C_{b(Pn)}$ is the amount of photosynthetically fixed C that is allocated below ground in a given period of time. *Pn* is the net photosynthetic rate as mmol C s^{-1} for the whole shoot system; $\%C_{BG}$ is the percentage of the total fixed C which is allocated below ground, over a given period of time; $\%C_{SR}$ is the percentage of the fixed C which was released via respiration in the shoot and t is the length of the daily light period, measured in seconds. The term $100\text{-}\%C_{SR}$ represents the total amount of C left after respiration.

Their second model expressed C^b as a function of the change in shoot mass, which would give an indication of C fluxes within the shoot:

$$\Delta C_{b(Wt)} = \Delta W_s \frac{\%C_{BG}}{\%C_{ST}}$$

Where ΔW_s is the mean increase in shoot mass over a given time period and $\%C_{ST}$ and $\%C_{BG}$ are the mean percentages of the C fixed and allocated to the shoot tissue and to the below ground components respectively.

The work of Koide and Elliott (1989) forms the backbone of mycorrhizal efficiency modeling, but they never tested their models experimentally. Therefore they have not defined the factors that affected each of the parameters involved in the different models. The models proposed by Jones *et al,*. (1991) elaborated on those of Koide and Elliott (1981) by defining C^b as a function of it's influencing factors, not just it's components. However, the expression of C^b in terms of photosynthetically fixed C can be misleading. It assumes that photosynthetic C is the only source of C available to the plant. It does not include structural and non-structural C that is already stored in the plant, which may be used and transported elsewhere in the plant. Similarly the expression of C^b in terms of the changes shoot mass assumes that the shoots are the only structures that will have an effect on below ground C, again ignoring other, pre-existing sources of C within the plant. This also neglects to take into account that VAM and non-VAM plants may allocate photosynthetic C in different proportions to different organs (Koide 1985; Smith 1980).

The above is made even more relevant when dealing with plants that store C for the following season's growth. *Vitis vinifera* is one such plant and has various sources of stored C (Buttrose, 1966 and McArtney and Ferree, 1998). In autumn the vines lose their leaves and consequently have no photosynthetic material at the start of the next growing

season. The lack of photosynthetic tissue means that there is no external source of C for the new growth that takes place and the vines must make use of the C stored within the plant. The vine can utilize C from the roots, the stem of the rootstock or the canes (Buttrose 1966; McArtney and Ferree, 1998).

2.3 Tissue construction cost and below ground respiration

Williams *et al,*. (1987) proposed a model which can be used to determine the construction cost of various tissues within a plant. They defined construction cost as the amount of glucose required to provide C skeletons, reductant and ATP for synthesizing the organic compounds in a tissue via standard biochemical pathways.

They calculated construction cost as:

$$C_w = \{(0.06968 \times \Delta H_c - 0.065)(1 - A) + \frac{kN}{14.0067} \times \frac{180.15}{24}\} \frac{1}{E_G}$$

Where C_w is the construction cost of the tissue (g glucose gDW^{-1}) and ΔH_c is the ash- free heat of combustion of the sample (kJ g^{-1}). A is the ash content of the sample (g ash gDW^{-1}); k is the reduction state of the N substrate (NO_3 was used, therefore k is +5) and E_G is the deviation of growth efficiency from 100%. E_G represents the fraction of the construction cost which provides reductant that is not incorporated into biomass. Williams *et al,*. (1987) determined the value of E_G to be 0.89.

Peng *et al,*. (1993) modified this equation and converted the g glucose into mmol C:

$$C_w = \{(0.06968 \times \Delta H_c - 0.065)(1 - A) + \frac{kN}{14.0067} \times \frac{180.15}{24}\} \frac{1}{0.89} \times \frac{6000}{180}$$

The units of construction cost are now mmol C gDW^{-1}. Peng *et al,.* (1993) use the construction cost to determine the growth respiration, which was defined as the respired C associated with the biosynthesis of new tissue:

$$R_{G(t)}=C_t-\Delta W_c$$

Where $R_{G(t)}$ is the growth respiration (μmol CO_2 d^{-1}) ; C_t (μmol CO_2 d^{-1}) is the C required for daily construction of new tissue. C_t was calculated by multiplying the root growth rate (ΔW_w, mgDW d^{-1}) by tissue construction cost (C_w). ΔW_c ((μmol d^{-1}) is the change in root C content and was calculated by multiplying the root C content and the root growth rate (ΔW_w, mg d^{-1}).

2.4 Aims

The above equations provide a means of evaluating the C economy of plants and their respective components. In deciduous plants like vines, which rely on stored C for the growth of new tissues in spring, the additional C-drain for AM fungal growth also imposes on the plant C reserves (McArtney and Ferree 1998; Buttrose 1966). AM fungal growth and nutrient acquisition also present a considerable C-drain on host plant reserves (Kucey and Paul 1982; Peng *et al.,* 1993; Johnson *et al.,* 1997; Black *et al.*, 2000) and would therefore affect the C reserve mobilisation during the initial growth stages of the vine. The aims of this study are to assess the host C economy during AM development and the subsequent C-costs of N and P uptake, as well as the effects of C costs on host growth. This will be evaluated in two separate experiments, one a fundamental study and

the other one a practical evaluation. The fundamental study will assess the symbiotic influence on the C costs of fungal establishment and nutritional benefits. The practical investigation will be aimed at evaluating the effects of the symbiosis on host growth and nutrient productivities.

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Chapter 3

Title: **Mycorrhizal C costs and nutritional benefits in developing grapevines**

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Chapter 3

Title: Mycorrhizal C costs and nutritional benefits in developing grapevines

Running title: C costs of AM vines

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ABSTRACT

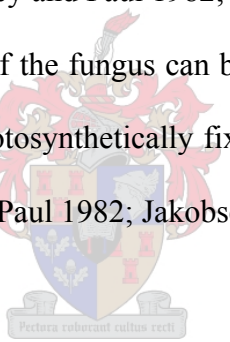
Arbuscular mycorrhizal (AM) C-costs in grapevines were investigated. Dormant vines rely on stored C for initial growth. Therefore AM colonisation costs would compete with plant growth for available C reserves. One-year old grapevines, colonised with *Glomus etunicatum* (Becker and Gerdemann), were cultivated under glasshouse conditions. The C-economy and P utilisation of the symbiosis were sequentially analysed. AM colonisation, during the 0-67 day growth period used more stem C relative to root C, which resulted in lower shoot growth. The decline in AM colonisation during the period of 67-119 days coincided with stem C replenishment and higher shoot growth.

Construction costs of AM plants and root C allocation increased with root P uptake. The efficiency of P utilisation were lower in AM roots. The reliance of AM colonisation on stem C declined with a decrease in colonisation, providing more C for the refilling of stem carbohydrate reserves and shoot growth. Once established, the AM symbiosis increased P uptake at the expense of the refilling of root C reserves. Although higher root C allocation increased the plant construction costs, AM roots were more efficient at P utilisation.

Key words: C-economy, arbuscular mycorrhiza, efficiency of P utilisation, grapevine

Introduction

Arbuscular mycorrhizal (AM) symbiosis may function to acquire soil nutrients for the host plant in exchange for soluble carbohydrates. Marschner and Dell (1994) showed that AM fungi supplied 80% of P and 25% of N for the host plants. The enhanced P nutrition of AM plants growing in phosphate limited soils, usually leads to higher plant growth rates than non-AM plants (Sanders and Tinker 1971; Smith 1982; Bolan 1991; Orcutt and Nilsen 2000). The dependency of the host plant on AM is balanced by the costs of maintaining the relationship. The costs of the symbiosis are in the form of organic carbon (C) derived from the host, which is transported below ground due to the sink effect of the fungus (Snellgrove *et al.,* 1982; Kucey and Paul 1982; Koch and Johnson 1984; Jakobsen and Rosendahl 1990). The C-costs of the fungus can be considerable and the fungus can receive up to 23% of the plant's photosynthetically fixed carbon (Snellgrove *et al* 1982; Koch and Johnson 1984; Kucey and Paul 1982; Jakobsen and Rosendahl 1990).



The C taken up by the fungus is incorporated into the growth and development of new fungal structures and spores. More than 90% of the root can be colonised by an AM fungus (Motosugi *et al,.* 2002) and can constitute up to 20% of the root dry mass (Harris and Paul 1987). Respiration of colonised roots was found to be between 6.6% and 16.5% (depending on fungal species) higher than uncolonised roots in cucumber plants (Pearson and Jakobsen 1993). The increased respiration rate contributes to the sink effect of the fungus and indicates that colonised roots have a higher metabolic activity than uncolonised roots.

The majority of studies to date have concentrated on quantifying the effects of AM and the C-costs of the symbiosis using photosynthetically fixed C (Black *et al.*, 2000; Wright *et al.,* 1998a; Fay *et al.,* 1996; Peng *et al.,* 1993; Koide and Elliot 1989). Few research projects focused on the mobilization and utilization of C from storage tissue for the development of the AM symbiosis. Merryweather and Fitter (1995) showed the reallocation of stored C for the growth of new roots and shoots in the geophyte *Hyacinthoides non-scripta* (L) Chouard ex Rothm. This study found that the C needed for the new season's growth was derived from C stored within the bulb. However, as the plant's photosynthetic tissue developed, its reliance on stored C declined, allowing for the C reserves to be replenished.

A number of factors will determine a plant's dependency on AM for nutrients, one being the type of root system. This is seen with coarse rooted plants species, such as vines, which are more dependent on AM for nutrient uptake than finer rooted species (Motosugi *et al.,* 2002; Merryweather and Fitter 1995; Eissenstat 1992). In deciduous plants like vines, which rely on stored C for the growth of new tissues in spring, the additional C-drain for AM fungal growth also taps into the plant C reserves (McArtney and Ferree 1998; Buttrose 1966). AM fungal growth and nutrient acquisition also present a considerable C-drain on host plant reserves (Kucey and Paul 1982; Peng *et al.,* 1993; Johnson *et al.,* 1997; Black *et al.*, 2000) and would therefore influence the C reserve mobilisation during the initial growth stages of the vine. The aim of this study was to assess the host C economy during AM development and the subsequent C-costs of N and P uptake once colonisation is established.

