Allelopathic effect of the weed *Cyperus esculentus* on the growth of young *Pinus patula* plantations

by

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Certification

This is to certify that, except where otherwise stated, the work reported in this thesis is that of the author, and that it has not been submitted for this degree at any other university or institution of higher education.

S.R. Bezuidenhout

June 2001

Dedication

To my parents, without whose continued support and interest I would not have had the determination to continue.

To my grandmother, whose love for knowledge inspired me to search for unanswered questions.

"It is more important to know where you are going than to get there quickly. Do not mistake activity for achievement. "

Abel Newcomer

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Abstract

Greenhouse trials were conducted at the University of Pretoria to ascertain whether interference from yellow nutsedge affected the growth and development of patula pine. The first trial was designed to investigate whether incorporated residues or root leachate of the weed was inhibitory towards growth. It was found that the incorporated residues inhibit not only growth but also the growth of mycorrhizae associated with patula pine. In the second trial aqueous extracts of the different growth stages of yellow nutsedge were prepared, and tested against the growth of lettuce and the mycorrhizae associated with the pine seedlings. It was concluded that at both the immature and mature growth stage of the weed, the most inhibition towards lettuce and mycorrhizal growth occurred. The third trial involved growth of the pine species together with yellow nutsedge. In the absence of the weed, pine growth was not affected compared to seedlings growing with the weed. Results from the pot trials were confirmed in a field trial. Seedlings growing in the presence of yellow nutsedge showed a significant growth reduction compared to those growing without weeds. This study showed that yellow nutsedge contains compounds, in especially the immature growth stage, that are inhibitory towards the growth of patula pine.

Introduction

The production of timber plays an important role in the economy of South Africa, and the demand for wood for paper, mining and building is increasing with an increasing population. Agriculture and forest development are subjected to changes in technology, market forces and political pressures. Major limitations to development are the physical constraints of climate. Therefore, the identification of areas suitable for afforestation is a challenging and formidable task.

The foregoing explains why the forestry industry is progressively acquiring land that was previously used for agricultural purposes (Noble & Schumann, 1993). These land areas are typically a mixture of natural vegetation (afforested soil) and old agronomic sites (oldland soil). Oldland soils may differ from afforested soils with respect to pathogens, weed spectrum and soil nutrition (Smith & Van Huyssteen, 1992). The weed community on oldlands consists virtually exclusively of annuals that commonly occur in annual crops, e.g., *Cyperus esculentus* (yellow nutsedge), *Bidens pilosa* (common blackjack) and *Conyza albida* (tall fleabane). On adjacent afforested soil, natural grass species dominate (Reinhardt, Khalil, Labuschagne, Claasens, & Bezuidenhout, 1996).

In Mondi Forest's nurseries, pine seedlings are grown in containers for six months before they are transplanted in the field. *Pinus patula* (patula pine) established on soil under natural vegetation typically do not experience any growth and developmental problems. However, when seedlings are established on old agronomic sites, growth and development abnormalities are encountered. According to Linde, Kemp & Wingfield (1994), mortality of the seedlings was greater than 95% in most

cases in the north-eastern Cape Province of South Africa. The pine seedlings on oldlands died approximately 4-5 months after they were established in the field. It was reported by Smith & Van Hyussteen (1992) and Schumann & Noble (1993) that the symptoms of poor seedling performance involved a minimally developed root system, stunting, lack of apical dominance, chlorosis and necrosis of fascicles and necrosis of the growth tips.

The disorder is referred to as the "oldland syndrome". Various attempts to solve or amend the problem were launched and included soil, pathogen and mycorrhizal studies (Marais, 1974; Noble & Schumann, 1993; Schumann & Noble, 1993; Linde *et al.*, 1994 and Viljoen, Wingfield & Marasas, 1994). However, the problem appears to be complex and probably can not be attributed to one factor alone.

The role of allelopathic weeds, in particular their effects on forestry and agroforestry, had not been investigated in terms of the oldland syndrome in forestry situations in South Africa prior to 1995. The impact allelopathic weeds can have on the growth of forestry species are known. Rietveld (1975) demonstrated the adverse allelopathic effect of grass residues on the germination and early growth of *P. ponderosa* (ponderosa pine). Gilmore (1985) noticed the erratic establishment of *P. taeda* (loblolly pine) on old fields covered with *Setaria faberii* (giant foxtail). Jobidon, Thibault, & Fortin (1989) investigated the potential harmful effects of straw of *Avena sativa, Hordeum vulgare* and *Triticum aestivum* on *Picea mariana* (black spruce) seedlings. Height growth was not affected, but manganese uptake was inhibited.

One weed species in particular, *Cyperus esculentus* (yellow nutsedge), was the common denominator in terms of the weed spectrum found on the oldlands in the forest plantations. The allelopathic potential of this weed on higher plant species, has been investigated by several researchers (Horowitz & Friedman, 1971; Tames, Getso & Vieitez, 1973; Meissner, Nel & Smit, 1979 & Drost & Doll, 1980).

Consequently, the main objective was to quantify the allelopathic effects of *Cyperus esculentus* on the growth and development of *P. patula* seedlings under controlled and field conditions.

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Chapter 1

Literature Overview

It is widely accepted that successful establishment of pine seedlings is dependent on favourable soil conditions, the presence of suitable ectomycorrhizae and pathogen-free pine seedlings. Soil, pathogen and ectomycorrhizal studies have indicated that each factor can contribute to the oldland syndrome.

1. SEEDLING DEVELOPMENT PROBLEMS IN RELATION TO SOIL CONDITIONS

The production of timber is dependent on optimum soil and environmental conditions for optimal yield. Unfavourable conditions can lead to devastating losses. When the oldland syndrome was noted as a problem in forestry it was thought that the answer was to be found in the chemophysical factors of the soil.

1. Soil and site conditions

Smith & Van Huyssteen (1992) evaluated the soil physical properties, site characteristics and planting techniques in relation to growth differences of *P. patula* between two types of soil, afforested and oldland soil, in the north eastern Cape. The extent of mortality, and differences between the soils, varied across a wide range of soils, and even within soil forms. The oldlands were characterised by a 15-30% clay content, while the topsoil was in a poor physical condition. Consistency was hard when dry and soft when wet. A plough pan occurred at a depth of 153-300 mm and the macro pores were depleted.

On the other hand, afforested soil was well aggregated due to the binding action of grass roots and a healthier micro and macro faunal population. The soil was therefore wetter even long after establishment due to improved infiltration of rainfall, better weed control and the absence of large air pockets and clods. Laboratory analysis of the soil samples showed little difference in organic carbon levels between the two types of soil, despite indications to the contrary in the field.

2. Effect of soil physical properties on root development

Smith & Van Huyssteen (1992) also concluded that trees growing on oldland soil have poor root development. Roots do not move out of the root plug, or they die back and weeds grow in the plug, taking advantage of the better growing conditions. Variations in soil strength from one region within the soil profile to the next have been shown to hinder root development. Due to the cloddy and loose nature of oldland soils, under-consolidation in the planting hole leads to undesirable air pockets around the root plug, and thus to poor root and soil contact.

3. Nutrition

Fisher (1987) mentioned poor nutrition and microclimate as major problems in pine seedling establishment. Schumann & Noble (1993) highlighted nutrient deficiencies in oldlands. Both hypothesised that nitrogen deficiencies in agricultural lands were due to inherent low mineralisable nitrogen and to competition from soil flora for inorganic nitrogen. Noble & Schumann (1993) found that the application of fertilizer to seedlings growing in pots resulted in significant increases in shoot growth compared with seedlings receiving no fertilization. Nitrogen immobilisation or allelopathy from crop residues and disease accumulation have often been linked

to these problems. The impact of the allelopathic potential of crop residues is less clear. Elliot & Cheng (1987) and Lynch (1987) stated that plant residues provide substrates for production of phytotoxic and other deleterious micro-organisms. Allelopathy is, however, not considered to be the dominant factor according to Noble & Schumann (1993).

Noble & Schumann (1992) visited the United States of America, where first-year mortalities on erodible oldlands are common. It was thought that fungal and insect pests, improper care and handling of seedlings, and drought stress, were factors that contribute to the problem. They concluded that, in South Africa, drought on soils with low water capacity, poor contact between the roots and soil, poor planting techniques, plough pans on ex-agricultural lands, root-feeding insects and charcoal root rot fungus were the most common reasons for pine mortality.

2. GROWTH AND DEVELOPMENT OF PINE SEEDLINGS IN RELATION TO PATHOGENS

Various pathogens were associated with pine seedling establishment failure in the United States. *Fusarium* spp. and *Macrophomina phaseolina* were especially prevalent (Huang & Kuhlman, 1990; Mitchell, Runion, Kelly, Gjerstad & Brewer, 1991). Therefore the failure of pines to become established in South Africa prompted pathogen studies.

1. *Fusarium*

Fusarium species are well-known pathogens in forestry nurseries around the world (Bloomberg, 1981; Viljoen, Wingfield & Crous, 1992). Diseases associated with *Fusarium* include seed decay, damping-off, root rot and stem cankers (Bloomberg,

1971; Barnard & Blakeslee, 1980; Bloomberg, 1981; Huang & Kuhlman, 1990). Container and bare-root seedlings of particularly conifer seedlings, pine and Douglas-fir, are subjected to *Fusarium oxysporum* (Bloomberg, 1981; Huang & Khulman, 1990). *F. subglutinans* was isolated from slash- (*P. elliotti*) and loblolly pine (*P. taeda*) seed and container seedlings of both species.

Viljoen, Wingfield & Marasas (1994) isolated *F. subglutinans* f.sp. *pini* and *F. oxysporum* from roots of diseased patula pine seedlings. *F. subglutinans* , however, occurred more. It developed in injured and wounded areas throughout the root system. Newly germinated pine seedlings were killed and associated symptoms included both pre- and post-emergence damping-off. When inoculated, established seedling mortality was lower than that of the newly germinated seeds. *F. subglutinans* was identified as a primary pathogen responsible for nursery disease.

2. ***Pythium***

Linde, Kemp & Wingfield (1994) consistently isolated *Pythium irregulare* from areas previously under agronomic production. It was, however, also isolated from dying pine seedlings. The pathogen was highly active when inoculated in four-month-old pine seedlings. It is therefore an important factor to consider in the establishment failures of patula pine. Abiotic factors such as water-logging, nutrient deficiencies, microbial populations and soil structure are also important.

Korf, Khalil, Labuschagne & Reinhardt (1997) concluded that, in the presence of the weeds *B. pilosa* (common blackjack), *Tagetes minuta* (khaki weed) and *C. albida* (tall fleabane), a 40% increase of *Pythium* in pine roots from both afforested and oldland soils occurred. They suggested that the seedlings were probably

predisposed by a stress factor, the weeds, rendering them more susceptible to pathogen infections.

3. GROWTH AND DEVELOPMENT IN RELATION TO MYCORRHIZAL INFECTION

Mycorrhizal fungi (literally fungus-root) form symbiotic relations with most terrestrial plants and occur throughout the world. According to Linderman (1988), mycorrhizae can be divided into three types, viz. ectomycorrhizae, endomycorrhizae and ectendomycorrhizae. The types are distinguished by the way the hyphae of the fungi are arranged within the cortical tissue of the root. Ectomycorrhizae roots are usually swollen and in some hosts, fungus combinations appear considerably more forked than non-mycorrhizal roots. They occur predominantly on forest species, mostly by mushroom- and puffball-producing basidiomycete and ascomycetes.

McCool (1988), and Erland & Sönderström (1990), reported that several factors are important for mycorrhizal colonization. Climate, especially temperature and light, nutrient status and moisture content of the soil can influence the degree of colonization. Fertilizer application, liming and ploughing can also affect the symbiotic association.

Marais (1974) conducted a study on the mycorrhizal effects on *P. patula* in South Africa. He concluded that mycorrhizae were essential for the growth and survival of the tree species. It enhances the phosphate status of the macrobiont and the mycobionts act as biological deterrents to root pathogens such as *Phytophthora cinnamoni*. Temperatures of 25°C stimulated growth, while pH did not significantly

influenced colonization. However, an increase in nitrogen caused a reduction in the incidence of mycorrhizal infection. Furthermore, the incidence of infection, rate of seed germination and seedling growth were all found to be positively correlated with the altitude of the origin of the seed source. Mycorrhizal species associated with *P. patula* in South Africa are *Boletus edulis* and *Amantia muscaria*.

1. Benefits of associated mycorrhizae

1.1 Nutrition

It has been shown by Schenck (1981), Tinker (1984) and Graham (1988) that mycorrhizae help plants to acquire mineral nutrients from the soil, especially immobile elements such as phosphate (P), zinc (Zn) and copper (Cu). In instances where plants have difficulty in obtaining these elements, the mycorrhizae will increase the efficiency of uptake, resulting in enhanced plant growth. Ectomycorrhizae can also form an association with other micro-organisms. According to Rambelli (1973), an association was observed between a fungus and nitrogen-fixing bacteria in *Pinus radiata*. Nitrogen fixed by the bacteria was available for the fungus and the pine tree. The bacteria in turn, derived nutrients from the fungal hyphae. The mycorrhizae obtain simple carbohydrates, vitamins and other growth factors.

When fertilizer is applied, mycorrhizal colonization apparently can decrease. Alexander & Fairley (1983) concluded that N-fertilization reduced mycorrhizal infection in Sitka spruce (*Picea sitchensis*) humus, and alters the relative mycorrhizal types. Newton & Pigott (1991) determined that fertilization tends to reduce ectomycorrhial infection in oak and birch seedlings, especially after the addition of nitrogen. Carlson (1994) found that the addition of fertilizers appears

to have no effect on the total number of mycorrhizal tips on patula pine (*P. patula*) roots, but the species abundance was altered.

Parke, Linderman & Black (1983) concluded that mycorrhizae can also increase the water uptake, and/or alter the plant's physiology, to reduce stress response to soil drought. According to Satin, Boyer & Gerdemann (1971), mycorrhizae also enhance water translocation in the plant. Marx & Bryan (1971) concluded that it can further reduce transplant injury and helps plants withstand high temperatures.

1.2 Pathogens

Mycorrhizae can be beneficial in terms of reducing the incidence of disease due to pathogen inoculation. It is clear from research done by Marx (1973) that ectomycorrhizae protect trees from root pathogen infection and reduce the effects pathogens can have on plants. This was shown for *Rhizoctonia solani* on loblolly pine and *Phytophthora cinnnamoni* on shortleaf pine. Uninoculated trees could not match the growth rate of those inoculated with the mycorrhizae *Pisolithus tinctorius* and *Cenococcum graniforme*. The number of pathogen propagules recovered from the soil from inoculated trees was also reduced. According to Marx (1969) and Schenck (1981), the protection effect is due to the antibiotic compounds that are produced by ectomycorrhizae. A mantle is formed around the roots which forms a mechanical barrier and thus protects the root against infection.

2. Allelopathic effects on mycorrhizal growth

Chu-Chou (1978) found that water extracts of roots of old *P. radiata* significantly inhibited the growth of a mycorrhizal fungus associated with *P. radiata*, and caused

root necrosis and wilting of the seedlings. Pellisier (1993) concluded that phenolic acids produced by species in a spruce forest inhibited the respiration of two spruce mycorrhizal species, *Laccaria laccata* and *Cenococcum graniforme*.

Perry & Choquette (1987) concluded that trees of some forestry species form different proportions of mycorrhizae types, depending on whether they are grown in soils from undisturbed forests or from clear cuts. They suggest that the observations are due to different characteristic chemical compounds in soil from different plant succession types or different types of disturbance which, in turn, influence the type of mycorrhizal formation on the seedling.

4. PLANT GROWTH AND DEVELOPMENT PROBLEMS IN RELATION TO INTERFERENCE BY WEEDS

Soon after germination the seedling must become independent of the parental resources associated with the seed. It must begin its existence as an individual and begin to extract from its surroundings the necessary resources for life. The success of that individual in its environment is often determined by its ability to obtain light, water and nutrients. Plant growth is fundamental to the understanding of plant functions and their interactions with the environment (Radosevich & Holt, 1984).

Interference

Sometimes an individual plant can have an inhibiting effect on the growth of its neighbours. It is therefore more common that a neighbouring plant will interact in a negative manner, where the emergence or growth of one or both is inhibited. Muller (1969) described the adverse effect of a neighbouring plant in association

with others and defined it as interference. According to Szczepanski (1977) the potential causes of interference include:

- Allelospoly (competition) ☞ the depletion of one or more resources required for growth;
- Allelopathy ☞ the addition of chemical toxins by one or more species in association, and
- Allelomediation ☞ selective harbouring of a herbivore that might selectively feed on one species, thus lending to the advantage of another.

Interference refers, therefore, to the overall effect of one plant upon another and encompasses both allelopathy and competition. Competition involves the **removal or diminution** of a shared resource, while allelopathy involves the **addition** of a chemical compound to the environment through different processes (Rice, 1984; Putnam, 1985). Confusion has occurred because some consider allelopathy to be part of competition. In addition, competition has been misused by many to describe interference. It is a specific mechanism for interference, but not the end result.

Allelopathy

The allelopathic effect of one plant upon another is so striking that competition for a common resource does not seem adequate to explain the observation. In organism communities, many species appear to regulate one another through the production and release of chemical attractants, stimulators or inhibitors (Putnam & Tang, 1986). According to Dakshini & Inderjit (1999), the release of chemicals that hinder the development and distribution of plants are not a new phenomenon. However, the recognition have been rather slow due to a lack of communication

between botanists and organic chemists, in addition to problems associated with isolation, purification and identification of these chemicals.

1. Definition

Allelopathy is derived from the Greek words *allelon* "of each other" and *pathos* "to suffer" (Rizvi, Haque, Singh & Rizvi, 1992). It therefore translates literally as mutual suffering. Allelopathy is described as the beneficial and deleterious biochemical interaction between plants and micro-organisms. Rice (1974) defines allelopathy as any direct or indirect effect by one plant, including micro-organisms, on another through the production of chemical compounds that escape into the environment and subsequently influence the growth and development of neighbouring plants. It includes both inhibitory and stimulative reciprocal biochemical interactions. The use of the term "allelopathy" may therefore be somewhat controversial. Chemicals found to inhibit the growth of a species at a certain concentration may stimulate the growth of the same species or another at a lower concentration (Putnam & Tang, 1986; Rice, 1995). Aldrich (1984) describes two types of allelopathy:

- True type ☞ the release into the environment of compounds that are toxic in the form in which they are produced, and
- Functional type ☞ the release into the environment of a substance that is toxic as the result of transformation by micro-organisms.

Many extremely important ecological roles of allelopathy may have been overlooked because of the focus on the detrimental effects of the added chemicals only.

