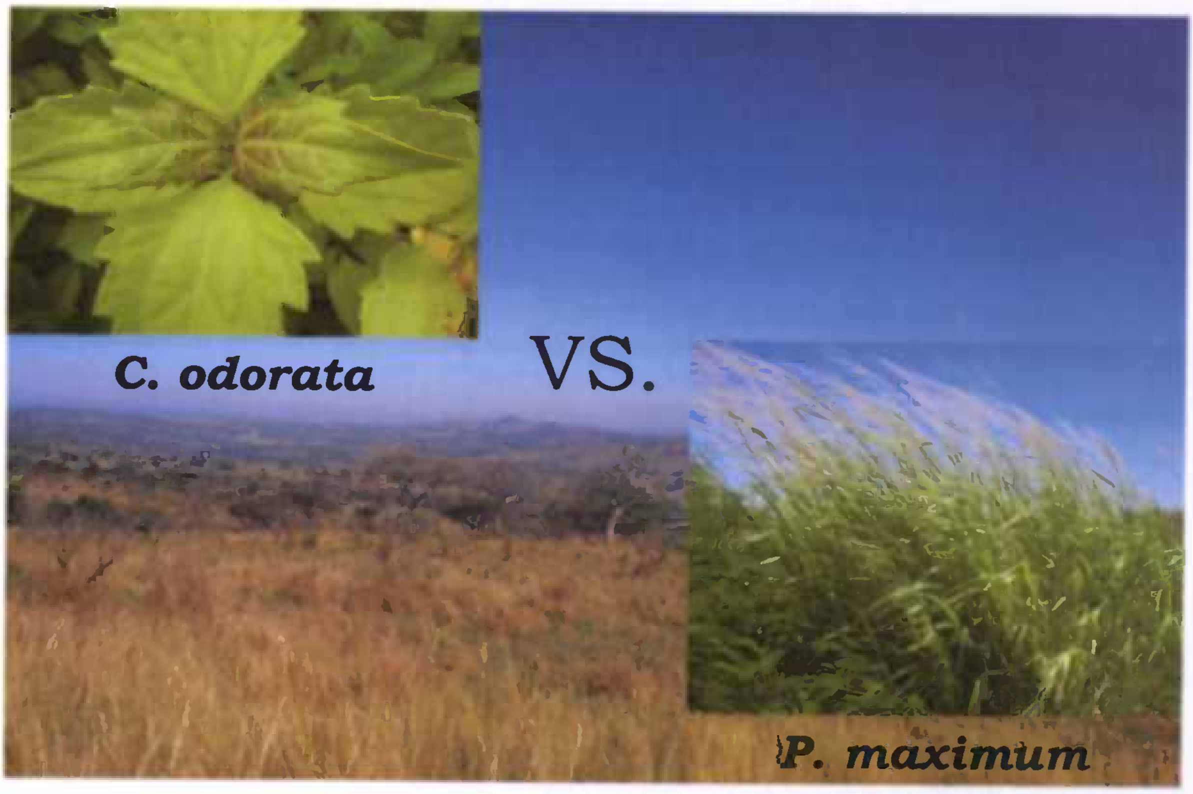
Effects of water availability on the competition of *Chromolaena odorata* and *Panicum maximum*.



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Abstract

Invasions of species form a major threat to biodiversity. *Chromolaena odorata* is one of these invasive species, native from Central America, but has invaded most tropical and subtropical area's around the world. In Africa it forms a major problem to ecosystems, lowering biodiversity and blocking the access to water.

First it mainly occupies river banks and the edges of woodlands but then slowly invades into the savannas. While invading it's competing with a native grass species, *Panicum maximum*. Field observations showed establishment of *C. odorata* if rainfall was high. At low rainfall it stayed restricted to the riverbanks and woodlands.

In this study we examine whether the successful spread and invasive capability of *C. odorata* in savannas depends on the water availability. A competition experiment was set up in the greenhouse in a complete randomized block design. Seeds were used of *C. odorata* of both Puerto Rico and South Africa to determine any differences between the native and invasive populations and seeds of *P. maximum* from South Africa. They had to compete in both an additive and a replacement design with low and high water availability. Biomass production was compared between mono and mixed cultures and Relative Crowding Coefficience (RCC) calculated to determine the competitive advantages and aggressiveness of each species.

In this experiment *P. maximum* has an advantage at both high and low water availability, it is more aggressive and out competes *C. odorata*. It has a better developed roots system, can extract more water and uses it more efficiently compared with *C. odorata*. *C. odorata* meanwhile invests more energy in leaves, resulting in a higher Specific Leaf Area (SLA), Leaf Area Ratio (LAR) and Leaf Weigh Ratio (LWR). This would indicate *C. odorata* is the superior competitor for light in the long term. A possible explanation for the fact that *C. odorata* is out competed by *P. maximum* could be the seed mass. Bigger/heavier seeds result in bigger/heavier seedlings. Although *P. maximum* doesn't have heavier seeds, it has significantly heavier seedlings.

Although *C. odorata* has a higher LAR and presumably a higher Relative Growth Rate, it needs time to make up for the disadvantage of lower seedling mass. In the experiment *P. maximum* remains taller and manages to over shade *C. odorata* during the entire experiment. To have a successful establishment a disturbance, like fire or herbivory, might be necessary during seedling stage, to take away the competition and give *C. odorata* the opportunity to outgrow *P. maximum*. In the field they grow under canopy, meaning a light limitation for *P. maximum* and less nutrients available (during the experiment we added enough nutrients to prevent limitation). Growth of *P. maximum* is highly dependable on nutrients and will be limited in the field, resulting in a decrease in length. The LAR, LWR and SLA of *C. odorata* decreases at low water availability, which means to succeed in establishment it needs to have high rainfall next to a disturbance as well.

Contents

Introduction

Invasion of species form a major threat towards national parks and conservation area's everywhere around the world. Biological invasions are a natural phenomena and have been occurring as long as species exist, but in the last few decades the number of invasions has been increasing substantially (Mack *et al.* 2000). This is creating a worldwide problem in conservation.

The increase in invasions is caused by the expanded human travelling in the last few decades. Ever since we started travelling and trading around the world we have been carrying other species with us, sometimes with a purpose in mind, but most of the time unknowing and unwillingly. Especially by ship, the most used invasion pathway (Wonham *et al.* 2005), many invasive species have managed to establish populations in area's they normally never would have reached (Vitousek *et al.* 1996, D' Antonio *et al.* 1992). Not all invasions are successful, most introduced and invaded plant species don't manage to establish themselves. They are out competed by the native species or can't cope with the environmental conditions in the new habitat and will disappear again (Lodge 2003, Richardson *et al.* 2006). But some of the species do manage to establish themselves and grow out to be a pest.
These species cause a lot of damage to ecosystems, and are even considered as the second greatest threat to biodiversity after habitat loss (Mack *et al.* 2000). This has stimulated the research on the effects of invasions around the world, gathering information to control them.

There are a lot of different theories why some of these invaded species become so successful in their new environment. Some focus on the invasibility of the ecosystem, others on the invasive species themselves.
An ecosystem is considered highly invasible when species richness is low (Richardson *et al.* 2006). In this case a lot of niches are available and nutrient availability is high. New introduced species can occupy an available niche immediately. This would, for example, be the case with islands. Islands are considered relatively species poor and have a lot of unoccupied niches. They leave a lot of resources available and together with low resource use efficiency, will it result in a high invasible ecosystem (Denslow 2003).
If an ecosystem has a high species diversity the resources will be used completely, leaving less for the invasive species. These ecosystems will be less invasible (Stachowicz *et al.* 2006).
Although a lot of experiments are consistent with this theory (Denslow 2003, Case 1990), a lot of experiments contradict the theory as well (Stachowicz *et al.* 2006, Robinson *et al.* 1995).

The susceptibility of an ecosystem for invasion also increases if an ecosystem has a lot of disturbances (Hierro *et al.* 2006, Keeley *et al.* 2003). The invasive species might cope better with the changing environmental conditions than the native species (Leishman *et al.* 2005, Daehler 2003).

More theories are known focussing on the species:
One is the escape of natural enemies. This is called the Enemy Release Hypothesis (Keane *et al.* 2002). In the native range the population growth is controlled by natural enemies, e.g. predators, herbivores, parasites and diseases. In the new environment these species may be absent, resulting in an uncontrolled population growth.
A second possible explanation is that some species have evolved into superior competitors.
This might be explained by several theories proposed in the last decades.
One theory is the Evolution of Increased Competitive Ability (EICA) which states that invaded species who escaped their natural enemies (the Enemy Release Hypothesis) don't need to allocate as much energy towards the defence anymore. They can use this energy for growth and reproduction and become more competitive (Rogers *et al.* 2004, Leger *et al.* 2003). Studies in recent years have both supported (Rogers *et al.* 2005) and contradicted (Wilkstrom *et al.* 2006, Lewis *et al.* 2006) this theory.
Another theory is a higher phenotypic plasticity, the same genotype can express several phenotypes depending on which one is most successful in that given environment. If a genotype with high plasticity has an advantage it will spread faster creating a rapid evolutionary change in the population. This would explain why invasive species can react to environmental changes faster and out compete the native ones (Richards *et al.* 2006).
Which theory is correct differs per invasion and might even be a combination of several theories.

After an invasive species established itself it can have tremendous influences on the functions of the ecosystems. It can change the structure and composition of the environment by altering fire regime, nutrient cycling, hydrology and energy budgets in the new ecosystem. The native species will be less adapted to these new environmental conditions, and this will give the invasive species a competitive advantage over the native species (Gordon 1998, D'Antonio *et al.* 1992). The native species will grow and reproduce less successful and may eventually even disappear (Vitousek 1990, Lodge *et al.* 2002).
Next to functional changes, an invading species has another big impact on the biodiversity. While spreading they are competing with the native species for the available resources. Usually light, nutrients or water.
If the invasive species can use the resource more efficient or manages to obtain more of the resource, it will leave less available for the native species.
All this leads to the decline of the establishment, survival and/or reproduction of the native species and eventually in a loss of biodiversity (Firbank *et al.* 1990, Blicker *et al.* 2003).

In this study we are looking at the competition of two plant species, *Chromolaena odorata* and *Panicum maximum. C. odorata* is invading the savannas and competes with *P. maximum*, a native species.

Chromolaena odorata (Asteraceae), also known as Siam weed, is an highly invasive species. It is listed among the top 100 of most invasive species. It's a fast growing perennial shrub forming dense tangled monospecific bushes 1.5-2.0 m in height (McFadyen et al 1996).

It is native in South and central America, but has invaded most of the tropical and subtropical area's around the world. It has invaded Asia, Africa and the Pacific and is still spreading rapidly. Weed scientists in Australia have identified *C. odorata* as the greatest threat to North Australia because of its damaging effects to agriculture and the environment (Michael 1989).
C. odorata was probably introduced into west Africa around the mid 1940s at Durban Harbour. It is thought to have come unintentionally with seed-contaminated packing material and started spreading South and Northwards (Goodall *et al.* 1995).

While invading new area's it became a major weed of crops, plantations, savannas and forests (Goodall *et al* 1995). Especially in protected area's, like the Hluhluwe Umfolozi Park in kwaZulu Natal, is it threatening the biodiversity. It is observed to form such dense woven canopy on riverbanks, large herbivores (except the elephant) can't penetrate it. This way it's blocking the access to the water supply.

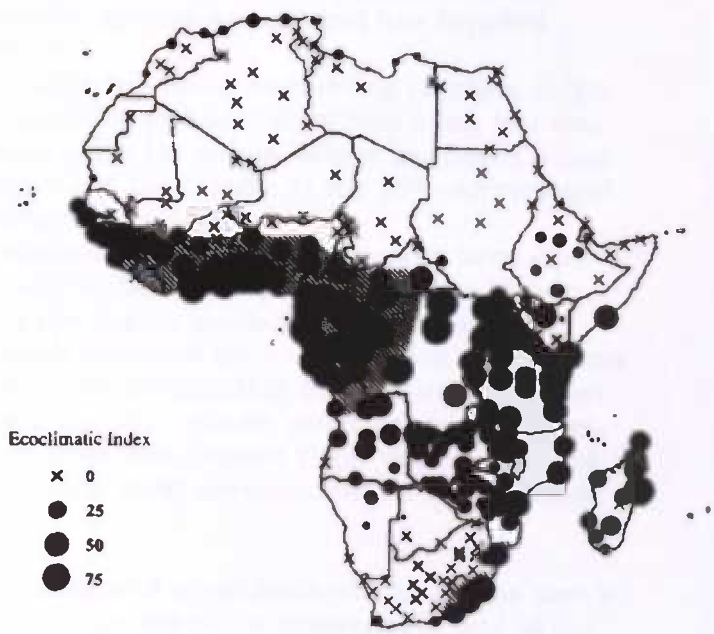


Figure 1: Locations in Africa which are predicted to be suitable for growth of *C. odorata*. Circles are proportional to the suitability of each area. The hatched area is the distribution in 1996. (McFadyen et al 1996)

In 1996 McFadyen created a model to predict the potential spread of *C. odorata* around the world. In figure 1 the potential distribution in Africa is shown. The grey area represents the distribution in 1996 and the circles are proportional to the suitability of each location. It is inhibited by frost (Goodall *et al.* 1995) and (less severely) rainfall but can invade even dry area's by growing on river sides.

Another negative effect of *C. odorata* in an ecosystem is that it's highly flammable. Next to river banks tends *C. odorata* to form stands along forest margins (Witkowski 2001). During a field fire it can carry the fire into the woods, thereby killing forest species.
Between 1990 and 1999 a lot of research has been done on the effects of fire and the invasion of *C. odorata* in subtropical grassland (Goodall *et al.* 2000). It was found that grassland without *C. odorata* infestations has a higher species richness than grasslands with *C. odorata* present. They also found that they could eliminate all existing *C. odorata*, using only fire. 5-7 years of annual burning eliminated all existing *C. odorata* (Goodall *et al.* 2000).