Materials and Methods

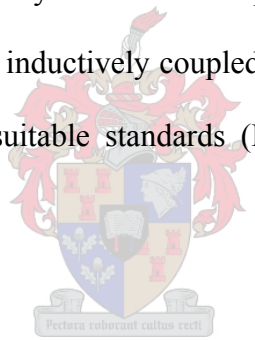
Plant growth and AM inoculation

One-year old grafted grapevines (*Vitis vinifera* L. cv. Pinotage, grafted onto Richter 99 rootstock) were planted in 20 litre pots containing river sand, between May and August 2002. The average grain size of the medium was 0.51mm with a pH of 7. The sand was sterilised in an autoclave for 1 hour at a temperature of 120 °C and a pressure of 200 kPa. The pots were placed in a north-facing glasshouse at the University of Stellenbosch, Stellenbosch, South Africa. The maximum daily photosynthetically active irradiance was between 600 and 700 µmol m^{-2} s^{-1} and the average day/night temperatures and humidities were 23/15°C and 35/75% respectively. The plants were watered with distilled water and every second week received Long Ashton nutrient solution, modified to contain only nitrate, 100µM phosphate and pH 6. The inoculum consisted of spores and hyphae from *Glomus etunicatum* (Becker and Gerdemann) (accession number J100092, Moss Herbarium, University of the Witwatersrand) and host root fragments in an inert clay-based granular support substrate. The experimental plants were inoculated and the control plants received a filtered inoculum solution, which was prepared by filtering the inoculum through a 37 µm mesh to remove the mycorrhizal fungal material.

Harvesting and nutrient analysis

The vines were pruned back to one-bud and allowed to grow until the respective harvests, which took place after 21, 43, 67, 95 and 119 days. Upon harvesting the plants were separated into different components, which consisted of new shoot tissue, woody scion tissue, stem and roots (new and old roots) and the fresh weight of each component was

recorded. Sub-samples of new root segments were obtained by cutting the new root material into 1 cm strips and then randomly selecting samples. These samples were stored in 50% ethanol in order to determine percentage AM fungal colonisation at a later stage. The harvested material was then placed in an oven, at 80 °C, for two days and dry weights were recorded. The dried plant material was milled using a 0.5 mm mesh (Arthur H Thomas, California, USA). In addition, a bulk sample was also made up by combining proportional sub-samples of each component, based on the percentage of each component in the whole plant (eg. where roots were 10% of the plant dry weight, then the bulk samples comprised 10% of the root material). These are indicated as "bulked plants" in the results. Milled samples were analysed for their respective C, N and P concentrations, by a commercial laboratory, using inductively coupled mass spectrometry (ICP-MS) and a LECO-nitrogen analyser with suitable standards (BemLab, De Beers Rd, Somerset West, South Africa).



Determination of percentage AM colonisation

Roots were harvested at 21, 43, 67, 95 and 119 days. Non-woody roots were cut into 1 cm segments and rinsed and cleared with 10% KOH for 5 minutes in an autoclave at 110^{O}C under steam pressure of 200 kPa. The KOH was rinsed off and the segments acidified with 2 N HCl for 10 min. Thereafter the roots were stained with 0.05% (w/v) analine blue for 10 min in an autoclave at 110^{O}C under steam pressure of 200 kPa and then destained in lactic acid overnight. Root segments were placed on slides and the colonisation components were determined according to Brundrett *et al,*. (1994).

Calculation of C-costs and AM efficiency

Root and stem C fluxes (mmolC/tissue type/d): This represents the C fluxes of the root and stem tissues for a given growth period. C flux is expressed as the rate of movement of an absolute amount of C in a specific tissue and calculated by dividing the change in C content of a specific tissue over time. (1)

Root construction costs (mmolC/gDW): Calculation of the tissue construction cost, modified from the equation used by Peng *et al.*, (1993).

$$Cw = [C + kN/14 \times 180/24] (1/0.89)(6000/180) \quad (2)$$

Where *Cw* is the construction cost of the tissue (mmolC/gDW), *C* is the carbon concentration (mmolC/g), *k* is the reduction state of the N substrate (NO_3 was used, therefore k is +5) and *N* is the organic nitrogen content of the tissue (g/g DW) (Williams *et al.*, 1987). The constant *(1/0.89)* represents the fraction of the construction cost which provides reductant that is not incorporated into biomass (Williams *et al.,* 1987, Peng *et al.*, 1993) and *(6000/180)* converts units of g glucose/g DW to mmolC/g DW.

Efficiency of P utilisation: The equation proposed by Koide and Elliott (1989) to calculate the quantity of C accumulated divided by the quantity of P accumulated for a given period of time.

$$\Delta C^r/\Delta P^r \quad (3)$$

ΔC^r is the C accumulated in the roots over a given time period and ΔP^r is the total P accumulated in the roots over the same time period. Similarly, the efficiency of shoot P utilisation was calculated using the C and P values of the shoots. It should be noted that a low efficiency value indicates that less C is required for the given amount of P utilised by the plant or plant component.

Growth respiration (mmol CO_2/gDW): Represents the C respired for the biosynthesis of new tissue, proposed by Peng *et al.*, (1993).

$$Rg(w) = Rg(t)/root\ gr \quad (4)$$

Rg(w) represents growth respiration based on dry weight, *root gr* is the root growth rate (gDW d^{-1}) and *Rg(t)* is the daily growth respiration (μmol CO_2 d^{-1}):

$$R_{G(t)} = C_t - \Delta W_c$$

C_t (μmol CO_2 d^{-1}) is the C required for daily construction of new tissue. C_t was calculated by multiplying the root growth rate (gDW d^{-1}) by tissue construction cost (C_w). ΔW_c (μmol d^{-1}) is the change in root C content and was calculated by multiplying the root C content and the root growth rate.

Statistical analysis

The differences between harvests (n = 6 for each treatment) for percentage AM colonisation, were separated using a *post hoc* Student Newman Kuels (SNK), multiple comparison test ($p \leq 0.05$) (SuperAnova). Different letters indicate significant differences between treatments. The percentage data were arcsine transformed (Zar, 1999). For each harvest, the difference between the means of AM and non-AM plants, was separated using a Student's *t*-test ("Statistica 6.0", StatSoft Tulsa, OK, USA) for independent samples by groups ($p \leq 0.05$). Different letters indicate significant differences between treatments.

Results

Growth and C-fluxes

Uninoculated plants remained non-mycorrhizal for the duration of the experiment. The percentage and rate of AM fungal colonisation (Figure 1 and Table 1) increased over the period of day 0-67 and declined during days 67-119. This coincided with a loss of C from the above ground components of AM plants, (Table 1) relative to non-AM plants. During the 0-67 day period of colonisation and above ground C loss, the shoot growth rate (Table 1) was lower in the AM plants. Furthermore, there was also a similar loss of C from AM and non-AM roots during this period (Table 1). For the 0-67 day phase, new root growth rate (Table 1) was lower in AM plants.

As the percentage and rate of AM fungal colonisation declined (Figure 1 and Table 1) during the 67-119 day period, refilling of above ground C reserves (Table 1) occurred in AM plants along with an increase in new root and shoot growth rates (Table 1). During the same period the non-AM roots had an increase in C flux, whilst the AM roots maintained a negative C flux (Table 1).

At day 67 when AM fungal colonisation was at a maximum (Figure 1), the AM shoot dry weight was lower, whilst the AM root dry weights were higher than the non-AM plants (Table 2). As AM fungal colonisation declined after day 67, there were no further differences between AM and non-AM dry weights for all the components (Table 2). However, the AM plants had a lower tissue construction cost (Cw) from 0 to 43 days, and a higher Cw from day 67 onwards (Figure 4).

Nutrient assimilation

No overall differences were found in the N and P concentrations of the shoots or the bulked plants between AM and non-AM plants for the duration of the experiment (Table 3). There were also no differences prior to day 67 in the root N and P concentrations between AM and non-AM plants, but subsequently the root N and P for AM plants were higher (Figure 2a, b). Concomitant with these increases (67-119 days), the growth respiration of new root tissue was higher in AM plants (Figure 3a). In spite of the higher growth respiration of new roots, the efficiency of P utilisation in AM roots was lower than non-AM roots (Figure 3b). This means that less C was used for P incorporation. It is likely that AM fungal tissue and not the new roots, accounted for the lower efficiency of root P utilisation, as found in the positive correlation ($y = 7.718x + 1187.379$, $r^2 = 0.983$) between efficiency P utilisation and AM fungal colonisation (data not shown).

Discussion

The roots and stem should be considered as regions of C storage for young developing grapevines (Buttrose 1966; McArtney and Ferree 1998), but in young AM inoculated grapevines, the source of C for the new growth of a developing AM symbiosis was unclear. The current findings show that above-ground C contributes significantly to the C budget, during the 0 to 67 day period of AM fungal colonisation, which concurs with the phase of rapid colonisation as described by Smith and Read (1997). The combined activity of new root growth and AM fungal colonisation required more C than was available in the root reserves alone, necessitating the above-ground C drain. The rapid loss of C from the colonised roots can be attributed to the relatively higher growth rate of

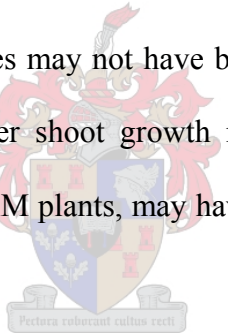
new fungal structures compared to the growth of new root tissue in uncolonised plants (Jakobsen and Rosendahl 1990). The AM C-drain from host reserves concurs with other findings of the sink effect of AM, albeit from photosynthetically fixed C which is supplied to the AM fungus and not C from stored plant reserves (Snellgrove *et al.,* 1982; Kucey and Paul 1982; Koch and Johnson 1984; Jakobsen and Rosendahl 1990).

The C drain imposed by the rapid phase of AM fungal colonisation had a negative impact on host growth, as evidenced by the lower new root and shoot growth rates. This may indicate that AM fungal colonisation was the preferential below ground sink, since colonised roots have higher metabolic rates (Pearson and Jakobsen 1993). The current data are inconsistent with other findings of higher root and shoot growth rates in AM plants (Linderman and Davis 2001; Estrada-Luna *et al,*. 2000). This possibly resulted from the current measurements being taken during the rapid phase (0 to 67 days) of AM development, whilst the other studies (Linderman and Davis 2001; Estrada-Luna *et al,*. 2000) had data from plants aged at 102 days (Linderman and Davis 2001) and 116 days (Estrada-Luna *et al,*. 2000). It is therefore likely that these studies (Linderman and Davis 2001; Estrada-Luna *et al,*. 2000) occurred during the plateau phase of AM development.

Once the plateau phase was reached (67-119 days), the established symbiosis no longer drained the above ground C reserves, allowing for a refilling of these reserves. A similar pattern of refilling was found in the bluebell (*Hyacinthoides non-scripta* (L.) Chouars ex Rothm), however the refilling occurred towards the end of the growth season (Merryweather and Fitter 1995). Once the AM symbiosis was established, the increased

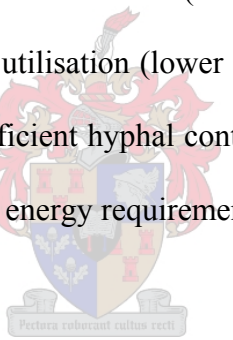
new root and shoot growth rates may have resulted from two major factors affecting the AM C-economy. Firstly, at the plateau phase, the decline in the AM fungal growth rate would have reduced the C-sink in the AM roots and secondly the AM roots were more efficient at using C for P utilisation during this period.