2. History

In a historical overview, Willis (1985) pointed out that allelopathy is not a new concept. Theophrastus (300 BC) first noticed the deleterious effect of cabbage on vine and suggested that it is due to odours. A common problem in both Greek and Roman times was the so-called soil sickness, the declining yields of fields. They did not understand that the condition could be caused by various factors such as mineral deficiencies, toxin accumulation, pathogens and the imbalance of micro-organisms. In the seventeenth and eighteenth centuries, botanists relied strongly on a comparative approach. They compared both plant form and function, particularly in relation to nutrition. The Dutchman Boerhoove suggested that root exudation may play a role in plants. Stephen Hales believed that root exudates facilitated excretion of used compounds. The theory of root excretions was a basis for the concept of allelopathy. Swiss botanist Auguste Pyrame de Candolle developed the plant interaction theory via root excretions. He was influenced by the increasing information on phytochemistry and the effects of diverse compounds on plant growth. Interest in the concept of allelopathy was rekindled at the close of the nineteenth century, principally for two reasons. The first was that careful agricultural experiments yielded results that could not adequately be explained by the exhaustion of soil nutrients. Secondly, improved techniques in chemistry allowed organic toxins to be identified from unproductive soils.

3. Proof of allelopathy

Many field studies implicate allelopathy, but isolation and identification of the chemical agents require a rigorous laboratory effort (Putnam & Tang, 1986). It is extremely difficult to prove that any deleterious effect is due to allelopathy rather than to competition for essential products. Numerous studies have provided

evidence, but seldom has a specific protocol been followed to achieve convincing proof (Putnam & Tang, 1986). These authors pointed out that the shortcomings of the discipline make it hard to differentiate between allelopathy and competition. These shortcomings include:

- A general lack of nomenclature to adequately describe the plant responses that occur in this manner;
- A dearth of techniques to separate allelopathic interactions from competition; and
- Failure to prove the existence of direct compared with indirect influences *via* other organisms/micro-environmental modification.

A considerable body of information has accumulated implicating allelopathy as an important form of plant interference. According to Willis (1985), Putnam & Tang (1986) and Cheng (1992) the methodology dictates that certain points in allelopathic research be established in order to suggest that it is operative:

- A pattern of inhibition of one species by another must be shown using suitable controls, describing the symptoms and quantitative growth reduction;
- The putative aggressor plant must produce a toxin;
- There must be a mode of toxin release from the plant to the environment and thus the target plant;
- Mode of toxin transport or accumulation in the environment must be evident;
- The afflicted plant must have some means of toxin uptake, be exposed to the chemical in sufficient quantities and time to cause damage, to show similar observed symptoms;
- The observed pattern of inhibition should not be explained solely by physical factors or other biotic factors, especially competition.

It is important to stress that the above points do not prove that allelopathy is operative, only that it offers the most reasonable explanation for the observed pattern. According to Cheng (1992), once the chemical enters the environment, a number of interacting processes will take place. These processes have been identified as:

- Retention ☞ the retarded movement of the chemical from one location to another, through soil, water and air;
- Transformation ☞ the change in form or structure of the chemical, leading to partial change or total decomposition of the molecule;
- Transport ☞ defines how the chemicals move in the environment.

Cheng (1992) pointed out that these processes are influenced by the nature of the chemical, the organisms present, the properties of the soil, and by environmental conditions. The fate of the chemicals depends on the kinetics and interactions of individual processes with time, at a particular site under a particular set of conditions.

Williamson (1990) noted that an allelopathic methodology, much the same as Koch's Postulate for microbiology, will provide an useful framework for experimental design, but he criticized the inherent analogy to microbiology. Weidenhamer (1999) concluded that if allelopathy is to be a scientifically credible hypothesis, rather than just a logical scenario, evidence must be provided. According to him, allelopathic effects are density-dependent in ways inconsistent with resource competition.

4. Allelochemicals

According to Putnam & Tang (1986) all alleged cases of allelopathy that have been studied appear to involve a complex of chemicals. No single phytotoxin was solely responsible for or produced as a result of interference by a neighbouring plant. Rizvi *et al.*, (1992) pointed out that the subject not only deals with the gross biochemical interactions and their effects on the physiological processes but also with the mechanism of action of allelochemicals at specific sites of action at the molecular level.

Few studies on allelopathy concentrate on the mechanisms and processes involved in the production of allelochemicals. Einhellig (1987) and Putnam & Tang (1986), raised the question whether alleged biochemical agents were in sufficient concentrations and with enough persistence in the environment to affect a neighbouring or succeeding plant. These chemicals could be transformed during the course of extraction. According to Cheng (1992), allelopathic symptoms may not be manifested at the time or site where plant damage has actually occurred.

4.1 Sources of allelochemicals

Radosevich & Holt (1984) stated that the primary effect of allelopathy seems to result from an association with plant litter in or on the soil. Rice (1984, 1995) and Putnam (1985) reported that allelochemicals are present in virtually all plant tissue, i.e. leaves, fruit, stems, and roots. These allelochemicals are released by such processes as volatilization, root exudation, leaching and decomposition of plant residues. Leaves may be the most consistent source, while roots are considered to contain fewer and less potent toxins. According to Aldrich (1984), allelochemicals must be concentrated in the leaves, stem or roots rather than in the fruit or

flowers. If it is concentrated in these latter organs it is unlikely that it could be available in time to interfere with neighbouring plants.

According to Rice (1984) and Putnam (1985), there are four ways in which the chemicals are released:

- Volatilization ☞ release into the atmosphere. It is only significant under arid or semi-arid conditions. The compounds may be absorbed in vapour by surrounding plants; be absorbed from condensate in dew, or may reach the soil and be taken up by the roots.
- Leaching ☞ rainfall, dew or irrigation may leach the chemicals from the aerial parts of plants that are subsequently deposited on other plants or on the soil. Leaching may also occur through plant residues. Their solubility will affect their mobility in soil water.
- Root exudation ☞ from plant roots into the soil environment. Whether these compounds are actively exuded, leaked or arise from dead cells sloughing off the roots is not clearly understood at this time.
- Decomposition of plant residues ☞ it is difficult to determine whether toxic substances are contained in residues and simply released upon decomposition, or produced instead by micro-organisms utilizing the residues.

4.2 Natural products identified as allelopathic agents

Alleged allelochemicals represent a myriad of chemical compounds from simple hydrocarbons and aliphatic acids to complex poly-cyclic structures. The secondary products could be classified in the following categories but it is impossible to enumerate each and every chemical identified as an allelochemical. Whittaker & Feeney (1971), Rice (1984, 1995) and Putnam & Tang (1986) divided allelochemicals

into various major chemical groups:

- Simple water-soluble organic acids
- Simple unsaturated lactones
- Long-chain fatty acids and polyacetylenes
- Naphthoquinone, anthroquinones and complex quinones
- Simple phenols
- Benzoic acid and derivates
- Cinnamic acid and derivates
- Flavonoids
- Tannins
- Terpenoids and steroids
- Amino acids and polypeptides
- Alkaloids and cyanohydrins
- Sulphides and glucosides
- Purines and nucleotides
- Coumarins
- Thiocyanates
- Lactones
- Actogenins

4.3 Mode of action of allelochemicals

Most of the allelochemicals are secondary metabolites and are produced as byproducts of primary metabolic pathways (Rice, 1984; Putnam & Tang, 1986 and Rizvi *et al.*, 1992). Secondary compounds have no physiological function essential for the maintenance of life (Aldrich, 1984). Reports most frequently identified effects which are readily observed in the field or under controlled conditions.

Delayed or inhibited germination and the stimulation or inhibition of root and shoot growth are often reported (Rizvi *et al.*, 1992). The major difficulty is to separate secondary effects from primary causes. An important question that always remains is whether the inhibitor reaches the site in the plant in sufficient concentration to specifically influence that reaction and whether other processes may be affected more quickly.

The mode of action of a chemical can broadly be divided into a direct and an indirect action (Rizvi *et al.*, 1992). Effects through the alteration of soil properties, nutritional status and an altered population or activity of micro-organisms and nematodes represent the indirect action. The direct action involves the biochemical/physiological effects of allelochemicals on various important processes of plant growth and metabolism. Processes influenced by allelochemicals involve:

- Mineral uptake ☞ allelochemicals can alter the rate at which ions are absorbed by plants. A reduction in both macro- and micronutrients are encountered in the presence of phenolic acids (Rice, 1974).
- Cytology and ultrastructure ☞ a variety of allelochemicals have been shown to inhibit mitosis in plant roots (Rice, 1974).
- Phytohormones and balance ☞ the plant growth hormones indoleacetic acid (IAA) and gibberellins (GA) regulate cell enlargement in plants. IAA is present in both active and inactive forms, and is inactivated by IAA- oxidase. IAA-oxidase is inhibited by various allelochemicals (Rice, 1974) Other inhibitors block GA-induced extension growth.
- Membranes and membrane permeability ☞ many biological compounds exert their action through changes in permeability of membranes. Exudation of

compounds from roots on root slices have been used as an index of permeability because plant membranes are difficult to study (Harper & Balke, 1981).

- Photosynthesis ☞ photosynthetic inhibitors may be electron inhibitors or uncouplers, energy-transfer inhibitors electron acceptors or a combination of the foregoing (Einhellig & Rasmussen, 1979; Patterson, 1981)
- Respiration ☞ allelochemicals can stimulate or inhibit respiration, both of which can be harmful to the energy-producing process (Rice, 1974).
- Protein synthesis ☞ studies utilizing radio-labelled C^{14} sugars or amino acids, and traced incorporation of the label into protein, found that allelochemicals inhibit protein synthesis (Rice, 1974).
- Specific enzyme activity ☞ Rice (1984) reported on a number of allelochemicals that inhibit the function of enzymes in the plant.
- Conducting tissue (Rice, 1974).
- Water relations (Rice, 1974).
- Genetic material (Rice, 1984, Aldrich, 1984).

Under natural conditions the action of allelochemicals seems to revolve round a fine-tuned regulatory process in which many such compounds may act together on one or more of the above processes (Rizvi *et al.*, 1992).

4.4 Methods for isolation, bioassay and identification

The concept of allelopathy is a matter of controversy (Aldrich, 1984) and is plagued with methodological problems, particularly those of the distinguishing effects of allelopathy from those of competition (Willis, 1985). Connell (1990) stated that no published field study has demonstrated direct interference by

allelopathy in soil while excluding the possibility of other indirect interactions with natural enemies, resources and other competitors. Only a few investigations have separated the components of interference because of the complexity of the ecological phenomenon (Fuerst & Putnam, 1983). The authors reported that evidence must be put forward before any attempt is made to determine the cause(s) of interference. The symptoms will vary from the most obvious germination and mortality responses to the more subtle plastic responses such as a reduction in size, mass or number of organs. Therefore observations and results are largely descriptive rather than analytical and provide only circumstantial evidence for allelopathy, leaving room for explanations other than allelopathy. Care must be taken to exclude competition as a factor. Competition can be selectively eliminated by adding limiting resources. According to Dakshini & Inderjit (1999) during the last one and a half decade, with the involvement of ecologists, ecophysiologists and microbiologists, the scope of allelopathic research has widened. The realization that in nature competition, allelopathy, microbial nutrient immobilization and mycorrhizal activity, directly or indirectly, affect mechanisms of allelopathic interference, had far reaching consequences in defining the potential of allelopathy. They concluded that any analysis of allelopathic interference has to be through a multifaceted approach.

The effects of allelopathy are manifested in the soil environment which provides a myriad of physical, chemical and biological processes that may interact with allelochemicals that could influence the study. It is impossible to prove that chemicals released by plants do not affect neighbouring plants. Harper (1977) proposed a rigorous protocol to search for the cause and effect. The cause-and-effect relationship cannot be established merely by observing the appearance of

phytotoxic symptoms, on the one hand, and showing the presence of chemicals of demonstrated toxicity in the vicinity of an affected plant, on the other.

According to Putnam & Tang (1986), most research activities on allelopathy were concentrated on apparent cases that were conspicuous under field conditions. Under controlled conditions, factors in competition may be segregated. It is possible to prove that chemical interactions are either totally or partially responsible for the interference observed. Since allelochemicals differ in terms of source and type, different methods have been devised for greenhouse and laboratory verification of their presence.

4.4.1 Extraction or leaching from plant tissue

Plant leachates have been collected to support the presence of extracellular bio-active compounds. Isolation of a compound involves collection in an appropriate solvent or adsorbent. According to Putnam (1985), a commonly used extract solvent is water or aqueous methanol in which dried or living plant material is soaked. After extracting the material for varying lengths of time, the exuded material is usually filtered or centrifuged before bioassay. In other cases the material is macerated together with distilled water.

Putnam (1985) also pointed out that under field conditions leaching may be caused by dew, rain or irrigation. Leachates do not include intracellular metabolites released because of physical damage inflicted during sample collection. In many cases, it is impossible to judge whether or not damage of the living tissue has occurred and the sample in a strict sense would be of doubtful origin.

4.4.2 Root exudates

According to Putnam & Tang (1986), several techniques for studing the effect of root exudates have been employed. Sand can be used in which both donor and recipient plants are present. The effects on early plant development before competition for growth factors occurs can then be evaluated. Also, donor plants can be grown in sand. The sand can then be leached and the leachate evaluated in terms of influence on recipient plants. Bell & Koeppe, (1972) devised a system where donor and recipient plants can be grown together in a system where the pots are arranged so that the nutrient solution flows from the donor to the recipient and back to a reservoir, flowing back and forth for varying periods of time. Tang (1999) reported on the development of a continuous root exudate trapping system. It is designed to collect rhizospheric organic compounds from the undisturbed growing plants. He used *Sorghum bicolor* as a test plant and identified more than 20 toxic compounds from the hydrophobic root exudate fraction.

4.4.3 Release from plant litter

Rice (1995) reported that soils collected in the field were used as sources of allelochemicals. Live or dead material can be placed on or in the soil for a selected period of time before receptor plants are planted directly in the soil for bioassay or the soil can be extracted for allelochemicals.

4.4.4 Volatile compounds

Muller, Muller & Haines (1964) germinated seed on filter paper sheets on a cellulose sponge placed in a large container adjacent to beakers containing the donor plants. The only contact between plant material and seed was aerial. Significant inhibition of germination occurred.

4.4.5 Bioassays

Bioassays are an integral part in all studies of allelopathy. They are necessary for evaluating the allelopathic potential of species and for following the activity during extraction, purification and identification of bio-active compounds. In their simplest form, bioassays, and the isolation and identification of allelochemical, are regarded by some as techniques for providing initial information only. Both these aspects of allelopathy research are important and should be used together. Failure to do so would make results inconclusive (Reinhardt, Khalil, Labuschagne, Claassens & Bezuidenhout, 1996). Bioassay techniques vary greatly and no researcher follows the same procedure. This is clearly demonstrated in the treatise by Rice (1995). The greatest problem with bioassays is the lack of standardized bioassays. Incomplete information on the allelochemical source, method of extraction, fraction concentrations and the absence of known compounds with demonstrated activity in bioassays are also hampering useful bioassays. Stowe (1979) challenged the validity of bioassays. He concluded that frequently little agreement between bioassay results and distinctive patterns of vegetation in the field is obtained. Brandsæter & Haugland (1999) reported that the variety of bioassay methods and experimental factors influences the results of bioassays. Furthermore, the lack of both knowledge about methods and factors and standardised bioassays, makes comparisons between different studies very difficult.

According to Putnam & Tang (1985) and Rice (1995) , the most widely used bioassay test is the influence on seed germination. Different types of techniques are used. All, however, include seed placed on substrate saturated with the test solution. Germination is often defined as the emergence of the radicle 2 mm beyond the seed coat and is scored over a period of time. Factors to consider are oxygen

availability, osmotic potential of the test solution, pH and temperature. Properly conducted bioassays of this nature have great value. They are simple to conduct and require a small quantity of test solution. However, according to Brandsæter & Haugland (1999), species differ in their response to bioassay tests. The root length of one species can be more affected that another, while osmotic potential can influence species differently. Therefore, confounding of germination and root length inhibition may give misleading results. Furthermore, the volume of the extracts and the amount of distilled water used, influence results considerably and therefore the conclusions of the bioassay. Inderjit & Weston (1999) concluded that each bioassay must be designed specifically to assess species interactions after careful consideration of their growth habits, biotic characteristics and ecophysiological factors.

The elongation of the hypocotyl or coleoptile can be used in conjunction with germination percentage. The elongation is, however, tedious to measure and instead dry mass can be used as a measure of growth (Bhowmik & Doll, 1984). Growth bioassays are often more sensitive than germination bioassays. When the quantity of test solution poses a problem, agar cultures can be used. Pre-germinated seed can be placed on the surface of the agar containing the allelochemicals.

4.4.6 Isolation and characterization of chemicals

Rice (1984) pointed out that chemical separation can be accomplished by partitioning the chemicals on the basis of polarity into a series of solvents. Compounds can also be separated by molecular size, charge or adsorptive characteristics. Various chromatography methods are utilized.

There is little doubt that plants do release significant amounts of substances into the environment. However, their fate remains poorly understood. Limited studies using C^{14}-labelled compounds suggest that most simple organic compounds such as phenolic acids are rapidly assimilated by soil micro-organisms or incorporated into humic acids (Willis, 1985). It may well be that addition of organic compounds to the soil environment is more important in determining the composition of the soil micro- flora and thus the effects of most allelopathic substances are probably indirect.

4.5 Factors affecting production of allelochemicals

Plants vary in their production of allelochemicals according to the environmental conditions to which they are exposed. Stress has a marked effect on the production of allelochemicals. According to Aldrich (1984) and Rice (1984), a variety of environmental conditions influence the quantity of chemicals produced:

- Light ☞ some allelochemicals are influenced by the amount, intensity and duration of light. The greatest quantities are produced during exposure to ultraviolet and long-day photoperiods. Thus under-storey plants will produce fewer allelochemicals because over-storey plants filter out the ultraviolet rays. At the peak plant growing period, it could be expected that more allelochemicals are produced than earlier or later in the growing season.
- Mineral deficiency ☞ more allelochemicals are produced under conditions of mineral deficiency.
- Drought stress ☞ under these conditions, more allelochemicals are produced
- Temperature ☞ in cooler temperatures, greater quantities are produce. The location and effects of allelochemicals within the plant seem to vary.

There are also numerous other factors influencing the production of

allelochemicals (Rice, 1995). The type and age of plant tissue during extraction is important since compounds are not uniformly distributed in plants. Production differs between species as well as within species.

Aldrich (1984) stated that environmental conditions that restrict growth tend to increase the production of allelochemicals. One could postulate that allelopathy may frequently be an accentuation of competition although not part of competition. If stress from competition increases the quantities of allelochemicals produced, it is conceivable that allelochemicals will inhibit the growth of some species and not others, thereby reducing the ability of the affected species to compete. The allelopathic plant and those affected by them are part of the ecosystem. If one factor changes, changes will occur in one or more factors. For example, light can be expected to interact with temperature and indirectly with soil moisture and other factors.

Much of the evidence indicates that several chemicals are released together and may exert toxicities in an additive or synergistic manner (Rice, 1995). Sometimes the allelopathic effect will be obvious and startling, but in the majority of cases the effects are subtle and thus more difficult to assess.

5. Roles of allelopathy in natural and manipulated systems

There is convincing evidence that allelopathic interactions between plants play a crucial role in natural as well as manipulated ecosystems. According to Rizvi *et al.*, (1992), studies of these interactions provided the basic data for the science of allelopathy. The data were applied to understand the problems of plant-plant, plant-microbe and plant-insect interactions and to exploit these in improving the

production of manipulated ecosystems.