Ambika (2002) examined the influence of environmental factors on the seedling growth of *C. odorata* and found it prefers bright sunlight, high soil moisture and high relative humidity (Ambika 2002). Especially a low level of soil moisture has a limiting effect on the growth (Witkowski *et al* 2001).
Field observations showed *C. odorata* mainly present at the edges of the grassland in coexistence with *Panicum maximum.* While competing it slowly starts invading the grasslands.

P. maximum, also known as guinea grass, is a coarse, perennial grass reaching heights of more than 2 m. It is native in Africa and occurs mainly in close proximity to trees. It is an highly invasive species as well and has invaded South America around the 17th century.
It slowly spread to Barbados and Jamaica travelling as bedding on slave ships. It was initially introduced in many countries as a source of bird seed, but was quickly considered as the best growing grass for animal forage purposes. From Jamaica it reached Central America around the middle of the 19th century and has spread up to the Mississippi (Parsons 1972).
P. maximum is very shade tolerant (Andrade 2004) and lives mostly beneath trees, where more water is available and water stress periods become shorter (Durr *et al.* 2003). A second reason is the higher availability of Nitrogen and Phosphate (Pieterse et al.1997). Beneath the trees an accumulation of nutrients occurs due to higher litter fall and the trees accumulate the nutrient rich dust from the air (Scholes *et al.* 1997). They can also extract nutrients from deeper layers and from farther away from the trees and deposit them underneath the tree (Scholes *et al.* 1997). This makes it the ideal environment for *P. maximum* to grow.

C. odorata is mainly present on riverbanks and woodlands which are the area's with highest soil moisture. Because both species occur underneath and at the edges of woodlands they start competing with each other for the water and nutrients. But field observations showed a slow invasion from riverbank and woodlands into savanna. During dry years *C. odorata* seems to stay restricted to the riverbanks and woodlands. In wet years *C. odorata* manages to stay and establish itself (personal observations M. te Beest and H. Olff). Once this happens, it can maintain through dry seasons as well, out competing *P. maximum.*
In this study we want to determine whether the survival and successful establishment of *C. odorata* seedlings in savannas depends on the water availability. We used seeds of *C. odorata* from two different sources, from Puerto Rico (native) and South Africa (introduced).
These will be competing with *P. maximum* from South Africa (native) and with each other. We want to answer two questions:

- What is the effect of the water availability on the competition of *C. odorata* and *P. maximum?*

- Will *C. odorata* from South Africa out compete *C. odorata* from Puerto Rico through competition for light?

Based on the field observations we expect *P. maximum* to grow better and out compete both introduced and native *C. odorata* at low moisture level.
These will have an advantage and out compete *P. maximum* at high moisture level in return.
We expect for the second question *C. odorata* from South Africa to out compete *C. odorata* from Puerto Rico at both high and low water availability. This is based on a previous study, the plant height of *C. odorata* from South Africa seems to increase faster and we expect it to over shade and out compete *C. odorata* from Puerto Rico.

Methods

1 Pilot studies

To determine the water availabilities we had to use in the experiment we performed two pilot studies. Each containing of a series of 10 different water availabilities, in triple, leading to 30 pots in total.
The water availabilities are between 10% and 70%, with 5-10% intervals. At 70% the soil was water logged.
The water availability is determined gravimetric and calculated with the following formula:

Water availability = gr of water/gr of dry soil * 100 %

Pot	water percentage
1	10%
2	15%
3	20%
4	25%
5	30%
6	35%
7	50%
8	60%
9	65%
10	70%(Water logged)

Table 1: The water availabilities.

The same soil as in the competition experiment was used. On each pot a *C. odorata* or *P. maximum* seedling was planted and covered with a thin layer of aluminium foil to prevent evaporation. The plants were watered twice a week and weight per pot measured before and after watering to determine the Water Use Efficiency (dry biomass (kg)/water used (g)).
After five weeks the pilot studies were harvested and dry biomass determined.

2 Experimental design

Seeds of *C. odorata* were collected in the field and germinated on sterile glass pearls.
Seeds from Puerto Rico had to be germinated in a germination chamber on 25°C with 75% light availability (daytime) and 17°C in the dark (night time). Each 24 hours was divided in 12 hours day and 12 hours night and humidity stayed constant at 65% (Ambika 2002).
The seeds from South Africa didn't germinate very well under the same conditions and had to be germinated in the greenhouse. The *P. maximum* seeds were collected in South Africa. Because germination highly depends on nutrient levels, they had to be germinated on potting soil in the same greenhouse as *C. odorata* from South Africa.

After germination the seedlings were planted in 90 pots (3.9L) containing a mixture of soil, half field ground and half potting soil. The field ground was sterilized at 110°C for 14 hours to kill all existing seeds and roots and the pots were filled with 2800 grams (2052 gr. dry weight) of the soil mixture.
To determine the water content of the mixed soil, 5 randomly chosen samples of 100 grams were dried at 120°C in 48 hours. The difference in wet and dry weight determines the water content.
Water was added to create the two desired water availabilities, 30 and 52 % (these two points were derived from the pilot studies). It was kept constant by watering the plants twice a week. To prevent nutrient limitation, nutrition's were added with the water.

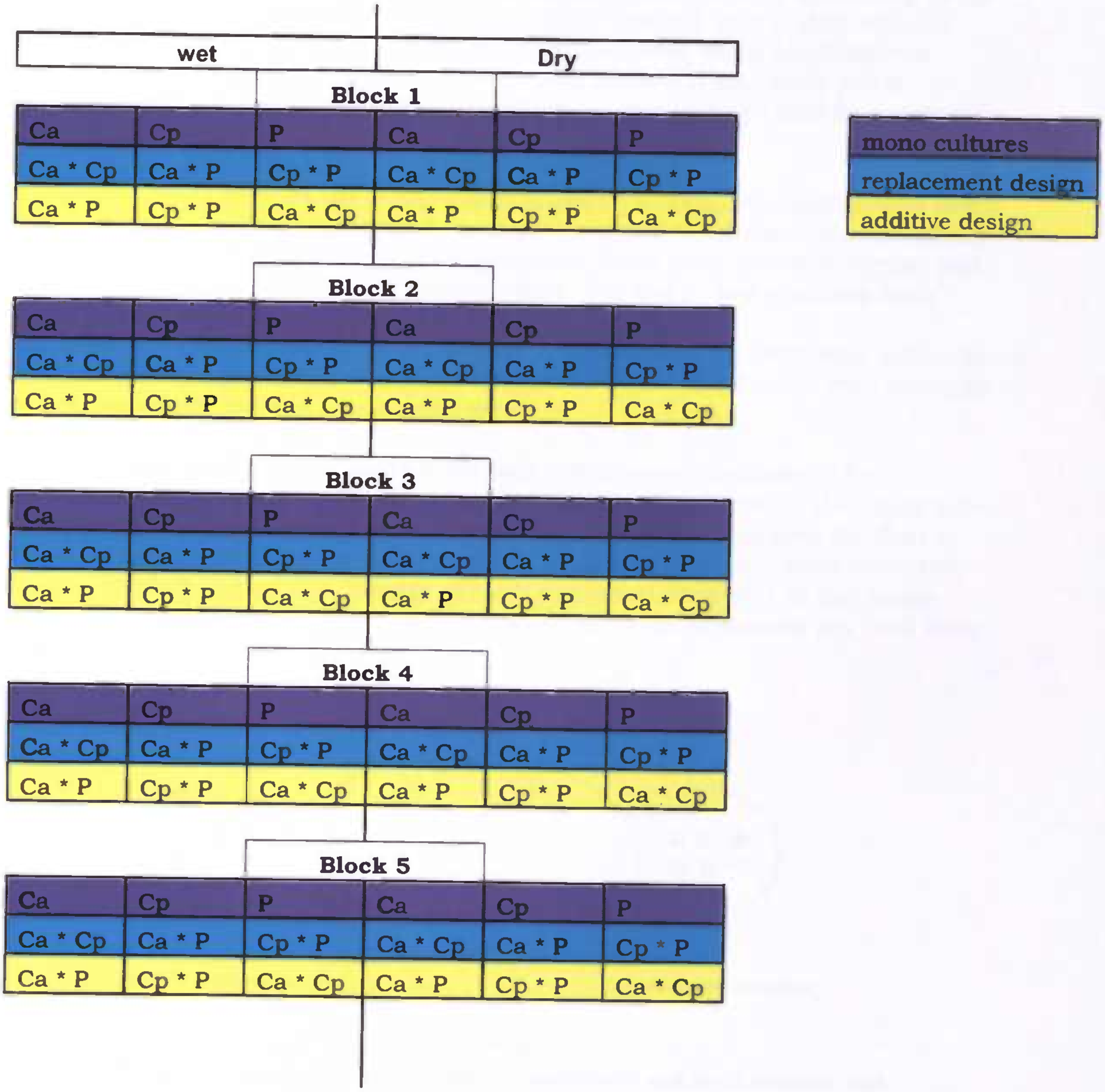


Figure 2: The Randomized block design in the greenhouse.
Each block containing 18 treatments: three seed sources, two water availabilities and mono- and mix cultures. The mixed cultures consist of both replacement and additive designs.

We started with 12,5 mL full strength Hoaglands (Hewitt 1966) the second week, after four weeks 25 mL nutrients and after six weeks 50 mL, according to the plant's needs (Olff *et al.* 2000). The pots were covered with a thin layer of aluminium foil to prevent evaporation. After planting, each seedling was surrounded by seedlings of the other species (figure 3) and each pot is surrounded by a cloth to simulate the plants surrounding them in a natural situation.

The experiment was set up in the greenhouse in a complete randomized block design (figure 2). Five blocks with each 18 treatments, all on a random place within a block. The 18 treatments consists of three seed sources, mono- and mixed cultures, and two water availabilities. For the mixed cultures both additive and replacement design were used.
Because the greenhouse isn't completely homogenised for light and temperature, the position of each block and each treatment inside the block, were changed to a new randomly chosen place every week.

In this experiment we are using both mono and mixed cultures. The monocultures consist of six individuals of the same species. In the replacement design (de Wit 1966) an equal number of one species is replaced for that of another species, leaving the total number of species per unit area constant.
In the additive design the density of each species is identical to the mono cultures, leading to a mixture with twice the amount of species per unit area (see figure 3).

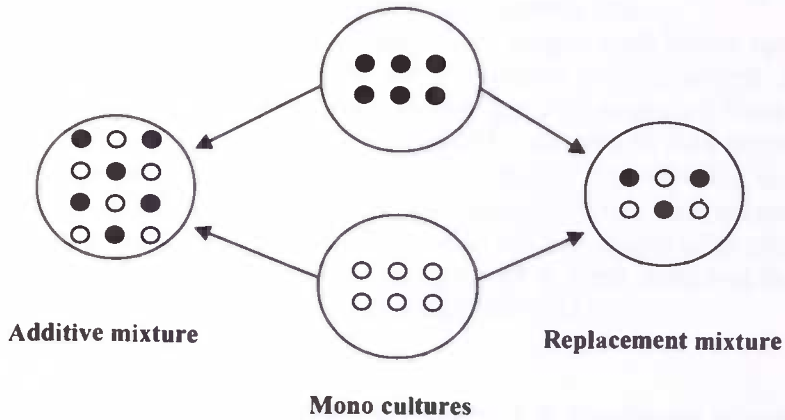


Figure 3. The planting arrangements of the monocultures and both additive and replacement mixtures.

Both replacement and additive design have been criticized on many occasions (Snaydon 1991, Hamilton 1994, Snaydon 1994). Snaydon argues the additive design is better because results are more easier to interpret compared to the replacement design. The interspecific competition remains the same, intraspecific competition is recognised easier (Snaydon 1991). On the opposite side Hamilton argues the replacement design is better because the total density remains the same and effects due to density will not interfere in the results.
To avoid methodological criticism, we decided to use both designs.

Harvest and Measurements

During the experiment several measurements were taken.

- Water use,
- Length and
- shade effect

The water use was determined by weighing the pots before and after watering, giving the exact amount of water used.
The water use efficiency (WUE) is calculated by dividing the dry biomass weight per pot (D) by the mass of the water used (W):

$WUE = D / W \quad (mg.g^{-1})$

The length was measured once a week of three individuals of each species in each pot.
C. odorata was measured from the roots to the top and *P. maximum* from the roots to the last internode, using the thickest branch.
The shade effect was determined three times, in week 5, 7 and 10, using a light meter to measure the difference between light under the canopy near the pot and under the light on the same height and position.

After 9 weeks the experiment was harvested. The biomass of the two species in mixtures were separated and the stem and leaves dried.
The biomass below ground was divided into upper and lower roots by dividing the pot horizontally in half and the roots washes and collected. Both layers were approximately 10 cm. All above and below ground samples were dried at 70°C for at least 48 hours and weighed. In mixed cultures it was impossible to separate the roots from each other. To determine the root dry weight per species we assume an equal root/shoot ratio in monocultures and mixed cultures.
The root weight in mono-cultures (M^r), the shoot weight of mono-cultures (M^s) and the shoot weight of the mixed cultures (O^s) were inserted in a formula to calculate the root weight in the mixed cultures (O^r):

$$O^r_p = \frac{M^r_p}{M^s_p} * O^s_p$$

(In this example the p of *P. maximum* is used).
(Berendse 1981)

The calculated root masses per pot were tested with a t-test to determine whether the calculated masses differ significantly from the measured root mass that was collected. There was no significant difference.

Before drying the leaves, a picture was taken of a part of the leaves of each species in each pot. With the computer program Sigmascan Pro 5.0 the surface of the leaves could be determined. Together with the dry biomass the exact SLA could be calculated.

SLA = leaf area/leaf dry mass ($cm^2.g^{-1}$)
(Garnier *et al.* 2001)

High SLA indicates relatively big thin leaves while a low SLA indicates small thicker leaves. Usually leaves grown in the shade will have higher SLA in comparison with leaves grown in the sun (Walck *et al.* 1999).

