=BOTANY HONOURS PROJECT 1989=

R. T. TSHIVHANDEKANO

FACTORS POSSIBLY INHIBITING GROWTH OF HERBACEOUS PLANTS IN THE UNDERSTORIES OF EUCALYPTUS CINEREA,

=BOTANY HONOURS PROJECT 1989=

R. T. TSHIVHANDEKANO

FACTORS POSSIBLY INHIBITING GROWTH OF HERBACEOUS PLANTS IN THE UNDERSTORIES OF EUCALYPTUS CINEREA,

Eucalyptus baxteri has been reported to produce a zone of suppression beneath its canopy when growing in coastal heath (De Moral, Willis and Ashton 1978). In their studies, investigations of ecophysiological parameters of soil water potential, soil nutrient levels and shading failed to account for suppression of understorey species. Their studies have shown that suppression of herbaceous layer species beneath the canopy of E. baxteri appear to be of an allelopathic rather than a competitive nature.

Although the suppression of growth of herbaceous layer species under the understoreys of so many different Eucalyptus species has been attributed to allelochemicals produced by these trees, it is possible that suppression is due to other factors rather than toxicity of soil beneath Eucalytus stands. The effects of plant competition for light, water and nutrients cannot totally be ignored when factors affecting the growth of herbaceous layer species under Eucalyptus understories are considered. For example, Lamont (1985) demonstrated that allelochemicals produced by leaves of Eucalyptus wandoo were not responsible for the suppression of herbaceous plants in the understorey of this plant species. From his study, it was found that competition for water at depth between the extensive lateral root system of E. wandoo trees and roots of adult shrubs was a more likely explanation for genesis of a suppression zone and location of its boundary.

Therefore, to determine the meaning of Eucalyptus undergrowth effects, careful chemical detective work may be necessary. One need to establish first that the effect is in fact chemical and

=BOTANY HONOURS PROJECT 1989=

R. T. TSHIVHANDEKANO

FACTORS POSSIBLY INHIBITING GROWTH OF HERBACEOUS PLANTS IN THE UNDERSTORIES OF EUCALYPTUS CINEREA,

Eucalyptus baxteri has been reported to produce a zone of suppression beneath its canopy when growing in coastal heath (De Moral, Willis and Ashton 1978). In their studies, investigations of ecophysiological parameters of soil water potential, soil nutrient levels and shading failed to account for suppression of understorey species. Their studies have shown that suppression of herbaceous layer species beneath the canopy of E. baxteri appear to be of an allelopathic rather than a competitive nature.

Although the suppression of growth of herbaceous layer species under the understoreys of so many different Eucalyptus species has been attributed to allelochemicals produced by these trees, it is possible that suppression is due to other factors rather than toxicity of soil beneath Eucalytus stands. The effects of plant competition for light, water and nutrients cannot totally be ignored when factors affecting the growth of herbaceous layer species under Eucalyptus understories are considered. For example, Lamont (1985) demonstrated that allelochemicals produced by leaves of Eucalyptus wandoo were not responsible for the suppression of herbaceous plants in the understorey of this plant species. From his study, it was found that competition for water at depth between the extensive lateral root system of E. wandoo trees and roots of adult shrubs was a more likely explanation for genesis of a suppression zone and location of its boundary.

Therefore, to determine the meaning of Eucalyptus undergrowth effects, careful chemical detective work may be necessary. One need to establish first that the effect is in fact chemical and

not one of competition between plants for light, water or nutrients. If it is possible, one must show that under natural conditions the quantitative relations of the chemical agents identified as they occur in the soil are adequate to produce the observed degree of inhibition of other plants which can be difficult to prove (De Moral et al. 1970).

In Cape Town (South Africa), below Table Mountain, there is a bare area that resulted from the removal of an Eucalyptus cinerea stand. The strange thing about this bare area, is that from the time the Eucalytus were clear-felled, it has been very difficult for plant species to regenerate in that bare area. The bare area has been apparent for about 5 years.

The main objective of this study was to try and isolate these factors which might have been responsible for the suppression of growth under Eucalyptus stands, and their subsequent effect on regrowth after clear-felling. Therefore this study was undertaken to test the following hypotheses.

(1) Allelochemical Hypothesis

Since the genus Eucalyptus has been shown to produce several volatile terpenes, of which several have been shown to be toxic to seed germination and seedling growth, Eucalyptus trees might have been produced allelochemicals that were leached into the soil. These allelochemicals might be the ones that are responsible for inhibiting regeneration of plant species, even after the Eucalyptus cinerea trees were removed.

(2) Topsoil Erosion Hypothesis

Since the understories of Eucalyptus were not rich in vegetation cover, the top soil, which is rich in nutrients and good for seedling establishment, was eroded down the slope. As a result, only the sub-soil was left. Plants were therefore unable to establish themselves in soils of such poor nutritional status with such low seed banks.

(3) Soil Nutrient Depletion Hypothesis

Eucalyptus might have depleted most of the soil nutrients before they were clear-felled. Therefore, even after their removal, the nutrient status of the soil was poor in such a manner that very few plant species could establish themselves.

STUDY AREA

The study site is one Table Mountain, Cape Town (34°50'S, 33°25'W) in South Africa. Soil samples were collected fom three sites, A,B and C. Site A and B are situated on Signal Hill. Site C and D (D=Eucalyptus cinerea removed area) are situated on the northern part of Table Mountain (see map).

CLIMATE. The general study area fall within the Mediterranean climate zone of the Soth western Cape. The climate is characterized by cool, wet winters and hot, dry summers.

Sites A and B are East-facing with A having a slope of 70° and B 60°. Both sites A and B are 350m above sea level with an annual rainfall of less than 889mm. Soil of both sites A and B is of granitic origin with a high proportion of clay and being less acidic. Sites C and D are North-facing with C having a slope of 50° and D being 60°. Site C is 340m above sea level whereas site is 360m above sea level, both with an annual rainfall of 1420mm. Soil in both site C and D is derived from Table Mountain sandstone with a high proportion of sand and being more acidic.