The further depletion of root C reserves in AM plants during the plateau phase (67-119 days) may reflect the C requirement of AM associated metabolism, as reported by Peng *et al* (1993), that AM roots have higher below ground respiration rates. This is congruent with the current findings of higher growth respiration in new roots of AM plants. The similar above-ground C-flux of the AM and non-AM plants during the same period of growth, indicate that these C reserves may not have been drained during the established AM symbiosis. However, the higher shoot growth rate, in spite of similar N and P concentrations in the AM and non-AM plants, may have resulted from the more efficient use of C for P utilisation.



During the plateau phase (67-119 days) the root nutritional benefits of the AM symbiosis also became apparent, in the higher N and P concentrations of AM roots. This may be because during the established symbiosis, the hyphal network of the AM fungus is more developed than in the former phase, thus providing a greater surface area for nutrient absorption (Jakobsen *et al.,* 1991; Smith and Read 1997). The cost of nutrient uptake is a significant C drain, as Baas *et al.,* (1989) found that 13% of fungal C was used for increased nutrient uptake and the remaining 87% for fungal respiration. However, the 87% fungal respiratory costs may be further subdivided into maintenance costs and

growth costs, both of which indirectly affect nutrient uptake by increasing and maintaining the hyphal network (Peng *et al.*, 1993). Therefore the percentage C used for nutrient acquisition may be larger than 13%. During the phase of rapid development a greater percentage C will be used for the growth of new fungal structures and only once the symbiosis is functional will more C be used for nutrient acquisition. In this regard, AM fungi are able to absorb P more efficiently than their hosts by increasing the absorptive area of the plant's root system and accessing P sources unavailable to the host roots (Bolan 1991; Smith and Read 1997). The AM fungal hyphae also have a higher affinity (lower km) for P than the host plants and the fungi are more efficient at competing with other soil microbes for nutrients (Bolan 1991; Smith and Read 1997). Furthermore, the lower efficiency P utilisation (lower tissue C:P ratio) for the AM roots can also be attributed to the more efficient hyphal contribution to P uptake (Bolan 1991; Smith and Read 1997) and the lower energy requirements for hyphal maintenance (Koide 1993).



The AM C drain and subsequent nutrient uptake costs may have resulted in the higher construction costs of the AM plants. These higher construction costs of the AM plants, did not negatively impact the bulk dry weights of hosts. It was shown when AM costs exceed the nutritional benefits to the host, negative growth responses can ensue (Graham and Eissenstat, 1998). This would indicate that, in the present study, the C-drain imposed by the AM fungus was possibly compensated for by increased photosynthetic rates, as previously reported (Wright *et al.*, 1998a and b; Fitter 1991).

In conclusion, the initial costs of AM fungal colonisation were borne by the above-ground C reserves, at the expense of new growth in host plants. These costs were offset once the plateau phase was reached, and the depleted reserves started to refill. Once established, the active symbiosis imposed a considerable below ground C sink on host reserves. In spite of these costs, the improved P nutrition of AM roots was achieved with a more efficient C-use. This concurs with other findings that, of the belowground C allocated to AM roots, a greater part is used by AM respiration and a smaller part for P uptake.

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Table 1. Colonisation rate, C flux and growth rate data. The colonisation rate of AM roots (% root length colonised d^{-1}), above ground (mmol C shoot and stem tissue^{-1} d^{-1}) and root C fluxes (mmol C root^{-1} d^{-1}) and new root and shoot growth rates (gDW d^{-1}) of grapevines colonised with *Glomus etunicatum*. Grapevines were grown in glasshouse conditions and harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n = 6) and uninoculated (-AM) (n = 6) plants were determined by Student's *t*-test for independent samples by groups. Significant differences ($p \leq 0.05$) between inoculated and uninoculated plants for each harvest, are indicated by different letters.

Parameters	Growth Period	
	0 to 67	67 to119
Colonisation rate (% root length colonised d^{-1})		
+ AM	1.41 b	-0.31 a
Above ground C flux (mmol C shoot and stem tissue^{-1} d^{-1})		
+ AM	-5.61 a	4.12 a
- AM	11.6 b	2.03 a
Root C flux (mmol C root^{-1} d^{-1})		
+ AM	-1.57 a	-3.37 a
- AM	-1.53 a	3.11 b
New root growth rate (gDW d^{-1})		
+ AM	0.024 a	0.131 b
- AM	0.043 b	0.102 a
Shoot growth rate (gDW d^{-1})		
+ AM	2.87 a	5.26 b
- AM	5.2 b	2.86 a

Table 2. Dry weight data. The dry weights (g) of grapevines colonised with *Glomus etunicatum.* Grapevines were grown in glasshouse conditions and harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n = 6) and uninoculated (-AM) (n = 6) plants were determined by T-test for independent samples by groups. Significant differences ($p \leq 0.05$) between inoculated and uninoculated plants for each harvest are indicated by different letters.

Dry Weights	Days of growth				
Components	21	43	67	95	119
Shoot (g)					
+AM	0.36 a	1.87 a	2.70 a	7.23 a	9.08 a
-AM	0.41 a	2.01 a	5.35 b	8.83 a	7.78 a
Stem (g)					
+AM	8.68 a	10.73 a	11.46 a	10.67 a	11.69 a
-AM	6.18 a	11.53 a	9.64 a	10.72 a	11.45 a
Scion (g)					
+AM	1.99 a	1.35 a	2.01 a	1.87 a	2.36 a
-AM	1.28 b	1.73 a	2.16 a	2.50 a	1.95 a
Root (g)					
+AM	8.00 a	7.04 a	10.72 a	7.02 a	10.26 a
-AM	7.20 a	6.85 a	6.76 b	11.48 a	8.55 a
Bulk (g)					
+AM	32.73 a	38.02 a	45.93 a	39.03 a	36.63 a
-AM	24.26 a	43.28 a	38.58 a	40.62 a	46.45 a

Table 3. Nutrient data. The shoot N (mmol N g DW^{-1}), shoot P (mmol P g DW^{-1}), bulk N (mmol N g DW^{-1}) and bulk P (mmol P g DW^{-1}) concentrations of grapevines colonised with *Glomus etunicatum.* Grapevines were grown in glasshouse conditions and harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n = 6) and uninoculated (-AM) (n = 6) plants were determined by T-test for independent samples by groups. Significant differences ($p \leq 0.05$) between inoculated and uninoculated plants for each harvest, are indicated by different letters.

Nutrient	Days of growth				
Concentration	21	43	67	95	119
N (mmol N g^{-1} dw)					
+AM shoot	2.50 a	1.71 a	1.19 a	1.08 a	0.95 a
-AM shoot	2.46 a	1.76 a	1.26 a	0.94 a	0.88 a
+AM bulk	0.48 x	0.46 x	0.51 x	0.53 x	0.53 x
-AM bulk	0.52 x	0.44 x	0.48 x	0.53 x	0.51 x
P (mmol P g^{-1} dw)					
+AM shoot	0.16 a	0.10 a	0.07 a	0.04 a	0.03 a
-AM shoot	0.16 a	0.10 a	0.05 a	0.03 a	0.03 a
+AM bulk	0.04 x	0.03 x	0.03 x	0.02 x	0.02 x
-AM bulk	0.03 x	0.03 x	0.02 y	0.02 x	0.02 x

Figure legends

Figure 1. The percentage arbuscular mycorrhizal (AM) (n = 6) colonisation of grapevines colonised with *Glomus etunicatum.* Grapevines were grown in glasshouse conditions and harvested at 21, 43, 67, 95 and 119 days. The differences between harvests (n = 6) were separated using a *post hoc* Student Newman Kuels (SNK), multiple comparison test ($p \leq 0.05$). Significant differences ($p \leq 0.05$) between each harvest, are indicated by different letters.

Figure 2. The root N concentration (mmolN gDW^{-1}) **(a)** and root P concentration (mmolP gDW^{-1}) **(b)** of grapevines colonised with *Glomus etunicatum.* Grapevines were grown in glasshouse conditions and harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n = 6) and uninoculated (-AM) (n = 6) plants were determined by *t*-test for independent samples by groups. Significant differences ($p \leq 0.05$) between inoculated and uninoculated plants, for each harvest, are indicated by astricts (*) and ns represents non-significance.

Figure 3. The new root growth respiration (mmolCO_2 gDW^{-1}) **(a)**, the efficiency P utilisation (C:P) **(b)** and the correlation between the percentage AM colonisation and efficiency P utilisation **(c).** Glasshouse-cultivated grapevines were colonised with *Glomus etunicatum* and harvested at 21, 43, 67, 95 and 119 days. For **(a)** and **(b)** differences between inoculated (+AM) (n = 6) and uninoculated (-AM) (n = 6) plants were determined by *t*-test for independent samples by groups. Significant differences ($p \leq 0.05$) between inoculated and uninoculated plants, for each harvest, are indicated by astricts

(*) and ns represents non-significance. For **(c)** the r^2 represents the coefficient of determination for each correlation.

Figure 4. The bulk construction costs of glasshouse-cultivated grapevines (mmolC gDW^{-1}). The grapevines were colonised with *Glomus etunicatum* and harvested at 21, 43, 67, 95 and 119 days. The differences between inoculated (+AM) (n = 6) and uninoculated (-AM) (n = 6) plants were determined by *t*-test for independent samples by groups. Significant differences ($p \leq 0.05$) between inoculated and uninoculated plants, for each harvest, are indicated by astricts (*) and ns represents non-significance.