With these measurements we could also determine several other plant characteristics:

- Leaf Area Ratio (LAR) leaf area per total plant biomass (cm^2 g^{-1})
- Leaf Weight Ratio (LWR) leaf mass per unit plant mass (g g^{-1})

Data analysis

All data was analysed using Statistica 7.0.
The distribution was tested for normality with the Kolmogorrov-Smirnov & Lilliefors test. To test whether two groups have the same variance and can be compared with each other the Levene's test was used.
To measure the competition between two species for the limited resource, the Relative Yield Total (RYT) had to be determined (de Wit 1960).

$RYT = (Y_{ij}/Y_{ii}) + (Y_{ji}/Y_{jj})$

Y_{ii} and Y_{jj} are the yield per pot of the monocultures of species I and J, Y_{ij} and Y_{ji} are the yield of the species in the mixed cultures.
With the RYT the 'de Wit' replacement graphs were made.
To compare both water treatments the RYT is tested with T-test for independent variables.

The Relative Crowding Coefficient was calculated to determine the ability of one species to obtain the limited resource in a mixed culture, compared with it's ability in monoculture.

$RCC_{ij} = (Y_{ij}/Y_{ii}) / (Y_{ji}/Y_{jj})$ (Harper 1977)

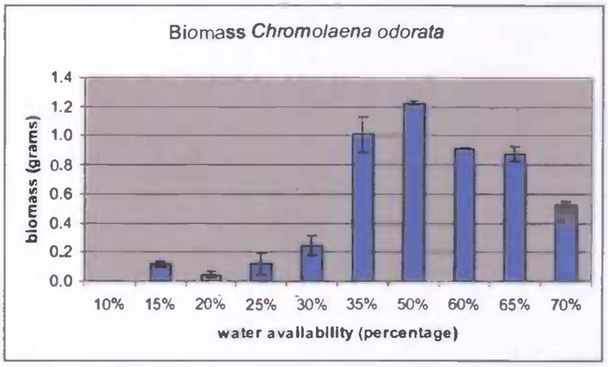
The same symbols are used as in the above equation.

Graphs of SLA, LAR and LMF were made and the differences are tested with a Nested design ANOVA. Block was included as a random factor.

Results

1. Pilot studies

First we had to determine which two water availabilities should be used at the experiment. To determine whether a species is growing successfully at a certain water level we set out the biomass production (root + shoot) against the water availabilities.
Figure 4a and b show the total biomass produced by respectively *C. odorata* and *P. maximum,* at a series of water availabilities from 10 to 70%. Both *C. odorata* (F (9,16) = 44.440, p = 0.000) and *P. maximum* (F(8,18) = 6.0062, p = 0.000787) have a biomass production depending significantly on the amount of water available.



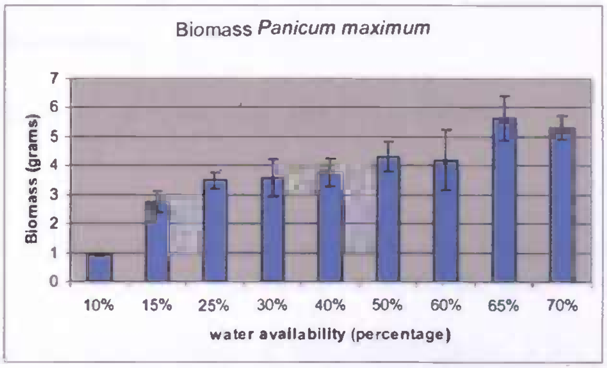


Figure 4a: The effect of water availability on the biomass production of *C. odorata* from South Africa.
Figure 4b: The effect of water availability on the biomass production of *P. maximum.*

C. odorata has a very low biomass production until it reaches 35%, at this point it increases up to an optimal production at 50%. After this point it decreases again. This means *C. odorata* has an optimal yield around 50%.
From 10-30% the yield is very low. Because all other resources are kept constant, *C. odorata* must be limited by the amount of water. Graph b shows an increase of biomass production of *P. maximum* if the water availability increases as well. It seems *P. maximum* can grow well at both low and high water availability.

Figure 5 shows the Water Use Efficiency (WUE) of *C. odorata* and *P. maximum.* The relation between WUE and the amount of water available of *C. odorata* is significant F (9,16)=10,08, p=0,01, it has an optimal WUE between 35 and 60% and a low WUE at < 35%. Although the WUE of *P. maximum* doesn't depend significantly of the water available F(8,18)=1.58, p= 0.24, it seems to show an optimum between 15 and 30% and low WUE higher than 30%.
In our experiment we want to simulate the natural situation. To simulate a dry season competition takes place at low water availability, making sure water is the limiting resource. To accomplice this, a water availability of 30% is used. Because growth of *C. odorata* is limited and WUE low, while *P. maximum* isn't limited and has a much higher WUE at this point, we would expect *P. maximum* to win the competition.

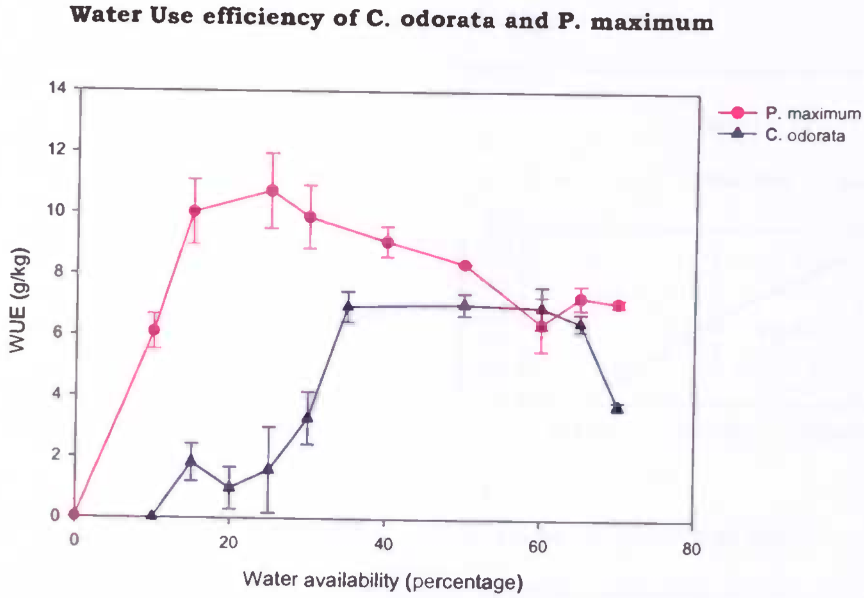


Figure 5: The effect of water availability on the WUE of C. *odorata* and *P. maximum.*

To simulate the wet season, enough water has to be provided to avoid limitation. At 50% *C. odorata* has an optimal yield and WUE is almost equal to the WUE of *P. maximum*. To optimize the competitive ability of *C. odorata*, this will be our wet treatment.
Although a high WUE indicates a competitive advantage it still depends on a lot of other factors, like resource allocation and Relative Growth Rate, before we can conclude anything about the outcome of this competition.

The competition experiment

With the Relative Yield and Relative Yield Total (RYT) we created 'De Wit' diagrams to determine which species would win the competition. Biomass production in mixed cultures are compared with biomass production in monocultures. Figure 6 and 8 show the results for the replacement design and additive design, for both wet and dry treatment. The red line represents the Relative Yield Total, the Total Yield produced per pot.
Values of 2.0 indicate no sharing of any limiting resource, there's no competition. A value of 1.0 would indicate an equally strong intraspecific as interspecific competition.
If the RYT is below 1.0, it indicates a stronger interspecific competition. It will experience a disadvantage growing in a mixture with the other species opposed to growing in a monoculture.
In each graph the relative yield per species is presented as well, indicating the success of each species in the mixture in comparison with it's monoculture.
Values of 0.5 indicate inter- and intraspecific competition are equal, while below 0.5 it means a stronger interspecific competition. Above 0.5 they experience a stronger intraspecific competition.

Replacement design

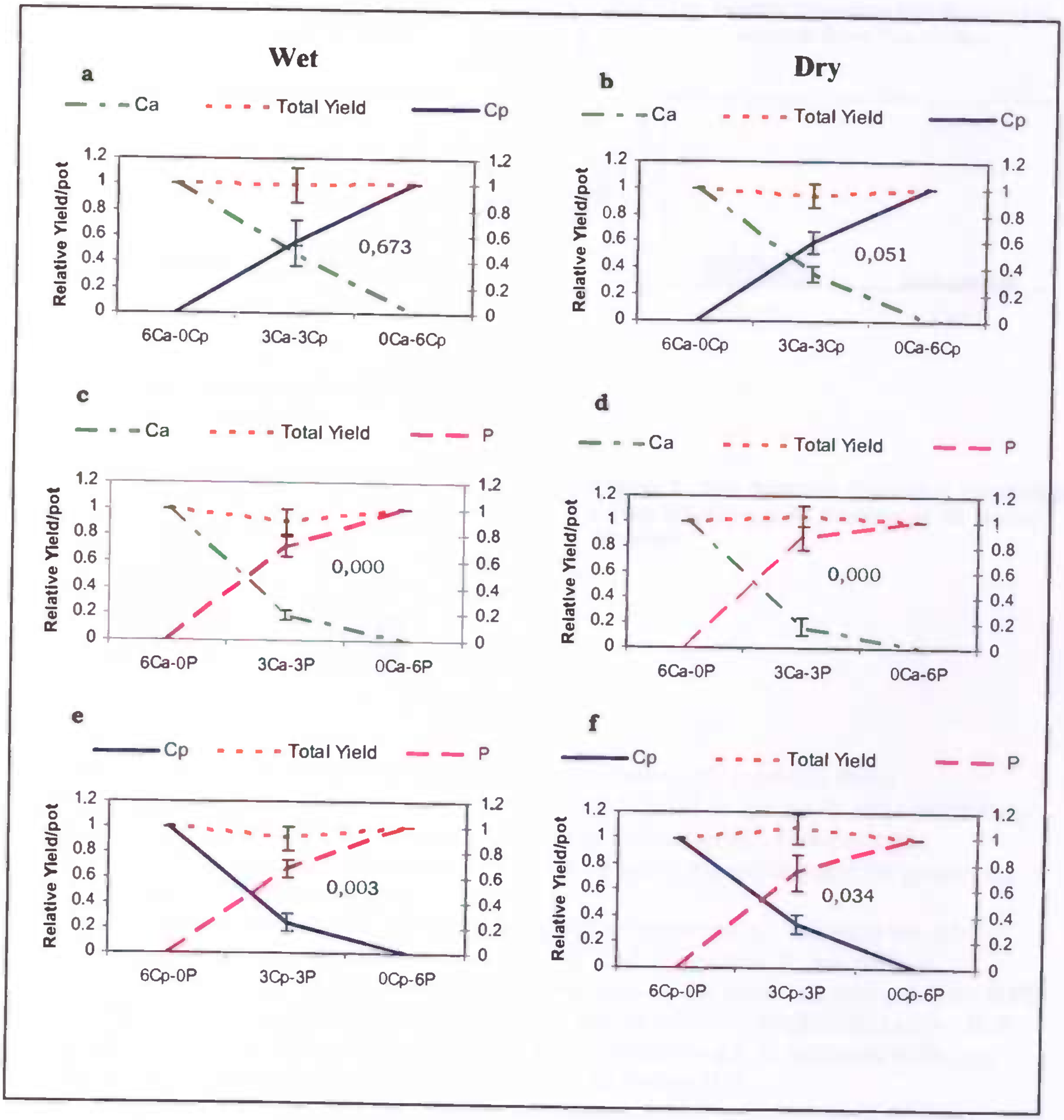
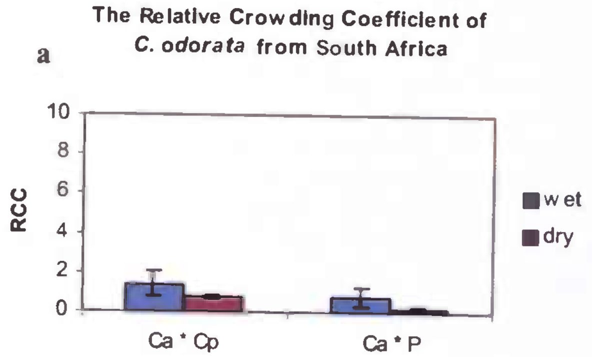


Figure 6: De Wit replacement graphs for the Relative Yield Total for the replacement design.
Competition between *C. odorata* from Puerto Rico (Cp) and South Africa (Ca) at wet (a) and dry (b) treatment,
competition between *P. maximum* (P) and *C. odorata* from South Africa (Ca) at wet (c) and dry (d) treatment and
the competition between *P. maximum* (P) and *C. odorata* from Puerto Rico (Cp) at wet (e) and dry (f) treatment.

The difference between the relative yield of both species in the mixtures were tested with t-test for independent values and the p-value is given in each graph.
Between wet and dry there's no significant differences (Appendix A).

a



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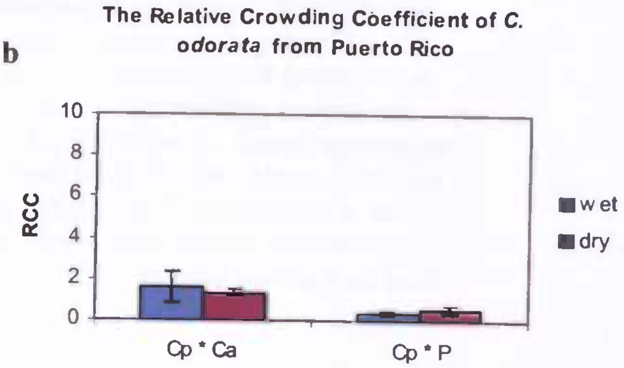

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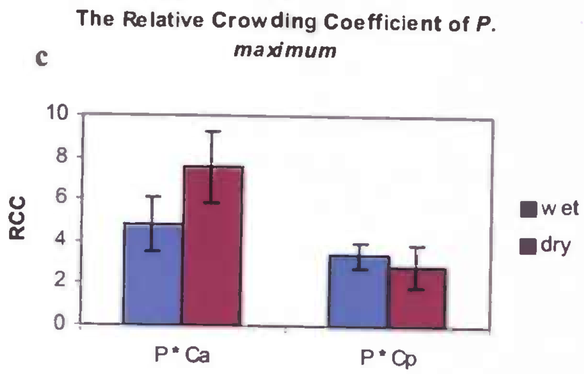

Figure 7. The Relative Crowding Coefficient of the Replacement designs of all three species.