4)MATERIALS AND METHODS

4.1)SOIL SAMPLING PROCEDURE

Soils were collected from three different sites (A, B, and C). Site A was a Eucalyptus cinerea stand as well as its adjacent fynbos stand from Signal Hill as was Site B. Site C was an E. cinerea and an adjacent control plot on the slopes of Table Mountain (see map). At site A, topsoil (0-2cm) and subsoil (2-5cm deep) were collected from the E. cinerea stand as well as its adjacent fynbos stand. This sampling was done on 28 March 1989. At site B and C, only topsoil samples (0-2cm) from the E. cinerea stand as well as from its adjacent fynbos stand were collected. Soil sampling at sites B and C was done on 15 August 1989. All the soil samples were sieved through a 2mm sieve prior to use in the bioassays.

4.2)TESTING HYPOTHESES

4.2.1ALLELOCHEMICAL HYPOTHESIS

In trying to test the allelochemical hypothesis, the following methods were employed:-

a) Heat destruction of the allelochemicals.

This was done by comparing the growth of bioassay radishes grown in autoclaved soil collected from the E. cinerea stand with the growth of bioassay radishes in non-autoclaved soil also collected from the E. cinerea stand (experimental details in section 4.3). The aim of autoclaving the soil was to denature allelochemicals if any was in the soil. Therefore, it was expected that if allelochemicals are present in the soil and autoclaving was able

to denature them, then bioassay plants grown in non-autoclaved soil should grow significantly smaller than bioassay plants grown in autoclaved soil.

b) Addition of allelochemicals in leachate watering.

Growth of bioassay radishes (grown in autoclaved soil from the Eucalyptus stand) watered with E. cinerea leaf extracts was compared with bioassay radishes (also grown in autoclaved soil from the E. cinerea stand) watered with distilled water. This was done to find out if watering the bioassay radishes with E. cinerea leaf extracts had any inhibiting effect on the growth of these bioassay species.

c) Comparing the growth of bioassay species in adjacent sites.

Growth of bioassay species (radishes and Eragrostis) in soil collected from the E. cinerea stand was compared with growth of the same bioassay species in soil collected from the fynbos stand. This was done to find out if E. cinerea trees have any negative effects on the soil, probably through allelochemicals that can be leached from the leaves of these trees into the soil (experimental details in section 4.3).

4.2.2 NUTRIENT DEPLETION HYPOTHESIS

This hypothesis was tested by adding fertilisers to soils collected from the E. cinerea stand. Growth of bioassay species in fertilized soil was compared with growth bioassay species in unfertilized soil. This was done to test if addition of fertilizers had any beneficial effect on the growth of bioassay species. The overall aim was to test if growth under E. cinerrea

stands is inhibited because of insufficient nutrients in the soil (experimental detail in section 4.3).

4.2.3 EROSION HYPOTHESIS

This hypothesis was tested by comparing the growth of bioassay species grown in topsoil with growth of bioassay species grown in subsoil. This test was done for both soil collected from the E. cinerea stand and from the fynbos stand (see section 4.3 for experimental details).

4.3 EXPERIMENTAL DETAILS

4.3.1 DIVISION OF SOILS INTO DIFFERENT TREATMENTS

Both topsoils and subsoil (from the E. cinerea and its adjacent fynbos stand) collected at site A was divided into three treatments. One treatment was fertilized, another one was autoclaved and the remaining one was the control (neither fertilized nor autoclaved).

Topsoil samples collected from site B and C (both from the E. cinerea stand and the fynbos stand) were divided into two treatments. One treatment was autoclaved whereas the remaining treatment was taken as the control.

4.3.2 PREPARATION OF DIFFERENT SOIL TREATMENTS AND LEAF LEACHATES

AUTOCLAVING- Soil was autoclaved for one hour at 96 ^{o}C and 14mmHG atmospheric pressure.

FERTILIZATION- In order to fertilize the soil, 10g of slow release nitrogen fertilizers containing 11% N, 7.3% P, 3.7% K,

9.4% Ca and 10% S was mixed with 1kg of soil.

LEAF LEACHATES- For preparation of leaf leachates, fresh leaves of E. cinerea was collected from site A. In the laboratory, 100g of these leaves were soaked in 5 litres of distilled water and shaken for one hour. After shaking, the leaves were filtered and the leachates were preserved for use in watering some of the bioassay species. The leaf leachate prepared at one time was used to water the bioassay species for 5 days after which it was renewed.

4.3.3 GROWTH MEDIUM AND GERMINATION OF SEEDS

Each soil treatment was placed into 250ml foamalite cups. For soil collected from site A, fertilized, control and autoclaved (both from the E. cinerea stand and its adjacent fynbos stand) soil treatments were replicated ten times (10 cups for each treatment except the autoclaved treatment from the E. cinerea stand which was replicated 20 times). In each cup, 3 radish seeds were planted.

For soil samples collected from sites B and C, each soil treatment was replicated 20 times (20 replicate cups for each treatment). Of these 20 replicate cups, 10 were planted with radish seeds (3 per pot) whereas the remaining 10 were planted with Eragrostis turf seeds (0.2 g of seeds per pot).

4.3.4 GROWTH CONDITIONS

All bioassay plant species were germinated and grown in a controlled growth room with 15h:9h light:dark photoperiod and at

an irradiance of 370-420 umol.m^{-1} s^{1} . Conditions during the light and dark periods were 29°C at 60% relative humidity and 24-34°C at 60% relative humidity respectively.

4.3.5 WATERING AND LENGTH OF GROWTH PERIOD

All the pots except 20 pots containing the autoclaved soil from the E. cinerea stand were watered with distilled water 3 times a week. The 20 pots containing the autoclaved soil from the E. cinerea stand (10 with topsoil and another 10 with subsoil and each pot having 3 radishes) were watered with leaf leachates extracted from fresh leaves of E. cinerea 3 times a week.