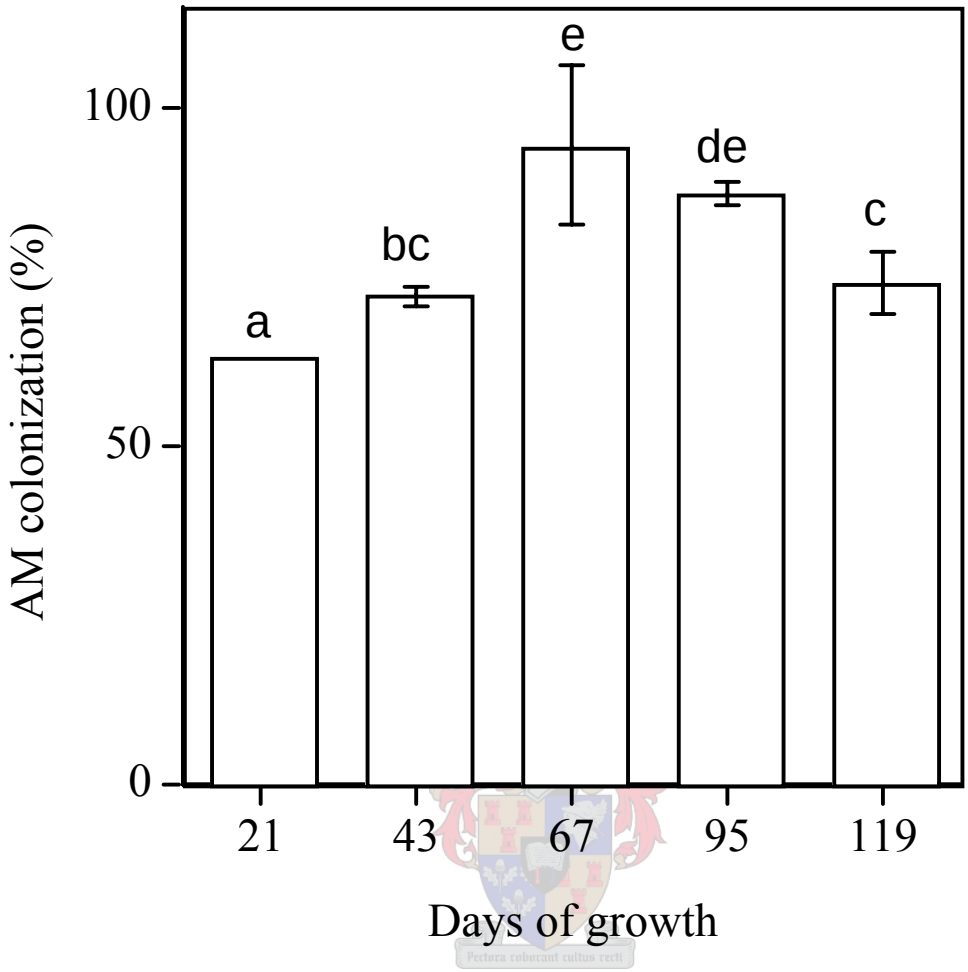
AM colonization (%)
100
50
0
a
bc
e
de
c
21
43
67
95
119
Days of growth

Figure 1

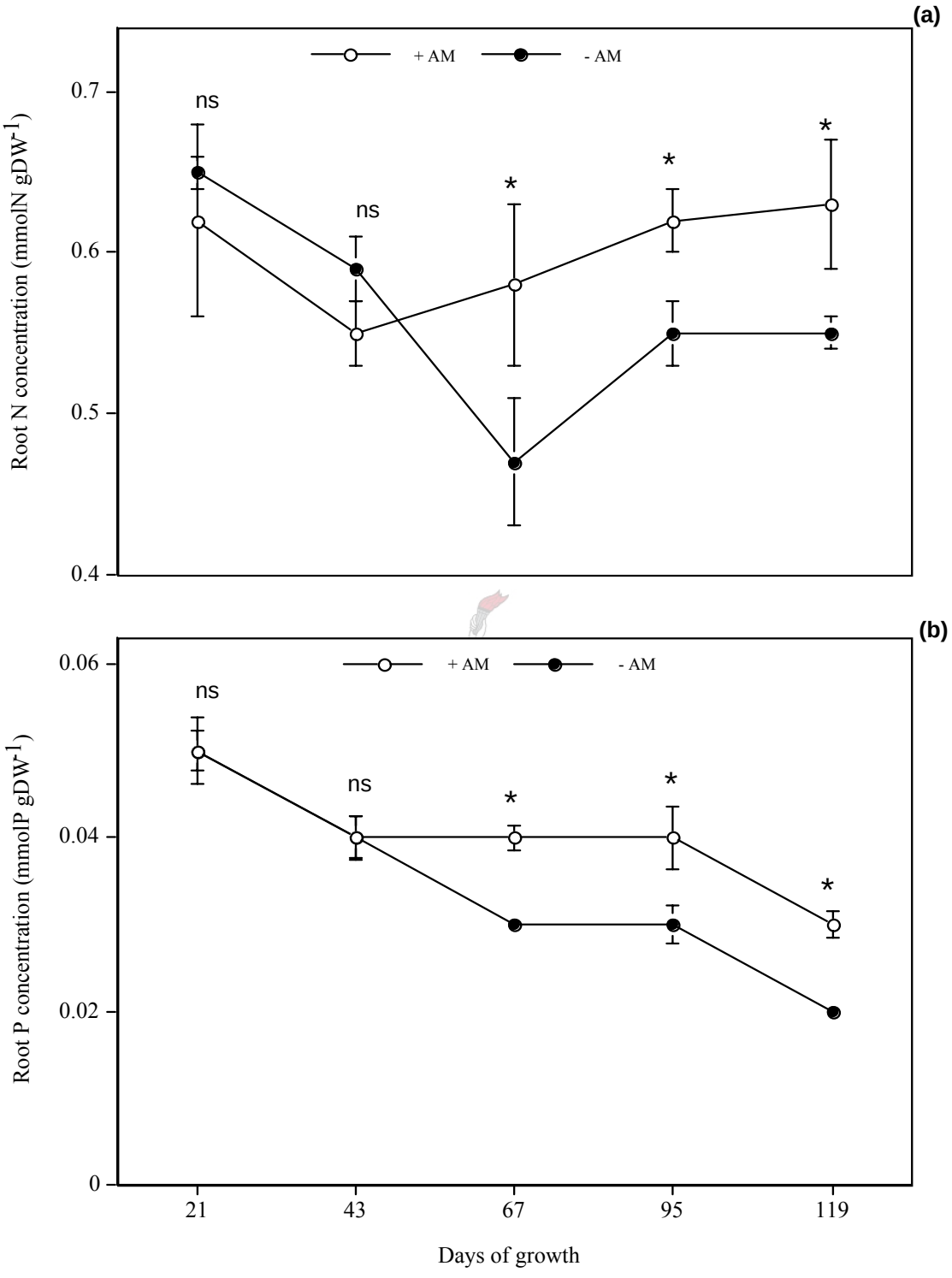
(a)
+ AM
- AM
Root N concentration (mmolN gDW-1)
0.7
0.6
0.5
0.4
ns
ns
*
*
*
(b)
+ AM
- AM
Root P concentration (mmolP gDW-1)
0.06
0.04
0.02
0
ns
ns
*
*
*
21
43
67
95
119
Days of growth

Figure 2

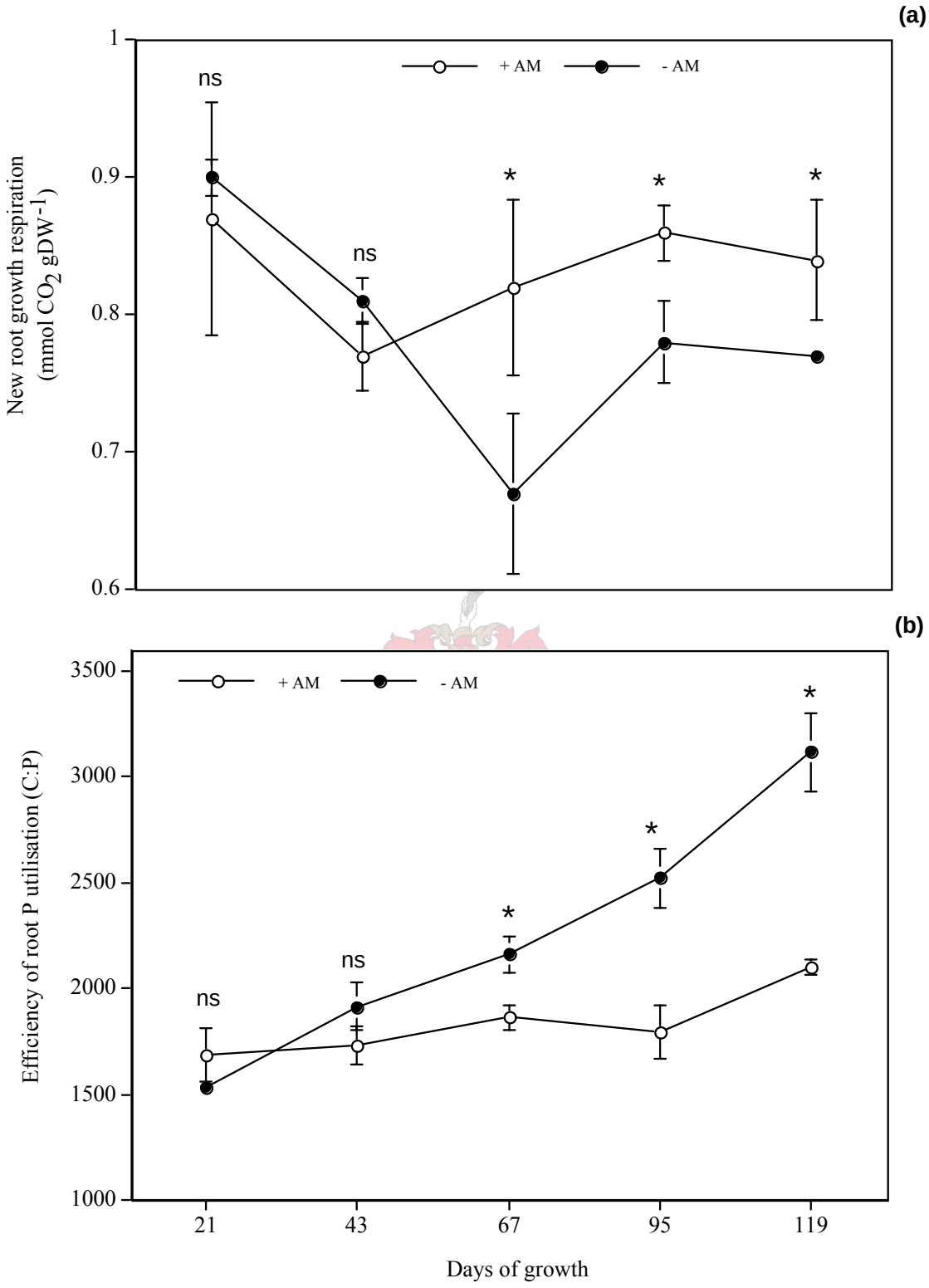
(a)
+ AM
- AM
New root growth respiration (mmol CO_2 gDW^{-1})
1
0.9
0.8
0.7
0.6
ns
ns
*
*
*
(b)
+ AM
- AM
Efficiency of root P utilisation (C:P)
3500
3000
2500
2000
1500
1000
ns
ns
*
*
*
21
43
67
95
119
Days of growth