Graphs 6 a and b represent the competition between *C. odorata* from South Africa and Puerto Rico. The Relative Yield Total is for both approximately 1.0, meaning no difference between inter- and intraspecific competition. Although *C. odorata* from Puerto Rico seems to have an advantage in graph b, the difference is not significant.
Figure 6 c-f show the results for the competition between *C. odorata* South Africa (c and d) and *C. odorata* Puerto Rico (e and f) against *P. maximum*. *P. maximum* seems to be winning the competition in all four situations. The RYT never differs significantly from 1,0 meaning no resource complementarity. But the relative yield per species shows both *P. maximum* and *C. odorata* differing significantly, *P. maximum* above and *C. odorata* below 0.5.
This indicates *P. maximum* experiences an advantage if grown together with *C. odorata* as opposed to grown with it's own species, the intraspecific competition is stronger than the interspecific competition. This results in a higher biomass production per plant of *P. maximum*. *C. odorata* experiences a negative effect of the presence of *P. maximum*, resulting in lower biomass production.
The Relative Crowding Coefficient is a measure to determine the aggressiveness of the competitors in mixed culture compared with the mono cultures. A value of 1.0 means an equal aggressiveness, >1.0 a higher aggression and <1.0 a lower aggression.
Figure 7 represents the RCC of all three species, using the data of the replacement design. Graph 7a shows *C. odorata* from South Africa against

both other species. It seems to win the competition from *C. odorata* Puerto Rico at wet treatment, but in graph 7b *C. odorata* from Puerto Rico shows the same result. Both have high standard errors meaning they both sometimes win sometimes lose, there is no better competitor if grown in a mixture together at wet treatment (see appendix B). In the dry treatment the differences are stronger, *C .odorata* from Puerto Rico is more aggressive (>1,0) and wins the competition from *C. odorata* from South Africa but this difference isn't significant either ($p=0,07$). In graph 7c *P. maximum* is in both competitions the better competitor and has a RCC value significantly higher than 1,0. It manages to out compete both *C. odorata* species at both water treatments.

Additive design

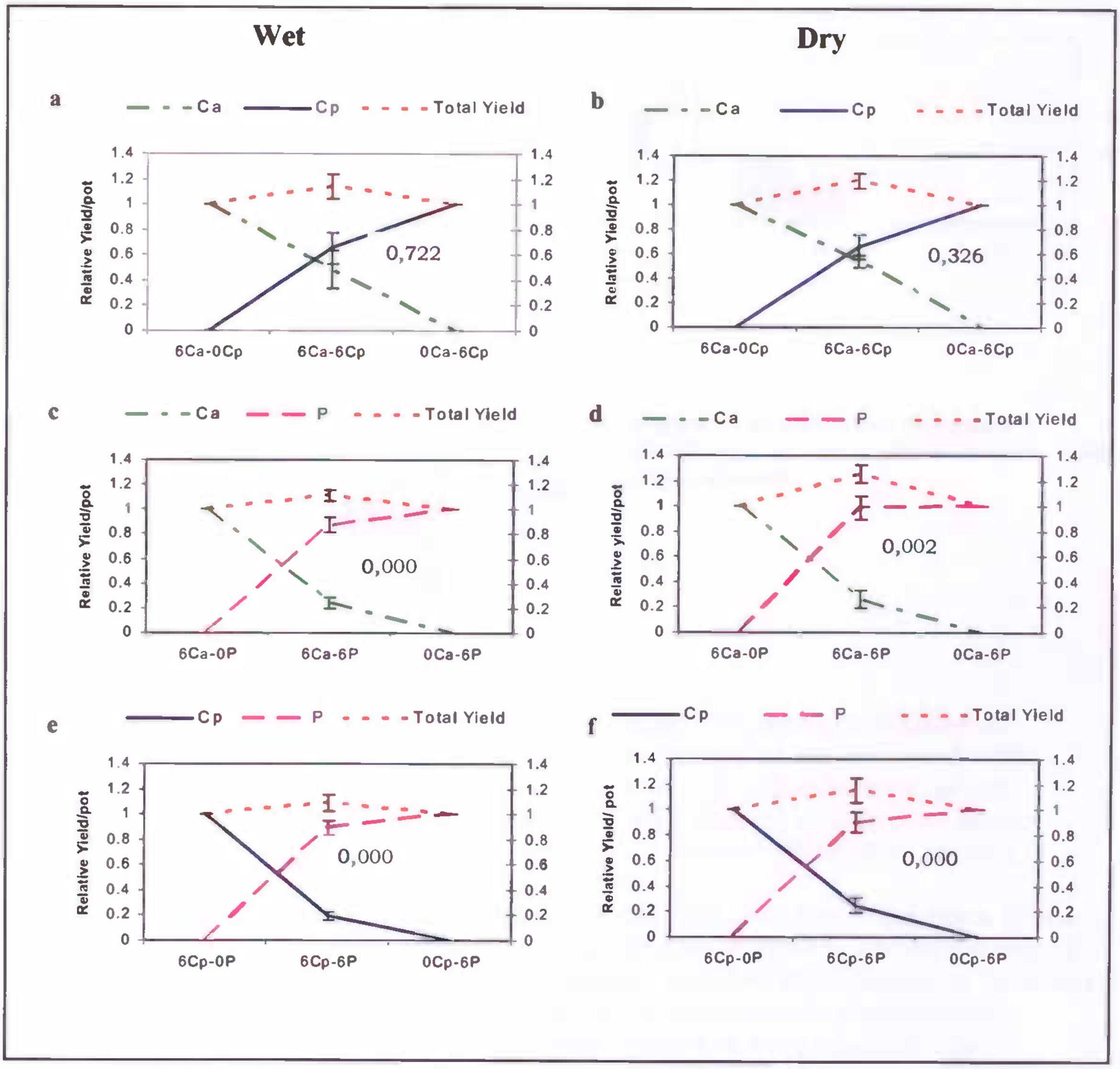

Figure 8: De Wit replacement graphs for the Relative Yield Total for the additive design. Competition between *C. odorata* from Puerto Rico and South Africa at wet (a) and dry (b) treatment,
competition between *P. maximum* and *C. odorata* from South Africa at wet (c) and dry (d) treatment and
the competition between *P. maximum* and *C. odorata* from Puerto Rico at wet (e) and dry (f) treatment.

The difference between the relative yield of both species in the mixtures were tested with t-test for independent values and the p-value is given in each graph.
Between wet and dry there's no significant differences (Appendix A).

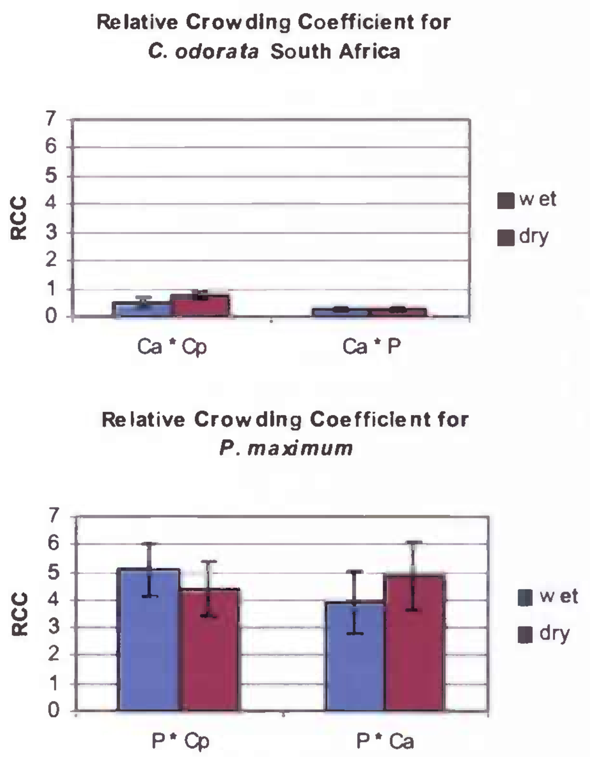

Figure 9. The Relative Crowding Coefficient of the Additive designs of all three species.

Figure 8 shows the results of the additive design. Because the density of the mixture in the additive design is twice the density of the monocultures, you can't interpret the 'de wit' diagrams the same. If niche differentiation occurs between the species theoretically the RYT should be four. Because the number of individuals has increased, other density dependent factors, like the self-thinning effect, play a role as well.
This way you can't conclude anything from the RYT, but the trend lines give a lot of information on what happens during the competitions. The advantage of the additive design is that you can distinguish whether the differences between mono and mixed cultures depends on intra- or interspecific competition. Because the number of individuals of each species is held constant, the intraspecific competition remains constant as well. If the biomass production in mixed differs from the monoculture it's caused by the interspecific competition. Again graphs a and b in figure 8 indicates no significant advantage for either *C. odorata* species. The intra- and interspecific competition is equal. *P. maximum* experiences an advantage grown in combination with *C. odorata*, both from Puerto Rico and South Africa. In this case it experiences a higher intraspecific competition as interspecific competition.

Figure 9 shows the RCC's of the additive design. The RCC's of *C. odorata* from South Africa in competition with *C. odorata* from Puerto Rico are not significantly different from 1,0 (see appendix B). If we look at the wet treatment it is almost significantly different, which means *C. odorata* from Puerto Rico expresses a higher aggressiveness and will have a competitive advantage if grown with *C. odorata* from South Africa. (Ca: p=0,0521 and Cp: p=0,0650, t-test with single value). RCC's of *P. maximum* are significantly higher than 1,0 while the RCC's of both *C. odorata* are significantly lower than 1,0 in competition with each other. This means *P. maximum* is more aggressive towards *C. odorata* from both South Africa and Puerto Rico and will out compete both. So far from both designs we can conclude that *P. maximum* out competes *C. odorata* from both seed sources and at both water levels.

Whether a species is a good competitor depends on several physiological traits, e.g. biomass allocation, root development, Specific Leaf Area etc. Figure 11 shows the biomass allocation to stems, leaves and roots of all three species. Each individual has a limited amount of energy available and has to distribute this between the stems, leaves and the roots, in a way that the plant can be as efficient as possible. This is also called a trade-off. *C. odorata* from Puerto Rico invests more energy in the leaves, while *P. maximum* invests more in stems and roots. The investment seems to differ between *C. odorata* from South Africa and Puerto Rico at wet treatment but this is due to a outlier. If the outlier is ignored, *C. odorata* from South Africa has the same biomass allocation as *C. odorata* from Puerto Rico.
If we look at the differences between wet and dry, we see *C. odorata* has to invest significantly more biomass in the roots at the expense of the leaves. This means water is the limiting resource at the dry treatment. The investment in the stems remains the same.
P. maximum invests more in the roots at the expense of the stems.
In appendix C the p-values of the differences between species are given as well. If you look at the differences between *C. odorata* from South Africa and Puerto Rico you see they're significant between roots and leaves at dry treatment but not at wet treatment. Although both invest more in roots at water shortage, the conclusion (together with figure 11) can be drawn that *C. odorata* from South Africa will invest significantly more energy in the roots while *C. odorata* from Puerto Rico keeps investing more in the leaves if water availability becomes low.

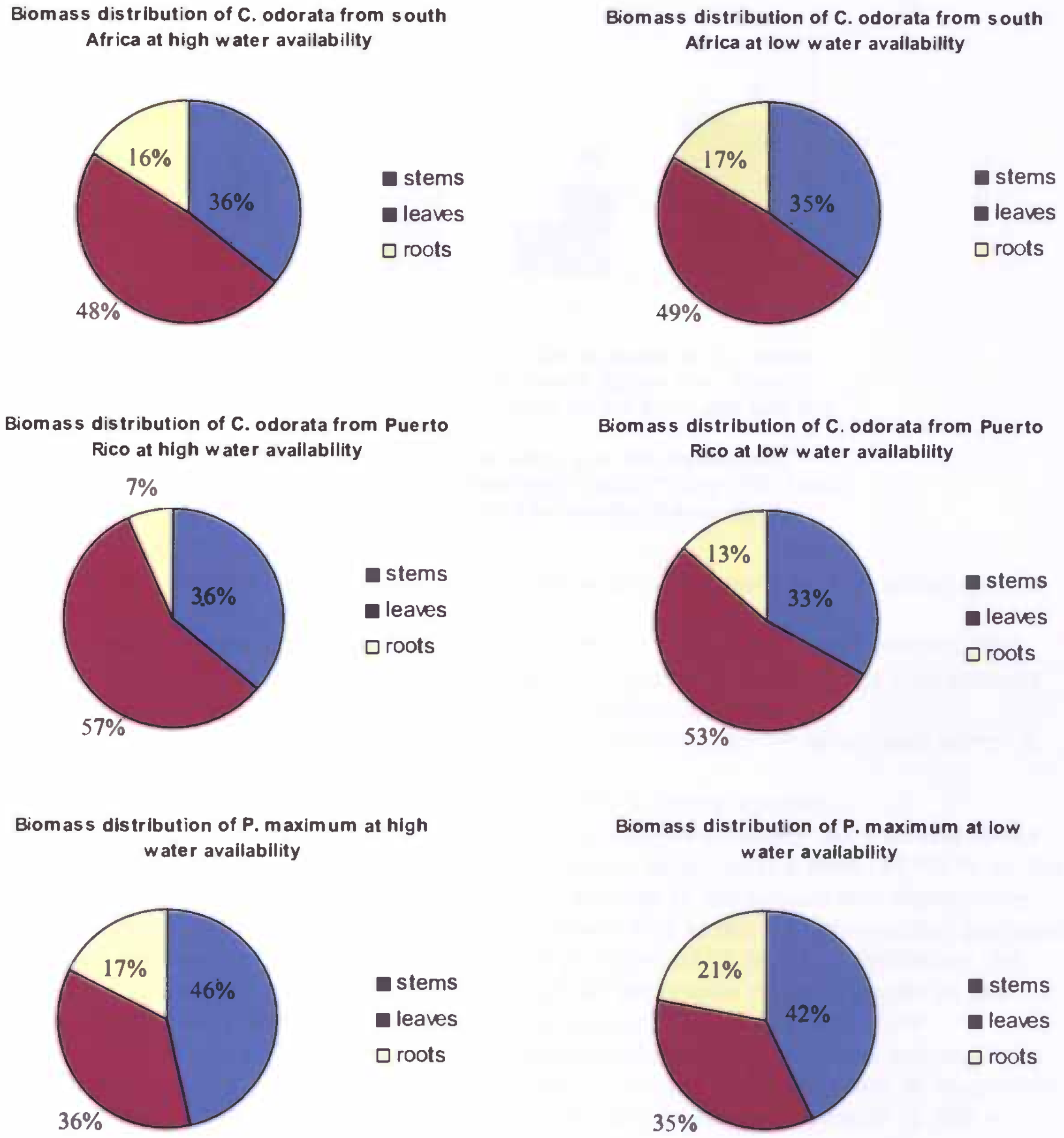

Figure 10: The biomass allocation in stems, leaves and roots. These graphs are based on the monocultures only.
With t-test of independent variables the differences between wet and dry have been tested.