4.3.6 HARVESTING TECHNIQUES

After 38 days and 42 days, radishes and Eragrostis tef were harvested respectively. In the case of radish, when harvesting, the roots were carefully washed and separated from the shoots. That is, for each individual radish plant, the shoot was separated from the roots.

In case of Eragrostis tef, roots were also carefully washed and separated from the shoots. As it is very difficult to separate each individual, all the plants in one pot was taken as one sample.

After separating the shoots from the roots (for both radishes and Eragrostis, both roots and shoots were oven-dried for 2 days at 80'C. After drying, both the roots and shoots were weighed on an AE 100 Mettler four decimal balance.

4.3.7 STATISTICAL ANALYSES

T-tests for independent samples were performed to determine if there was any significant difference in growth of bioassay species in similar treated soils from different stands (E. cinerea and fynbos stand).

For, example, using this test, growth of species in autoclaved soil from the E. cinerea stand was compared with growth of similar bioassay species in autoclaved soil from the fynbos stand, etc.

The same test (T- test for independent samples) was performed to determine if there was any significant difference in growth of bioassay species in fertilized soil and unfertilized soil, autoclaved and non-autoclaved soil, topsoil and subsoil, leachate-watered soil and distilled-watered soils. All results are presented as means and their standard errors.

Table 1. Growth response of radishes and Eragrostis plants to autoclaved soil and non autoclaved soil from E. cinerea stands situated on Signal Hill (site A and B) and Table Mountain. The data presented is the mean plant dry mass ± standard error.

SITE OF SOIL COLLECTION	BIOASSAY SPECIES	AUTOCLAVED Plant dry mass (g)	UNAUTOCLAVED Plant dry mass (g)	
Signal Hill (A)	Radishes	0.60±0.06	0.40±0.03	NS
Signal Hill (B)	Radishes	0.28±0.03	0.31±0.03	NS
" " "	Eragrostis	1.01±0.09	1.21±0.03	NS
Table Muontain	Radishes	0.20±0.01	0.20±0.01	NS
" "	Eragrostis	0.67±0.04	0.65±0.03	NS

NS= non significant (P>0.05) ; t-test.

Table 2. Growth response of radishes grown in heated topsoil and watered with either distilled water or leaf leachates extracted from the fresh leaves of E. cinerea. Presented on the table is the mean total plant dry masses of bioassay radishes plants ± standard error

DISTILLED WATER	LEACHATES	SIGNIFICANCE
Plant dry mass(g)	Plant dry mass (g)	
0.64±0.06	1.01±0.06	** (p < 0.05)

** = significanc (p< 0.05) ; t-test.

5) RESULTS

5.1 ALLELOCHEMICAL HYPOTHESIS

a) HEAT DESTRUCTION OF ALLELOCHEMICALS.

For both soil collected from Signal Hill and Table Mountain (from the E. cinerea stand), neither radishes nor Eragrostis tef showed any significant growth response to autoclaving. That is, the growth of both these plants (E. tef and radishes) grown in autoclaved soil was not significantly different from the growth of the same bioassay species grown in non-autoclaved soil (Table 1).

b) WATERING WITH LEACHATES

Radishes grown in autoclaved soil from the E. cinerea stand and watered with E. cinerea leaf extracts were significantly larger than the growth of the same bioassay species grown in autoclaved soil from the E. cinerea stand, but watered with distilled water. This shows that watering the radishes with the E. cinerea leaf extracts had a beneficial effect rather than an inhibitory effect on their growth (Table 2).

c) COMPARING GROWTH OF BIOASSAY SPECIES IN ADJACENT SITES.

c) (i) Signal Hill (A)- Growth of radishes in fertilized soil from the E. cinerea stand was significantly higher than the growth of radishes in fertilized soil from the fynbos stand. However, in the case of autoclaved and control soil treatments, there was no significant difference in the growth of radishes grown in soil from Eucalyptus and those grown in fynbos soil (see Table 3).

Table 3. Response of plant size of radishes and eragrostis grown in diiferently treated topsoil from E.cinerea stand and the adjacent fynbos stands situated in three different sites, namely A, B, C.(see section 1 for more detail about the sites.) . Both Radishes and Erasgrostis plants were harvested and the mean plant dry masses ± standard errors of these bioassay plants are shown.

SITE OF SOIL COLLECTION	BIOASSAY SPECIES	TREATMENTS	EUCALYPTUS STAND Plant dry mass (g)	FYNBOS STAND Plant dry mass (g)	
Signal Hill(A)	Radishes	Fertilized	1.30±0.08	0.82±0.05	**
	aa	Autoclaved	0.60±0.06	0.37±0.06	NS
	aa	Control	0.31±0.03	0.17±0.02	NS
Signal Hill (B)	Radishes	Autoclaved	0.28±0.03	0.49±0.04	**
	aa	Control	0.31±0.01	0.26±0.03	NS
	Eragrostis	Autoclaved	1.39±0.09	1.02±0.10	NS
	aa	Control	0.68±0.10	1.21±0.08	**
Table Mountain	Radishes	Autoclaved	0.20±0.01	0.41±0.04	NS
	aa	Control	0.20±0.01	0.19±0.01	NS
	Eragrostis	Autoclaved	0.68±0.04	1.04±0.09	**
	aa	Control	0.65±0.03	0.46±0.03	NS

**= Significant ($p<0.05$); t-test.
NS= Not significant ($p>0.05$) ; t-test.

Table 3. Response of plant size of radishes and eragrostis grown in diiferently treated topsoil from E.cinerea stand and the adjacent fynbos stands situated in three different sites, namely A, B, C.(see section 1 for more detail about the sites.) . Both Radishes and Erasgrostis plants were harvested and the mean plant dry masses + standard errors of these bioassay plants are shown.