Figure 3

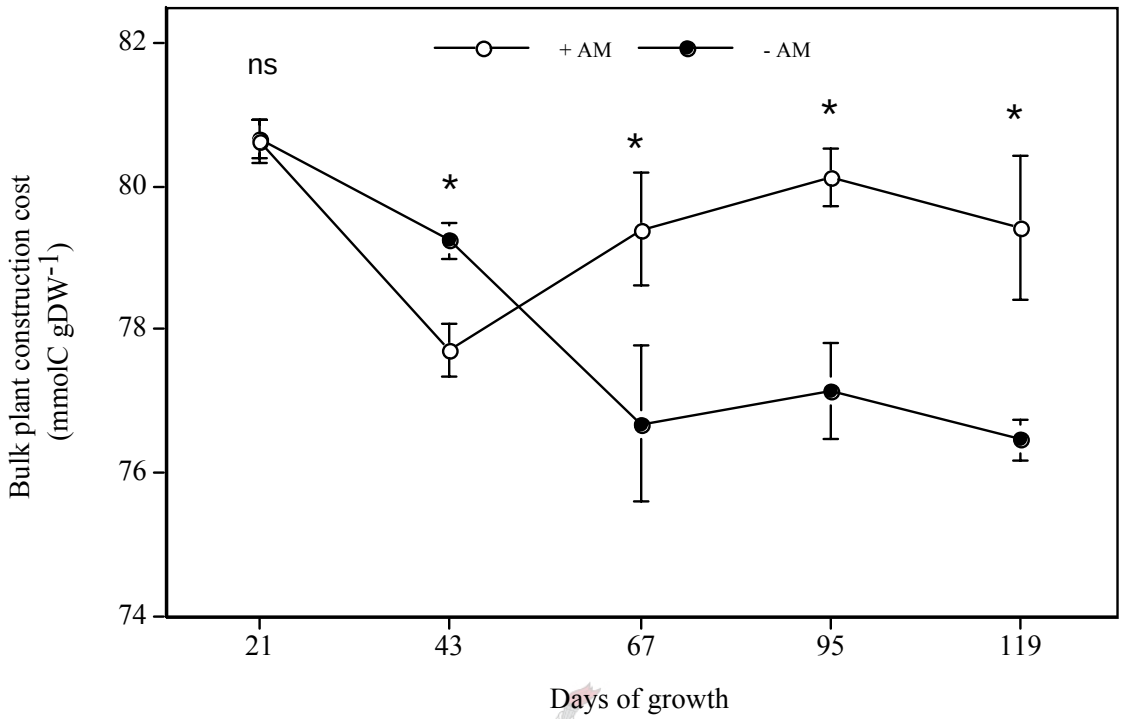

Figure 4

Chapter 4

Title: **The effect of mycorrhizal developmental stages on host growth**

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Chapter 4

Title: The influence of mycorrhizal developmental stages on host growth

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ABSTRACT

The effects of different stages of arbuscular mycorrhizae (AM) development on the growth of grapevines were investigated. The different growth stages of the fungus use varying amounts of host carbohydrates for AM fungal development and nutrient acquisition and would compete with host growth for available C. One-year old grapevines, colonised with *Glomus etunicatum* (Becker and Gerdemann), were cultivated under glasshouse conditions. The growth and nutrient utilisation rates of the grapevines were sequentially analysed. The residual effects of the AM fungal C drain during the rapid phase of colonisation decreased host root and shoot growth at days 67 and 95. Subsequently, by day 119 root and shoot development increased to levels higher than

those of the non-AM plants. Higher root P levels were correlated with lower initial root growth. In AM plants, this lower root growth (67 and 95 days) resulted in lower shoot N utilisation rates in the AM plants. Although, the C costs of the AM fungal development may have initially impacted negatively on root growth and shoot development, the fully established AM symbiosis improved host growth to a greater extent than in non-AM plants.

Key words**:** mycorrhizal development, root growth, N and P utilization, shoot development

Introduction

The colonisation of grapevine roots by an arbuscular mycorrhizal (AM) fungus can enhance the nutritional status of the vine, especially under low soil P conditions (Nikolaou *et al,.* 2002). Schreiner (2003) demonstrated that vines with high levels of AM colonisation had greater fine root density and increased above ground vigor. However, the extent with which the vines benefit from the symbiosis is affected by the species of AM fungi, the soil conditions and the rootstock cultivar (Schubert *et al,.* 1988; Linderman and Davis 2001; Schreiner 2003).

Vines rely on stored carbohydrates for the growth of new shoot and root material (Buttrose 1966; Crossen 1997). At the onset of growth carbohydrate reserves are remobilized and used to sustain growth until the plant is photosynthetically active (Buttrose 1966; Crossen 1997; McArtney and Ferree 1998). Buttrose (1966) found that it takes four to five weeks of shoot growth before there is any nett gain in vine dry weight. The carbohydrate reserves are stored mainly in the stem of the young vines but as the vine matures the roots also become a major storage component of carbohydrates (McArtney and Ferree 1998).

Therefore the development of the AM symbiosis will compete with the growth of new plant tissues for carbohydrates and can result in plant growth depression (Peng *et al,.* 1993). Despite the possible growth depression, vines show improved growth and vigor when colonised by an AM fungus (Linderman and Davis 2001; Motosugi *et al,.* 2002).

However, these studies did not look at the early effect that the symbiosis had on the vines. Therefore we aim to evaluate the effects that the AM fungus *Glomus etunicatum* (Becker and Gerdemann) has on the growth of grapevines, through the early developmental stages of both the AM fungus and the vines.

Materials and Methods

Plant growth and AM inoculation

One-year old grafted grapevines (*Vitis vinifera* L. cv. Pinotage, grafted onto Richter 99 rootstock) were planted in 20 litre pots containing sterilized river sand, between May and August 2002. The pots were placed in a north-facing glasshouse at the University of Stellenbosch, Stellenbosch, South Africa. The maximum daily photosynthetically active irradiance was between 600 and 700 µmol m^{-2} s^{-1} and the average day/night temperatures and humidities were 23/15°C and 35/75% respectively. The plants were watered with distilled H_2O and every second week received Long Ashton nutrient solution, modified to contain 100µM phosphate and pH 6. The experimental plants were inoculated with *Glomus etunicatum* (Becker and Gerdemann) (accession number J100092, Moss Herbarium, University of the Witwatersrand) and the control plants received a filtered inoculum solution. This solution was prepared by filtering the inoculum through a 37 µm mesh, which removed all fungal material.

Growth data and nutrient analysis

The vines were pruned back to one-bud and allowed to grow until the respective harvests, which took place after 21, 43, 67, 95 and 119 days. Every second day shoot length and

leaf expansion was recorded by measuring the maximum width and longitudinal axis of the leaf. Measurements were made on the 4th leaf from the apex. Upon harvesting the plants were separated into different components, which consisted of new shoot tissue, woody scion tissue, stem and roots (new and old roots) and the fresh weight of each component was recorded. Sub-samples of root segments were stored in 50% ethanol in order to determine percentage AM fungal colonisation at a later stage. The harvested material was then placed in an oven, at 80 °C, for two days and dry weights were recorded. The dried plant material was milled using a 0.5 mm mesh (Arthur H Thomas, California, USA). A bulk sample was made up in addition to the existing components by combining sub-samples of each component proportionally to the percentage that each component made to the whole plant. The milled samples were analyzed for their respective N and P concentrations, by a commercial laboratory, using inductively coupled mass spectrometry (ICP-MS) and a LECO-nitrogen analyzer with suitable standards (BemLab, De Beers Rd, Somerset West, South Africa).

Determination of percentage AM colonisation

Roots were harvested at 3-week intervals. Non-woody roots were cut into 1 cm segments and rinsed and cleared with 10% KOH for 5 minutes in an autoclave at 110^{O}C under steam pressure of 200 kPa. The KOH was rinsed off and the segments acidified with 2 N HCl for 10 min. Thereafter the roots were stained with 0.05% (w/v) analine blue for 10 min in an autoclave at 110^{O}C under steam pressure of 200 kPa and then destained in lactic acid overnight. Root segments were placed on slides and the colonisation components were determined according to Brundrett *et al*. 1994.

Growth, efficiency, utilisation and shoot extension costs

Shoot growth rate (mm d^{-1}): The daily increase in shoot length, determined by dividing the change in length by the change in days. (1)

Leaf area (mm^2): This is the average leaf area, calculated by multiplying the maximum width and longitudinal axis of the leaf, as described by Montero *et al,*. (2000). (2)

Leaf expansion rate (mm^2 d^{-1}): The daily increase in leaf area, calculated by dividing the change in leaf area by the change in days. (3)

Nutrient utilisation rate (mmol mm^{-1} d^{-1}): Determines the daily incorporation of a respective nutrient in 1 mm of growth. This is calculated by dividing the nutrient use efficiency by the change in days. (4)

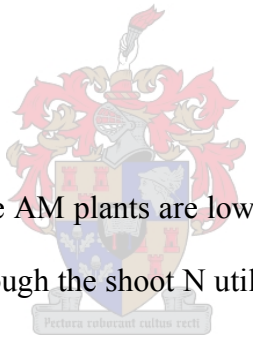
Statistical analysis

The differences between harvests, for percentage AM colonisation, were separated using a *post hoc* Student Newman Kuels (SNK), multiple comparison test ($p \leq 0.05$) (SuperAnova). Different letters indicate significant differences between treatments. The percentage data were arcsine transformed (Zar, 1999). For each harvest, the difference between the means of AM and non-AM plants, was separated using a Student's T-test ("Statistica 6.0", StatSoft Tulsa, OK, USA) for independent samples by groups ($p \leq 0.05$). Different letters indicate significant differences between treatments.

Results

Roots

The percentage AM fungal colonisation increased until day 67 and then leveled off, reaching a maximum of 89% root colonisation (Figure 1a). The AM colonisation per gram new root fresh weight was at a maximum at day 21, after which it declined until day 95 before leveling off (Figure 1b). The fresh weight of the AM colonised new roots was initially lower than the non-AM plants, but subsequently increased to greater levels than the non-AM plants by day 119 (Figure 2a). There was a correlation between root FW and root P concentration, with a more negative slope in the non-AM plants compared to AM plants (Figure 2b).



Shoots

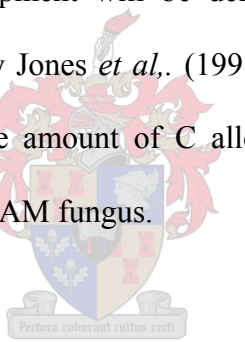
The shoot P utilisation rates for the AM plants are lower over the periods of 21 to 43 and 67 to 95 days (Figure 3a) and although the shoot N utilisation rates are initially higher for the AM plants they are lower over the period of 43 to 95 days (Figure 3b). The shoot:bulk ratios are lower for the AM plants over the period of 67 to 95 days, but by day 119 the AM plants had increased their shoot:bulk ratios to levels higher than that of the non-AM plants (Figure 4a). The AM vines also had lower shoot growth rates over the period of 43 to 67 days (Figure 4b). The leaf expansion rates (figure 5a) and the average leaf areas (Figure 5b) of the AM and non-AM plants did not differ for the duration of the experiment.

Discussion

The pattern of AM colonisation is in accordance with Smith and Read (1997), who described AM colonisation occurring in three stages, which are: the lag phase, phase of rapid colonisation and plateau phase. The latter phase commences during the period of 67 to 119 days. The decline in the amount of colonisation per gram new root fresh weight is due to the rise in new root growth in the AM vines over time. This may be due to root production exceeding fungal colonisation of the host. The effect of this lower AM colonisation as the root production increases, is the negative relationship between root fresh weight and root P concentration. This occurrence of higher root P concentrations with lower root fresh weights is due to the higher AM colonisation at the lower root growths. AM hyphae are more efficient than roots at acquiring P from the medium (Smith and Read 1997) and therefore a negative relationship would ensue as new root development outpaces the AM colonisation.