5.1 Patterning of vegetation and succession

Natural successions of plants occur in nature (Aldrich, 1984; Rice, 1995). Plants modify the environment, thus leading to a predictable succession, with the early colonizers being those species that rely upon large numbers of seed, and late entrants those species that rely on their competitive ability. Perennial species concentrate offshoots around a parent and allelopathy could thus be beneficial to the spread of such species. The fact that dense colonies of some perennials frequently occur essentially as pure stands in itself implicates allelopathy (Aldrich, 1984). The explanation for a specific vegetational pattern has mostly been given to competition. In recent times, evidence is accumulating that points to the fact that, apart from competition, allelopathy does play an important role. According to Rizvi *et al.* (1992), allelopathic plants affect the patterning of vegetation in their immediate vicinity.

5.2 Allelopathy and agriculture

The effect of weeds on crops, crops on weeds and crops on crops have invariably been emphasized (Rice, 1995). Results obtained so far clearly demonstrate that some of the findings on allelopathic control of weeds, elimination of deleterious allelopathic effects of crops on crops, or exploitation of beneficial interactions in a rotation or mixed cropping system have a direct bearing on crop production (Rizvi *et al.*, 1992). According to Wu, Pratley, Lemerle & Haig (1999) there are several ways in which allelopathy can be used in a crop-weed situation. The most important one is for weed management. The use of allelopathic crops can reduce the amount of herbicides used in agriculture.

C. esculentus (yellow nutsedge) is a herbaceous perennial that is considered as one of the world's worst weeds (Holm, Plucknett, Puncho, & Herberger, 1977). It is a problem in cropping systems in tropical and temperate climates, where it causes large losses in crop yields. The weed is characterized by prolific vegetative activity which produces a complex underground system of basal bulbs, rhizomes and tubers. Stoller, Wax & Slife (1979) investigated the competition effect of *C. esculentus* on maize (*Zea mays*). They identified a relationship between nutsedge density (shoot/m^2) and percentage reduction in crop yield. An 8% yield reduction was achieved for every 100 shoots/m^2. Yield reduction of 41% occurred when no weed control was carried out in a field initially infested with 1200 shoots/m^2.

C. esculentus and *C. rotundus* (purple nutsedge) are known for their allelopathic abilities. Drost & Doll (1984) concluded that extracts and residues of *C. esculentus* have an inhibitory effect on the growth of soyabeans (*Glycine max*) and maize. Tames, Getso & Vieitez (1973) found compounds in *C. esculentus* tubers that were inhibitory to oat coleoptiles and seed germination of other crops. Horowitz & Friedman (1971) dried *C. esculentus* tubers and mixed it with soil. The root and top growth of barley planted in the soil were significantly reduced. Meissner, Nel & Smit (1979) grew *C. rotundus* in sterilised, well-fertilized soil. Growth of barley, cucumber and tomato on the soil were considerably reduced.

5.3 Allelopathy and forestry

Allelopathic interactions have been demonstrated to play a crucial role in natural and man-made forests (Rice, 1995). Such interactions are pivotal in determining the composition of the vegetation growing as under-storey vegetation in forest regeneration (Rizvi *et al.*, 1992). It can, however, not be used as an universal

explanation for regeneration failures or poor stand growth. Rice (1995) described various trials conducted to gain information on the allelopathic effects, not only of woody species, but herbaceous species as well.

5.3.1 Allelopathy of woody species

Thobiessen & Werner (1980) reported that hardwood seedlings do not grow under *P. resinosa* but do grow under *P. sylvestris* in spite of the fact that *P. resinosa* has a higher light intensity and the soil a higher nitrate level. Kil & Yim (1983) expanded research on the allelopathic potential of *P. densiflora* (red pine). They found that toxic substances inhibited seed germination and growth of the species in the forest. These substances were released in fresh and fallen leaves, roots, pine forest soil and pine pollen rain. Kil (1989) studied the allelopathic potential of five species of the Pinaceaes, *viz. P. densiflora, P. thunbergii, P. rigida, Larix leptolepis* and *Cedrus deodora.* All five species inhibited germination of test species, but the most severe inhibition in all cases was on dry-mass growth of the test species.

5.3.2 Allelopathy of herbaceous species on woody species

Hollis, Smith & Fisher (1982) tested foliar leachates and extracts from partially decomposed leaves of nine abundant, under-storey herbaceous species for their allelopathic effect on germination, radicle extension and shoot growth of *Pinus elliotii* (slash pine) and *P. taeda* (loblolly pine). They concluded that foliar leachates from *Eupatorium capillifolium* (dogfennel) and *Lyonia lucida* (fetterbush) strongly inhibited germination and radicle extension in both pine species. Rietveld (1975) demonstrated the adverse allelopathic effect of *Festuca arizonica* grass residues on germination and early growth of *P. ponderosa* (ponderosa pine). Drew (1988)

examined the influence of under-storey species in the growth of *Prunus serotina* (black cherry). *Aster acuminatus* (whorled wood aster) and *Dennstaedtia punctilobula* (hayscented fern) were the dominant herbaceous species. Complete removal of the two species stimulated height growth and species diversity increased after two growing seasons. Four years after removal, *A. acuminatus* had no further significant inhibitory effect. Jobidon, Thibault, & Fortin (1989) investigated the potential harmful effects of straw of *Avena sativa, Hordeum vulgare* and *Triticum aestivum* on *Picea mariana* (black spruce) seedlings. Height growth was not affected, but manganese uptake was inhibited. Fisher & Adrian (1981) noticed a strong effect of *Paspalum notatum* (Bahia grass) on *P. elliottii* (slash pine). As the percentage of grass increased, the height growth decreased markedly. The authors concluded that Bahia grass competes with the trees, but that allelopathy could not be ruled out. Gilmore (1985) noticed the erratic establishment of *P. taeda* (loblolly pine) on old fields covered with *Setaria faberii* (giant foxtail). Water extracts of *S. faberii* inhibited germination and radicle elongation of pine seedlings in petri dishes. Extracts from dried foxtail tops were the most inhibitory, while those from fresh tops and roots were less inhibitory.

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Chapter 2

Allelopathic influence of residues of the agronomic weeds *Cyperus esculentus*, *Bidens pilosa* and *Conyza albida* on the early growth and development of *Pinus patula*

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Chapter 2

Allelopathic influence of residues of the agronomic weeds, *Cyperus esculentus*, *Bidens pilosa* and *Conyza albida* on the early growth and development of *Pinus patula*

1. Introduction

The failure of *P. patula* to become established on previously cultivated lands (oldlands) in South Africa has resulted in considerable financial loss. Differences in pine stands on oldlands and afforested soil were evident on the Giant's Castle Estate in Mooi River, KwaZulu-Natal. Due to a history of crop production in certain areas, various annual weeds infest the pine plantations. Of these weeds, *Cyperus esculentus* (yellow nutsedge) is the dominant species during spring and early summer. It occurs in early spring with tubers sprouting after the first rain, and lasts through the winter as a residue on the soil surface. In January, when nutsedge matures, *Bidens pilosa* (common blackjack) and *Conyza albida* (tall fleabane) are growing actively.

According to Radosevich & Holt (1984), numerous naturally occurring chemicals are present in plant material, and when residues are left on the soil surface after harvest, or plowed in, the chemicals can be released by rain or by micro-organisms during decomposition. Subsequent mortality or growth suppression do not have to be related directly to the release of a toxic organic substance from the plant material. The modification in micro-environments could account for the phytotoxic response. Another source of allelochemicals is the production and release by

growing plants of toxins that ultimately inhibit development of adjacent plants. Wardle, Nicholson & Rahman (1993) found that residual matter of roots and shoots of *Carduus nutans* (musk thistle) and the stems of dead plants were especially inhibitory to shoot and root biomass of certain grasses and legumes. Ahmed & Wardle (1994) used tissue decomposition bioassays and aqueous extracts of *Senecio jacobaea* (ragwort) to demonstrate strong allelopathic effects on pasture species.

Mycorrhizal fungi are obligate symbionts which derive nutritional benefits from plants and contribute to plant nutrition (Tinker, 1984). According to Agrios (1988), they do not prevent disease, but the absence of mycorrhizae in certain fields results in plant stunting and poor growth. Marx (1969) concluded that in instances where plants have difficulty in obtaining nutrients, mycorrhizae will increase the efficiency of uptake, resulting in enhanced plant growth. Parke, Linderman & Black (1983) stated that mycorrhizae can also increase water uptake and/or alter the plant's physiology to reduce stress response to drought. The work of Marais (1974) confirmed that mycorrhizal infection is essential for the growth and survival of *P. patula*. He reported enhanced phosphate status of the macrobiont and that the microbionts act as biological deterrents to root pathogens such as *Phytophthora cinnamoni*.

2. Aims

1. To evaluate the effect of different weed residues on the growth of *P. patula* seedlings.
2. To determine whether the residues could influence the mycorrhizal colonization on *P. patula* roots.

3. Materials and Methods

Experiment 1: Influence of *C. esculentus* residues on growth of *P. patula* seedlings and mycorrhizal colonization

Site and growth medium description

In January 1996, *C. esculentus* tubers collected at the Giant's Castle Estate of Mondi Forests at Mooi River, KwaZulu-Natal (29°59'S; 29°12'E; altitude 1400m), were planted in quartz sand in Mitscherlich pots (250 mm diameter x 250 mm deep). The pots were kept in a temperature-controlled glasshouse (25°C ± 3 max. and 15°C ± 3 min.) at the University of Pretoria's experimental farm (25 °45'S; 28°15' E; altitude 1320 m). Every second day the pots received 250 ml of a complete nutrient solution (Nitch, 1972).

Foliage of flowering *C. esculentus* growing on the Mondi Forests estate was collected by cutting the plants at their base. The material was left in the sun to dry and then milled to a coarse texture (particles about 2 mm long) with a WileyArthut H. Thom Asco mill. Mitscherlich pots that drained freely were filled with 6 kg of a 50:50 soil mixture consisting of oldland soil (clay loam) and pure quartz sand. Oldland soil was collected on the Estate at a site where, for several years, *P. patula* seedlings had failed to grow properly. The soil properties were as follows: pH (H_2O) 4.87; Bray I P 7.93 and exchangeable Ca, Mg, K and Na of 77.90; 111.40; 151.40 and 20.10 mg kg^{-1} respectively. "Dilution" of the natural soil with quartz sand was done so that allelopathic effects were less likely to be masked by adsorption of allelochemicals on soil colloids such as clay minerals and organic matter. The milled *C. esculentus* foliage was incorporated on a 2 % m/m basis into the soil mixture. This weed residue concentration was estimated to simulate reasonably accurately, on the basis of amount of weed material per $1m^2$, the natural

C. esculentus infestation of mature plants at the study site.

Experimental design

The experiment consisted of three treatments:

- Control, with no weed material added;
- *P. patula* seedlings growing in the soil mixture containing incorporated foliage material of *C. esculentus*;
- Leachate, collected from the root zone of immature *C. esculentus* plants in the glasshouse, applied to *P. patula* seedlings.

P. patula seedlings, about 50-60 mm in height, were obtained from the Mondi Forest nursery in Pietermaritzburg. A day after pots were filled with the soil mixture, two *P. patula* seedlings were planted about 50 mm apart in the pots. Special care was taken not to disturb the root systems unduly. After transplanting, pots received water to about 80% of field capacity. Afterwards, depending on the treatment, seedlings received 250 ml of the nutrient solution or 250 ml of the root leachate. The latter was obtained by pouring the nutrient solution over *C. esculentus* plants which were grown separately in the Mitscherlich pots.

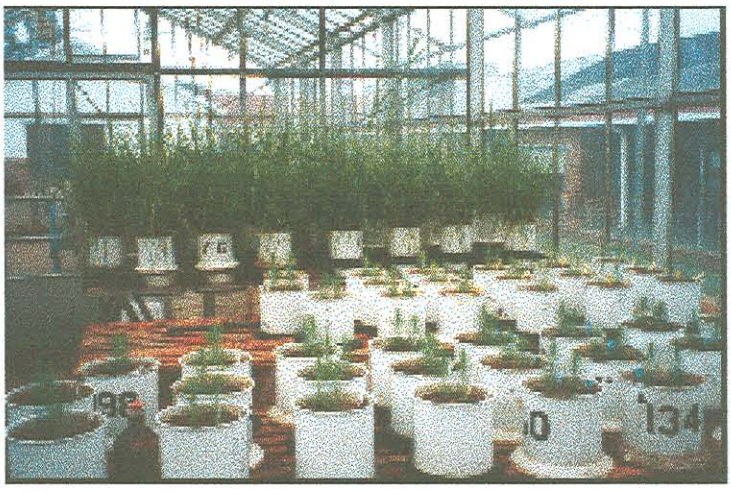

Figure 1 View of the pot experiment. Root leachate was collected from the Mitscherlich pots in the background

Two weeks after transplanting, height and stem diameter measurements were taken. Thereafter, growth measurements were made fortnightly. The growth parameters of height and stem diameter are frequently used to define growth of tree seedlings (Knowe, Nelson, Gjerstad, Zutter, Glover, Minogue & Dukes Jr., 1985; Bacon & Zedaker, 1987). Height was measured from a constant point, 20 mm from the soil surface, to the apical growth point, while the stem diameter was taken from a fixed point at the base of the stem. Seedlings were harvested nine months after they were transplanted to the pots and foliage dry mass was determined after the foliage had been dried in an oven at 70°C for three days.

After nine months' growth, the colonization of ectomycorrhizae on the roots of seedlings was measured. Ectomycorrhizal development on the root system of the pine seedlings originated from natural inoculum present in the pine bark medium used for growing seedlings in the nursery. Fifty randomly selected root pieces per treatment were analysed. Cleaned root pieces were placed in a petri dish with water and scanned under a stereoscope at 10X magnification. The number of ectomycorrhizal morphological types cm^{-1} of root was recorded. Rating numbers 0.5, 1.0, 2.5, and 6.0 were multiplied by the corresponding number of ectomycorrhizal morphological types, *viz.* monopodial, bifurcated, coralloid (3-5 tips) and coralloid (>5 tips), respectively. This rating system was used to determine more accurately the total number of ectomycorrhizal tips cm^{-1} of root.

Statistical analysis

Each treatment was replicated ten times. A completely randomized design was used. Analysis of variance was carried out to determine the effect of the different *C. esculentus* treatments on the percentage height and stem diameter increase of the seedlings. Percentage growth increase was calculated as the final height or

stem diameter minus the initial height or stem diameter divided by the initial height or stem diameter multiplied by 100. Differences between treatment means were identified using the Least Significant Difference Test at P=0.05.

Experiment 2: Influence of incorporated residues of *B. pilosa* and *C. albida* on the growth of *P. patula* seedlings and mycorrhizal root colonization on two types of soil

Site and growth medium description

Above-ground parts of actively growing *B. pilosa* and *C. albida* were collected at the Hatfield experimental farm of the University of Pretoria. The same procedure for weed material preparation was used as that in Exp. 1. Two types of soil, afforested and oldland soil, were collected at Mondi's Giant's Castle Estate (KwaZulu-Natal). The two collection sites were about 30 m apart. Soil properties are given in Table 1.

Table 1 Soil properties of afforested and oldland soil collected at the Giant's Castle Estate of Mondi Forests at Mooi River

	pH (H_2O)	Bray I mg kg^{-1}	Ca mg kg^{-1}	Mg mg kg^{-1}	K mg kg^{-1}	Na mg kg^{-1}
Afforested Soil	4.48	5.12	99.70	102.70	109.40	14.40
Oldland Soil	4.87	7.93	77.90	111.40	151.40	20.10

The soil was left in the sun to dry for three days and then sifted through a 2 mm sieve. Plastic pots, 160 mm in height and 195 mm in diameter, with holes in the base for drainage, were filled with 2.2 kg of each type of soil. The milled weed materials were incorporated into the soil on a 1% (m/m) basis, and again 1.5 months into the experiment. Therefore the total weed material added was 2% (m/m). No weed

material was present in the control. The weed residue concentration of 2% was estimated to represent a field infestation of approximately 90 mature plants m^{-2}.

Experimental design

The experiment consisted of six treatments:

- Control, with no weed material added;
- Incorporated *B. pilosa* material into both types of soil, and
- Incorporated *C. albida* material into both types of soil.

The same batch of *P. patula* seedlings as in Exp.1 was used. A single *P. patula* seedling was transplanted to each pot early in February 1996, a day after the pots were filled with soil. The seedlings received 250 ml of the same complete nutrient solution every second day. Growth parameters were the same as in Exp. 1. The seedlings were harvested and the foliage dry mass determined, twelve weeks after seedling transplant.

Ectomycorrhizal colonization was measured, as described for Exp. 1.

Statistical analysis

Each treatment was replicated ten times in a factorial design with factors treatment and soil type. Beginning height and stem diameter data of seedlings growing on afforested soil required transformation to squared values and analysis of variance was conducted on the transformed data.

4. Results and Discussion

Experiment 1: Influence of *C. esculentus* residues on growth of *P. patula* seedlings and mycorrhizal colonization

1. Height

Data for the percentage increase in seedling height are not presented as there

were no significant effects (ANOVA appears in Table 1 in Appendix A).

2. Stem diameter

The control seedlings had a significantly higher growth rate than seedlings watered with *C. esculentus* root leachate (Table 2). The differences in percentage stem diameter increase between the two weed treatments were not significant. Seedlings treated with root leachate of *C. esculentus* had a significantly greater stem diameter than the other treatments at the onset of the experiment. Despite this, the distinct significant growth difference between plants exposed to the root leachate of *C. esculentus* and the control plants strongly suggests the presence of inhibitory compounds that were released from the weed material into the soil.

Table 2 Stem diameter increase of *Pinus patula* seedlings at different *Cyperus esculentus* treatments (ANOVA in Table 2, 3 and 4 in Appendix A)

Treatment	Final Diameter (mm)	Initial Diameter (mm)	Growth Increase (%)
Control	11.50 a	1.38 b	740.20 a
Applied leachate	11.50 a	1.63 a	611.76 b
Incorporated material	10.54 b	1.41 b	670.25 ab
Standard error	0.189	0.048	30.94
CV (%)	6.55	12.68	17.95

Means followed by the same letter are not significantly different at P=0.05.

Allelochemicals were probably continuously applied to the pine seedlings through the leachate, while with the incorporated material, once it was degraded, it was not

applied again. Also different types of allelochemicals and/or varying concentrations of these compounds could have been involved. This could explain the difference in growth inhibition between the weed treatments.

Information on the allelopathic abilities of *C. esculentus* and *C. rotundus* (purple nutsedge) are available. Tames, Getso & Vieitez (1973) and Drost & Doll (1980) concluded that compounds in *C. esculentus* extracts were inhibitory to seed germination and growth of certain crop species. Meissner, Nel & Smit (1979) grew *C. rotundus* in sterilised, well-fertilized soil and showed that the growth of *H. vulgaris* (barley), *Cucumis sativus* (cucumber) and *Lycopersicum esculentum* (tomato) were considerably reduced after the weed was removed. *C. esculentus* was near maturity when collected for incorporation into the soil, while the leachate was periodically obtained from plants growing actively. Growth stage can be vital for the release of growth-inhibiting allelochemicals. Yakle & Cruse (1984) stated that large quantities of crop residues are left on the soil surface due to non-tillage, but that reductions in maize root and shoot mass were more pronounced when fresh residues were incorporated.