SITE OF SOIL COLLECTION	BIOASSAY SPECIES	TREATMENTS	EUCALYPTUS STAND	FYNBOS STAND	
			Plant dry mass (g)	Plant dry mass (g)	
Signal Hill(A)	Radishes	Fertilized	1.30±0.08	0.82±0.05	**
	"	Autoclaved	0.60±0.06	0.37±0.06	NS
	"	Control	0.31±0.03	0.17±0.02	NS
Signal Hill (B)	Radishes	Autoclaved	0.28±0.03	0.49±0.04	**
	"	Control	0.31±0.01	0.26±0.03	NS
	Eragrostis	Autoclaved	1.39±0.09	1.02±0.10	NS
	"	Control	0.68±0.10	1.21±0.08	**
Table Mountain	Radishes	Autoclaved	0.20±0.01	0.41±0.04	NS
	"	Control	0.20±0.01	0.19±0.01	NS
	Eragrostis	Autoclaved	0.68±0.04	1.04±0.09	**
	"	Control	0.65±0.03	0.46±0.03	NS

**= Significant ($p<0.05$); t-test.
NS= Not significant ($p>0.05$) ; t-test.

Table 4. Growth response of Radish bioassay plants to addition of fertilizers in the top and subsoil samples from E.cinerea stand. Presented on the table is the plant dry mass+ standard error.

DEPTH	UNFERTILIZED	FERTILIZED	
	Plant dry mass	Plant dry mass	
Topsoil	0.39±0.03	1.30±0.08	**
Subsoil	0.12±0.01	0.50±0.03	**

**= Significant ($p<0.05$); t-test.

c) (ii) Signal Hill (B)- Growth of radishes in autoclaved soil from the E. cinerea stand was significantly lower than the growth of radishes in autoclaved soil from the fynbos stand. There was no significant difference in the growth of radishes in control soil from the E. cinerea stand and in soil from the fynbos stand (Table 3).

The response of Eragrostis tef (monocot) was different from that shown by the radishes (dicots). The growth of Eragrostis tef in autoclaved soil from the E. cinerea stand was not significantly different from their growth in autoclaved soil from the fynbos stand. On the other hand, growth of Eragrostis tef in control soil from the E. cinerea stand was significantly lower than their growth in control soil from the fynbos stand (Table 3).

c) (iii) Table Mountain- In both treatments (heated and control), the growth of radishes in soil from the E. cinerea stand was not significantly different from their growth in soil from the fynbos stand (Table 3). Growth of Eragrostis tef in autoclaved soil from the E. cinerea stand was significantly lower than their growth in autoclaved soil from the fynbos stand. There was no significant difference in growth of Eragrostis tef in control soil from the E. cinerea stand and their growth in control soil from the fynbos stand (Table 3).

5.2 NUTRIENT DEPLETION HYPOTHESIS.

There was a positive plant growth response in addition to fertilizers to both topsoil and subsoil from the E. cinerea stand. The mean total plant dry mass of plants grown in fertilized soil from the E. cinerea stand was significantly

Table 4. Growth response of Radish bioassay plants to addition of fertilizer in the top and subsoil samples from E.cinerea stand. Presented on the table the plant dry mass± standard error.

DEPTH	UNFERTILIZED	FERTILIZED	
	Plant dry mass	Plant dry mass	
Topsoil	0.39±0.03	1.30±0.08	**
Subsoil	0.12±0.01	0.50±0.03	**

**= Significant ($p<0.05$); t-test.

Table 5. Growth response of radish bioassay plants in topsoil and in subsoil from E.cinerea stand.The differences in growth of plants between similar treated topsoil and subsoil is also shown. Presented on the table is the plant dry mass ±1SE

TREATMENTS	TOPSOIL Plant dry mass (g)	SUBSOIL Pant dry mass (g)	
Fertilized	1.30±0.08	0.51±0.03	**
Autoclaved	0.64±0.06	0.14±0.01	**
Control	0.39±0.03	0.12±0.01	**

** = Significant ($p<0.05$) ; t-test.

Table 1. Growth response of radishes and Eragrostis plants to autoclaved soil and non autoclaved soil from E. cinerea stands situated on Signal Hill (site A and B) and Table Mountain. The data presented is the mean plant dry mass ± standard error.

SITE OF SOIL COLLECTION	BIOASSAY SPECIES	AUTOCLAVED Plant dry mass (g)	UNAUTOCLAVED Plant dry mass (g)	
Signal Hill (A)	Radishes	0.60±0.06	0.40±0.03	NS
Signal Hill (B)	Radishes	0.28±0.03	0.31±0.03	NS
" " "	Eragrostis	1.01±0.09	1.21±0.03	NS
Table Muontain	Radishes	0.20±0.01	0.20±0.01	NS
" "	Eragrostis	0.67±0.04	0.65±0.03	NS

NS= non significant (P>0.05) ; t-test.

Table 2. Growth response of radishes grown in heated topsoil and watered with either distilled water or leaf leachates extracted from the fresh leaves of E. cinerea. Presented on the table is the mean total plant dry masses of bioassay radishes plants ± standard error

DISTILLED WATER	LEACHATES	SIGNIFICANCE
Plant dry mass(g)	Plant dry mass (g)	
0.64±0.06	1.01±0.06	** (p < 0.05)

** - significanc (p< 0.05) ; t-test.

higher than the mean total plant dry mass of radishes grown in unfertilized soil from the E. cinerea stand. These results shows that addition of fertilizers to soil from the E. cinerea stand had a beneficial effect on the growth of radishes (Table 4).

5.3 EROSION HYPOTHESIS.

Growth of radishes in topsoil from the E. cinerea stand was significantly higher than the growth of this bioassay species in subsoil from the same stand (Table 5).