The less negative relationship between root fresh weight and root P in AM roots than the non-AM roots, may be due to AM hyphae assisting the new roots in P uptake. The effects of the lower new root growth may also have resulted in the lower shoot N utilization rate in AM vines. Since AM hyphae aids in P acquisition to a greater extent than N (Marschner and Dell 1994; Smith and Read 1997), the lower root growth would have affected the shoot N utilization rate more than the shoot P utilization rate.

AM growth can impose a considerable C drain on host reserves (Snellgrove *et al* 1982; Koch and Johnson 1984; Kucey and Paul 1982; Jackobsen and Rosendahl 1990), which was the likely cause of the decline of new root growth at days 67 and 95. The lower root growth of the AM plants at 67 and 95 days may be the residual effect of the AM fungal C drain during the stage of rapid development. Wright *et al,.* (1998) reported that the C drain imposed on photosynthesis by the AM fungus increases as percentage colonisation increases and then levels off. Furthermore Jones *et al,.* (1991) found a similar pattern of C allocation to AM roots at about 60 days. Although the AM fungus has already entered the plateau phase at day 67, it is possible that the host response to a change in the AM sink strength during fungal development will be delayed. This has been previously confirmed by the results reported by Jones *et al,.* (1991) and Mortimer *et al,.* (2004, *in press*) that there is a change in the amount of C allocated below ground during the different developmental stages of an AM fungus.



Furthermore, the AM sink effect on host growth is also evident in the decline of shoot investment, in terms of fresh weight, relative to the whole plant for the growth at days 67 and 95. This is accordance with Peng *et al,.* (1993) who reported a growth depression in mycorrhizal plants after 52 and 92 days. However, by day 119 this investment increased above that of the non-AM plants. Greater AM shoot development has been reported by a number of studies in the past (Estrada-Luna *et al,.* 2000; Linderman and Davis 2001; Nikolaou *et al,.* 2002). However, the AM sink effects on shoot growth were not apparent in the leaf expansion and leaf areas. The similar leaf expansion and leaf areas between the AM and non-AM vines are in contrast with past work describing greater leaf areas and

leaf expansion for AM colonised plants (Estrada-Luna *et al,*. 2000; Bray *et al,*. 2003; Nikolaou *et al,*. 2003).

The C costs of the AM fungal phase of rapid development can be seen as negative to root growth and shoot development. These negative effects may continue for a period of time, even during the plateau phase of fungal development. Once the AM symbiosis is fully established, the host growth and development is then improved to a greater extent than in non-AM plants.

Acknowledgements

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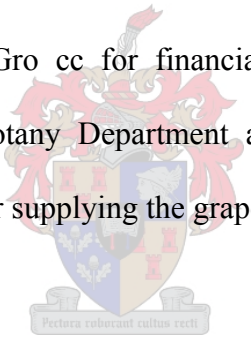

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Figure legends

Figure 1. The percentage arbuscular mycorrhizal (AM) colonisation (a) and the AM colonisation per g root FW (b) of grapevines colonised with *Glomus etunicatum.* Grapevines were grown under glasshouse conditions and harvested at 21, 43, 67, 95 and 119 days. Significant differences ($p<0.05$) between inoculated and uninoculated plants, for each harvest, are indicated by different letters or astricts (*) and ns represents non-significance. (n = 6)

Figure 2. The new root fresh weights (g) (a) and the correlation between the root P concentrations and new root fresh weights (b) of grapevines colonised with *Glomus etunicatum.* Grapevines were grown in glasshouse conditions and harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n = 6) and uninoculated (-AM) (n = 6) plants were determined by T-test for independent samples by groups. Significant differences ($p<0.05$) between inoculated and uninoculated plants, for each harvest, are indicated by astricts (*) and ns represents non-significance.

Figure 3. The shoot P utilisation rate (mmolP mm^{-1} d^{-1}) (a) and the shoot N utilisation rate (mmolN mm^{-1} d^{-1}) (b) of grapevines colonised with *Glomus etunicatum.* Grapevines were grown in glasshouse conditions and harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n = 6) and uninoculated (-AM) (n = 6) plants were determined by T-test for independent samples by groups. Significant differences ($p<0.05$) between inoculated and uninoculated plants, for each harvest, are indicated by astricts (*) and ns represents non-significance.

Figure 4 . The shoot:bulk ratio (a) and the shoot growth rate (mm d^{-1}) (b) of grapevines colonised with *Glomus etunicatum.* Grapevines were grown in glasshouse conditions and harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n = 6) and uninoculated (-AM) (n = 6) plants were determined by T-test for independent samples by groups. Significant differences ($p<0.05$) between inoculated and uninoculated plants, for each harvest, are indicated by astricts (*) and ns represents non-significance.

Figure 5. The leaf expansion rate (mm^2 d^{-1}) (a) and average leaf area (mm^2) (b) of grapevines colonised with *Glomus etunicatum.* Grapevines were grown in glasshouse conditions and harvested at 21, 43, 67, 95 and 119 days. Differences between inoculated (+AM) (n = 6) and uninoculated (-AM) (n = 6) plants were determined by T-test for independent samples by groups. Significant differences ($p<0.05$) between inoculated and uninoculated plants, for each harvest, are indicated by astricts (*) and ns represents non-significance.

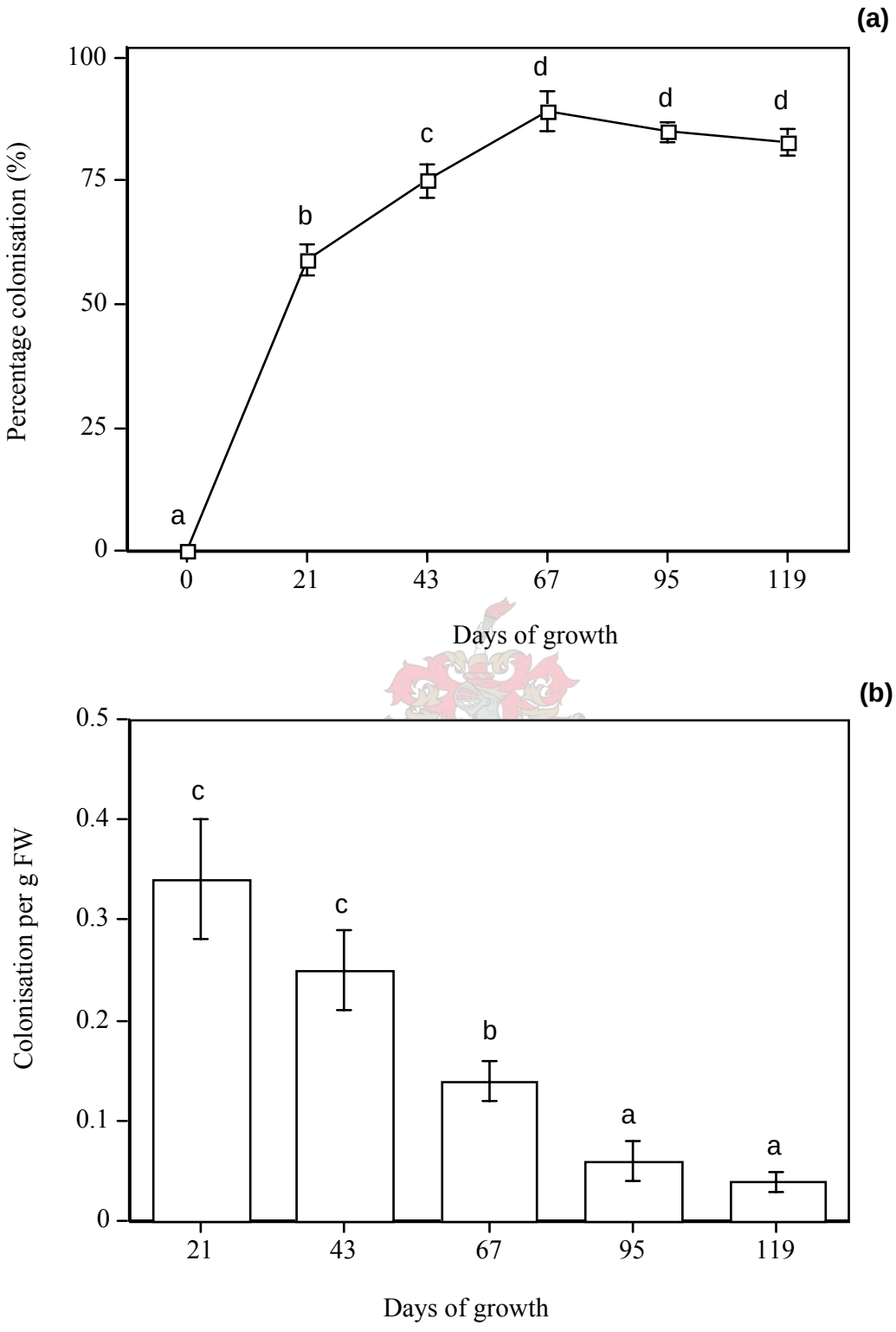

(a)
Percentage colonisation (%)
100
75
50
25
0
a
b
c
d
d
d
0
21
43
67
95
119
Days of growth
(b)
Colonisation per g FW
0.5
0.4
0.3
0.2
0.1
0
c
c
b
a
a
21
43
67
95
119
Days of growth