p-values	Stems	Leaves	Roots
Ca with outlier	0,423	0,585	0,433
Ca without outlier	0,115	0,032	*0,001*
Cp	0,266	0,027	*0,005*
P	0,056	0,590	*0,020*

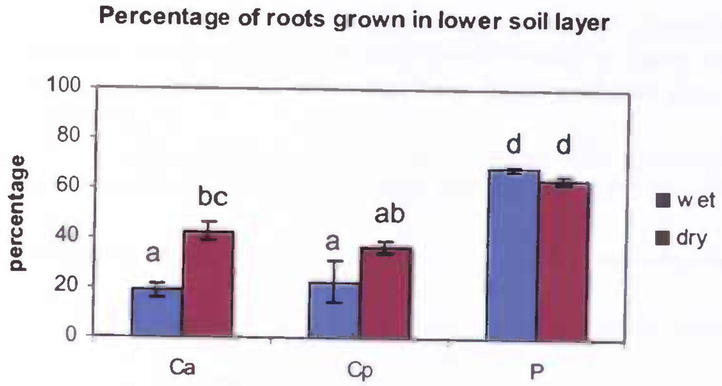

Figure 11: The relative root biomass in the lower soil layer of *C. odorata* South Africa (Ca), Puerto Rico (Cp) and *P. maximum* (P) for both wet and dry treatment.
Means with the same letter are not significant, different letters indicate p < 0,05 (Tukey HSD test). This graph is based on the monocultures only.

Next to investment, has rooting depth also a major impact on the competition for water.
Roots on the same depth will have to compete for the resources present. But roots on two different depths will not have to compete, the species can coexist without effecting each others growth (niche differentiation).
Figure 11 shows the relative amount of roots present in the lower soil layer. It differs significantly
(Two-factor ANOVA, F (2,26)= 50.7572 p=0.00) between species.
P. maximum is present in both layers and has approximately 60% in the lower layer. *C. odorata* is mainly present in the upper layer with a max. of 40 % in the lower layer (at low water availability). This means *P. maximum* will experience low levels of competition for water in the lower soil layer. An interaction between species and water (Two-factor ANOVA, F (2,26)=6.2112 p=0,01) indicates the effects of water differs per species. *C. odorata* increases rooting depth at low water availability significantly, while *P. maximum* shows no difference.
Graphs 12 and 13 show the total root biomass found in the upper respectively lower soil layer. *C. odorata* has significantly less root biomass than *P. maximum* in both upper (F (2,26) = 24,22651 p=0,000) and lower soil layer (F (2,26) = 0,615887 p=0,000).

P. maximum seems to have a competitive advantage because more roots are produced and are rooted deeper, giving it the opportunity to take up more water compared to *C. odorata.* Between *C. odorata* from South Africa and Puerto Rico are no significant differences.
Because the roots were impossible to separate in the mixed cultures only the measurements of the monocultures were used for figures 10, 11, 12 and 13.

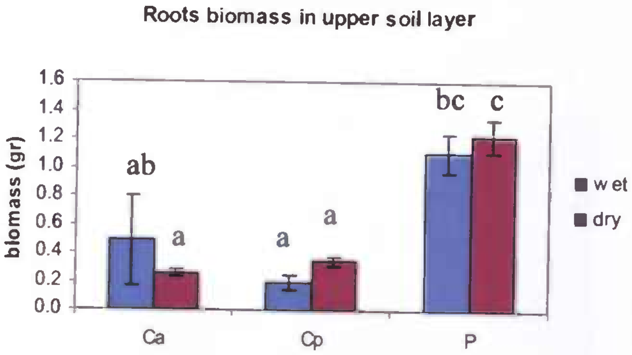

Figure 12: The root biomass in upper soil layer of *C. odorata* South Africa (Ca), Puerto Rico (Cp) and *P. maximum* (P) for both wet and dry treatment.
Means with the same letter are not significant, different letters indicate p < 0,05 (Tukey HSD test). This graph is based on the monocultures only.

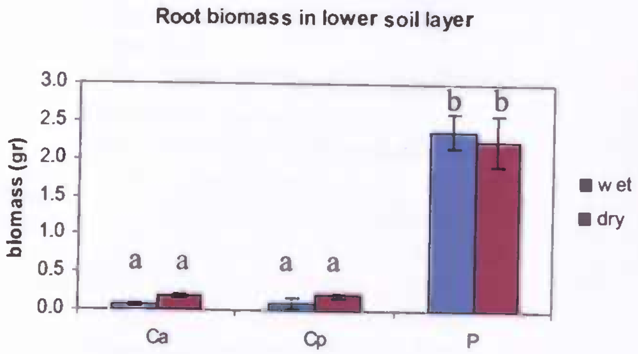

Figure 13: The root biomass in lower soil layer of *C. odorata* South Africa (Ca), Puerto Rico (Cp) and *P. maximum* (P) for both wet and dry treatment.
Means with the same letter are not significant, different letters indicate p < 0,05 (Tukey HSD test). This graph is based on the monocultures only.

The water use efficiency (WUE) determines how much water a species has to absorb to produce a certain amount of biomass. A low WUE indicates more water has to be absorbed to produce the same amount of biomass as a species with high WUE. Whether *P. maximum* really has the advantage (because of more, deeper roots) is determined by the WUE. Or it can extract more water and produce more biomass or it simply needs to take up more water to produce the same amount.

The differences are significant between species F(2,75)=305,829 p=0,0000, water treatment F(2,75)=63,423 p=0,001347 and mono- or mixed cultures F(2,75)=71,912 p=0,001060. Figure 14 shows the Water Use Efficiency (WUE) of all three species in monocultures. In Appendix E the graphs are shown for both mono and mixed cultures as well. If you look at the monocultures you see *P. maximum* has a much higher water use efficiency compared to *C. odorata* at both high and low water availability. If these two species would be competing for water in the field, it means *P. maximum* will need less water to produce the same amount of biomass. Together with better developed roots we can conclude *P. maximum* has the ability to extract more water, use it more efficiently and produce more biomass per unit water. This will give *P. maximum* an advantage especially at low water availability. The WUE is significantly higher at low water availability meaning a higher biomass production per unit water if water is in limiting supply. In the competition between *C. odorata* from South Africa and Puerto Rico, the WUE doesn't differ at either water availabilities.

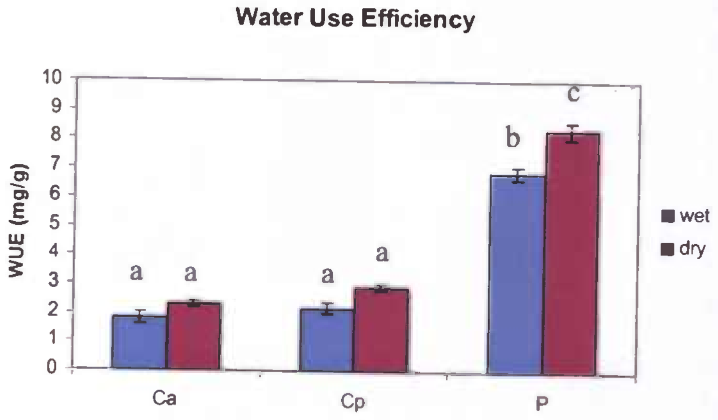

Figure 14: Water use Efficiency of *C. odorata* from South Africa (Ca) Puerto Rico (Cp) and *P. maximum* (P) in monocultures. Means with the same letter are not significant, different letters indicate p < 0,05 (Tukey HSD test). This graph is based on both mono and mixed cultures.

Usually a competition for water is in combination with a competition for light. This is because a limit supply of water results in a decrease of light use efficiency due to higher energy investment in roots. If they manage to extract more water it will grow faster, over shade the other one and out compete them. This means the competitive ability of a species highly depends on the amount of light the leaves can absorb and the efficiency of the light used to produce biomass as well.
To compare the competitive ability of two species is usually done with the Relative Growth Rate (RGR). It's a measure that determines the biomass growth per unit plant biomass, measured in time.
A high RGR usually indicates a superior competitor, because it grows faster and will be able to over shade the species with low RGR.
The RGR depends of a morphological trait (LAR) and a physiological trait (NAR).

The Net Assimilation rate (NAR) is the biomass growth per unit leaf area per day, but couldn't be determined in this experiment. The Leaf Area Ratio (LAR) is the leaf area per total plant mass. A high LAR means it has a relatively big leaf surface area in comparison with the total plant.

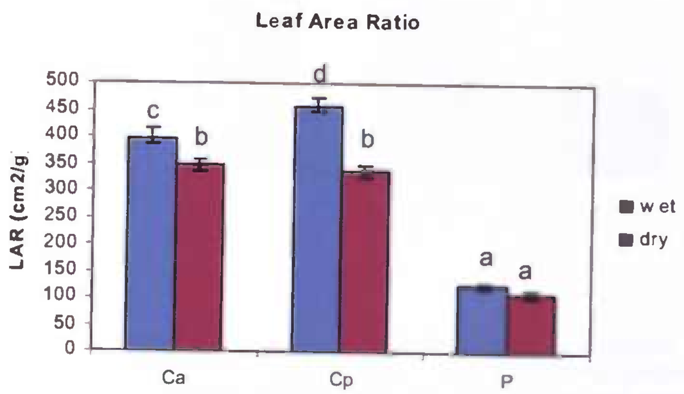

Figure 15: Differences between Leaf Area Ratio of *C. odorata* from South Africa (Ca), Puerto Rico (Cp) and *P. maximum* (P).
Means with the same letter are not significant, different letters indicate p < 0,05 (Tukey HSD test). This graph is based on both mono and mixed cultures.

Figure 15 shows the LAR of *C. odorata* and *P. maximum*. The LAR differs significantly between species F (2,70)=196,179 p=0,0000 and between wet and dry F (1,70)=24,168 p=0,00002. *C. odorata* has a higher LAR which indicates it has relatively more photosynthetic surface (leaf area) relative to the plant's biomass. An interaction between species and water (F (5,70)= 8,192 p=0,000435) indicates it doesn't differ significantly between water treatments for all three species.
The LAR decreases at dry treatment for *C. odorata*, but not for *P. maximum*. You can also see that *C. odorata* from Puerto Rico has significantly higher LAR at wet treatment compared to *C. odorata* from South Africa.

The LAR is determined by two factors, the amount of biomass allocated to the leaves (Leaf Weight Ratio) and the leaf area constructed with this biomass (Specific Leaf Area). A high LWR means the plant has invested a lot of biomass into the leaves. Figure 16 shows the LWR of the three species. It differs significantly between species F (2,70)= 209,16 p=0,000 and water availability F (1,70)=10,55 p=0,00146.
As shown in graph 16 has *C. odorata* higher LWR, it invests more biomass in the leaves.
The biomass allocation in figure 11 shows the same result.

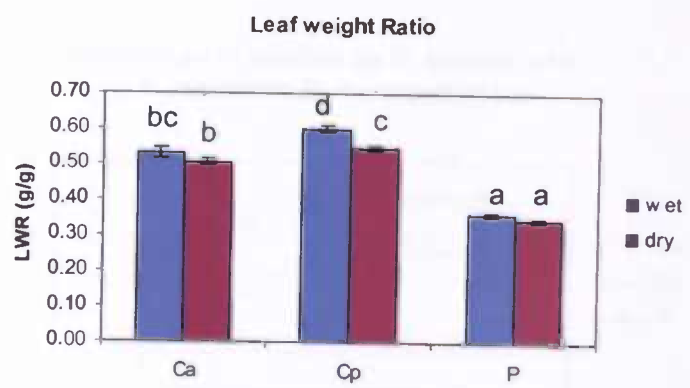

Figure 16: Differences between Leaf Weight Ratio of *C. odorata* from South Africa (Ca), Puerto Rico (Cp) and *P. maximum* (P).
Means with the same letter are not significant, different letters indicate p < 0,05 (Tukey HSD test). This graph is based on both mono and mixed cultures.

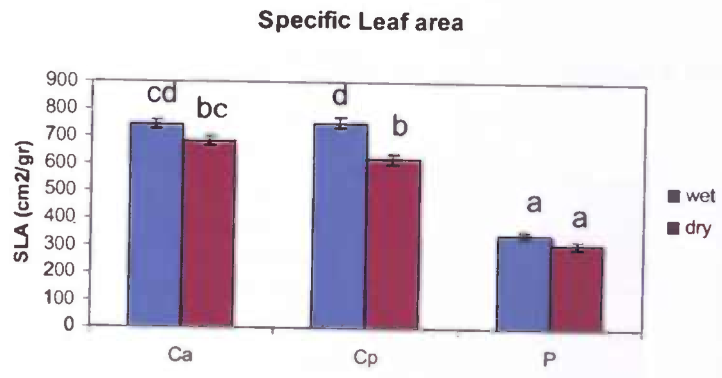

Figure 17: the Specific Leaf Area of *C. odorata* and *P. maximum*. The differences are significant between the species Means with the same letter are not significant, different letters indicate p < 0,05 (Tukey HSD test). This graph is based on both mono and mixed cultures.