6)DISCUSSION

6.1) ALLELOCHEMICAL HYPOTHESIS.

a) Heat destruction of allelochemicals

Both radishes (dicots) and Eragrostis tef (monocots) grown in autoclaved soil collected from the Eucalyptus stands were not significantly different from those grown in soil collected from the fynbos stand.The idea of autoclaving the soil was to see if , by autoclaving, allelochemicals that might be in the soil might be denatured. Christensen and Muller (1975) observed high germination and seedling growth in a growth suppression zone located in Adenostoma chaparral. Since Adenostoma chaparral is known for its allelopathic inhibition of other plants, germination of most suppressed plants after a fire has been attributed to denaturing of growth inhibiting chemicals in the soil. In this study, autoclaving the soil did not have any beneficial effect on the growth of bioassayed plants. These results can mean that the soil was not heated enough to denature the allelochemicals. That is why there was no significant difference in growth of plants grown in autoclaved soil and non-autoclaved soil.

Even if plants grown in autoclaved soil could have grown bigger than plants grown in non-autoclaved soil, it would not have been totally wise to attribute the better growth response of autoclaved grown plants to the denaturing of growth inhibiting chemicals, since heating the soil can also result in more nutrient release into the soil, resulting in better growth. Therefore, better growth of plants in autoclaved soil can also be due to the higher availability of nutrients. I think that before

the idea that heating can denature allelochemicals can be expected to be true, more work should first be done in this field. The possible way would be to identify if there is any presence of allelochemicals in the soil before heating, and also, do the same procedure after the soil has been heated and see if the allelochemicals will still be in the soil or not. If after heating allelochemicals are no longer in the soil, then it can be concluded that heating denature allelochemicals in the soil.

b) Watering with extracts

Lack of vegetation in Eucalyptus understories has been attributed to allelochemicals that are leached from the leaves of Eucalyptus into the soil by rain water. Laef leachates of Eucalyptus (hybrid) were found to inhibit the growth and germination of certain food crops (Raw and Reddy 1984). Leaf extracts of Eucalyptus camaldulensis were also found to be inhibiting to the growth of certain plants (De Moral and Muller 1970). In this study, radish plants that were watered with leaf extracts of Eucalyptus cinerea were found to be significantly larger than radish plants that were watered with distilled water. Since the genus Eucalyptus have demonstrated to produce several volatile terpenes that are toxic to seed germination and seedling growth of numerous species of plants (De Moral and Muller 1970), it was expected in this experiment that leaf leachates of Eucalyptus will reduce the germination and also the growth of radish seedlings. However, the opposite situation was found. The leaf extracts of Eucalyptus cinerea promoted growth of radish seedlings as compared to ones watered with distilled water. The

possible factor causing Eucalyptus cinerea leaf leachates to enhance growth of radish seedlings can be, additional supply of nutrients leached from leaves of Eucalyptus cinerea leaves. Tukey (1966) have shown that a whole range of substances including nutrients, amino acids, various carbohydrates and organic acids, among them phenolics, are leached from the above-ground plant surfaces to the soil in quite significant amounts.

The aim of doing this experiment was to test if growth of herbaceous layer species in the understories of Eucalyptus stands is inhibited by allelochemicals that are leached from the leaves of these trees into the soil. Since watering with extracts of Eucalyptus leaves did not show any negative effects on the growth of radishes that were used as bioassays, the question to ask is whether failure of Eucalyptus cinerea leaf leachates to inhibit growth of radishes demonstrated the absence of allelochemicals in the leaves of Eucalyptus cinerea or not.

Since leaves of Eucalyptus cinerea were only soaked in distilled water for one hour, that could have been insufficient time to extract allelochemicals from the leaves of Eucalyptus. Allelochemicals of one plant species cannot inhibit the growth of all plant species. That is why even under the Eucalyptus stand there are some plant species that manage to survive. Therefore, inability of Eucalyptus cinerea leaf leachates to inhibit growth of radishes cannot only show that leaves of Eucalyptus cinerea have no allelochemicals, but can also show that radish plants are not inhibited by allelochemicals produced by the leaves of

Eucalyptus. As far as I am concerned failure of Eucalyptus leaf leachates to inhibit radish growth cannot disqualify the hypothesis that understories of Eucalyptus stands are devoid of vegetation due to allelopathic effects. I suggest further tests need to be done. It could have been much more meaningful if species that are found adjacent to Eucalyptus stands, but fail to establish themselves under Eucalyptus trees were used as bioassay species. Only when those plant species showing to be very sensitive to growing under Eucalyptus stands in the field have been tested as bioassays, and been found not to be inhibited by Eucalyptus leaf extracts; would I then reject the hypothesis that allelochemicals leached from the leaves of Eucalyptus cinerea are inhibiting plant growth in E.cinerea understories.

Apart from being leached from leaves, allelochemicals can also be volatilized from the leaves into the atmosphere, and finally concentrate into the soil and inhibit plant growth. They can also be released into the soil as the leaf litter decompose or washed into the soil by rain water (Baker 1966, De Moral and Muller 1970, De Moral and Ashton 1978, Rao and Reddy 1984).

Since watering the radish plants with leaf extracts of Eucalyptus cinerea failed to show any role that Eucalyptus leaf leachates might play in inhibiting plant growth in their understories, I strongly suggest that more research work should be done to find out if the lack of vegetation under Eucalyptus understories is not due to allelopathic effects. I think the following can be done to validate the presence or absence of allelochemicals in

the soil:-

(1) An analysis of soils from the Eucalyptus stand to find out if there are any substances that are known to be toxic. If these are known to be in the Eucalypytus leaves, this could answer the allelochemical suppression hypothesis.

(2)Attempt to isolate terpenes from leaves of Eucalyptus cinerea as these were found to inhibit growth of other plants. This would be another way of showing if allelopathy is contributing to growth inhibition in Eucalyptus understories. This should also be attempted on the leaf litter under Eucalyptus stands.