3. Dry mass

Data for the dry mass of seedlings are not presented as differences between the means were not significant (ANOVA in Table 5 in Appendix A).

4. Ectomycorrhizal growth

The presence of *C. esculentus* residues and the application of root leachate from live plants significantly inhibited colonization of ectomycorrhizae on roots of *P. patula* seedlings (Table 3). The treatment effect was significant in the case of

each EM morphological type.

Table 3 Ectomycorrhizal colonization of *Pinus patula* roots in soil containing root leachate or leaf material (2% m/m) of *Cyperus esculentus*

	EM morphological types cm^{-1} root				
Treatment	**Monopodial**	**Bifurcated**	**Coralloid (3-5 tips)**	**Coralloid (>5 tips)**	**Rated Total ***
Control	2.44 ab	1.72 b	0.88 a	2.68 a	20.98 b
Leachate	1.80 b	3.00 b	0.96 a	0.52 b	9.33 a
Leaf tissue	3.52 a	4.60 a	0.28 b	0.20 b	8.28 a

Means sharing a common letter are not significantly different at P=0.05

* EM rating: Monopodial = 0.5 x value; Bifurcated = 1.0 x value; Coralloid 3-5= 2.5 x value
> 5= 6.0 x value

According to Perry & Choquette (1987), allelopathic effects on ectomycorrhizae vary not only with allelochemical concentration, but are highly specific to fungal species and the nature of the allelochemical. They found that different water soluble leachates from various types of plant litter and at different concentrations significantly inhibited ectomycorrhizae colonization. Several fulvic acids and soil extracts stimulated growth of *Pisolithus tinctorius*, a fungus that was also inhibited by leachates from different plant litters. Chu-Chou (1978) found that water extracts of old *P. radiata* roots completely inhibited the growth of a mycorrhizal fungus associated with the pine species.

The significant inhibition of the mycorrhizal growth on the roots of the *P. patula* seedlings in the present study can probably be attributed to the presence of

allelochemicals in the incorporated *C. esculentus* material and those contained in the root leachate. The inhibition of *P. patula* seedling growth on oldlands could therefore be indirectly due to insufficient colonization of mycorrhizae and thus be ascribed to the loss of benefits provided by the symbiont to the tree species.

Experiment 2: Influence of incorporated residues of *B .pilosa* and *C. albida* on the growth of *P. patula* seedlings and mycorrhizal root colonization on two types of soil

1. Height

The interaction Treatment x Soil type was not significant for *P. patula* height increases (ANOVA appears in Table 6 in Appendix A). The percentage height increase of *P. patula* seedlings was significantly influenced by the incorporation of *B. pilosa* and *C. albida* on both types of soil (Tables 4 and 5).

Table 4 Effect of incorporated *Bidens pilosa* and *Conyza albida* on the percentage height increase of *Pinus patula* seedlings on afforested soil (ANOVA for each parameter presented appears in Table 7, 8 and 9 of Appendix A, respectively)

	Final Height (mm)	Initial Height (mm)	Growth Increase %
Control	20.20 b	9.81 b	102.40 b
Bidens pilosa	26.65 a	12.90 a	106.29 b
Conyza albida	19.34 b	7.80 c	150.13 a
Standard Error	1.55	0.43	14.90
CV (%)	22.29	24.81	36.39

Means followed by the same letter are not significantly different at P=0.05.

Table 5 Effect of incorporated *Bidens pilosa* and *Conyza albida* on the percentage height increase of *Pinus patula* seedlings on oldland soil (ANOVA for each parameter presented appears in Table 10, 11 and 12 in Appendix A, respectively)

	Final Height (mm)	Initial Height (mm)	Growth Increase %
Control	15.89 b	10.43 b	53.34 b
Bidens pilosa	20.30 a	11.65 a	74.18 ab
Conyza albida	17.94 ab	9.55 c	87.01 a
Standard Error	0.92	0.22	7.61
CV (%)	15.28	6.62	32.38

Means followed by the same letter are not significantly different at P=0.05.

Incorporated *C. albida* material significantly increased the height growth of *P. patula* seedlings on both soil types (Table 4 and 5). Although not significant, *B. pilosa* also showed a tendency to increase growth. According to Rice (1984), allelopathy encompasses both stimulatory and inhibitory effects, depending on the concentration of allelochemicals present. If the concentration is low, growth increase may occur, while at a higher concentration the same allelochemicals can cause inhibition of growth. Based on current data the foregoing explanation seems the most plausible for the effects shown in Tables 4 and 5, although it is realized that only much more fundamental work could provide concrete evidence of actual growth stimulatory allelopathic effects.

2. Stem diameter

The interaction Treatment X Soil type was not significant (ANOVA appears in Table 13 in Appendix A). As shown in Table 6 and 7, the incorporated weed material

significantly increased the stem diameter of the pine seedlings.

Table 6 Effect of incorporated *Bidens pilosa* and *Conyza albida* on the percentage stem diameter increase of *Pinus patula* seedlings on afforested soil (ANOVA for each parameter presented appears in Table 14, 15 and 16 in Appendix A, respectively)

	Final Diameter (mm)	Initial Diameter (mm)	Growth Increase %
Control	8.43 b	1.43 a	489.83 b
Bidens pilosa	9.81 a	1.15 b	539.55 b
Conyza albida	9.00 b	1.55 a	694.66 a
Standard Error	0.22	0.04	22.49
CV (%)	7.68	12.41	12.41

Means followed by the same letter are not significantly different at P=0.05.

Table 7 Effect of incorporated *Bidens pilosa* and *Conyza albida* on the percentage stem diameter increase of *Pinus patula* seedlings on oldland soil (ANOVA for each parameter presented appears in Table 17, 18 and 19 in Appendix A, respectively)

	Final Stem Diameter (mm)	Initial Stem Diameter (mm)	Growth Increase %
Control	6.86 b	1.42	394.08 b
Bidens pilosa	8.83 a	1.48	514.41 a
Conyza albida	9.28 a	1.35	554.02 a
Standard Error	0.24	ns	26.72
CV (%)	8.71	13.70	16.56

Means followed by the same letter are not significantly different at P=0.05.

The incorporated *C. albida* and *B. pilosa* material caused significant seedling stem diameter increases compared to the control. Meissner, Nel & Beyers (1986) reported the inhibitory effect of *B. pilosa* on certain crop species. The significance of the increase in growth is that it indicates that the inhibitory compounds were either absent or present at concentrations too low to retard growth of seedlings.

Small differences in the nutrient status of the two types of soil (Table 8), which were measured at the end of the trial, are unlikely to have been responsible for the difference in growth between the different treatments (Table 4, 5 ,6 and 7).

Table 8 Soil analysis for the two different types of soil after seedlings grew for a period of twelve weeks

Soil	pH (H_2O)	Bray I P mg kg^{-1}	Mg mg kg^{-1}	K mg kg^{-1}	Na mg kg^{-1}
Afforested					
Control	4.57	24.23	299.5	930.6	128.4
Bidens pilosa	4.72	28.96	141.5	926.6	126.9
Conyza albida	4.62	28.63	302.5	459.6	121.3
Oldland					
Control	4.42	16.62	296.5	1004.6	124.1
Bidens pilosa	4.62	18.58	318.5	521.6	124.2
Conyza albida	4.66	29.21	357.5	884.6	113.2

3. Dry mass

Only main effects were significant (ANOVA appears in Table 20 in Appendix A). A significant greater dry mass was obtained for pine seedlings with incorporated

weed material on both soils (Table 9). This is in accordance with height and diameter data obtained.

Table 9 Dry mass for *Pinus patula* seedlings growing on two types of soil with incorporated *Bidens pilosa* and *Conyza albida* foliage material (ANOVA in Table 21 and 22 in Appendix A)

	Foliage Dry Mass	
	Afforested Soil	**Oldland Soil**
Control	15.72 b	10.15 b
Bidens pilosa	23.64 a	18.89 a
Conyza albida	19.51a	17.18 a
Standard Error	1.125	0.588
CV (%)	18.12	12.22

Means sharing a common letter are not significantly different at P=0.05

4. Ectomycorrhizal growth

Only main effects were significant. The effects the weed treatments had on mycorrhizal growth are shown in Table 10.

Table 10 Ectomycorrhizal colonization of *Pinus patula* roots on both afforested and oldland soil containing *Bidens pilosa* and *Conyza albida* residues (ANOVA appears in Tables 23 and 24, Appendix A)

	EM morphological types cm^{-1} root	
	Afforested Soil	**Oldland Soil**
Control	15.3 a	11.2
Bidens pilosa	8.9 b	8.9
Conyza albida	7.7 b	9.7
Standard Error	3.95	NS
CV (%)	37.2	

Means sharing a common letter are not significantly different at P=0.05

The incorporated weed foliage had a significant inhibiting effect on mycorrhizal growth on both types of soil. Due to optimal growth conditions, the inhibition of mycorrhizae probably did not manifest in poor pine growth. As mentioned earlier, mycorrhizae help the host acquire nutrients and water, and it is thus conceivable that, with optimal supply of both growth factors, the benefits of mycorrhizae would have been masked in the present study. It is evident that both weed treatments suppressed the EM colonization on pine roots.

5. Conclusions

Incorporation of residues of different weed species had different effects on the growth of pine seedlings. *C. esculentus* was deleterious towards pine growth, while *B. pilosa* and *C. albida* were not. It is therefore likely that inhibition of seedling growth can be expected in new plantations where *C. esculentus* has grown for some time. However, it is also possible that, with time, *B. pilosa* and *C. albida* could also

inhibit pine growth, given situations where weed infestations have become well established over several years. Inhibitory effects may be more pronounced in the field situation where, with every growth season, residues build up. Because all the weed treatments significantly inhibited EM colonization on pine roots, it is suggested that pine seedling growth will be indirectly impeded as a result, particularly under field conditions where the bionts' vitality is closely linked. It is concluded that compounds released from the foliage of *C. esculentus* were responsible for inhibition of both seedling growth and mycorrhizal colonization on seedling roots, but the possible stimulatory effect of *B. pilosa* and *C. albida* warrants further investigation.

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Appendix A

Cyperus esculentus

1.Height

Table 1 Analysis of variance of the percentage height increase of *Pinus patula* seedlings at different *Cyperus esculentus* treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	1867.696	933.848	0.13	0.8817
Error	42	310591.773	7395.042		
Total	44	312459.569			

R^2.100 = coefficient of determination

R^2 = 0.0600

2.Diameter

Table 2 Analysis of variance of the percentage stem diameter increase of *Pinus patula* seedlings at different *Cyperus esculentus* treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	124055.868	62027.934	4.24	0.0211
Error	42	614928.461	14641.154		
Total	44	738984.330			

R^2 = 0.1679

Table 3 Analysis of variance of the initial stem diameter increase of *Pinus patula* seedlings at different *Cyperus esculentus* treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	0.556	0.278	7.97	0.0012
Error	42	1.467	0.035		
Total	44	2.023			

R^2 = 0.2750

Table 4 Analysis of variance of the final stem diameter increase of *Pinus patula* seedlings at different *Cyperus esculentus* treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	9.152	4.576	8.53	0.0008
Error	42	22.539	0.537		
Total	44	31.691			

R^2 = 0.2888

3. Mass

Table 5 Analysis of variance of seedling dry mass due to different *Cyperus esculentus* treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	20.725	10.363	0.67	0.5189
Error	42	653.075	15.549		
Total	44	673.800			

R^2 = 0.0308

Bidens pilosa and Conyza albida

1. Height

Table 6 Analysis of variance of *Pinus patula* seedling height increase on both types of soil growing containing incorporated *Bidens pilosa* and *Conyza albida* material

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	22191.278	11095.134	9.23	0.0004
Time	1	28833.274	28833.274	23.98	0.0001
Treatment X Soil	2	2520.806	1260.403	1.05	0.3579
Error	52	62532.349	1202.545		
Total	57	116076.708			

R^2 = 0.4613

Table 7 Analysis of variance of the initial height of *Pinus patula* seedlings on afforested soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	58333.231	29166.615	39.47	0.0001
Error	27	19952.223	738.971		
Total	29	78285.454			

R^2 = 0.7451

Table 8 Analysis of variance of the final height of *Pinus patula* seedlings on afforested soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	319.261	159.630	6.60	0.0046
Error	27	653.269	24.195		
Total	29	972.530			

R^2 = 0.3283

Table 9 Analysis of variance of the percentage height increase of *Pinus patula* seedlings on afforested soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	14048.999	7024.499	3.16	0.0583
Error	27	59946.898	2220.255		
Total	29	73995.897			

R^2 = 0.1899

Table 10 Analysis of variance of the initial height of *Pinus patula* seedlings on oldland soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	22.254	11.127	22.88	0.0001
Error	27	13.131	0.486		
Total	29	25.285			

R^2 = 0.6289

Table 11 Analysis of variance of the final height of *Pinus patula* seedlings on oldland soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	92.130	46.065	6.11	0.0069
Error	25	188.521	7.541		
Total	27	280.651			

$R^2 = 0.3283$

Table 12 Analysis of variance of the percentage height increase of *Pinus patula* seedlings on oldland soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	5516.121	2758.060	5.29	0.0121
Error	25	13035.331	521.413		
Total	27	1855.452			

$R^2 = 0.2973$

2. Diameter

Table 13 Analysis of variance of *Pinus patula* seedling stem diameter increase on both types of soil growing containing incorporated *Bidens pilosa* and *Conyza albida* material

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	337696.614	168848.307	29.54	0.0001
Time	1	110392.796	110392.796	19.31	0.0001
TreatmentxSoil	2	32137.941	16068.970	2.81	0.0693
Error	52	297214.201	5715.658		
Total	57	777441.551			

$R^2 = 0.6178$

Table 14 Analysis of variance of the initial stem diameter of *Pinus patula* seedlings on afforested soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	214801.190	107400.595	21.23	0.0001
Error	27	136605.156	5059.450		
Total	29	351406.346			

$R^2 = 0.6113$

Table 15 Analysis of variance of the final stem diameter of *Pinus patula* seedlings on afforested soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	9.618	4.809	9.89	0.0006
Error	27	13.130	0.486		
Total	29	22.748			

$R^2 = 0.4229$

Table 16 Analysis of variance of the percentage stem diameter increase of *Pinus patula* seedlings on afforested soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	214801.190	107400.595	21.23	0.0001
Error	27	136605.156	5059.450		
Total	29	351406.346			

$R^2 = 0.6113$

Table 17 Analysis of variance of the initial stem diameter of *Pinus patula* seedlings on oldland soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	0.0847	0.042	1.12	0.3397
Error	27	1.017	0.038		
Total	29	1.102			

$R^2 = 0.0769$

Table 18 Analysis of variance of the final stem diameter of *Pinus patula* seedlings on oldland soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	31.878	15.939	30.70	0.0001
Error	25	12.980	0.519		
Total	27	44.858			

R^2 = 0.7106

Table 19 Analysis of variance of the percentage stem diameter increase of *Pinus patula* seedlings on oldland soil

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	133289.497	66644.748	10.37	0.0005
Error	25	160677.163	6427.087		
Total	27	293966.670			

R^2 = 0.4534

3. Mass

Table 20 Analysis of variance of *Pinus patula* seedling dry mass on both types of soil containing incorporated *Bidens pilosa* and *Conyza albida* material

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	712.605	356.303	43.30	0.001
Soil	1	267.267	267.267	32.48	0.001
TreatmentxSoil	2	28.477	14.238	1.73	0.187
Error	52	427.860	8.228		
Total	57	1434.195			

R^2 = 0.701

Table 21 Analysis of variance of seedling dry mass pine seedlings growing on oldland soil containing incorporated *Bidens pilosa* and *Conyza albida*

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	413.198	206.599	59.70	0.0001
Error	25	86.511	3.460		
Total	27	499.710			

R^2 = 0.8268

Table 22 Analysis of variance of seedling dry mass pine seedlings growing on afforested soil containing incorporated *Bidens pilosa* and *Conyza albida* foliage

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	313.825	156.912	12.41	0.0002
Error	27	341.509	12.648		
Total	29	655.334			

R^2 = 0.4789

Table 23 Analysis of variance of ectomycorrhizal colonization of *Pinus patula* roots in afforested soil containing *Bidens pilosa* and *Conyza albida* residues

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	168.11	84.06	5.40	0.021
Error	12	186.93	15.58		
Total	14	355.04			

R^2 = 0.569

Table 24 Analysis of variance of ectomycorrhizal colonization of *Pinus patula* roots in oldland soil containing *Bidens pilosa* and *Conyza albida* residues

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	2	13.63	6.82	0.28	0.757
Error	12	287.32	23.94		
Total	14	300.95			

R^2 = 0.661

Chapter 3

Growth stage of *Cyperus esculentus* influences its allelopathic effects on ectomycorrhizal growth and higher plant species

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Chapter 3

Growth stage of *Cyperus esculentus* influences its allelopathic effects on ectomycorrhizal growth and higher plant species

1. Introduction

The genus *Cyperus* comprises over 600 species, which occur mainly in tropical and temperate regions of the world, causing large reductions in crop yields (Holm, *et al.*, 1977; Gifford & Bayer, 1995). *Cyperus esculentus* (yellow nutsedge) is a herbaceous perennial weed that is characterized by prolific vegetative activity which produces a complex underground system of rhizomes and tubers (Gifford & Bayer, 1995). It can spread asexually by the formation of rhizomes that end in the production of underground tubers (Wills, Hoagland & Paul, 1980; Stoller & Sweet, 1987; Gifford & Bayer, 1995). Tubers are recognized as the primary dispersal unit. Flowering is variable and many populations do not flower after a cropping season's growth. Sometimes mature, viable seed will develop, but seedlings from seed apparently lack the vigour required for survival (Stoller & Sweet, 1987).

Stoller, Wax & Slife (1979) investigated the competition effect of *C. esculentus* with *Zea mays* (maize). An 8% yield reduction was achieved for every 100 shoots m^{-2}. Yield reduction of 41% occurred when no weed control was done on a field initially infested with 1200 shoots m^{-2}.

C. esculentus and *C. rotundus* (purple nutsedge) are also known for their allelopathic abilities. Drost & Doll (1980) concluded that extracts and residues of *C. esculentus* have an inhibitory effect on growth of *Glycine max* (soyabeans) and *Z. mays*

(maize). Tames, Getso & Vieitez (1973) found compounds in *C. esculentus* tubers that were inhibitory to *Avena fatua* (oat) coleoptiles and seed germination of other crops. Horowitz & Friedman (1971) dried *C. esculentus* tubers and mixed them with soil. The root and top growth of *Hordeum vulgaris* (barley) planted in the soil were significantly reduced. Meissner, Nel & Smith (1979) grew *C. rotundus* in sterilized, well-fertilized soil and showed that the growth of *H. vulgaris* (barley), *Cucumis sativus* (cucumber) and *Lycopersicum esculentum* (tomato) were considerably reduced after the weed was removed and the crops established.