If we look at graph 17 we see a higher SLA for *C. odorata* as well. The difference between species ((F (2,70)=105,3 p=0,000002) and the water treatment (F(1,70)=20,3 p=0,0107) are significant. This means *C. odorata* has relative more and bigger, thinner leaves. With the same leaf biomass, it will have a higher surface area and will catch more sunlight. It is capable of producing more biomass with the same amount of light.

The interaction between species and water F (5,70)=8,4292 p=0,01072 shows the water treatment has an effect on the Specific Leaf Area only at specific species. Looking at the graph it means the water only has a significant affect on the SLA of *C. odorata* from Puerto Rico and not on *C. odorata* of South Africa or *P. maximum*.

During the experiment the percentage of light blocked by the species were measured as well. In Figure 20 inhibition of light is set against the time. These measurements were taken in week 5, 7 and 9. Only data of monocultures is used because the light inhibition of mixed cultures can't be separated per species.

C. odorata inhibits more light than *P. maximum*. In week 5 it already inhibits between 60 and 80 % and after 9 weeks this has risen to approximately 90%. Canopy over shaded by *C. odorata* would have a lot of trouble to survive. *P. maximum* leaves more light through, starting between 40 and 60% in week 5 and increasing to approximately 80% in week 9.

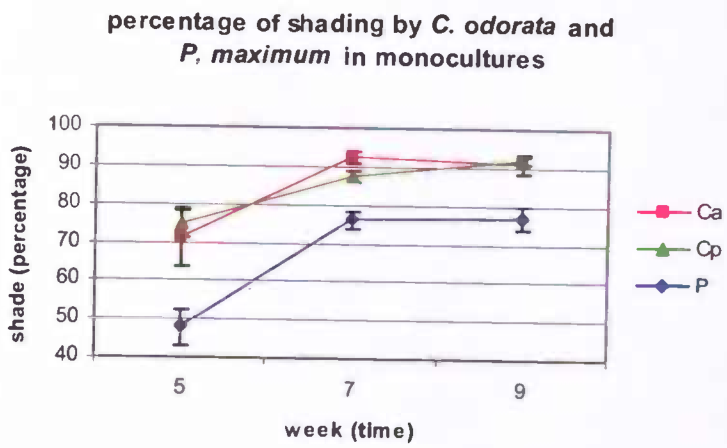

Figure 18: The percentage of light inhibited by *C. odorata* from South Africa (Ca) Puerto Rico (Cp) and *P. maximum* (P). this graph is based on the monocultures only.

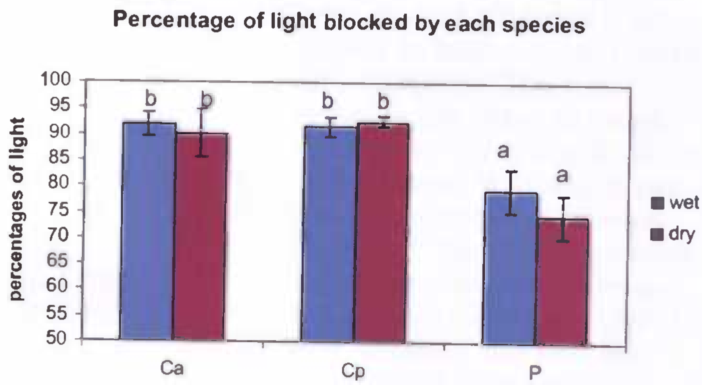

Figure 19: The percentage of light blocked by *C. odorata* from South Africa (Ca) Puerto Rico (Cp) and *P. maximum* (P). Means with the same letter are not significant, different letters indicate p < 0,05 (Tukey HSD test). This graph is based on the monocultures only.

In time the trend remains the same. If we take a closer look at week 9 we see no significant differences between native and invasive *C. odorata*. Between wet and dry treatment the percentage of light blocked remains the same as well. It only differs between *C. odorata* and *P. maximum*. The amount of light blocked by *P. maximum* is a lot less compared to the amount blocked by *C. odorata*. If *C. odorata* manages to over shade *P. maximum*, not a lot of light would remain available for *P. maximum*. Whether the amount of light blocked has any effect depends on which species is taller, over shading the other one.
During the experiment we observed that *P. maximum* already started over shading *C. odorata* from day one.

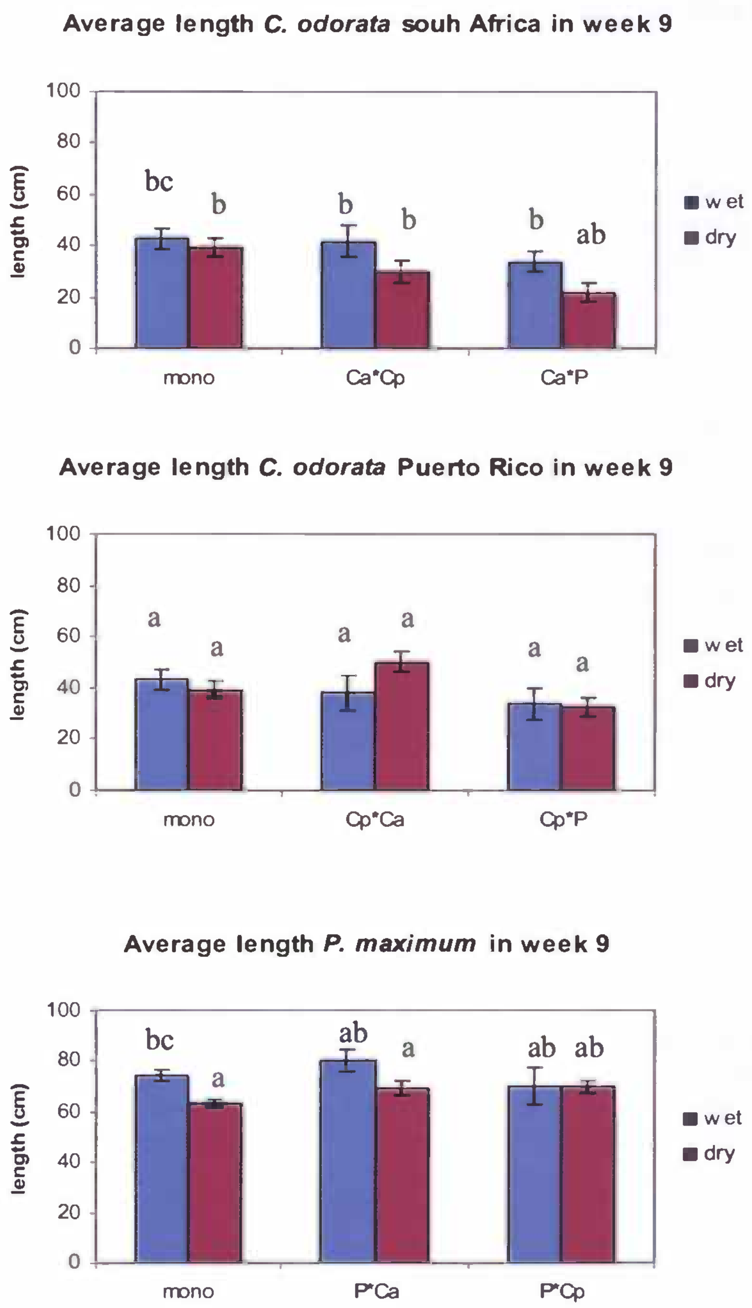

Figure 20: Plant height of *C. odorata* from South Africa (Ca), Puerto Rico (Cp) and *P. maximum* (P) at week 9.
Means with the same letter are not significant, different letters indicate p < 0,05 (Tukey HSD test).

In Appendix I the lengths from week 4 till week 9 are shown in both monocultures and mixtures. The trend remains the same through time so we are going to focus only at week 9 at which point the differences are greatest. In week 9 the differences are significant between species $F\ (2,\ 120)=19{,}958$ $p=0{,}0007$ and between wet and dry $F\ (1{,}120)=8{,}936$ $p=0{,}036$. *P. maximum* grows significantly higher compared to *C. odorata*. Between both *C. odorata* species there's no differences.
Only *P. maximum*. is influenced significantly by water treatment at the mono cultures. This is probably caused by the high uptake of water by 6 individuals of *P. maximum*. In stead of competing with 2 other individuals, each plant has to compete with 5 individuals of *P. maximum*. Differences between wet and dry become stronger, which results in a significant difference.
C. odorata doesn't seem to be effected by the water treatment that much. Although height seems to decline in presence of *P. maximum*, this difference is not significant.

Conclusion & Discussion

In a competition for water between *Chromolaena odorata* and *Panicum maximum* we expected *P. maximum* to out compete *C. odorata* at low water availability while *C. odorata* would win at high water availability (based on personal field obs. M. te Beest and H. Olff). In this experiment The 'de wit' replacement graphs (figures 6 and 8) indicate *P. maximum* experiences a higher intraspecific competition, it has lower biomass production per plant if grown with it's own species as opposed to grown with *C. odorata*. Meanwhile *C. odorata* is experiencing a higher interspecific competition, biomass production decreases in presence of *P. maximum*. This is the same at both high and low water availability.
All this indicates *P. maximum* as the superior competitor independent of the water availability, it out competes *C. odorata* at both high and low water availability. In the natural environment this would mean *P. maximum* should be out competing *C. odorata*, making an establishment of *C. odorata* in the savanna impossible. Meanwhile field observations showed a survival and establishment of *C. odorata* at high water availability.
This contrast might be explained if we look at the physiological traits of both species.

A savanna is a relative dry area and competition for water determines mostly the biodiversity and ecosystemal structure (Knoop *et al.* 1985).
For *C. odorata* to invade the savanna it needs to compete for water successfully against *P. maximum*. According to the pilot studies (graphs 4 and 5), *C. odorata* has a low Water Use Efficiency (WUE) and low biomass production if water availability is low as well.
At high water availability (50-60%) *C. odorata* has an optimal growth and high Water Use Efficiency. *P. maximum* is less effected by low water availability, it manages to produce a high amount of biomass even at 15% water availability (graph 5).
These data are consistent with the hypothesis that *P. maximum* would win the competition at low water availability and *C. odorata* at high water availability. But next to using water efficient it needs to be able to obtain water successful as well. Figure 11 shows the biomass allocation of both species. *P. maximum* invests relatively more energy in roots. It has more roots, in both upper and lower soil layer, and has greater rooting depth (figures 12 and 13). This means *P. maximum* will be able to extract more water and together with a high Water Use Efficiency (figure 14) will it be able to out compete *C. odorata* in a competition for water. All this indicates *P. maximum* as the superior competitor for water.

A competition for water between two species is usually combined with a competition for light (Köchy *et al.* 2000). At seedling stage the species have to compete for the available nutrients and water, but as they grow taller they will start over shading each other as well, resulting in a competition for light. Grace (1990) found that in many cases the ability to use one resource very efficiently is negatively correlated with the ability to use another resource. In this case *P. maximum* has a high allocation towards the roots to obtain more water, but leaves less allocation towards leaves to intercept light.

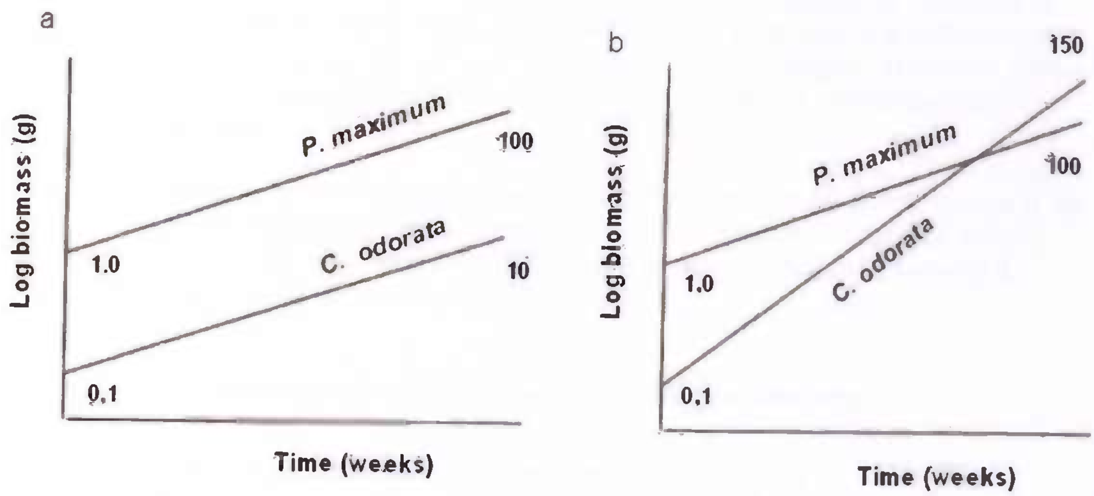

Figure 21: Relative Growth Rate between both species is a) equal or b) *C. odorata* has a higher RGR.

C. odorata in contrast invests more in leaves and less in roots (figure 10). Grotkopp (2002) tested with 29 spine species which physiological traits show a positive correlation with the invasive success of the species. He came to the conclusion the Relative Growth Rate (RGR) has the most positive correlation with the invasive success.

To compete successfully for light a species needs to over shade the others and therefore needs to have a high biomass production to become taller than the other. Although we haven't determined the RGR in this experiment, we do know *C. odorata* has higher Leaf Weight Ratio (LWR, figure 16) and Specific Leaf area (SLA, figure 17). Several studies showed the RGR is strongly correlated with the Specific Leaf area (SLA) (Poorter H *et al.* 1990, Poorter L *et al.* 2004, Grotkopp E *et al.* 2002). This would mean *C. odorata* has a higher RGR and will be able to produce more biomass per unit time. The lower WUE also indicates a higher SLA and RGR, because a plant with a lot of stomata will evaporate a lot of water, reducing the WUE.