(3) I also suggest that the natural fynbos species that show the tendency of avoiding Eucalyptus stands be used as bioassays instead of quickly growing crop species. Using several kinds of plants as bioassays could also be of great assistance in answering the allelochemical suppression hypothesis.

c) Comparing growth in adjacent stands

Radish growth in soils from the E. cinerea and fynbos stands did not show any significant differences (Table 3). If growth inhibiting allelochemicals had been leached from the fresh leaves and leaf litter, or exuded from the roots of E. cinerea into the soil beneath them, then soil collected from the E. cinerea stand would be have an inhibitory effect on the growth of radishes. However, the results of this study show this not to be the case. Absence of significant inhibitory effects of soil from the E. cinerea stand on the growth of radishes may be interpreted in the following way:-

There are no growth inhibiting allelochemicals that are

transferred from E. cinerea trees into the soil in one or more of the above mentioned ways. This interpretation must be treated with caution since growth inhibiting allelochemicals can be present in the soil but just unable to inhibit the growth of radishes either because radishes are more tolerant to these allelochemicals than plant species that are inhibited in the field or because the negative effects of allelochemicals were cancelled by the favourable growth conditions in the laboratory. This second possibility could be tested by growing radishes under E. cinerea and in fynbos stands (in the field) and see if the same results as those found in the laboratory are found.

Of great interest is the significantly higher growth of Eragrostis tef bioassay plants grown in control soil from one fynbos stand (Signal Hill (B)) as compared to the growth of the same bioassay species grown in soil from its adjacent E. cinerea stand (Signal Hill (B)). On the other hand, growth of the same bioassay plant species (Eragrostis) in the soil from a second stand (Table Mountain) was not significantly different from that of the same bioassay species grown in its adjacent E. cinerea stand soil.

Reduction of growth of Eragrostis bioassays in control soil from the E. cinerea stand can be due to allelochemicals that are present in the soil. As explained in the case of radishes that allelochemicals might be present in the soil but failing to inhibit radishes because of their greater tolerance, Eragrostis (monocots) plants are possibly more sensitive to these allelochemicals and those may have resulted in a significant

growth reduction.

If the growth reduction of Eragrostis in control soil from the E. cinerea stand situated on Signal Hill is due to the presence of allelochemicals produced by E. cinerea trees, why then is the growth of Eragrostis in control soil from the E. cinerea stand situated on Table Mountain not reduced when compared to their growth in control soil from fynbos? A possible explanation that I consider to explain the discrepancy shown by Eragrostis tef bioassays is that even though Eucalyptus stands on Signal Hill and Table Mountain are composed of similar Eucalyptus species that produce similar allelochemicals, the effects of these allelochemicals can also be largely determined by the type of soil into which they are leached. Certain soil types are able to absorb allelochemicals so that their concentration is high enough to suppress growth of other plants whereas accumulation of allelochemicals in other soil types may be difficult. This may be due to the physical structure of the soil (i.e. clay content) and even the presence of soil microorganisms that may play a role in the denaturation of these allelochemicals . It was found that loam soils are able to retain a high proportion of toxic factors from infiltrating solutions than do sands (De Moral and Muller 1970). Muller (1966) found allelopathy to also be intensified by drought. Soil from Signal Hill with its higher clay content probably accumulates higher concentrations of allelochemicals than the more sandy Table Mountain sandstone soil.
If this is the case, why then are the understories of both Eucalyptus stands deviod of vegetation? This question forces one

to doubt that the presence of allelochemicals is the only factor responsible for inhibition of herbaceous plant growth in the understories of Eucalyptus stands.

If by autoclaving the soil growth inhibiting allelochemicals were denatured as was expected, growth of bioassay species in autoclaved soil from E. cinerea stands should not have been lower than growth of the same bioassay species in autoclaved fynbos stand soil. However, radish and Eragrostis bioassays grown in soil from E. cinerea stands (on Signal Hill and Table Mountain respectively) were significantly lower than their growth in autoclaved soils from fynbos stands. Autoclaving the soil also had beneficial effects on the growth of bioassy species in soils from fynbos stands. This positive growth response could be due to the addition of available nutrients that are released into the soil after heating of the organic matter. If autoclaving also has a beneficial effect on the growth of plants in soil that is non-toxic, then it is unreasonable to ascribe positive growth of plants grown in autoclaved Eucalyptus soil to be totally due to the denaturing of growth inhibiting allelochemicals in the soil. This positive growth response may solely be the result of nutrients released into the soil from the heated organic matter. I do not think that the results obtained by growing bioassay species in soil samples from E. cinerea and their adjacent fynbos stands showed any clear effects that E. cinerea might be having on the soils. In order to confirm that allelochemicals are transferred from E. cinerea to the soils beneath, determinations of allelochemicals known to be produced by Eucalyptus trees

(terpenes) should be undertaken for soils collected beneath E. cinerea. If allelochemicals could have been isolated from these soils, they could have been used directly to test inhibition of growth in the bioassay species. It would also be more meaningful to use plant species that appear to avoid growing inder E. cinerea stands in the field as bioassays, as this would provide ecologically relevant information.

d)Allelopathy in relation to the bare area (Eucalyptus removed)

Even though the presence of allelopathy in this study was not demonstrated, assuming that Eucalyptus produce chemicals that inhibit the growth of their understorey plants, the following can be the way in which allelopathic effects have resulted in the inability of plants to occupy the bare area even after Eucalyptus have been remonved.

(1) The first can be even if Eucalyptus are removed, the allelochemicals that were leached into the soils before Eucalyptus tree removal are long-lived, and are still having negative effects on the growth of many other plant species.

(2) If allelochemicals of Eucalyptus are short lived, then they only have an indirect effect on the growth of other plant species after removal of the Eucalyptus trees. This could possibly happen his way:- Before removal of Eucalyptus trees, allelochemicals produced by these trees inhibited the growth of herbaceous layer species, thereby resulting in poor vegetation cover. After removal of the Eucalyptus trees, the soil left with poor vegetation cover was more subjected to severe soil erosion, and as a result, topsoil was eroded away. The absence of topsoil can

result in plant species not being able to establish themselves because of poor conditions for growth and germination of seeds.
(3) Allelochemicals produced by certain plant species are able to inhibit some nitrogen-fixing bacteria (Rice and Blum 1969, Wilson and Rice 1968). It can also be possible that allelochemicals produced by E.cinerea are also able to inhibit nitrogen fixing bacteria. If this is true, then this can strongly affect succession of plant species even after removal of E.cinerea. These species that are able to survive in low nitrogen conditions will be the ones that first occupy the area after removal of E.cinerea. It can take a long time before they can increase the amount of nitrogen in the soil such that later successional species can establish themselves.