Figure 1

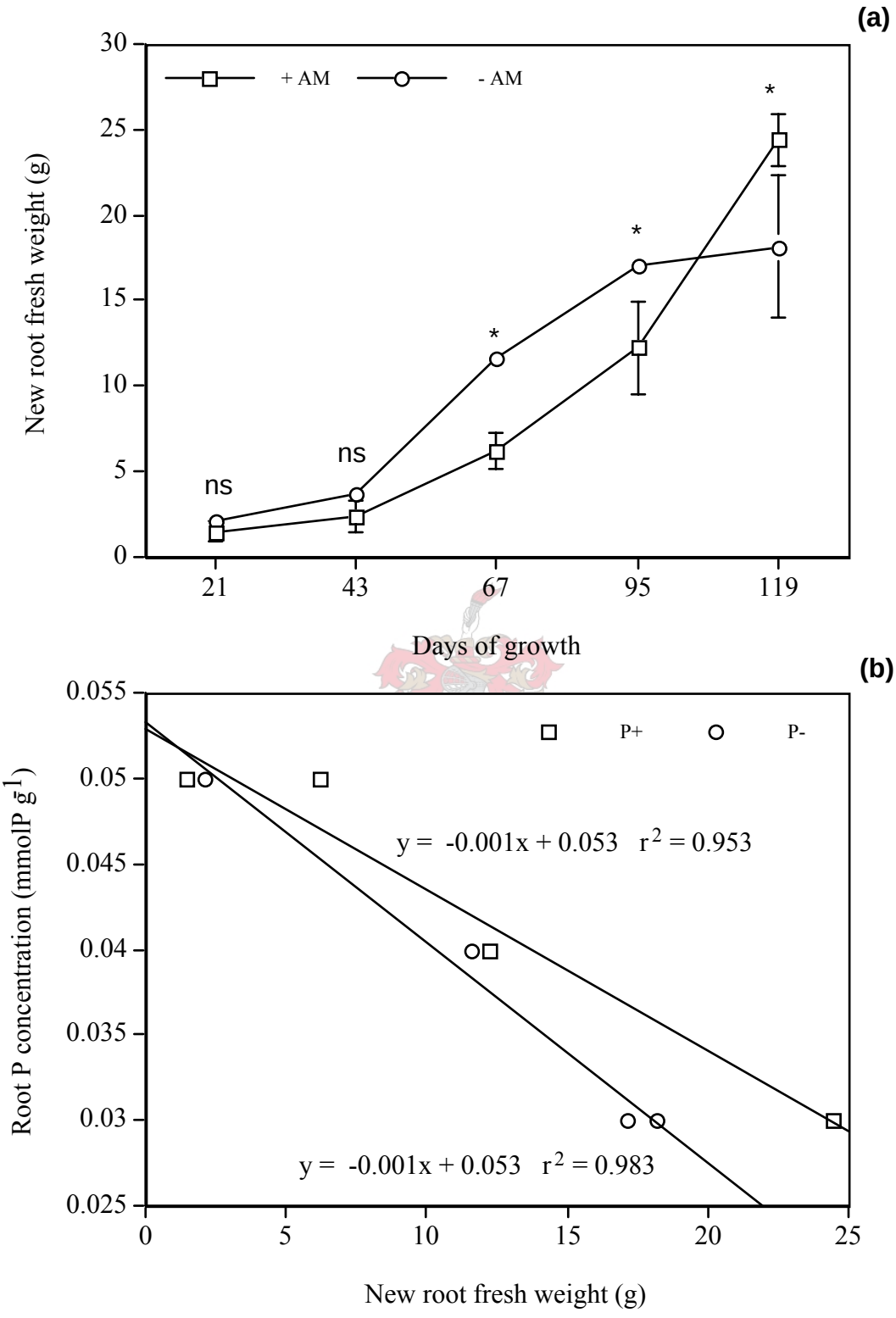

(a)
+ AM
- AM
New root fresh weight (g)
30
25
20
15
10
5
0
ns
ns
*
*
*
21
43
67
95
119
Days of growth
(b)
P+
P-
Root P concentration (mmolP g-1)
0.055
0.05
0.045
0.04
0.035
0.03
0.025
y = -0.001x + 0.053 r2 = 0.953
y = -0.001x + 0.053 r2 = 0.983
0
5
10
15
20
25
New root fresh weight (g)

Figure 2

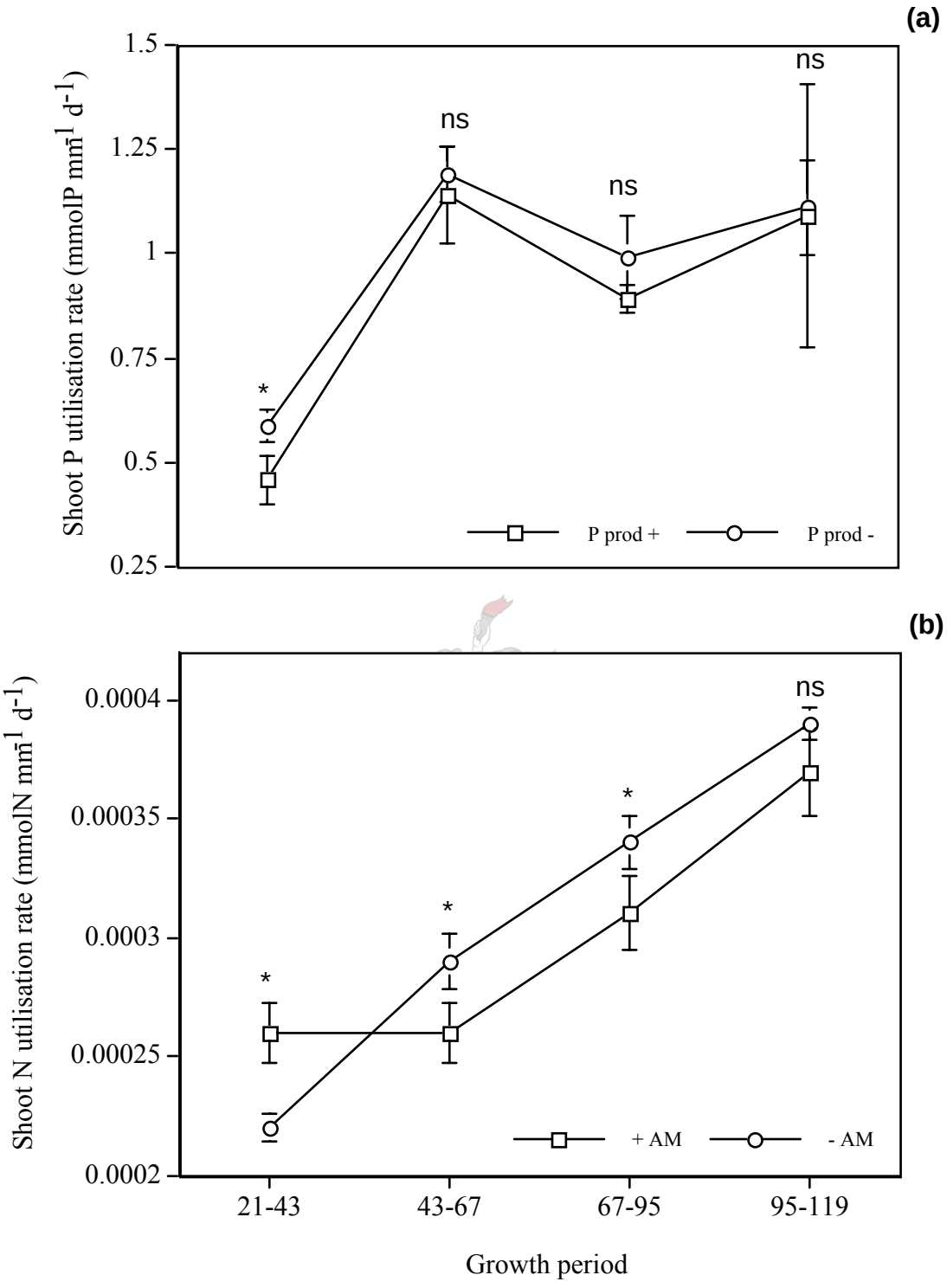

(a)
Shoot P utilisation rate (mmolP mm^{-1} d^{-1})
1.5
1.25
1
0.75
0.5
0.25
ns
ns
ns
*
P prod +
P prod -
(b)
Shoot N utilisation rate (mmolN mm^{-1} d^{-1})
0.0004
0.00035
0.0003
0.00025
0.0002
ns
*
*
*
+ AM
- AM
21-43
43-67
67-95
95-119
Growth period

Figure 3

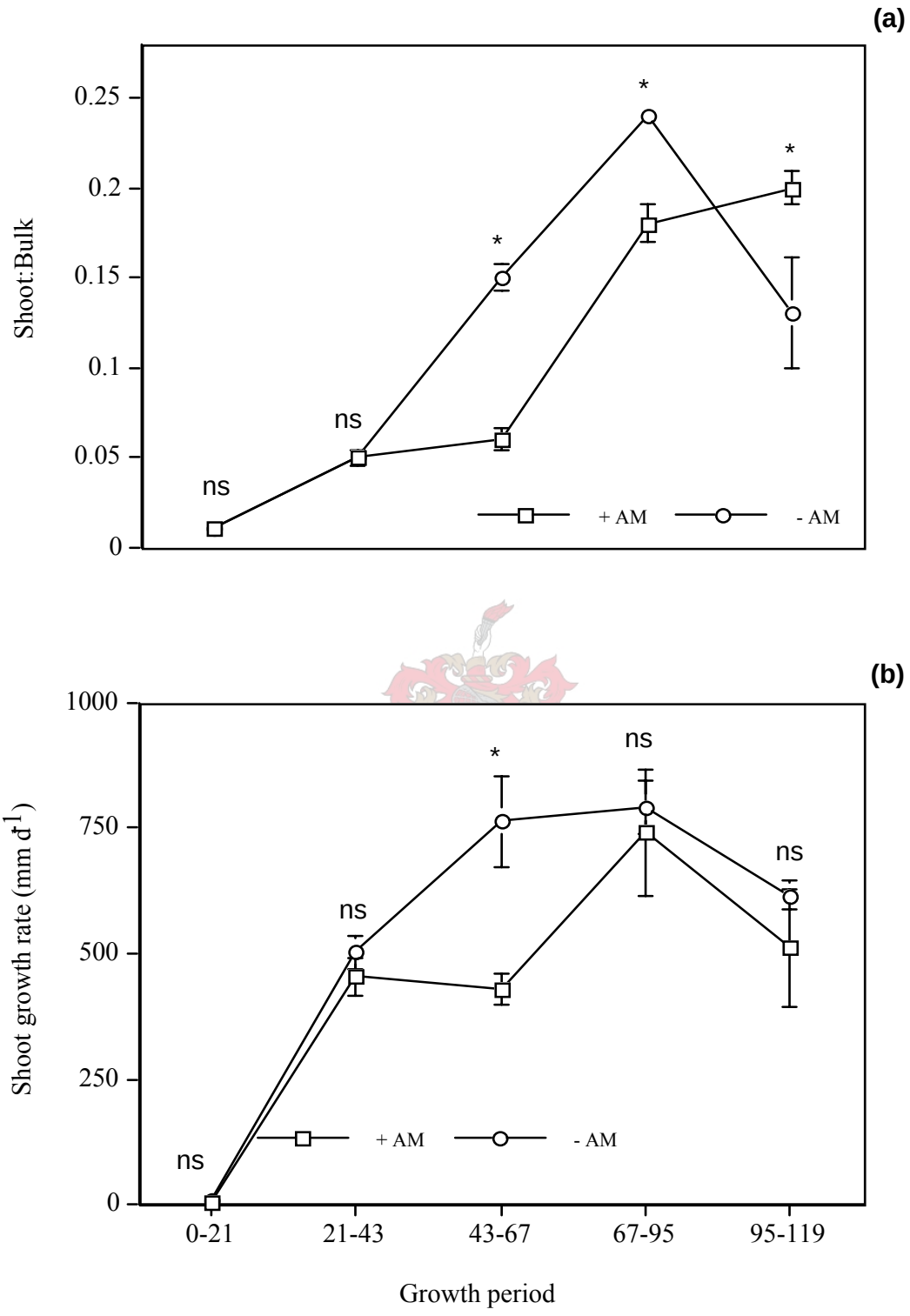

(a)
Shoot:Bulk
0.25
0.2
0.15
0.1
0.05
0
ns
ns
*
*
*
+ AM
- AM
(b)
Shoot growth rate (mm d-1)
1000
750
500
250
0
ns
ns
*
ns
ns
+ AM
- AM
0-21
21-43
43-67
67-95
95-119
Growth period