Despite a higher RGR, *C. odorata* wasn't capable of out competing *P. maximum* in this experiment. If we focus at average plant height, we see *P. maximum* over shading *C. odorata* during the whole experiment (Appendix I) giving *P. maximum* the ability to out compete *C. odorata*. This might be due to the seed and seedling masses of both species. Big seeds result in bigger/heavier seedlings. Large-seeded species have in average a competitive advantage at the seedling stage (Turnbull *et al.* 1999).

If both species would have the same RGR the ratio of biomass would remain the same. But differences between the biomasses would increase through time (see figure 22a). If *P. maximum* would start at ten times the amount of biomass it would have ten times as much biomass at week 9 as well, but the difference between 0,10 and 1,0 gram or 10 and 100 grams is a lot bigger. It would mean the species with the bigger seeds will keep and increase it's advantage in a competition for light.

Figure 23 shows the biomass of the seeds and seedlings of all three species. As you can see has *P. maximum* significantly heavier seedlings. The seed mass seems to be heavier as well, but this difference is only significant between *P. maximum* and *C. odorata* from Puerto Rico.

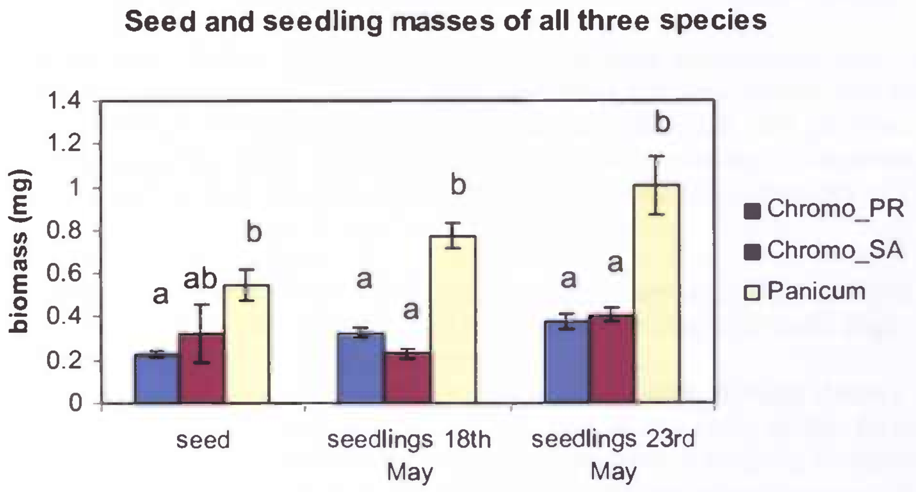

Figure 22. Seed and seedling mass of *P. maximum* and both *C. odorata* from Puerto Rico and South Africa. Seedling masses are measured at the 18th of may and 5 days later on the 23rd of may.
The differences are tested per group. Means with the same letter are not significant, different letters indicate $p < 0,05$ (Tukey HSD test).

Because *C. odorata* has higher RGR it should eventually overgrow *P. maximum* (see figure 22b), but to overcome these disadvantages at seedling stage (i.e. the regeneration niche), it needs time. Because *P. maximum* over shades *C. odorata,* it limits the light availability, decreasing the advantage of *C. odorata* and disenabling the successful establishment.

Human disturbances can have a positive effect on the invasibility of ecosystems (Leishman *et al.* 2005, Daehler 2003, Hierro *et al.* 2006, Keeley *et al.* 2003). If an disturbance occurs, e.g. fire, herbivory, the competitive effect of *P. maximum* might be decreased or eliminated long enough for *C. odorata* to overcome it's initial seedling disadvantages. Once it would have overgrown *P. maximum* it will manage to establish itself. *C. odorata* leaves block more of the light, after 5 weeks it already stops between 60 and 80 %. Once *C. odorata* has overgrown *P. maximum* it will limit the light available for *P. maximum*.

Because water deficiency has a negative effect on the LAR, LWR and SLA, it has a negative effect on the RGR as well (figures 15, 16 and 17). This would explain why *C. odorata* can only invade the savanna at high rainfall when water isn't limiting the growth.

Another reason why *P. maximum* is so successful in this experiment is because growth highly depends on the nutrients available (Durr *et al.*2003). Because savannas usually are quite nutrient poor (Saito 2004) *P. maximum* might not reach these lengths in the field.
It mainly grows at the edges of the savanna under canopy (Durr *et al.* 2003, Witkowski 2001) because nutrient levels are a little bit higher under the canopy, but this means growth will be limited by light as well. Because *C. odorata* is a better light competitor it will have an extra advantage at this point.
Once it has overgrown *P. maximum,* it will probably win the competition.

We conclude by posing that *C. odorata* will only become successful and establish itself in a savanna, if rainfall was high and a disturbance has taken place. Because other environmental conditions will influence this process as well, still more research needs to be done to eventually manage to control the invasion of *C. odorata.* But this study might have solved some peaces of the puzzle..

In the competition between *C. odorata* from South Africa and Puerto Rico we expected *C. odorata* from South Africa to have an advantage at both high and low water availability, due to light competition.
In the 'de wit' graphs of both the replacement and additive design there's no significant difference in the competitive ability. But if you look at the Relative Crowding Coefficients of the additive design, it indicates a slightly competitive advantage of *C. odorata* from Puerto Rico, but only at low water availability. The difference between designs is probably due to the density difference. If more individuals are present but the same amount of resources, they will have to compete more aggressively. The species with a disadvantage will experiences more pressure and produce even less biomass.

One of the theories why invasive species are expected to be so successful is the higher phenotypic plasticity (Richards *et al.* 2006). But if we compare the native and invasive *C. odorata* we actually see a decline in phenotypic plasticity. The native ones react to lower water availability by increasing the biomass allocation to the roots (figure 10). But if water is more available, it reduces the biomass allocated to the roots and increases the biomass allocated to the leaves. This is also seen in figures 15, 16 and 17. At wet treatment the Leaf Area Ratio (LAR), the Leaf Weight Ratio (LWR) and the Specific Leaf Area (SLA) increase significantly for the native ones. The invasive *C. odorata* meanwhile reacts less to the increase of the water availability. Although the LAR increases significantly, the increase is still lower than the increase of the native ones.
During the experiment differences in leaf morphology were observed as well, *C. odorata* from Puerto Rico (native) produces extra leaves on the stems of the main leaves and the invasive species doesn't. But as soon it has to compete for light it stops producing these extra leaves and start growing in height faster, until it's no longer over shaded. At this point it starts producing the extra leaves

again. The native *C. odorata* seems to have both physiological and morphological higher phenotypic plasticity.

This contradiction might be explained by the beginning of the invasion. The theory is that the current population derived from a small number of seeds, arriving on package material in Durban (Goodall *et al.* 1995). A hugh bottle neck must have taken place on the genotypic variation.
If all these seeds have derived from this small number of seeds the genotypic variation is very low, decreasing the phenotypic plasticity of the population. Due to low genetic variation, traits become dominant or disappear a lot faster. Characteristics different from the native species can accumulate very fast, changing the characteristics of the whole population.
A second explanation would be a theory proposed by Bossdorf (2004). It is called the ERCA (evolutionary Reduced Competitive Ability) hypothesis, indicating a species loses it's competitive ability if in the new environment less competition occurs. Especially if the competitive ability of the species are high cost, natural selection might act against it.

A nice follow up study would be to copy this experiment but set it up in the savannas itself. By creating your own disturbances this might give you some clear information under which conditions *C. odorata* manages to establish itself and when establishment is disenabled.

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Appendix A: The p-values of the t-tests for the 'de Wit' replacement diagrams.

Replacement design:

replacement design		Ca vs. 0,5	Cp vs. 0,5	P vs. 0,5	Ca vs. Cp	Ca vs. P	Cp vs. P	Tot Yield vs. 1,0
Ca * Cp	wet	0.596	0.834		0.673			0.975
Ca * P	wet	0.000		0.033		0.000		0.391
Cp * P	wet		0.022	0.075			0.003	0.393
Ca * Cp	dry	0.069	0.346		0.051			0.605
Ca * P	dry	0.007		0.018		0.000		0.642
Cp * P	dry		0.084	0.165			0.034	0.547

wet vs. dry	Ca vs. Ca	Cp vs. Cp	P vs. P	Tot Yield vs. Tot Yield
Ca * Cp	0.640	0.962		0.760
Ca * P	0.915		0.154	0.315
Cp * P		0.366	0.667	0.294

Additive design:

additive design		Ca vs. 0,5	Cp vs. 0,5	P vs. 0,5	Ca vs. Cp	Ca vs. P	Cp vs. P	Tot Yield vs. 1,0
Ca * Cp	wet	0.953	0.517		0.722			0.381
Ca * P	wet	0.012		0.008		0.000		0.073
Cp * P	wet		0.001	0.002			0.000	0.263
Ca * Cp	dry	0.467	0.183		0.326			0.031
Ca * P	dry	0.023		0.006		0.002		0.022
Cp * P	dry		0.011	0.008			0.000	0.159

wet vs. dry	Ca vs. Ca	Cp vs. Cp	P vs. P	Tot Yield vs. Tot Yield
Ca * Cp	0.880	0.725		0.491
Ca * P	0.691		0.316	0.069
Cp * P		0.427	0.902	0.207

Both t-test for independent samples and t-test for single means were used.

Appendix B: p-values of t-tests with independent sample. Tested for the difference of the Relative Crowding Coefficients (RCC) compared with 1,0.

additive design		dry	wet
Ca*Cp	Ca	0,2664	0,0521
	Cp	0,1744	0,0650
Ca*P	Ca	0,0013	0,0031
	P	0,0356	0,0988
Cp*P	Cp	0,0007	0,0000
	P	0,0231	0,0121

replacement design		dry	wet
Ca*Cp	Ca	0,0703	0,5662
	Cp	0,2107	0,4873
Ca*P	Ca	0,0018	0,6794
	P	0,0195	0,0344
Cp*P	Cp	0,0509	0,00528
	P	0,1365	0,0197

Appendix C: p-values of t-tests to compare the differences of biomass allocation between the species (figure 11).

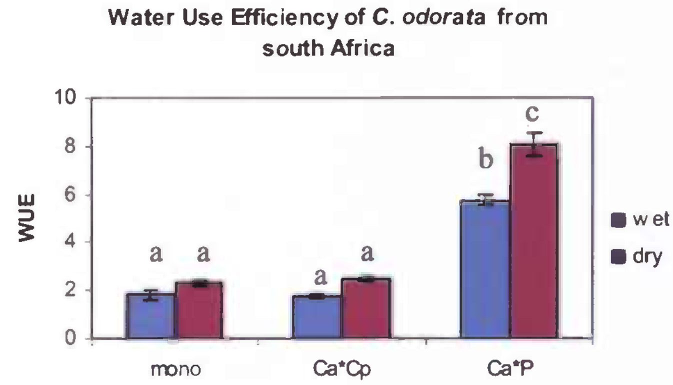
biomass	water treatment	Ca vs. Cp	Ca vs. P	Cp vs. P
stems	wet	0,672	*0,002*	*0,001*
leaves	wet	0,119	*0,006*	*0,000*
roots	wet	0,263	0,392	*0,000*
stems	dry	0,366	*0,000*	*0,000*
leaves	dry	*0,004*	*0,000*	*0,000*
roots	dry	*0,020*	*0,000*	*0,001*

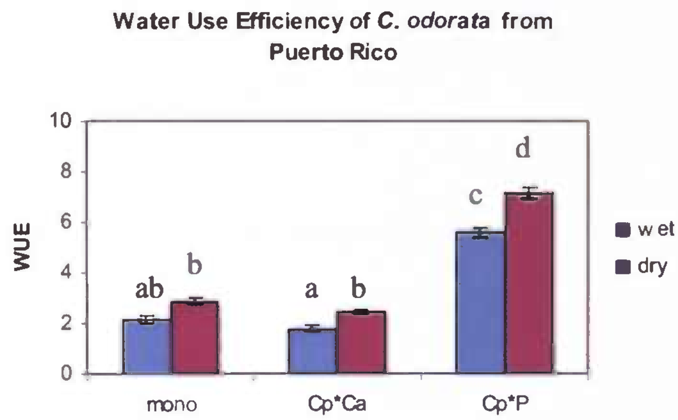
Appendix D: Analysis of variance (ANOVA). All effects tested for the Water Use Efficiency.

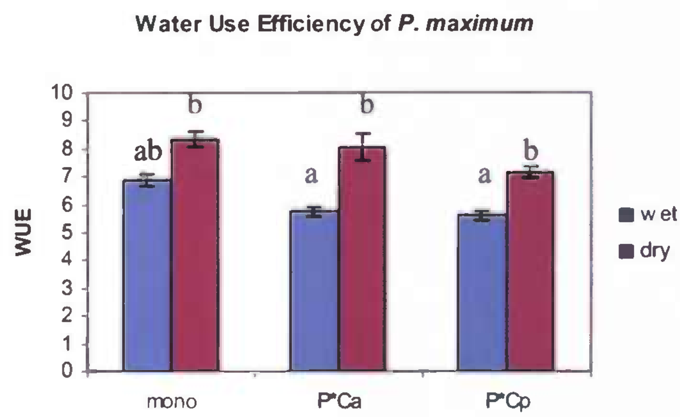
Univariate Tests of Significance for WUE (data per species per pot) Over-parameterized model Type III decomposition

	Effect	SS	Degr. of	MS	Den.Syn.	Den.Syn.	F	p
Intercept	Fixed	2028.498	1	2028.498	4.00010	1.456011	1393.188	0.000003
{1}block	Random	5.824	4	1.456	3.19987	0.776918	1.874	0.307669
{2}species	Fixed	303.682	2	151.841	8.01228	0.496491	305.829	0.000000
{3}monomix	Fixed	28.363	1	28.363	4.00037	0.394411	71.912	0.001060
{4}water	Fixed	34.756	1	34.756	4.00026	0.548000	63.423	0.001347
block*species	Random	3.969	8	0.496	7.29970	0.311753	1.591	0.272486
block*monomix	Random	1.578	4	0.394	4.18549	0.375426	1.051	0.478237
species*monomix	Fixed	51.107	2	25.553	8.04959	0.123381	207.109	0.000000
block*water	Random	2.192	4	0.548	6.90314	0.538571	1.017	0.460507
species*water	Fixed	3.016	2	1.508	8.02130	0.286526	5.263	0.034669
monomix*water	Fixed	2.246	1	2.246	4.00041	0.349858	6.421	0.064388
block*species*monomix	Random	0.984	8	0.123	8.00000	0.097423	1.263	0.374741
block*species*water	Random	2.289	8	0.286	8.00000	0.097423	2.937	0.074285
block*monomix*water	Random	1.399	4	0.350	8.06034	0.097783	3.578	0.058469
species*monomix*water	Fixed	0.513	2	0.256	8.06264	0.097796	2.623	0.132640
1*2*3*4	Random	0.779	8	0.097	90.00000	5.082792	0.019	0.999998
Error		457.451	90	5.083				

Appendix E: The graphs for the WUE of *C. odorata* from South Africa, Puerto Rico and *P. maximum*, both mono and mixed cultures.