6.2) NUTRIENT DEPLETION HYPOTHESIS

One of the possible explanations to the lack of herbaceous layer plant species under Eucalyptus stands can be due to lack of enough nutrients. Plants that were grown in fertilized soils from Eucalyptus grew significantly larger than plants that were grown in unfertilized soils. The high growth rate of radish plants in fertilized Eucalyptus soils as compared to the unfertilised Eucalyptus soil seem to support the hypothesis that herbaceous layer plants might be unable to grow under Eucalyptus stands due to lack of enough nutrients. Eucalyptus trees might be taking more nutrients from the soil, to such an extent that the nutrient condition of the soil under these trees becomes unfavourable for herbaceous plants to grow.

When comparing soil nitrogen mineralization in a Eucalyptus plantation and a natural Acacia forest in Senegal, Bernhand-Reversat (1988) found the mineral N, measured by in-vitro incubation to be lower in Eucalyptus than in the Acacia stand.

It can also be possible that the mineralization rate of nitrogen under E.cinerea stands that were studied were also low, as compared to the adjacent natural fynbos stand. Low mineralization rate can reduce the amount of nitrogen that is available for plant use in the soil, and as a result, herbaceous plant species might be unable to grow in such soil with low nitrogen content. Since Eucalyptus leaves are very sclerophyllous, with a high content of tannins, it can take a very long time for the leaf litter under Eucalyptus trees to decompose. Low decomposition rate can result in low release of minerals into the soil. This can result in a low nutrient content of the soil. Therefore, the low decomposition rate of Eucalyptus leaf litter can be one of the factors that are causing soil in the Eucalyptus stands to be very nutrient poor.

However, I do not believe that the positive growth response of plants through fertilized Eucalyptus plants has fully demonstrated the hypothesis that E.cinerea soils are nutrient depleted. I think that this could have been much more meaningful if it was supported by additional work on soil nutrient analysis. I feel that if nutrient analysis of the soil from the Eucalyptus stand was done, and compared to that of its adjacent fynbos stands, much more reliable results could have been obtained.

Furthermore, if the depletion of nutrients below the Eucalyptus

stands is the factor that is causing establishment of herbaceous plants to be difficult, what about those fynbos and some herbaceous plants that are more tolerant to less fertile environments. Certainly they should have been able to colonize the Eucalyptus understories, and their absence in the Eucalyptus understories suggest that there are more factors other than poor nutrient status of the soil that are playing a role in inhibiting growth of plants in Eucalyptus understories.

However,the hypothesis that Eucalyptus soils can be nutrient depleted can serve to explain why even after removal of Eucalyptus trees, soil beneath them remained devoid of vegetation for a very long period. If before they were removed, they extracted more nutrients from the soil, even after their removal, the nutrient status of the soil will remain poor. Also, if their present mineralization rate of nitrogen was very low, then even after their removal the amount of mineralized nitrogen in the soil will still be very low. Therefore, after clear-felling of the Eucalyptus trees, the bare area which remained was very poor in nutrients, and only those species that are able to survive in severely nutrient-depleted soils will be able to regenerate. It is only after the bare area has been colonised and ameliorated by species that are more tolerant to low nutrient conditions, that those species that are more sensitive to high nutrient conditions can be able to establish themselves (Conell and Slatyer 1977). This process can take a long time and this can be the reason why the area remained bare for a long time after the removal of the Eucalyptus stands.

6.3) SOIL EROSION HYPOTHESIS

Radish plants grown in topsoil collected from the Eucalyptus stands grew significantly larger than radish plants grown in subsoil collected from the same stand. This is possibly due to the fact that the topsoil has more organic matter as compared to the subsoil, resulting in them having a better nutrient status than the subsoil. The high organic matter content of the topsoils can also result in them having a better aeration and better water-holding capacity than subsoils. Because of favourable conditions for germination and growth supplied by the topsoil, most plants will generally need the topsoil in order to germinate and grow well.

Erosion of the topsoil after removal of the Eucalyptus trees can be one of the best possible explanations of difficulties that plants encounter, resulting in them not being able to regenerate in the bare soil after the removal of Eucalyptus trees. Before the removal of the Eucalyptus trees, the understories of the Eucalyptus was devoid of any vegetation as they generally are, due to many possible factors that I will attempt to explain. After removal of Eucalyptus trees, the bare soil, which was devoid of vegetation, was more subjected to soil erosion. After the topsoil was eroded away by rain water, it was very difficult for the plants to establish themselves, as the bottom soil which was left, was unfavourable for both germination and growth. Factors such as poor nutrient status of the soil, and the poor

water-holding capacity can result in inhibition of plant growth. As already explained in the previous section, it is those species that are more tolerant to less nutrient, and lower moisture that will be able to colonize those bare soils. After colonising and adding more nutrients to the soil through litter decomposition, it is then that more species can establish themselves (Connell and Slatyer 1977). Since this process can take a long time, it can be the possible reason why those cleared Eucalyptus areas have been bare for as long as 5 years.