According to Graham (1988), mycorrhizal colonization begins after seedling germination, when the radicle is growing rapidly. Several researchers have reported that mycorrhizae help plants to acquire mineral nutrients from the soil, especially immobile elements such as phosphorous (P), zinc (Zn) and copper (Cu) (Marais, 1974; Schenck, 1981; Tinker, 1984; Graham, 1988). Marx & Bryan (1971), concluded that mycorrhizae can further reduce transplant injury and help plants to withstand high temperatures. It is also clear from research done by Marx (1973) that ectomycorrhizae protect trees from root pathogen infection. Robinson (1972) demonstrated that run-off from roots of living and raw humus of *Calluna vulgaris* (heather) contained a factor toxic to several mycorrhizal fungi. The data also showed that the inhibitor may have prevented infection of *Calluna* by certain pathogenic fungi. Aqueous extracts of *Populus temula* leaves inhibited the growth of several species of *Boletus*, a mycorrhizal fungus. They also have a weaker inhibitory effect on litter-decomposing species of *Marasmius*. The inhibitors was identified as catechol and benzoic acid.

According to Rice (1995), phenols, benzoic acid and derivates have a mixed origin. Some compounds originate directly from dehydoshikimic acid, others from acetate, but apparently most are derived from cinnamic acid. These compounds have been the most commonly identified allelopathic chemicals produced by higher plants. *p*-Hydroxybenzoic acid and vanillic acid are the most commonly identified benzoic acid derivates involved in allelopathy.

2. Aims

1. To verify that *C. esculentus* is inhibitory towards ectomycorrhizal growth.
2. To investigate the effects of aqueous extracts of *C. esculentus* of varying age on seed germination and early seedling growth of certain crop species.
3. To evaluate the effect of compounds identified in *C. esculentus* on seed germination and early seedling development.

3. Materials and Methods

Experiment 1: Effect of aqueous extracts of *C. esculentus* tubers and parts of varying age on ectomycorrhizal growth

Preparation of extracts

In May 1998, *C. esculentus* foliage and tubers were collected at the Giant's Castle Estate of Mondi Forests (Pty) Ltd. KwaZulu-Natal province (29°59'S; 29°12'E; altitude 1400m). Foliage from immature and mature plants were kept fresh by cooling it and cutting it separately into 20 mm lengths. Fifty grammes of foliage material and tubers were mixed with 1000 ml distilled water and macerated in Waring Commercial blender for 30 seconds. The suspension were filtered through Whatman no. 1 filter paper and the supernatant was used for testing allelopathic potential.

Ectomycorrhizal growth

A modified Melin-Norkrans (MMN) agar medium (Marx, 1969) was prepared for mycorrhizal growth. After autoclaving the mixture for 20 minutes at 121 °C, the pH of the medium was adjusted to between 5.5 and 5.7. Sterilized petri dishes (90 mm in diameter) were filled with 30 ml of MMN medium. One method involved placing the ectomycorrhiza (EM) inoculum (*Boletus maxaria*) in the middle and applying 1 ml of the different *C. esculentus* extracts, filtered through a 0.2 μm micropore filter and a sterilized syringe, around it in four holes in the agar medium. In the second method, 1 ml extract was spread evenly over the agar surface and the inoculum placed in the middle. For the control, 1 ml of distilled water was applied. After three weeks the colony diameter was calculated, diagonally and across.

Statistical analysis

A factorial design (2 X 4) design was used with two application methods and four *C. esculentus* growth stages. Each treatment was replicated five times. Analysis of variance was done to determine the effect of the different *C. esculentus* extracts on the growth of the EM. Mean separation was done by the Least Significant Difference (LSD) test at P=0.05.

Experiment 2: Effect of aqueous extracts of *C. esculentus* tubers and plants of varying age on the germination of *L. sativa*

Preparation of extracts

The same weed material, extraction method and treatments were used for this experiment as described above. Two extract concentrations (2% and 5% (m/v)) were used.

Seed germination test

Thirty *L. sativa* seeds were randomly placed on two pieces of Whatman no. 1 filter paper in a covered petri dish, 90 mm in diameter. Treatments were kept moist by applying 5 ml of the appropriate extract once, while the control only received 5 ml of distilled water. All treatments were incubated in a growth chamber at the University of Pretoria's phytotron at a day/night temperature regime of 30/20°C with a 12/12h day/night interval. Each day, for a week, the number of seeds that had germinated in each treatment was recorded and expressed as a percentage of the total number of seeds sown. Germination was considered to be when the radicle had extended at least 2 mm.

Statistical analysis

A factorial design (2 X 4) design was used with two extract concentrations and four *C. esculentus* growth stages. A completely randomized design was used with five replicates. Analysis of variance was done on data expressed as percentage seed that had germinated. Germination data for the 2% extract concentration required transformation to logarithm values and analysis of variance was conducted on transformed data. Mean separation was done by the Least Significant Difference (LSD) test at P=0.05.

Experiment 3: Effect of extracts of *C. esculentus* tubers and plants of varying age on the growth of *L. sativa*

Preparation of extracts

The same extract procedure and treatments were used as described in Exp. 2. Only the 5% extract concentration was tested.

Growing seedlings

L. sativa var. Great Lakes was used as test species. Water was used as growth

medium to eliminate the potential confounding influence of soil factors. *P. patula* seedlings were not used because they are not suited for use in hydroponics. Plastic pots (125 mm diameter) were lined with plastic bags and filled with 800 ml of a complete nutrient solution (Nitch, 1972).

The pots were kept in a temperature-controlled glasshouse (25/15°C) at the University of Pretoria's phytotron. *L. sativa* seedlings, approximately two weeks old, were carefully removed from the seed trays so as not to disturb the root system and washed in water to remove the growth medium. Roots of *L. sativa* seedlings were then suspended in the nutrient solution through a polystyrene lid fitting on the pot. The combination of seedling, lid and nutrient solution was weighed to ascertain the initial mass of the test system. After a week of growth, the whole system was weighed every second day. A maximum of 50 ml of the different extracts was applied to replace the water lost through evaporation/transpiration, and the balance was made up with nutrient solution. At the end of the growth period (14 days), the plants' fresh and dry foliage mass were determined. The seedlings were dried in an oven at 70°C for 72 h and their individual mass recorded.

Statistical analysis

Each treatment was replicated ten times. A completely randomized design was used. Analysis of variance was done to determine the effect of the different extracts on the fresh and dry mass of the test species. Mean separation was done by the Least Significant Difference test at (P=0.05).

Experiment 4: Effect of *C. esculentus* on the emergence rate of *Z. mays* on soil

Growth of seedlings

In October 1998, pots (195 mm diameter x 200 mm deep), with holes in the base

for drainage, were filled with 2.5 kg soil collected at the University of Pretoria's Hatfield experimental farm. The soil was classified as a sandy loam. The first and second treatment involved sowing *C. esculentus* tubers and leaving it for 28 days to grow (day minus 28). In treatment one, the tubers were removed when the maize seed was planted (day 0). In the second treatment, the *C. esculentus* plants were left undisturbed when the maize seed was planted. The *C. esculentus* tubers and maize seed were planted at the same day (day 0) and left for 28 days to grow in treatment four, before the *C. esculentus* plants were removed. In the fourth treatment the maize and *C. esculentus* tubers were also planted on the same day (day 0), but the weed was allowed to grow undisturbed. No *C. esculentus* plants or material were present in the soil of the control treatment at any stage. All pots received 250 ml of a complete nutrient solution (Nitch, 1972), on alternate days, for the duration of the trail. Crop emergence in each treatment was measured by counting the number of coleoptiles that had emerged from the soil. Measurements commenced 8 days after seeding of the crop, *i.e.* on the day the first coleoptiles emerged from the soil, and were made on each of the next three days. Data were expressed as the percentage seedlings that emerged out of a total number of seeds sown in each pot.

Statistical analysis

Each treatment was replicated 10 times in a completely randomized design. Data for the emergence of seeds sown were subjected to analysis of variance. Mean separation was done by the Least Significant Difference (LSD) test at P=0.05.

Experiment 5: Influence of an allelochemical identified in *C. esculentus* growth media on the germination of *L. sativa* and *Z. mays*

Preparation of extracts

The aim was to identify natural compounds in *C. esculentus* which could be biologically active against *P. patula* or its associated mycorrhizae. Four soil samples were studied. They were respectively termed:

A = Quartz sand in which *C. esculentus* was grown;

B = Oldland soil kept weed-free;

C = Oldland soil with no weed control;

D = Oldland soil with only *C. esculentus* present.

Each sample was separated into four parts and extracted with water and ethanol. Subsequently, biochemical analysis and biological tests were done.

I. Extractions:

1.1 Water extraction

A sample of 100 g dry soil or quartz sand was stirred at 100 rpm for 2 min. in 400 ml of demineralized water, and then incubated (dark, 15°C) for 14 hours. After filtration, a part of the extract was used for chemical analysis. It was mixed with 150 ml of ether and the supernatant collected. This operation was performed three times. The etheric fraction was then evaporated at room temperature during the night and compounds were solubilized in 5 ml acetaldehyde for analysis.

1.2 Ethanol extraction

Twenty grammes of dry soil and 30 g quartz sand were incubated in 50:50 (v/v) methanol:water solution at 35 °C for 20 min. After filtration, the extract was passed through a rotavator in order to remove water and alcohol. Compounds were then solubilized in 5 ml acetaldehyde for analysis.

II. Biochemical analysis

1. Total phenols

Seven ml of the water extract were added to 1 ml of Folin-Ciocalteu and 2 ml Na_2CO_3 and incubated for 20 min at 40°C. Absorbency was then read at 760 nm. Quantities were obtained with standard gallic acid.

2. H.P.L.C.

This method gave the identity and quantity of phenolic compounds. The compounds identified in the water extract are presented in Table 1. The data for the ethanolic extracts are not presented, as the same compounds were identified.

Table 1 Phenolic compounds identified in water extracts of artificial and natural growth media for *C. esculentus*

	Quartz sand µg/g	Oldland weed-free µg/g	Oldland with weeds µg/g	Oldland soil with only *C. esculentus* µg/g
***P*-OHbenzoic acid**	0.012	0.003	0.004	0.008
***P*-OHbenzaldehyde**	0.005	0.001	0.001	0.001
Vanillic acid	0.141	0.056	0.071	0.183
Syringic acid	0.000	0.000	0.015	0.000
Vanillin	0.000	0.014	0.015	0.003
***P*-coumaric**	0.000	0.000	0.000	0.000
Ferulic acid	0.000	0.000	0.000	0.000

Vanillic acid was identified in *C. esculentus* growth media in the highest concentrations and therefore, it was decided to work with this compound. It was obtained from Merck laboratories and a concentration range was prepared with

distilled water, to give: 0, 0.04, 0.08, 0.12, 0.16, 0.2 and 0.24 mg l^{-1}.

Seed germination test

Ten ml of vanillic acid were added to lettuce seeds while 6-ml, 8-ml or 10-ml volumes were applied to the maize seeds. Fifteen lettuce or ten maize seeds were placed on Whatman no. 3 filter paper, in sterilized petri dishes. The maize seeds were surface sterilized with 1.5% sodium hypochlorite for 1 min. All these steps were performed in a laminar flow cabinet. The pH (H_20) of the solutions was between 4.93 and 5.03. All treatments were incubated at 25°C throughout the trail in the dark at the University of Pretoria's phytotron. Seeds were considered germinated when radicles were at least 2 mm in length and also healthy in appearance.

Statistical analysis

Treatments were replicated ten times in a completely randomized design. Data for the number of seeds germinated were subjected to analysis of variance, as described in Experiment 1.

4. Results and Discussion

Experiment 1: Effect of aqueous extracts of *C. esculentus* tubers and parts of varying age on ectomycorrhizal growth

The first method, where *C. esculentus* extracts were applied to holes in the growth medium, was unreliable because the EM grew over the growth medium cavity. Data obtained using the second method appears in Table 2. All the extract treatments caused significant inhibition of EM growth. The inhibition caused by the mature *C. esculentus* plant extracts was significantly greater than that effected by the tuber extracts (Table 2). The influence of the tuber and immature plant extract was not significantly different. This indicates that all the extracts contained

growth inhibitory substance(s).

Table 2 Effect of the different *Cyperus esculentus* extracts on ectomycorrhizal (*Boletus maxaria*) growth on artificial medium (ANOVA appears in Table 1, Appendix B)

Extract	Ectomycorrhizal diameter growth (mm)
Control	7.20 a
Tubers	6.33 b
Immature plants	6.00 bc
Mature plants	5.83 c
Standard Error	0.141
CV (%)	9.92

Means followed by the same letter are not significantly different at P=0.05.

The inhibition of growth of *B. maxaria* in the present study was less pronounced than that reported by Reinhardt, Khalil, Labuschagne, Claassens & Bezuidenhout (1996) for unidentified mycorrhizal types found on the roots of *P. patula* seedlings, which were exposed to *C. esculentus* and two other agronomic weeds. They concluded that the inhibition of mycorrhizae, which are essential for successful pine establishment (Marais, 1974), represents an indirect allelopathic effect on the pine seedlings. This may be crucial in determining their resistance to other stress factors.

Bioassays using microorganisms have been employed to evaluate allelochemical effects. Rice (1984) describes such a method, whereby saturated paper disks with material elaborated by marine blue-green algae were used. It was placed on agar

impregnated with yeast and their action, according to the extent of an inhibition zone, was evaluated. Nilson, Hogberg, Zackrisson & Wang (1993) found that aqueous extracts of the shrub *Empetrum hermaphroditum* impaired the growth of the mycorrhiza, *Paxillus involutus*, associated with *Pinus sylvestris* (scots pine). The extract also impaired the mycorrhizal growth in culture.

Experiment 2: Effect of aqueous extracts of *C. esculentus* tubers and plants of varying age on the germination of *L. sativa*

Germination rates for seeds exposed to the different aqueous extracts of *C. esculentus* are shown in Figures 1 and 2.

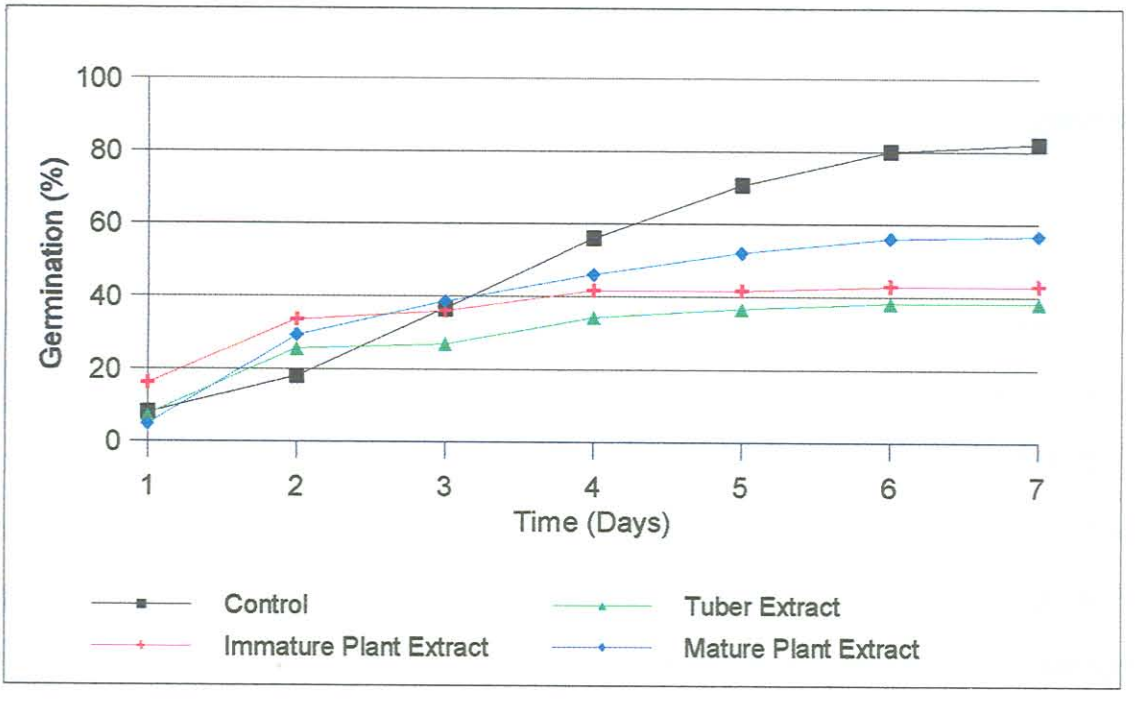

Figure 1 Germination of *Lactuca sativa* seed exposed to different *Cyperus esculentus* extracts at the 2% concentration level

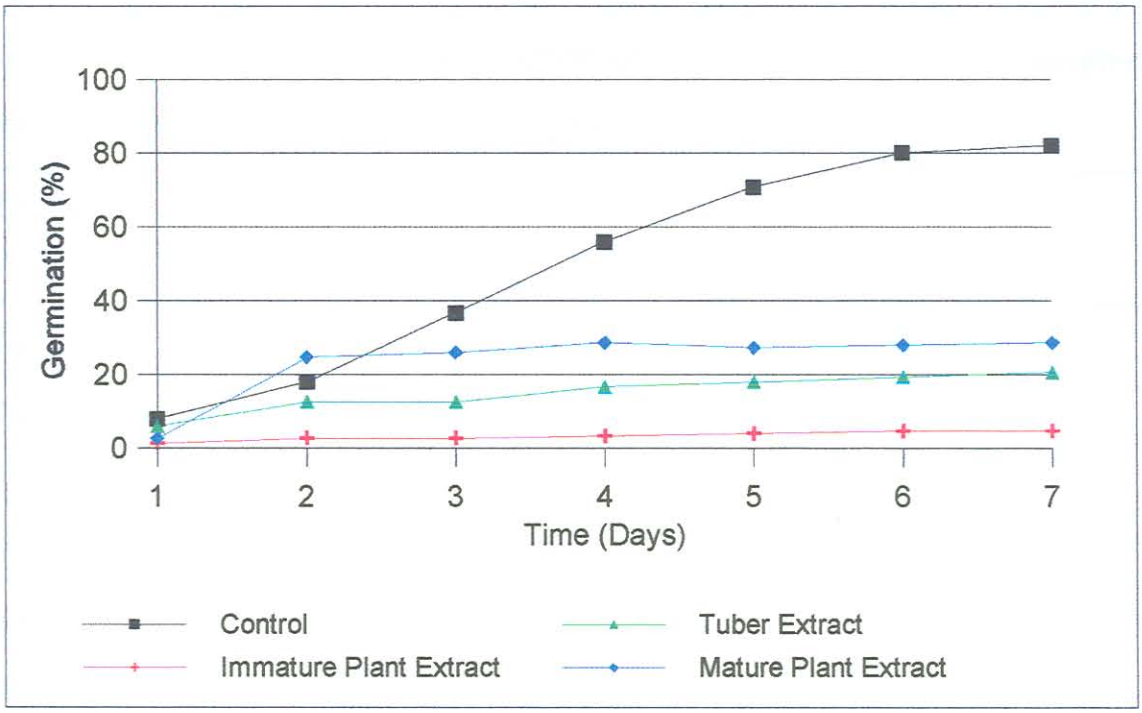

Figure 2 Germination of *Lactuca sativa* seed exposed to different *Cyperus esculentus* extracts at the 5% concentration level

The interaction Concentration X Treatment was not significant. After seven days of exposure to the extracts, at the 2% extract concentration, only the mature plant extract did not significantly inhibit seed germination, while all the 5% extracts significantly inhibited seed germination, compared to the control (Table 3). These observations correspond with the results of Exp. 1. Although not significantly different from the other treatments, the immature *C. esculentus* extract had the most inhibitory effect on seed germination. This shows that the inhibitory compounds were either different or, the same compounds more concentrated in the immature plant material than in the tubers and mature plants.