Appendix F: Analysis of variance (ANOVA). All effects tested for the Leaf area Ratio (LAR) and Leaf Weight Ratio (LWR)

Univariate Tests of Significance for LAR Over-parameterized model Type III decomposition

	SS	Degr. of	MS	F	p
Intercept	7850869	1	7850869	2214.523	0.000000
species	1390976	2	695488	196.179	0.000000
monomix	25470	1	25470	7.184	0.008250
water	85680	1	85680	24.168	0.000002
species*monomix	11883	2	5941	1.676	0.190923
species*water	58086	2	29043	8.192	0.000435
monomix*water	577	1	577	0.163	0.687196
species*monomix*water	1875	2	937	0.264	0.768067
Error	489234	138	3545		

Univariate Tests of Significance for LWR Over-parameterized model Type III decomposition

	SS	Degr. of	MS	F	p
Intercept	22.00502	1	22.00502	11911.56	0.000000
species	0.77280	2	0.38640	209.16	0.000000
monomix	0.00442	1	0.00442	2.39	0.124088
water	0.01948	1	0.01948	10.55	0.001462
species*monomix	0.00438	2	0.00219	1.18	0.309094
species*water	0.00703	2	0.00352	1.90	0.152942
monomix*water	0.00008	1	0.00008	0.04	0.833262
species*monomix*water	0.00006	2	0.00003	0.02	0.984955
Error	0.25494	138	0.00185		

Appendix G: Analysis of variance (ANOVA). All effects tested for the Specific Leaf Area.

Univariate Tests of Significance for SLA (data per species per pot) Over-parameterized model Type III decomposition

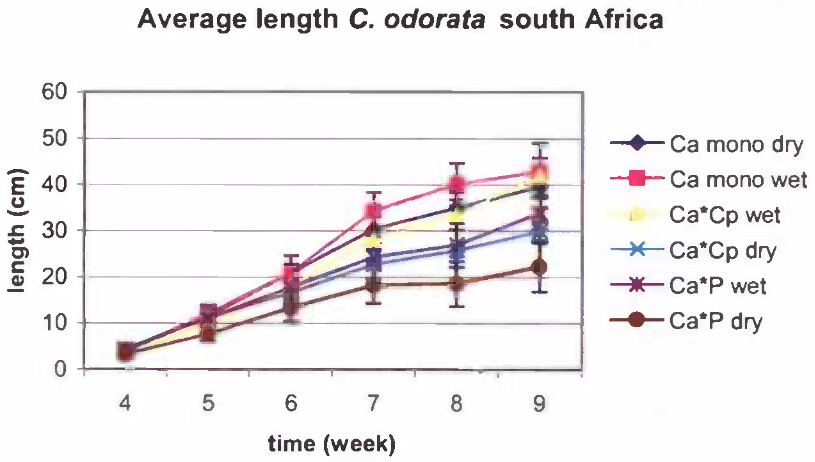
	Effect	SS	Degr. of	MS	Den.Syn.	Den.Syn.	F	p
Intercept	Fixed	30318402	1	30318402	4.00000	48032.97	631.1999	0.000015
{1}block	Random	192132	4	48033	4.69546	16315.78	2.9440	0.140687
{2}species	Fixed	2751626	2	1375813	8.00035	13065.83	105.2986	0.000002
{3}monomix	Fixed	42147	1	42147	4.00001	8104.94	5.2001	0.084751
{4}water	Fixed	127998	1	127998	4.00002	6298.81	20.3209	0.010757
block*species	Random	104532	8	13067	9.88902	8345.38	1.5657	0.249929
block*monomix	Random	32420	4	8105	6.60435	6383.20	1.2697	0.370277
species*monomix	Fixed	14422	2	7211	8.00084	5474.52	1.3172	0.320261
block*water	Random	25195	4	6299	6.12267	5679.33	1.1091	0.431077
species*water	Fixed	80425	2	40213	8.00097	4770.66	8.4292	0.010725
monomix*water	Fixed	583	1	583	4.00004	2808.81	0.2075	0.672343
block*species*monomix	Random	43797	8	5475	8.00000	1899.99	2.8814	0.077822
block*species*water	Random	38166	8	4771	8.00000	1899.99	2.5109	0.107219
block*monomix*water	Random	11235	4	2809	8.00234	1900.13	1.4782	0.295136
species*monomix*water	Fixed	13540	2	6770	8.00242	1900.14	3.5628	0.078237
1*2*3*4	Random	15200	8	1900	90.00000	3843.74	0.4943	0.857354
Error		345937	90	3844				

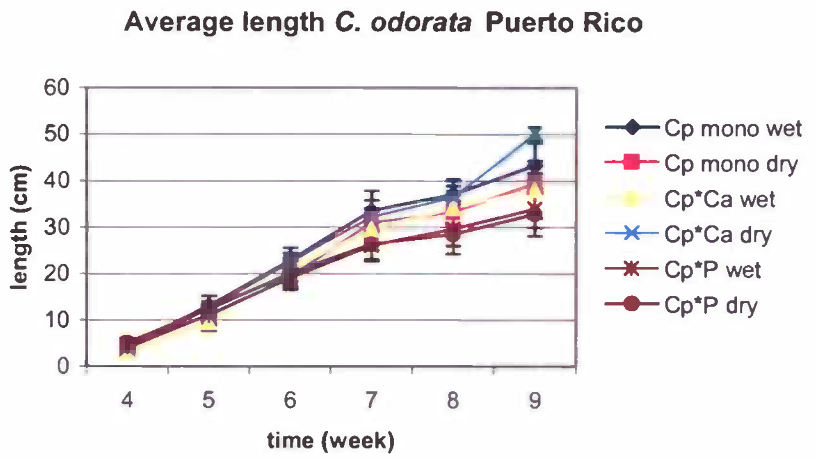
Appendix H: Analysis of variance (ANOVA). All effects tested for the shade (week 9).

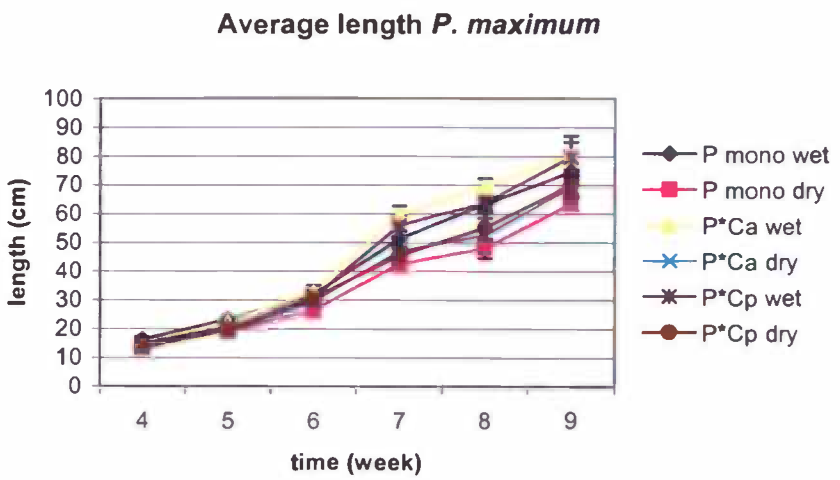
Univariate Tests of Significance for shade3 (calculations) Over-parameterized model Type III decomposition

	Effect	SS	Degr. of	MS	Den.Syn.	Den.Syn.	F	p
Intercept	Fixed	883383.7	1	883383.7	4.00018	402.0403	2197.252	0.000001
{1}block	Random	1608.3	4	402.1	0.90551	88.8427	4.526	0.360684
{2}monomix	Fixed	28.1	1	28.1	4.00333	27.8168	1.009	0.371908
{3}species	Fixed	1735.5	2	867.7	8.02514	28.6881	30.248	0.000183
{4}water	Fixed	46.5	1	46.5	4.02829	2.6339	17.640	0.013494
{5}nr plants	Fixed	40.0	1	40.0	4.00204	150.2084	0.266	0.633032
block*monomix	Random	111.2	4	27.8	1.43433	28.9189	0.962	0.597813
block*species	Random	229.3	8	28.7	1.20994	37.1505	0.771	0.700374
monomix*species	Fixed	635.7	2	317.9	8.01957	38.2140	8.318	0.011072
block*water	Random	10.5	4	2.6	0.98831	42.2801	0.062	0.983971
monomix*water	Fixed	17.7	1	17.7	4.00316	29.3321	0.603	0.480604
species*water	Fixed	53.3	2	26.6	8.02920	24.7131	1.077	0.385023
block*nr plants	Random	601.0	4	150.2	4.66660	67.4342	2.228	0.209424
monomix*nr plants	Fixed		0		0.00000	0.0000		
species*nr plants	Fixed	146.3	2	73.2	8.10108	36.3621	2.012	0.195253
water*nr plants	Fixed	14.1	1	14.1	4.00562	54.5449	0.259	0.637582
block*monomix*species	Random	305.5	8	38.2	8.00000	38.5985	0.989	0.505790
block*monomix*water	Random	117.3	4	29.3	8.01985	38.6208	0.759	0.579751
block*species*water	Random	197.5	8	24.7	0.00000			
monomix*species*water	Fixed	37.4	2	18.7	8.01937	38.6203	0.484	0.633154
block*monomix*nr plants	Random		0		0.00000	0.0000		
block*species*nr plants	Random	290.0	8	36.2	8.00000	23.3143	1.555	0.273378
monomix*species*nr plants	Fixed		0		0.00000	0.0000		
block*water*nr plants	Random	218.1	4	54.5	8.13677	23.4486	2.326	0.142592
monomix*water*nr plants	Fixed		0		0.00000	0.0000		
species*water*nr plants	Fixed	12.1	2	6.1	8.15739	23.4687	0.259	0.778172
1*2*3*4	Random	308.8	8	38.6	60.00000	72.5001	0.532	0.827489
Error		4350.0	60	72.5				

Appendix I: the length in mono and mixed cultures of *C. odorata* from South Africa (Ca), Puerto Rico (Cp) and *P. maximum* (P) at both wet and dry treatment from week 4 till week 9.







Appendix J: Analysis of variance (ANOVA). All effects tested for the length (week 9).

Univariate Tests of Significance for week 9 (length) Over-parameterized model Type III decomposition

	Effect	SS	Degr. of	MS	Den.Syn.	Den.Syn.	F	p
Intercept	Fixed	835478.1	1	835478.1	3.5295	207.967	4017.365	0.000002
block	Random	886.1	4	221.5	1.1572	1780.458	0.124	0.954508
plant	Fixed	50160.9	2	25080.5	8.0954	1241.940	20.195	0.000714
content	Fixed	4747.1	5	949.4	20.0678	437.645	2.169	0.098440
waterlevel	Fixed	2694.8	1	2694.8	4.1092	284.857	9.460	0.035781
block*plant	Random	10103.2	8	1262.9	3.9257	753.470	1.676	0.327134
block*content	Random	8780.5	20	439.0	2.4558	782.136	0.561	0.810743
block*waterlevel	Random	1149.9	4	287.5	18.9747	190.373	1.510	0.239113
plant*content	Fixed	118.6	1	118.6	4.0331	740.567	0.160	0.709369
plant*waterlevel	Fixed	899.5	2	449.7	283.0000	327.671	1.373	0.255150
content*waterlevel	Fixed	1661.8	5	332.4	46.6788	225.263	1.475	0.215943
block*plant*content	Random	2977.8	4	744.4	283.0000	327.671	2.272	0.061696
block*content*waterlevel	Random	3618.1	19	190.4	283.0000	327.671	0.581	0.918540
plant*content*waterlevel	Fixed	1133.4	1	1133.4	283.0000	327.671	3.459	0.063949
Error		92730.8	283	327.7				

Appendix K: the Tuckey HSD tests of initial seed and seedling masses of *P. maximum* and both *C. odorata* species (Figure 23).

Seed masses:

Tukey HSD test; variable seed (initielezaailinggewichten) Approximate Probabilities for Post Hoc Tests Error: Between MS = .07633, df = 27.000

	Species	{1}	{2}	{3}
1	Panicum		0.040120	0.190109
2	Chromo_PR	0.040120		0.714833
3	Chromo_SA	0.190109	0.714833	

Seedling masses on 18th of May:

Tukey HSD test; variable seedling 1 (initielezaailinggewichten) Approximate Probabilities for Post Hoc Tests Error: Between MS = .01581, df = 27.000

	Species	{1}	{2}	{3}
1	Panicum		0.000127	0.000127
2	Chromo_PR	0.000127		0.224284
3	Chromo_SA	0.000127	0.224284	

Seedling masses on 23rd of May:

Tukey HSD test; variable seedling 2 (initielezaailinggewichten) Approximate Probabilities for Post Hoc Tests Error: Between MS = .06878, df = 27.000

	Species	{1}	{2}	{3}
1	Panicum		0.000156	0.000185
2	Chromo_PR	0.000156		0.968534
3	Chromo_SA	0.000185	0.968534	