6.4)OTHER POSSIBLE FACTORS THAT MIGHT BE LIMITING THE GROTH OF PLANTS IN THE UNDERSTORIES OF EUCALYPTUS CINEREA

a) Competition for water (moisture content)

It is possible that Eucalyptus trees are more competitive for water, and as a result, the soil under Eucalyptus stands are poor in water content. This lower soil moisture content can lead to herbaceous plants being unable to establish themselves under Eucalyptus trees. What I observed in watering plants grown in soil collected from the Eucalyptus stand is that when you pour water on the top of the soil, the water will make some balls. Therefore, it was very difficult for water to penetrate the soil. The reason why water made some balls on top of the soil collected from the Eucalyptus stands, can be that being aromatic terpene plants, Eucalyptus trees produce a lot of terpenes and tannins into the soil and this makes it difficult for water to penetrate (Morrow, pers. com). The poor penetration of water due to these

tannins can result in low moisture in the soil. Therefore, it can be very difficult for herbaceous plants to establish themselves in such low moisture conditions, whereas Eucalyptus trees have long tap roots that are able to exploit the water deep down in the water table, where the roots of herbaceous plants cannot reach.

The possible way to find out of the lack of moisture is responsible for inhibiting plants in Eucalyptus understories, can be to measure the water potential of similar plant species in the Eucalyptus stand, and from a fynbos stand and note if there is any difference. Another way is to compare the Eucalyptus soil moisture content with that of the fynbos soil moisture content.

b) Absence of seed banks

The absence of seed banks under Eucalyptus stands can be one of the factors that is causing the Eucalyptus-cleared area not to regenerate. Because so few plants are growing under Eucalyptus stands, there will be less seeds that are buried in the soil. Therefore, even after removal of Eucalyptus stands, few plants will be able to regenerate, since there will not be enough seeds for germination. Therefore, for the Eucalyptus-cleared area to be regenerated, wind-dispersed seeds from other places must first be blown onto these bare areas. This process can take a long time, as it is just a matter of chance for wind-blown seeds to land on that bare area. After these wind-blown seeds have germinated, and grown big enough to produce seeds, it is then that seed banks can start to be formed in the bare areas. As time goes on, more

plants will grow from seeds, and after a long time, it is then that the whole area can be regenerated. This can simply be tested by collecting soil from the bare area and plant seeds from natural fynbos like e.g. Proteas, and see if they would germinate. Another way of doing this can be to try and germinate seeds of natural fynbos plants in the field and note if they germinate or not.

7) CONCLUSIONS

Among the possible reasons why after the removal of Eucalyptus stands (Table Mountain) few plants germinated and grew, includes the following:-

(1) The bareness of the Eucalyptus stands before removal has resulted in severe topsoil erosion, which resulted in less germination and growth for plants since growth conditions were not favourable. The bareness of the Eucalyptus stands can be attributed to the following factors; a) a lower amount of nutrient in the soil b) a lower soil moisture content and c) allelochemicals that are produced by the Eucalyptus trees.

Not much work has been done in order to understand why Eucalyptus understories are devoid of vegetation. However, I think that this should be taken seriously, because removal of these trees does not lead to rapid recovery of the site cleared. Since Eucalyptus are grown in most parts of South Africa, this can result in these trees changing the soil structure of the most productive lands, in such a way that , even after these trees are no longer needed, it will be very difficult to use the soil to grow other crops. My suggestion is that serious attention be paid to factors that are inhibiting the growth of herbaceous plants under Eucalyptus trees, because with an understanding of that, it can be very easy to know why after removal of Eucalyptus trees soils are no longer productive, and how that problem can be solved.

ACKNOWLEDGEMENTS

This work has been made possible by funding from the Council for Scientific and Industrial Research CSIR). Special thanks are due to the following people for their help and advice.

DR. W.D. STOCK

DR. W. BOND

MR. D. BARNES

MR. T.L. MANUEL

REFERENCES

Baker , M.G. (1966) . Volatile growth inhibitors produced by Eucalyptus globulus . Madrono 18: 207-210

Bernhard-Reversat , F.B. (1988) . Soil nitrogen mineralization under a Eucalyptus plantation and a natural Acacia forest in Senegal. Forest Ecology and Management 23: 233-244.

Blum , U. & Rice , E.L. (1969) . Inhibition of symbiotic nitrogen-fixation by gallic and tannic acid and possible role in old-field succession . Bull. Torrey. Bot. Club 96: 531-544

Christensen , N.L. & Muller , C.H. (1975). Effects of fire on factors controlling plant growth in Adenostoma chaparral. Ecological Monographs 45: 29- 55.

Conell , J.H. & Slatyer , R.O. (1977). Mechanisms of succession in natural communities and their role in community stability and organisation . American Naturalist 111: 1119-1141.

del Moral , R. & Muller , C.H. (1969). Fog drip: A mechanism of toxin transport from Eucaluptus globulus . Bull. Torrey. Bot. Club 96 (4) : 467 -475

del Moral , R. & Muller , C.H. (1970). The allelopathic effects of toxin transport from Eucalyptus globulus . Bull. Torrey. Bot. Club 96: 467-475.

del Moral , R. ; Willis , R.J. & Ashton , D.H. (1978) . Suppression of coastal heath vegetation by Eucalyptus baxteri . Australian Journal of Botany 26: 203-219.

Lamont , B. (1985). Gradient and zonal analysis of understorey suppression by Eucalyptus wandoo. Vegetatio 63: 49-66

Muller , C.H. (1966). The role of chemical inhibition in vegetational composition . Bull. Torrey. Bot. Club 93: 332-351

Rao , N.S. & Reddy , P.C. (1984). Studies on the inhibitory effects of Eucalyptus (hybrid) leaf extracts on the germination of certain food crops. Indian Forester 110: 218-222.

Silander , J.A. ; Trenbath , B.R. & Fox , L.R. (1983). Chemical interference among plants mediated by grazing insects. Oecologia (Berlin) 58: 415-417.

Tukey , H.B. (1966) . Leaching of metabolites from above ground plant parts and its implications. Bull. Torrey. Bot. Club 93: 385-401.

Wilson , R.E. & Rice , E.L.(1968). Allelopathy as expressed by Helianthus annuus and its role in old-field succession. Bull. Torrey. Bot. Club 95(5) : 432-448.

Zar, J.H. (1984). Biostatistical Analysis. Prentice-Hall, New Jersey.