Figure 4

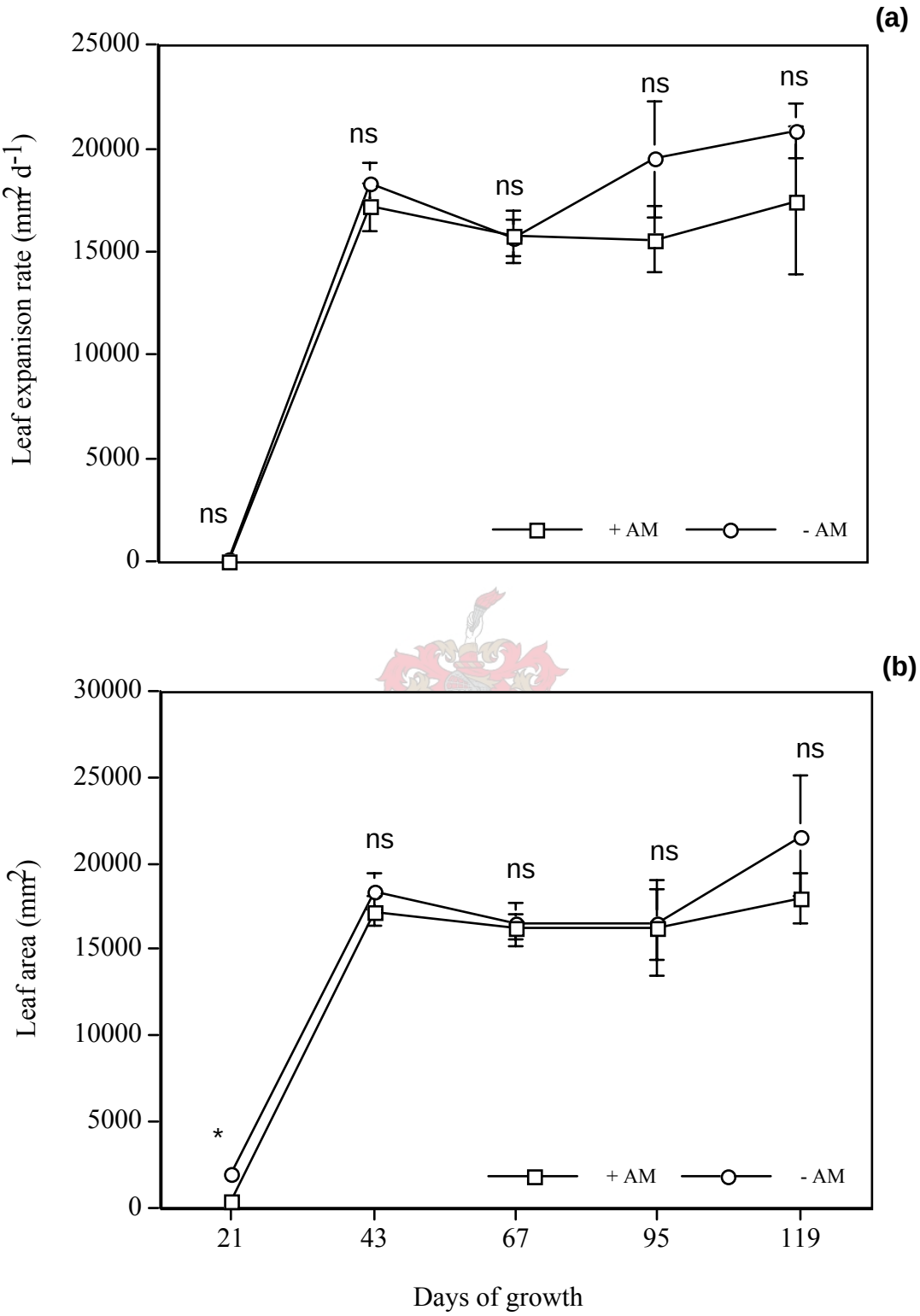

(a)
Leaf expanison rate (mm² d⁻¹)
25000
20000
15000
10000
5000
0
ns
ns
ns
ns
ns
+ AM
- AM
(b)
Leaf area (mm²)
30000
25000
20000
15000
10000
5000
0
*
ns
ns
ns
ns
+ AM
- AM
21
43
67
95
119
Days of growth

Figure 5

Chapter 5

General Discussion and Future Prospects

5.1 General Discussion

This study has shown that during the initial stages of colonisation AM acts as a C sink, competing with the host for available C. Past work on the AM sink effect has focused mainly on the movement of photosynthetic C below ground to support the AM fungus (Snellgrove *et al.,* 1982; Kucey and Paul 1982; Koch and Johnson 1984; Jakobsen and Rosendahl 1990; Black *et al,.* 2000). This however, does not take into account the effect that stored C will have on the C economy of the plant and symbiosis. The role of stored C becomes even more crucial when working with deciduous plants that rely on stored C for new growth at start of a growing season. Merryweather and Fitter (1995) reported that stored C in AM plants is remobilized at the start of a growing season and then the C reserves are refilled towards the end of the season, when the plants enter dormancy.

In Chapter 3 the results indicated that the C costs of mycorrhizal development were borne primarily by above ground C, stored in the stem. This was confirmed in Chapter 4 by the lower initial shoot and root growth of the AM colonised plants. However, this growth depression did not last and by the end of the experiment the root and shoot growth of the AM plants was higher than that of the non-AM plants. AM induced growth depression has been reported for AM colonised plants and can be attributed to the AM fungal C drain, but usually occurs when the plants are grown under high P conditions (Son and Smith 1988; Peng *et al*,. 1993).

The higher new root growth and shoot development of AM colonised vines at day 119 (Chapter 4) occurred in spite of the initial negative effects on host growth and the sink effect that the AM fungus imposes on the host. These benefits became apparent when the symbiosis was fully established and is consistent with past work on the benefits of the AM symbiosis (Estrada-Luna *et al,*. 2000; Linderman and Davis 2001; Nikolaou *et al,*. 2002). The benefits of the symbiosis can be attributed to improved P nutrition of the AM vines (Sanders and Tinker 1971; Smith 1982; Bolan 1991 and Orcutt and Nilsen 2000). However, by day 119 only the AM roots had higher P concentrations and not any of the other plant components. This may be because more time was needed before the full effect of the symbiotic benefits become apparent.

Once the plateau phase of fungal development had been reached the plant began to refill its C reserves that were depleted during the early stages of plant and AM fungal growth (Chapter3). This explains the residual effects of the C drain which were still apparent during the AM fungal plateau phase and resulted in decreased root growth and shoot development (Chapter 4). Merryweather and Fitter (1995) reported that in spite of the majority of plant C reserve refilling taking place towards the end of the growing, a small percentage occurs earlier on in the growing season.

The results obtained in this study revolve around the C sink of the AM fungus and the subsequent effects on plant growth and nutrition. However, the key processes involved in the sink effect imposed by the fungus still remain to be elucidated. Muller *et al,*. (1999)

proposed the theory that the ability of trehalose to induce the expression of SuSy (mimic sucrose) might be a means of the AM inducing a sink in the tissue surrounding it. Ravnskov *et al,.* (2003) reported that AM colonisation induced the expression of SuSy in host roots to upregulate the amount of sucrose supplied to colonised, especially during the early stages of AM colonisation. The enhanced SuSy expression was apparent before any other mycorrhizal effects could be detected. Blazquez *et al,.* (1993) found that trehalose-6-phoshpate inhibits hexokinase activity, thus regulating the influx of glucose into glycolysis, creating a higher substrate availability for the production of trehalose, further strengthening the sink effect of the fungus.

5.2 Future Prospects

Proposed future project: Investigate the changes in the concentrations of the key metabolites and the activities of the key enzymes involved in trehalose synthesis during the establishment of the AM symbiosis.

5.2.1 Project outline

5.2.1.1 New root experiment

A time course experiment, determining metabolite changes and enzyme activities during predetermined time intervals.

- % AM
- Pi content and concentrations in new root tissue
- GR and RGR of new roots

- Change in the following metabolite concentrations over time: Trehalose, sucrose, glucose, fructose and UDP-glucose
- Activities of the following enzymes over time: SuSy, invertase, Trehalose-6-Phoshphate phosphatase (TPP), Trehalose-6-phosphate synthase (TPS).
- Correlate enzyme activities with metabolite changes
- Correlate metabolite changes with changes in AM infection.

5.2.1.2 High and low P treatment

Experimental and control plants grown at high and low P to determine if P levels will influence the metabolite changes. Determine if the sink strength is the same under non-stressful conditions (high P).

- % AM infection
- Pi levels in new root tissue
- Below ground sink strength

5.2.2 Below ground sink strength and proposed models for assessing C-fluxes in grapevines

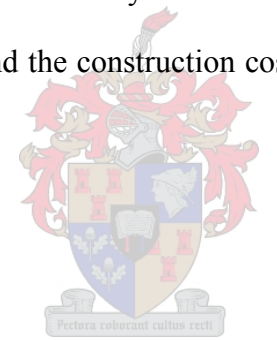
There is increased allocation of C to mycorrhizal roots due to the sink effect of the fungus. Below is a proposed model to determine the strength of this sink effect, taking into account storage tissues (stem and old roots) as well as photosynthetic tissue (shoots):

$C_{ss} = ((\text{shoot C}/\text{stem C}) \times (rt_n/rt_o))/C_{tot}$

Where C_{ss} is the below ground C sink strength (mmolC/g DW), *shoot C* is the C concentration of the new shoot tissue (mmolC/gDW) and *stem C* is the C concentration of

the woody stem tissue (mmolC/gDW). rt_n and rt_o represent the new and old root tissues respectively (mmolC/gDW) and C_{tot} is the total plant C (mmolC/g DW).

Pearson and Jakobsen (1993) calculated the efficiency of the mycorrhizal relationship as the amount of C used per unit of P transported; below ground C use per unit P transported; P transported per meter of hyphae and hyphal C use per meter of hyphae. These are all different ways of expressing the relationship between C and P and are useful when investigating the C/P exchange or the contribution that the hyphae make to P uptake. With the aid of the models proposed by Koide and Elliott (1989), Jones *et al,*. (1991), Williams *et al,*. (1987), Pearson and Jakobsen (1993) and the proposed sink strength model, the efficiency of the mycorrhizal relationship; the movement and allocation of new and stored C and the construction cost of different plant tissues can be accurately determined.



5.3 References

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