Table 3 Effect of different concentrations of aqueous *Cyperus esculentus* extracts on the percentage germination of *Lactuca sativa* after a seven-day exposure period (ANOVA appears in Tables 2 and 3, Appendix B)

Extracts	Germination	
	(2%)	(5%)
Control	82.00 a	82.00 a
Tubers	38.00 b	20.67 b
Immature plants	42.67 b	4.67 c
Mature plants	60.67 ab	28.67 b
Standard error	0.225	5.48
CV (%)	18.91	36.06

Means followed by the same letter are not significantly different at P=0.05.

Experiment 3: Effect of extracts of *C. esculentus* tubers and plants of varying age on the growth of *L. sativa*

The effects of the different *C. esculentus* extracts on the fresh and dry mass of *L. sativa* seedlings are presented in Table 4. There were no significant differences among the different *C. esculentus* treatments. All extracts significantly reduced the fresh and dry matter yield of the *L. sativa* seedlings relative to the control. In contrast to the germination results, there were no significant differences in the effect of the immature plant extract compared with the other plant part extracts, although it caused the lowest fresh and dry mass yield of the test species. This could be because the enzymatic processes involved in germination are more sensitive to the extracts than seedling development (Putnam, 1985). Brandsæter & Haugland (1999) concluded that the volume of extracts and distilled water used in bioassays influenced the results considerably and hence the conclusion of studies

where bioassays are used.

Table 4 Effects of different aqueous *Cyperus esculentus* extracts on the fresh and dry mass of *Lactuca sativa* seedlings (ANOVA appears in Table 4, Appendix B)

Extracts	Fresh Mass (g)	Dry Mass (g)
Control	93.36 a	8.26 a
Tubers	40.53 b	4.31 b
Immature plants	34.02 b	3.43 b
Mature plants	47.65 b	4.64 b
Standard error	5.92	0.56
CV (%)	34.72	34.54

Means followed by the same letter are not significantly different at P=0.05.

Experiment 4: Effect of *C. esculentus* on the emergence rate of *Z. mays* on soil

Z. mays emergence was significantly retarded when *C. esculentus* tubers were established 28 days prior to crop seeding and then either totally removed or left (Table 5). This suggests that allelochemicals, which were released into the soil by actively growing *C. esculentus* plants, reduced the rate of emergence and probably the rate of germination as well. The finding demonstrates the need to recognize the risk of poor crop establishment on fields with current or recent infestations of the weed. Weed tubers planted on the day of crop sowing did not affect the rate of crop emergence. This is in accordance with the findings of Meissner, Nel & Smith (1979). They found that the growth of *H. vulgaris* (barley), *Cucumis sativus* (cucumber) and *Lycopersicum esculentum* (tomato) were considerably reduced when, after *C. rotundus* grew in sterilized, well-fertilized soil, the weed was removed.

Burgos & Talbert (1996) reported an emergence reduction of 43-63% of *Zea mays* var. *Rugosa* when the crop was planted into residues of allelopathic rye and wheat. According to Tollenaar, Mihajlovic & Vyn (1993) removal of the above rye and wheat phytomass before maize planting, generally did not influence the delay in development and reduction in yield of the subsequent maize crop.

Table 5 Percent *Zea mays* seedlings that emerged from soil into which *Cyperus esculentus* tubers were planted either before or at sowing of the crop (Day=0) (ANOVA for each day presented appears in Table 6, 7, 8 and 9 of Appendix B, respectively)

Timing of *Cyperus esculentus* tuber planting relative to *Zea mays* seeding	*Cyperus esculentus* presence	Days after seeding			
		7	8	9	10
Control	Absent	90.00 a	95.00 a	95.00 a	95.00 a
Day 0 minus 28*	Undisturbed growth	12.50 b	37.50 b	70.00 b	92.50 a
Day 0 minus 28	Removed on day 0	17.50 b	35.00 b	75.00 b	92.50 a
Day 0 plus 28**	Undisturbed growth	95.00 a	97.50 a	97.50 a	97.50 a
Day 0 plus 28	Removed on day 28	82.50 a	97.50 a	97.50 a	97.50 a
Standard Error		6.739	8.019	7.169	NS
CV (%)		35.82	34.98	26.06	

Means followed by the same number are not significantly different at P=0.05.

* Day minus 28: Planting of weed tubers, 28 days prior to crop seeding.

** Day 0: Sowing of maize seed and planting of weed tubers.

Experiment 5: Influence of an allelochemical identified in *C. esculentus* growth media on the germination of *L. sativa* and *Z. mays*

Although not significant, there was a tendency for germination inhibition when concentrations of vanillic acid were increased (Table 6). However, some anomalies were observed. Only from 0.16 mg L^{-1} onwards, germination percentages was reduced. Therefore, if the concentration range had included higher concentrations, significant differences could have been possible. According to Willamson & Weidenhamer (1990), it is likely that the toxicity of allelochemicals is a function of both concentration (static availability at a given point in time) and flux rates (dynamic availability based on the total amount of chemical moving in and out of a system over a period of time).

Table 6 Percentage *L. sativa* germination with added vanillic acid (ANOVA appears in Table 10, Appendix B)

Concentration (mg l^{-1})	Germination (%)
0	62.23 ab
0.04	65.56 a
0.08	65.56 a
0.12	60.37 ab
0.16	62.44 ab
0.2	59.24 ab
0.24	53.96 b
Standard error	2.3
CV (%)	20.55

Means followed by the same number are not significantly different at P=0.05.

The effects of the different volumes and concentrations on the germination of maize are presented in Table 7. Anomalies were apparent at the 0.04 and 0.16 mg l^{-1} concentrations. At the control treatment, the high volume (10 ml) reduced germination significantly compared to the lowest volume (6 ml). It is concluded that the volume of solution the seeds were exposed to had a greater effect on germination than the concentrations of vanillic acid tested.

Table 7 Effect of different volumes of the concentration range of vanillic acid on the percentage germination of *Zea mays* after three days (ANOVA appears in table 11, Appendix B)

	Volume of solution added		
Concentration	**6ml**	**8ml**	**10ml**
0	84 a	75 ab	54 c
0.04	72ab	74 ab	74 ab
0.08	76 ab	79 ab	60 c
0.12	69 b	81 a	50 c
0.16	79 ab	78 ab	71 ab
0.2	78 a	68 b	49 c
0.24	81 a	83 a	53 c
Standard Error		21.47	
CV (%)		4.81	

Means followed by the same number are not significantly different at P=0.05.

5. Conclusions

Results of Exp. 1 suggest that inhibitory allelopathic effects from *C. esculentus* on mycorrhizae could be pivotal in interactions between the weed and higher plant

species associated with the symbiont. The growth inhibition on the mycorrhizae associated with *P. patula* could at least partly explain the establishment problems of new pine seedlings on former crop fields infested with *C. esculentus*.

The finding that mature weed extract had a greater inhibitory effect on the mycorrhizal growth than the other extracts was not confirmed in Exp. 2 where lettuce was used as indicator species. This apparent anomaly may, in fact, indicate that differential test species' responses should be considered in allelopathy research. With the elimination of soil as factor in the hydroponics experiment (Exp. 3), it is evident that compounds contained in *C. esculentus* material have an inhibitory effect on the growth of *L. sativa*. Different *C. esculentus* plant parts at different development stages have differential allelopathic effect on mycorrhizae and crop species. It is therefore important that the growth stage of weeds be considered in assessments of their allelopathic potential.

It is impossible to extrapolate results from the maize emergence experiment (Exp. 4 & 5) to field conditions, but it is conceivable that, under conditions favouring the production and release of allelochemicals in high concentrations by *C. esculentus*, maize seedlings would be placed under chemical stress that might weaken their resistance to other environmental stress factors.

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Appendix B

1. Mycorrhizal Growth

Table 1 Analysis of variance of ectomycorrhizae growth on agar treated with different *Cyperus esculentus* extracts

Source	Df	Sum of Square	Mean Squares	F	Pr > F
Model	3	21.307	7.102	17.83	0.0001
Error	72	28.688	0.398		
Total	75	49.995			

R^2.100=coefficient of determination

R^2 = 0.4262

2. Germination test

Table 2 Analysis of variance of *Lactuca sativa* germination with a 2% *Cyperus esculentus* extract concentration applied

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	3	3.061	1.020	4.03	0.0258
Error	16	4.046	0.253		
Total	19	7.107			

R^2=0.4307

Table 3 Analysis of variance of *Lactuca sativa* germination with a 5% *Cyperus esculentus* extract concentration applied

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	3	16853.333	5617.778	37.38	0.0001
Error	16	2404.444	150.278		
Total	19	19257.777			

R^2 =0.8751

3. Mass

Table 4 Analysis of variance of the effects of different *Cyperus esculentus* extracts on the fresh mass of *Lactuca sativa* seedlings

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	3	21702.745	7234.248	20.67	0.0001
Error	36	12599.455	349.984		
Total	39	34302.00			

$R^2 = 0.6327$

Table 5 Analysis of variance of the effects of different *Cyperus esculentus* extracts on the dry mass of *Lactuca sativa* seedlings

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	3	136.042	45.347	14.28	0.0001
Error	36	114.321	3.176		
Total	39	250.363			

$R^2 = 0.5434$

Table 6 Analysis of variance of the percent *Zea mays* seedlings that emerged seven days after seeding from soil into which *Cyperus esculentus* tubers were planted either before or at sowing of the crop

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	4	66925	16731.25	36.84	0.0001
Error	45	20437.5	454.16		
Total	49	87362.5			

$R^2 = 0.823$

Table 7 Analysis of variance of the percent *Zea mays* seedlings that emerged eight days after seeding from soil into which *Cyperus esculentus* tubers were planted either before or at sowing of the crop

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	4	43875	10968.75	17.83	0.0001
Error	45	27687.5	615.28		
Total	49	71562.5			

$R^2 = 0.797$

Table 8 Analysis of variance of the percent *Zea mays* seedlings that emerged nine days after seeding from soil into which *Cyperus esculentus* tubers were planted either before or at sowing of the crop

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	4	8175	2043.75	4.41	0.006
Error	45	20875	463.89		
Total	49	29050			

$R^2 = 0.775$

Table 9 Analysis of variance of the percent *Zea mays* seedlings that emerged ten days after seeding from soil into which *Cyperus esculentus* tubers were planted either before or at sowing of the crop

Source	Df	Sum of Squares	Mean Square	F	Pr>F
Model	4	250	62.5	0.59	0.956
Error	45	4750	105.56		
Total	49	5000			

$R^2 = 0.767$

Table 10 Analysis of variance of the percentage *L. sativa* germination with added vanillic acid

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Concentration	6	2924.501	487.416	3.07	0.0069
Time	2	78789.191	39394.596	247.88	0.0001
Concentration X Time	12	708.226	59.019	0.37	0.9721
Error	189	30036.626	158.924		
Total	209	112458.545			

$R^2 = 0.732$

Table 11 Analysis of variance of the effect of different volumes of the concentration range of vanillic acid on the germination of *Zea mays* after three days

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Concentration	6	2619.048	436.508	1.89	0.0851
Volume	2	15482.857	7741.429	33.45	0.0001
Concentration X Volume	12	6603.810	550.317	2.38	0.0071
Error	189	43740.000	231.429		
Total	209	68445.714			

$R^2 = 0.361$

Chapter 4

The influence of boron and allelochemicals released from *Cyperus esculentus* on the growth and development of *Pinus patula* seedlings

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Chapter 4

The influence of boron and allelochemicals released from *Cyperus esculentus* on the growth and development of *Pinus patula* seedlings

1. Introduction

According to Fisher (1980), a rapidly growing body of data suggests that allelopathy is important in the survival and growth of trees in both plantations and natural stands. The widespread occurrence of woody species that are allelopathic to other species, and sometimes to themselves are extensively researched. Unfortunately very little research has been done on the allelopathic effects of herbaceous angiosperms in forestry.

Fisher, Wood and Glavicic (1978) found that *Solidago* (goldenrod) and *Aster* inhibit the establishment and growth of sugar maple in old fields in Ontario. Horsley (1977) examined regeneration failure in *Prunus serotina* (black cherry) on old fields. He ruled out the effects of browsing, microclimate and competition and concluded that allelopathic inhibition by *Dryopteris novebaracensis* (New York fern) *Brachyelytrum erectum* (shorthusk grass) and *Aster* was the main cause. Poor nutrition and microclimate can be barriers to the establishment of tree species on old fields but allelopathy is often an additional adversity that must be overcome.

Cyperus esculentus is a troublesome weed in many countries, reducing crop yields through competition and allelopathy (Horowitz & Friedman 1971; Tames, Getso & Vieitez, 1973; Stoller, Wax & Slife, 1979 and Drost & Doll, 1980). It can spread

asexually by the formation of rhizomes that end in the production of underground tubers, which are recognized as the primary dispersal unit (Wills, Hoagland & Paul, 1980; Stoller & Sweet, 1987; Gifford & Bayer, 1995). According to Stoller, Nema & Bhan (1972) and Thullen & Keeley (1975), tubers may sprout several times before exhausting its energy supply. Therefore, it can be competitive for the entire season.

Boron (B) is an essential micro-element, and the plant's requirements are the greatest during leaf development, flowering and fruit setting. According to Shorrocks (1984), boron deficiency can cause abnormal cell division, i.e., the cell's longitudinal walls remain short and are thus incomplete and irregular. Leaf expansion, distorted leaf development and the lack of internode elongation can also occur. Eventually, shoot and root apical meristems die or become moribund and, after the loss of apical dominance, stunted side shoots develop from the auxiliary meristems. It was reported by Smith & Van Hyussteen (1992) and Schumann & Noble (1993) that the symptoms of poor pine seedling performance on oldlands in South Africa, involved a minimally developed root system, stunting, lack of apical dominance, chlorosis and necrosis of fascicles, and necrosis of the growth tips.

2. Aims

The influence of *C. esculentus* and boron on *P. patula* were evaluated. Specific aims were:

1. To investigate the possibility that boron deficiency can lead to the observed growth problems of *P. patula*
2. To determine the effect of an aqueous *C. esculentus* extract on *P. patula* seedling growth.

3. To measure the effect *C. esculentus* suppression by pine needles have on the growth of *P. patula*.
4. To evaluate the growth of *P. patula* seedlings growing with *C. esculentus* on three types of soil.

3. Materials and Methods

Experiment 1: Effect of added boron, an aqueous *C. esculentus* extract and pine needles on the *P. patula* seedlings grown with, or without, the weed.

Site and growth medium description

The experiment was conducted from October 1997 to December 1998 at the University of Pretoria's experimental farm. The topsoil (0-0.5 m) of two sandy loam soils, afforested and "oldland" soil, were collected in September 1997 at the Giant's Castle Estate of Mondi Forests (Pty) Ltd. KwaZulu-Natal. Afforested soil was collected from a site where no abnormal growth of existing pines was evident. The oldland soil was collected from an adjacent area (approximately 30 m apart), previously used for production of annual crops. Soil properties are given in Table 1.

Table 1 Soil properties of afforested and oldland soil collected in September 1997 at the Giant's Castle Estate of Mondi Forest at Mooi River KwaZulu-Natal

Soil	pH (H_2O)	Bray I P $mgkg^{-1}$	Ca $mgkg^{-1}$	K $mgkg^{-1}$	Mg $mgkg^{-1}$	B $mgkg^{-1}$	C %
Afforested	5.26	2.85	203.3	159	188.5	0.84	3.6
Oldland	4.9	12.54	254.3	276	158.5	1.05	3.7

Upon arrival at the experimental farm, the soils were left in the sun to dry and subsequently sieved. Solid fertilizer in the form of 2:3:2 (24) at the equivalent rate of 200 kg ha^{-1} was added to the soil prior to planting of the pine seedlings. A boron treatment involved addition of boracic acid to oldland soil at the equivalent rate of 10 kg ha^{-1}. Three kilogram of soil was placed into drained plastic pots (195 mm diameter x 160 mm deep) and were kept in a temperature-controlled glasshouse (25°C/ 15°C).

C. esculentus leaf litter were collected from a natural infestation on the oldland. An aqueous extract was prepared by cutting the leaf litter in 20 mm lengths. Fifty grammes of material were mixed with 1000 ml distilled water and macerated in a Waring commercial blender for 30 seconds. The suspension was filtered through Whatman no. 1 filter paper and the supernatant was used to test for allelopathic potential. The extract was kept at 5°C until it was used.

Experimental design

The experiment consisted of four treatments:

1. Afforested soil with no added boron or *C. esculentus* extract (T 1)
2. Oldland soil as described for the afforested soil treatment (T 2)
3. Oldland soil with only boron added (T 3)
4. Oldland soil with added boron and *C. esculentus* leaf litter extract (T 4).

Afforested soil was included in the experiment as it served as a comparison for expected normal seedling growth. *P. patula* seedlings, approximately 80-100 mm in height were obtained from the Mondi Forests nursery at Pietermaritzburg, KwaZulu-Natal. Three days after pots were filled with the soil, a single *P. patula* seedling was transplanted to each pot. Special care was taken not to disturb the

root system . Pots received 39% tap water to field capacity, where after watering was done every third day. At each watering, the extract treatment (T 4) received 150 ml water and 100 ml extract. The other treatments received 250 ml tap water. After a week, vigorous leaf development at apical meristems of the pines indicated that they had established successfully, and seedling height and stem diameter were measured. The stem diameter was measured approximately 20 mm from the soil surface. A mark was made on the stem for recurrent diameter measurements. Height was measured from a constant reference point at the soil surface to the apical growing point. Growth parameters were measured fortnightly.

During the first two weeks, herbaceous weeds, especially *C. esculentus* had sprouted on all the oldland soil but not on afforested soil. The *C. esculentus*, was allowed to grow at the three oldland soil treatments, but all other weeds were removed as they emerged. Light competition between the pine seedlings and *C. esculentus* was limited by cutting *C. esculentus* at about 100 mm from the soil surface.

The *C. esculentus* extract was applied for only 14 weeks. Thereafter, all the remaining *C. esculentus* plants were removed from this treatment and subsequently kept weed-free for the rest of the trial period.

Twenty weeks after initial fertilization, 250 ml complete nutrient solution (Nitch, 1972) was administered every third day to all the treatments. Boron was not again added to the treatments.

During September 1998, pine needles collected at the Estate, were placed on the

soil surface of treatment T 3 as a groundcover to suppress *C. esculentus* growth.

Harvesting commenced in December 1998. Seedlings were cut at the soil surface and the top-growth placed in a drying oven at 70°C for three days after which the dry mass of the seedlings was determined.

Statistical analysis

Each treatment was replicated ten times. A completely randomized design was used. Analysis of variance was done to determine the effect of the different treatments on the percentage height and stem diameter increase of seedlings. Percentage growth increase was calculated as the final height or stem diameter minus initial height or stem diameter divided by initial height or stem diameter multiplied by 100. Differences between treatment means were identified using the Least Significant Difference test at P=0.05.

Experiment 2: Growth of *P. patula* seedlings on three different types of soil in the presence of *C. esculentus*

Site and growth medium description

The experiment commenced in February 1998 and ended the following February. Three types of soil were collected in December 1997 at the Giant's Castle Estate- soil from a field where no plantation species were cultivated (grassland soil), afforested- and oldland soil. Afforested and oldland soil were collected about 30 m apart. All soils were classified as a sandy loam with soil properties given in Table 2.

Table 2 Soil properties of three types of soil collected in December 1997 at the Giant's Castle Estate

Soil	pH (H_2O)	Bray I P $mgkg^{-1}$	Ca $mgkg^{-1}$	Mg $mgkg^{-1}$	K $mgkg^{-1}$	B $mgkg^{-1}$	C %
Grassland	4.66	4.87	122	71	94	0.26	4.6
Afforested	5.23	1.64	171	251	114	0.46	5.2
Oldland	5.3	13.55	245	47	85	0.3	3.9

The soil was left in the sun to dry for three days and then sifted through a 2 mm sieve. Plastic pots, 160 mm in height and 195 mm in diameter with holes in the base for drainage, were filled with 3 kg of each type of soil. The pots were kept in a temperature-controlled glasshouse (25°C/ 15°C) at the experimental farm.

Experimental design

The experiment consisted of soil type as factor (three levels), with or without *C. esculentus*. *P. patula* seedlings, approximately 80-100 mm in height, were obtained from the Mondi Forests nursery at Pietermaritzburg. A day after pots were filled with the soil, a single *P. patula* seedling was transplanted to each pot. Special care was taken so as not disturbed the root system. Approximately 50 g *C. esculentus* tubers collected at the Estate, were planted together with the seedlings. Pots were watered to 39% field capacity with a complete nutrient solution (Nitch, 1972), where after seedlings received a total of 250 ml solution, every second day. *C. esculentus* was cut at intervals to reduce competition for light between the weed and the pine seedling.

Growth parameters were the same as in Exp. 1. Seedlings were harvested twelve months after transplant. They were cut at the soil surface and placed in a drying

oven (70°C) for three days where after the dry mass was determined.

Statistical analysis

Each treatment was replicated eight times in a completely randomized design. The same statistical analysis was used for data from both experiments.

4. Results and Discussion

Experiment 1: Effect of added boron, an aqueous *C. esculentus* extract and pine needles on the *P. patula* seedlings grown with, or without, the weed.

In Figure 1 and 2 the height and stem diameter increases of the seedlings over the 14 month growth period is displayed. During the winter, height growth was retarded but during spring and summer, growth increments increased (Figure 1).

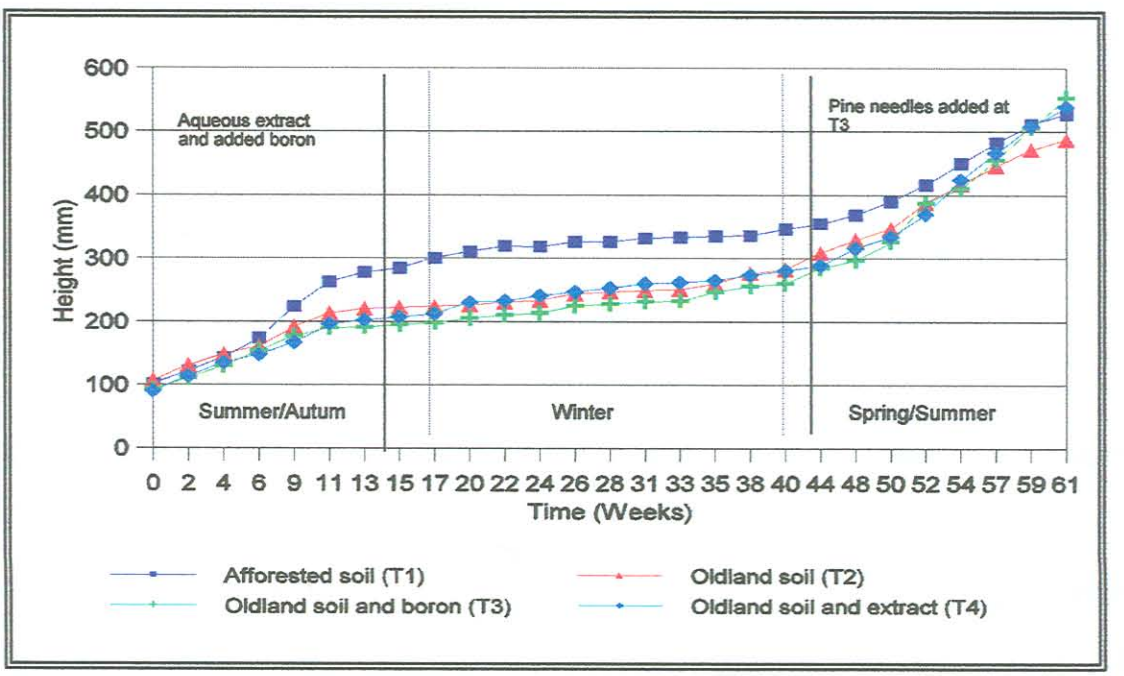

Figure 1 Effect of different treatments on the height growth of *Pinus patula* seedlings during the 14 month growth period

Stem diameter growth is considered to be a more reliable parameter of growth as

the stem diameter of the seedlings was not so severely retarded during winter as was observed with height (Figure 2).

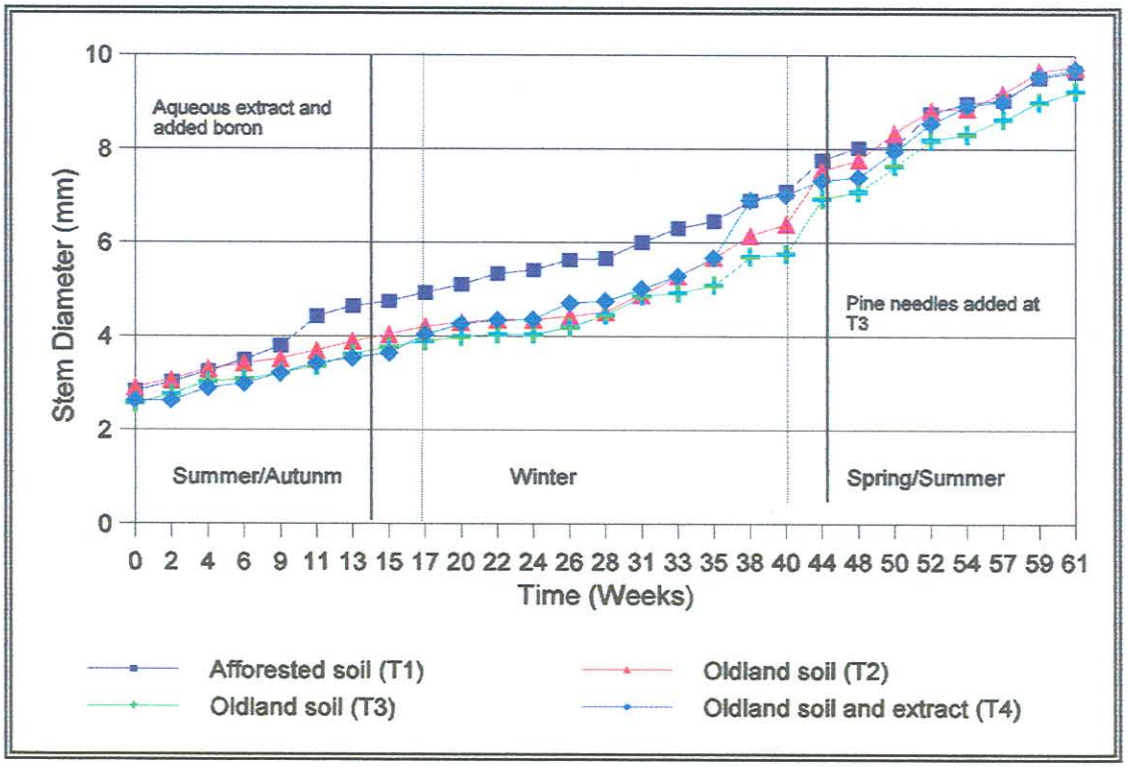

Figure 2 Effect of different treatments on the stem diameter growth of *Pinus patula* seedlings over the 14 month growth period

Experiment 1: Treatment 1: Effect of added boron and an aqueous *C. esculentus* extract on the growth of *P. patula* seedlings over a 14 week period.

Four weeks after being transplanted, seedlings on the oldland soils appeared chlorotic and were noticeably smaller than those on the afforested soil. It is unlikely that these symptoms were due to nutrient deficiencies because all the treatments received the same and adequate nutrition (Table 3).

Table 3 Soil analysis of afforested and oldland soil 14 weeks after the addition of boron and an aqueous *C. esculentus* extract

Treatment	pH (H_2O)	Bray I P $mgkg^{-1}$	Ca $mgkg^{-1}$	K $mgkg^{-1}$	Mg $mgkg^{-1}$	Na $mgkg^{-1}$
Afforested soil	5.22	3.87	505	195	382	45
Oldland soil	5.71	7.67	685	261	430	59
Oldland + Boron	5.77	7.87	721	285	445	60
Oldland +Boron+Extract	5.3	12.42	436	324	310	53

Ten weeks after the chlorotic appearance of the pine seedlings was noticed, the seedlings regained a healthy green colour.

4.1.1 Height

The percentage height increase of seedlings was significantly influenced by the different treatments, 14 weeks after transplant (Table 4). Seedlings growing on afforested soil had a significantly higher height increase than those growing at all three of the oldland soil treatments. This can be attributed to the interference from *C. esculentus* present on the oldland. There were no differences in pine height growth between seedlings growing on the three oldland soil treatments.

Table 4 Effect of added boron and an aqueous *Cyperus esculentus* extract on the percentage height increase of *Pinus patula* seedlings growing in the presence of weeds at 14 weeks after transplant (ANOVA appears in Tables 1, 2 and 3 in Appendix C)

	Final Height (mm)	Initial Height (mm)	Growth Increase (%)
Afforested soil	284.2 a	101.5 a	187.42 a
Oldland soil	222.5 b	106.9 a	112.27 b
Oldland + Boron	194.0 b	92.4 a	112.68 b
Oldland + Boron + Extract	208.5 b	93.0 a	131.10 b
Standard Error	16.01	NS	14.15
CV (%)	13.93		32.94

Means followed by the same letter are not significantly different at P=0.05.

4.1.2 Stem diameter

Seedlings on afforested soil had a significantly higher percentage stem diameter increase than seedlings on oldland soil (Table 5). This result is similar to that for height growth. Stem diameter of pine seedlings in the presence of *C. esculentus* on oldland soils, were reduced by approximately 39% compared to those growing on afforested soil (Table 5). According to Buchanan & Burns (1970), the critical period of weed competition occurs six to eight weeks after agronomic crop emergence. Keeley & Thullen (1975) found *C. esculentus* capable of reducing yields of furrow irrigated cotton when allowed to compete for periods of four weeks or more. Jooste & Van Biljon (1980), reported reduced maize yields from 11.4% to 23.9% with heavy infestations of *C. esculentus*.

Table 5 Effect of added boron and *Cyperus esculentus* extract on the percentage stem diameter increase of *Pinus patula* seedlings growing in the presence of weeds at 14 weeks after transplant (ANOVA appears in Tables 4, 5 and 6 in Appendix C)

	Final Stem Diameter (mm)	Initial Stem Diameter (mm)	Growth Increase (%)
Afforested soil	4.74 a	2.82 ab	69.00 a
Oldland soil	4.00 b	2.92 a	37.55 b
Oldland + Boron	3.76 bc	2.55 b	49.02 b
Oldland + Boron + Extract	3.60 c	2.62 b	38.21 b
Standard Error	0.1	0.09	4.52
CV (%)	8.14	11.26	29.52

Means followed by the same letter are not significantly different at P=0.05.

The applied *C. esculentus* extract had no significant effect on the growth of seedlings. According to Cheng (1992), it is unlikely that extracted allelochemicals from plant material, are those that actually reach the host plant in nature. These chemicals can be transformed during the course of extraction, therefore, allelopathic symptoms may not be manifested at the time or site where plant damage has actually occurred. By the time symptoms are observed, the chemicals may no longer be present. As none of the typical boron deficiency symptoms was evident, and differences between seedlings growing with or without added boron on the oldland soil were not significant, *P. patula* growth abnormalities cannot be ascribed to boron deficiencies in this experiment.

4.2. Experiment 1: Treatment 2: Effect of *P. patula* seedlings growth with or without *C. esculentus* present.

After 14 weeks growth, the extract was no longer applied. *C. esculentus* was removed from this treatment and it was kept weed-free for the rest of the trial period. However, *C. esculentus* was still present on the remaining two oldland soil treatments (T2 and T3).

4.2.1 Height

After eight months, a significant difference in percentage height increase was established between the seedlings growing on afforested soil and seedlings on the oldland soil kept weed-free (Table 6). However, this time seedlings growing on oldland soil had a higher percentage growth increase than seedlings growing on afforested soil. During this time it was winter and relatively no *C. esculentus* were present.

Table 6 Height increase of *Pinus patula* seedlings eight months after *Cyperus esculentus* was removed from one oldland soil treatment but remained in the other two (ANOVA appears in Tables 7, 8 and 9 in Appendix C)

	Final Height (mm)	Initial Height (mm)	Growth Increase (%)
Afforested soil	368.5 a	300.0 a	22.83 b
Oldland soil	328.5 ab	224.0 ab	46.65 a
Oldland soil	296.0 b	198.5 b	49.12 a
Oldland without *C. esculentus*	316.0 b	219.0 b	44.29 a
Standard Error	14.35	10.8	6.06
CV (%)	13.87	14.51	45.78

Means followed by the same letter are not significantly different at P=0.05.

The differences in percentage height increase between seedlings growing at the different oldland treatments were not significant.

4.2.2 Stem diameter

The seedlings growing at all three oldland soil treatments had a significant higher percentage stem diameter increase than seedlings growing on afforested soil (Table 7).

Table 7 Stem diameter increase of *Pinus patula* seedlings eight months after *Cyperus esculentus* was removed from one oldland soil treatment but remained in the other two (ANOVA appears in Tables 10, 11 and 12 in Appendix C)

	Final Stem Diameter (mm)	Initial Stem Diameter (mm)	Growth Increase (%)
Afforested soil	8.02 a	4.91 a	63.71 b
Oldland soil with *C. esculentus*	7.76 ab	4.15 b	86.98 a
Oldland soil with *C. esculentus*	7.07 c	3.88 b	82.45 a
Oldland without *C. esculentus*	7.39 bc	4.04 b	84.00 a
Standard Error	0.2	0.11	4.21
CV (%)	8.44	8.29	16.78

Means followed by the same letter are not significantly different at P=0.05.

Differences in percentage stem diameter increase between seedlings growing on different oldland soils, were not significant. With no herbaceous weed competition, pine seedlings had no interference from *C. esculentus*. William & Warren (1975) suggested that *C. rotundus* must be controlled within three weeks after non-competitive crops emerged, because during the absence of the weed, the crop is likely to suffer little yield reductions.

4.3. Experiment 1: Effect of added pine needles on the growth of *P. patula* seedlings with, or without, the weed

During September 1998, pine needles was placed on the soil surface of the treatment T 3 to suppress *C. esculentus* growth.

4.3.1 Height

The differences in percentage height increase among seedlings growing on afforested and oldland soil, are displayed in Table 8.

Table 8 Height increase of *Pinus patula* seedlings three months after pine needles were placed on the soil surface (ANOVA appears in Tables 12, 13 and 14 of Appendix C)

	Final Height (mm)	Initial Height (mm)	Growth Increase (%)
Afforested soil	554.3 a	386.5 a	36.23 b
Oldland soil	477.3 a	346.0 ab	39.46 b
Oldland + pine needles	552.5 a	325.0 b	70.70 a
Oldland without *C. esculentus*	538.5 a	326.0 b	65.94 a
Standard Error	NS	15.18	5.4
CV (%)		13.87	32.15

Means followed by the same letter are not significantly different at P=0.05.

Pine seedlings growing on oldland soil with *C. esculentus* suppression, had a significantly higher height increase than seedlings growing on afforested soil and oldland soil with weeds. No significant differences in height growth were evident between the treatments with *C. esculentus* suppression and where no weeds were present.

4.3.2 Stem diameter

As with height, the removal or suppression of herbaceous vegetation, resulted in a significant higher percentage stem diameter increase compared to seedlings growing with *C. esculentus* (Table 9). The suppression of *C. esculentus* vegetation

with pine needles did not result in a significant increase in the stem diameter of seedlings compared to those growing without the weed. Although no weeds were present on the afforested soil, seedlings had a significant lower percentage stem diameter increase than those on oldland soil without weeds present.

Table 9 Stem diameter increase of *Pinus patula* seedlings after the pine needles was placed on the soil surface (ANOVA appears in Tables 16, 17 and 18 in Appendix C)

	Final Stem Diameter (mm)	Initial Stem Diameter (mm)	Growth Increase (%)
Afforested soil	9.45 a	8.23 a	14.93 b
Oldland soil	9.52 a	8.34 a	14.23 b
Oldland + pine needles	9.25 a	7.48 b	22.52 ab
Oldland without *C. esculentus*	9.55 a	7.59 b	26.09 a
Standard Error	ns	0.2	2.85
CV (%)		8.04	46.28

Means followed by the same letter are not significantly different at P=0.05.

Results obtained are in accordance with those by Nelson, Pedersen, Autry, Dudley & Walstad (1981) and Bacon & Zedaker (1985). In their studies, they concluded that height, stem diameter and biomass of pine seedlings increased when competing vegetation were removed. Schumann, Little & Eccles (1995) concluded that of *Eucalyptus grandis*, *P. patula* and *Acacia mearnsii*, *P. patula* leaf and branch residues had the greatest suppressive effect on the establishment of four weed species. Water extracts of the these residues also resulted in significant suppression of

weed establishment, suggesting allelopathic effect.

4.4. Dry mass

The differences in the dry mass of seedlings were not significant (ANOVA appears in Table 19 in Appendix C).

Experiment 2: Growth of *P. patula* seedlings on three types of soil in the presence of *C. esculentus*

1. Height

Percentage height increase was significantly influenced by the different treatments 12 months after transplant. Differences between seedlings growing with or without weeds on grassland or afforested soil, were not significant. Seedlings growing without weeds on the oldland soil had a significant greater height increase than seedlings with weeds on both grassland - and oldland soil (Figure 3). This could be due to the absence of *C. esculentus*.

Seedlings on oldland soil without weeds, showed the highest percentage height increase (182.74%). A significant difference was obtained between the percentage height increase of seedlings on oldland soil and grassland soil both without weeds. Height differences between height of seedlings growing with weeds on the different soils, were not significant.

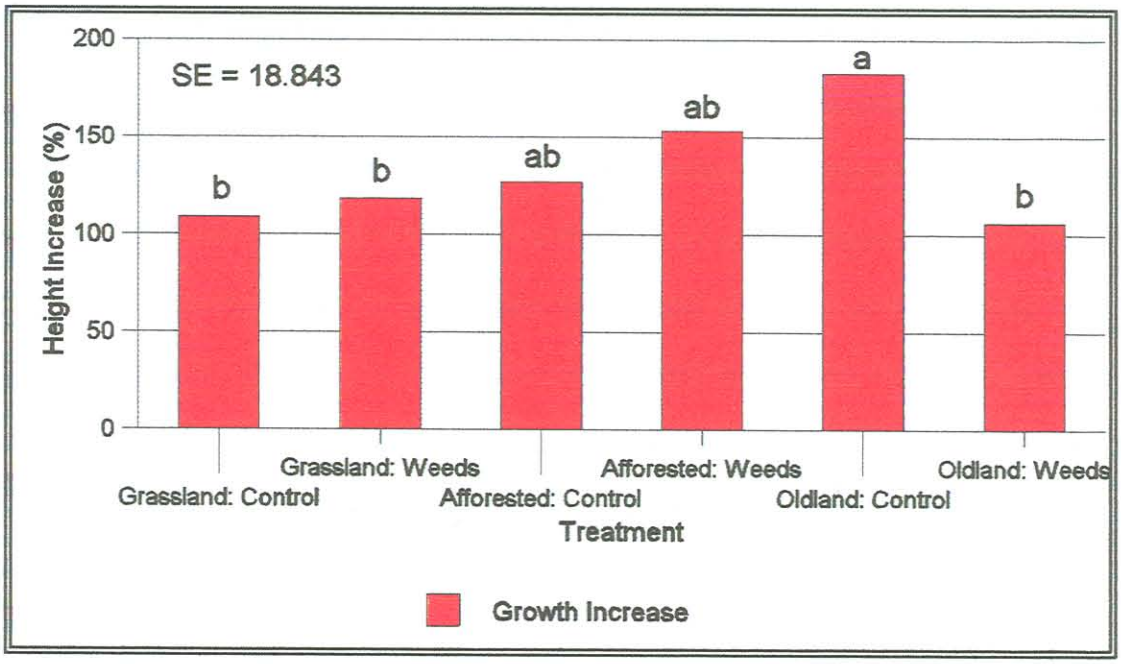

Figure 3 Effect of three types of soil with or without *Cyperus esculentus* on the percentage height increase of *Pinus patula* seedlings (ANOVA appears in Tables 20, 21 and 22 in Appendix C)

2. Stem diameter

The percentage stem diameter increase between seedlings at the different treatments was not significant (ANOVA appears in Tables 23, 24 and 25 in Appendix C).

3. Dry mass

Differences between the dry mass of seedlings at the different treatments were not significant (ANOVA appears in Table 26 in Appendix C).

5. Conclusions

The significant negative response of *P. patula* seedlings in the presence of *C. esculentus*, indicates the existence of an underlying biotic component in the poor growth of seedlings on oldland soil. *C. esculentus* was actively growing when the soil was collected and therefore the concentration of allelochemicals in the soil would have been considerably higher when the weed was actively growing with the seedlings, having a more inhibitory effect on seedling growth. Partial recovery from the detrimental effects of these compounds was observed during the winter when no weeds were present. In a similar fashion, seedlings had a significant higher growth increase when the growth of *C. esculentus* was suppressed by the addition of pine needles as a groundcover, or when the weed was removed. The results obtained from the experiment also indicate that boron deficiency is probably not responsible for abnormal growth of tree seedlings on oldland soil, as none of the deficiency symptoms were observed. Results suggests that *P. patula* seedlings can be successfully established on oldland soil, provided that *C. esculentus* control is practiced.

6. References

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Appendix C

1. Added boron and aqueous *Cyperus esculentus* extract

Table 1 Effect of added boron and an aqueous *Cyperus esculentus* extract on the beginning height of *Pinus patula* seedlings 14 weeks after transplant

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	1470.100	490.033	1.34	0.2762
Error	36	13149.800	365.272		
Total	39	14619.900			

$R^2 = 0.1006$

Table 2 Effect of added boron and an aqueous *Cyperus esculentus* extract on the final height of *Pinus patula* seedlings 14 weeks after transplant

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	47229.800	14743.267	15.70	0.0001
Error	36	36092.600	1002.572		
Total	39	83322.400			

$R^2 = 0.5668$

Table 3 Effect of added boron and an aqueous *Cyperus esculentus* extract on the percentage height increase of *Pinus patula* seedlings 14 weeks after transplant

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	37755.547	12585.182	6.28	0.0015
Error	36	72121.036	2003.362		
Total	39	109876.583			

$R^2 = 0.3436$

Table 4 Effects of added boron and *Cyperus esculentus* on the beginning stem diameter of *Pinus patula* seedlings 14 weeks after transplant

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	0.887	0.296	3.14	0.0372
Error	36	3.393	0.094		
Total	39	4.280			

$R^2 = 0.2072$

Table 5 Effects of added boron and *Cyperus esculentus* on the final stem diameter of *Pinus patula* seedlings 14 weeks after transplant

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	7.627	2.542	23.66	0.0001
Error	36	3.868	0.107		
Total	39	11.495			

$R^2 = 0.6635$

Table 6 Effects of added boron and *Cyperus esculentus* on the percentage stem diameter increase of *Pinus patula* seedlings 14 weeks after transplant

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	6488.235	2162.745	10.57	0.0001
Error	36	7368.013	204.667		
Total	39	13856.248			

$R^2 = 0.4683$

2. Treatment kept weed-free

Table 7 Beginning height of *Pinus patula* seedlings eight months after the aqueous *Cyperus esculentus* extract was no longer applied and the treatment kept weed-free

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	59336.875	19778.958	16.96	0.0001
Error	36	41982.500	1166.181		
Total	39	101319.375			

$R^2 = 0.5856$

Table 8 Final height of *Pinus patula* seedlings eight months after the aqueous *Cyperus esculentus* extract was no longer applied and the treatment kept weed-free

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	28062.500	9354.167	4.54	0.0084
Error	36	74135.000	2059.306		
Total	39	102197.500			

$R^2 = 0.2746$

Table 9 Percentage height increase of *Pinus patula* seedlings eight months after the aqueous *Cyperus esculentus* extract was no longer applied and the treatment kept weed-free

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	4563.131	1521.044	4.14	0.0127
Error	36	13217.112	367.142		
Total	39	17780.243			

$R^2 = 0.2566$

Table 10 Beginning stem diameter of *Pinus patula* seedlings eight months after the aqueous *Cyperus esculentus* extract was no longer applied and the treatment kept weed-free

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	6.265	2.088	16.88	0.0001
Error	36	4.454	0.124		
Total	39	10.719			

$R^2 = 0.5845$

Table 11 Final stem diameter of *Pinus patula* seedlings eight months after the aqueous *Cyperus esculentus* extract was no longer applied and the treatment kept weed-free

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	5.206	1.735	4.26	0.0113
Error	36	14.670	0.408		
Total	39	19.876			

$R^2 = 0.2619$

Table 12 Percentage stem diameter increase of *Pinus patula* seedlings eight months after the aqueous *Cyperus esculentus* extract was no longer applied and the treatment kept weed-free

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	3338.632	1112.877	6.29	0.0015
Error	36	6373.236	177.034		
Total	39	9711.868			

$R^2 = 0.3438$

3. Pine needles as groundcover

Table 13 Beginning height of *Pinus patula* seedlings two months after pine needles was placed on the soil surface of one of the treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	24815.000	8271.667	3.59	0.0228
Error	36	82945.000	2304.028		
Total	39	107760.000			

R^2 = 0.2303

Table 14 Final height of *Pinus patula* seedlings two months after pine needles was placed on the soil surface of one of the treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	39445.900	13148.633	1.44	0.2471
Error	36	328615.200	9128.200		
Total	39	368061.100			

R^2 = 0.1072

Table 15 Percentage height increase of *Pinus patula* seedlings two months after pine needles was placed on the soil surface of one of the treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	9453.414	3151.138	10.82	0.0001
Error	36	10485.623	291.268		
Total	39	19939.067			

R^2 = 0.4741

Table 16 Beginning stem diameter of *Pinus patula* seedlings two months after the pine needles was placed on the soil surface

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	5.746	1.914	4.74	0.0069
Error	36	14.550	0.404		
Total	39	20.296			

R^2 = 0.2831

Table 17 Final stem diameter of *Pinus patula* seedlings two months after the pine needles was placed on the soil surface

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	1.007	0.336	0.42	0.7376
Error	36	28.551	0.793		
Total	39	29.558			

R^2 = 0.0340

Table 18 Percentage stem diameter increase of *Pinus patula* seedlings two months after the pine needles was placed on the soil surface

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	1010.589	336.953	4.16	0.0125
Error	36	2914.754	80.965		
Total	39	3925.613			

R^2 = 0.2575

Table 19 Dry mass of *Pinus patula* seedlings 14 months after the seedlings were transplanted

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	3	89.156	29.719	0.71	0.5530
Error	36	1508.922	41.914		
Total	39	1598.078			

R^2 = 0.0558

4. Growth on three types of soil

Table 20 Effect of three types of soil with or without *Cyperus esculentus* on the beginning height of *Pinus patula* seedlings

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	4872.917	974.583	3.28	0.0137
Error	42	12475.000	297.034		
Total	47	17347.917			

R^2 = 0.2809

Table 21 Effect of three types of soil with or without *Cyperus esculentus* on the final height of *Pinus patula* seedlings

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	47040.104	9408.021	1.47	0.2205
Error	42	269084.375	6406.771		
Total	47	316124.479			

R^2 = 0.1488

Table 22 Effect of three types of soil with or without *Cyperus esculentus* on the percentage height increase of *Pinus patula* seedlings

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	35593.688	7118.738	2.51	0.0450
Error	42	119297.021	2840.405		
Total	47	154890.709			

R^2 = 0.2298

Table 23 Effect of three types of soil with or without *Cyperus esculentus* on the beginning stem diameter of *Pinus patula* seedlings

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	0.309	0.062	1.55	0.1964
Error	42	1.676	0.040		
Total	47	1.985			

R^2 = 0.1554

Table 24 Effect of three types of soil with or without *Cyperus esculentus* on the final stem diameter of *Pinus patula* seedlings

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	9.408	1.882	0.67	0.6517
Error	42	118.745	2.827		
Total	47	128.153			

R^2 = 0.0734

Table 25 Effect of three types of soil with or without *Cyperus esculentus* on the percentage stem diameter increase of *Pinus patula* seedlings

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	54498.523	10899.705	1.67	0.1632
Error	42	274260.824	6530.020		
Total	47	328759.347			

R^2 = 0.1658

Table 26 Dry mass of *Pinus patula* seedlings 12 months after the seedlings were transplanted

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	5	621.290	124.258	1.56	0.1934
Error	42	3353.511	79.846		
Total	47	3974.812			

R^2 = 0.1563

Chapter 5

Inhibition of *Pinus patula* seedling growth by *Cyperus esculentus*, and other weed species in the field

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Chapter 5

Inhibition of *Pinus patula* seedling growth by *Cyperus esculentus*, and other weed species in the field

1. Introduction

According to Noble & Schumann (1993) the forestry industry is progressively acquiring land that was previously used for agricultural purposes. These land areas are typically a mixture of natural vegetation (afforested soil) and old agronomical sites (oldland soil). Smith & Van Huyssteen (1992) reported that oldland soils may differ from afforested soils with respect to pathogens, weed spectrum and soil nutrition. Reinhardt, Khalil, Labuschagne, Claasens, & Bezuidenhout (1996) concluded that the weed community on oldlands consists virtually exclusively of annuals that commonly occur in annual crops, e.g. *Cyperus esculentus* (yellow nutsedge), *Bidens pilosa* (common blackjack) and *Conyza albida* (tall fleabane). On adjacent afforested soil, grass species dominate .

Where pine seedlings have been established on old agronomical sites, growth and development abnormalities were encountered. According to Linde, Kemp & Wingfield (1994) mortality of the seedlings was greater than 95% in most cases in the North eastern Cape province in South Africa. The pine seedlings on oldlands died approximately 4-5 months after they were established in the field. It was reported by Smith & Van Huyssteen (1992) and Schumann & Noble (1993) that the symptoms

of poor seedling performance involved a minimally developed root systems, stunting, lack of apical dominance, chlorosis and necrosis of fascicles, and necrosis of the growth tips. Various attempts to solve or amend the problem were launched which included soil and pathogen studies (Smith & Van Huyssteen, 1992; Schumann & Noble, 1993 and Viljoen, Wingfield & Marasas, 1994).

Interfering vegetation decreases the growth of pine seedlings (Nelson, Pedersen, Autry, Dudley & Wellstead, 1981; Bacon & Zedaker, 1985; Knowe, Gjerstad, Glover, Nelson, Zutter, Minogue & Dukes, 1985; Zutter, Gjerstad & Glover, 1986). Height, stem diameter and biomass of pine seedlings increased when competing vegetation was removed. Zutter *et al.*, (1986) reported that interfering vegetation had a significant effect on biomass, fascicle type distribution, fascicle morphology and leaf area of loblolly pine seedlings. Miller, Zutter, Zedaker, Edwards, Haywood & Newbold (1991) investigated the influence of woody and herbaceous competition on early loblolly pine growth. After five years, the tree volume was increased by an average of 67% with woody control while with herbaceous control volume increased by 171%.

2. Aim

The main objective was to determine the effect of *C .esculentus* and naturally occurring broadleaf and grass weeds on the height and stem diameter growth of pine seedlings growing on an oldland site.

3. Materials and Methods

Site description

A field trial was established in October 1998 at the Giant's Castle Estate of Mondi Forest (Pty) Ltd. at Mooi River KwaZulu-Natal. The site was previously under agronomic production and the soil is classified as a clay loam with: pH (H_2O) 5.71 and exchangeable Ca, Mg, K and Na content of 685; 261; 430 and 59 mg kg^{-1} respectively. Herbaceous vegetation on the site was dominated by the weeds *C. esculentus, B. pilosa, C. albida* and *Tagetes minuta* (kaki weed).

Experimental design

The trial consisted of three weed treatments: (1) only *C. esculentus* present, (2) only broadleaf species present, and (3) a mixture of both broadleaf spp., grass spp. and *C. esculentus*. No weeds were present at the control treatment. These different weed infestations were obtained by spraying herbicides before seedling establishment. The control treatment was sprayed with atrazine, 2,4-D and glyphosate at the manufacture's recommended rates. It was kept weed-free for the duration of the trial by hoeing. For the treatment consisting of only *C. esculentus*, 2,4-D was sprayed and all other weed species were removed by hand. For only broadleaves to be present, (treatment 2), alachlor was sprayed and hoeing was used for the duration to control the grasses. No herbicides were sprayed at treatment (3) for all the weed species to flourish. Standard silviculture procedures for soil preparation were followed with trial initiation. *P. patula* seedlings were transplanted by staff of Mondi Forests (Pty) Ltd. At transplant, each seedling received 2 L tap water and solid fertilizer in the form of 2:3:2 at a equivalent rate of 200 kg/ha. Seedlings were planted 1.5 m in and between rows. Due to frost damage, it was necessary to replant seedlings in January

1999. Each plot consisted of seven rows, 50 m in length. Due to replant problems, initial measurements of randomly selected seedlings were made in September 1999. Measurements included height growth from the stem base to the apical growth point, and stem diameter measured at 50 mm above the soil surface.

Statistical analysis

A block design was used and the four treatments were assigned to six blocks. Analysis of variance was done to determine the effect of the different weed treatments on the height and stem diameter growth of seedlings. Differences between means were identified by the Least Significant Difference test at P=0.05.

4. Results and Discussion

The presence of weeds caused significant reductions in both pine seedling height and stem diameter compared to the control (Figure 1 and 2).The height inhibition caused by the presence of *C. esculentus,* was significantly greater than the reduction by only broadleaf spp. (Figure 1). Although height reductions by the mixture of weed species were significantly greater compared to the control, it was not significantly different from the reductions caused by *C. esculentus.*

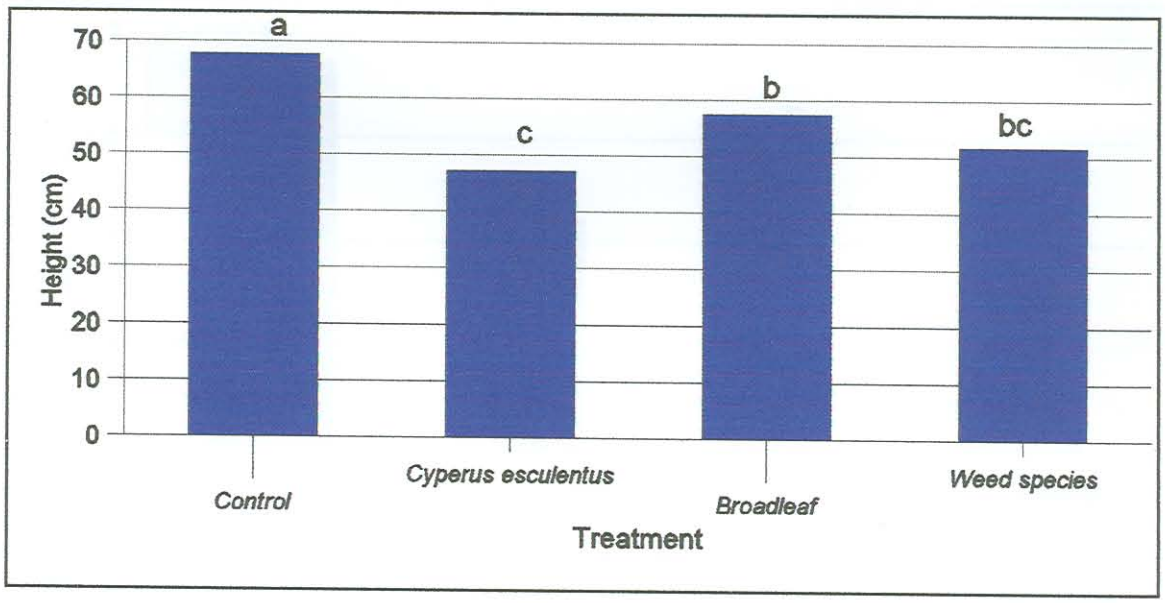

Figure 1 The influence of different weed treatments on the height growth of *Pinus patula* seedlings. Means followed by the same letter are not significantly different at P=0.05. (ANOVA appears in Table 1 in Appendix D)

All three weed treatments caused a significant seedling stem diameter reduction compared to the control (Figure 2). *C. esculentus* and the weed species treatment, caused a greater reduction in stem diameter than the treatment with only broadleaf species.

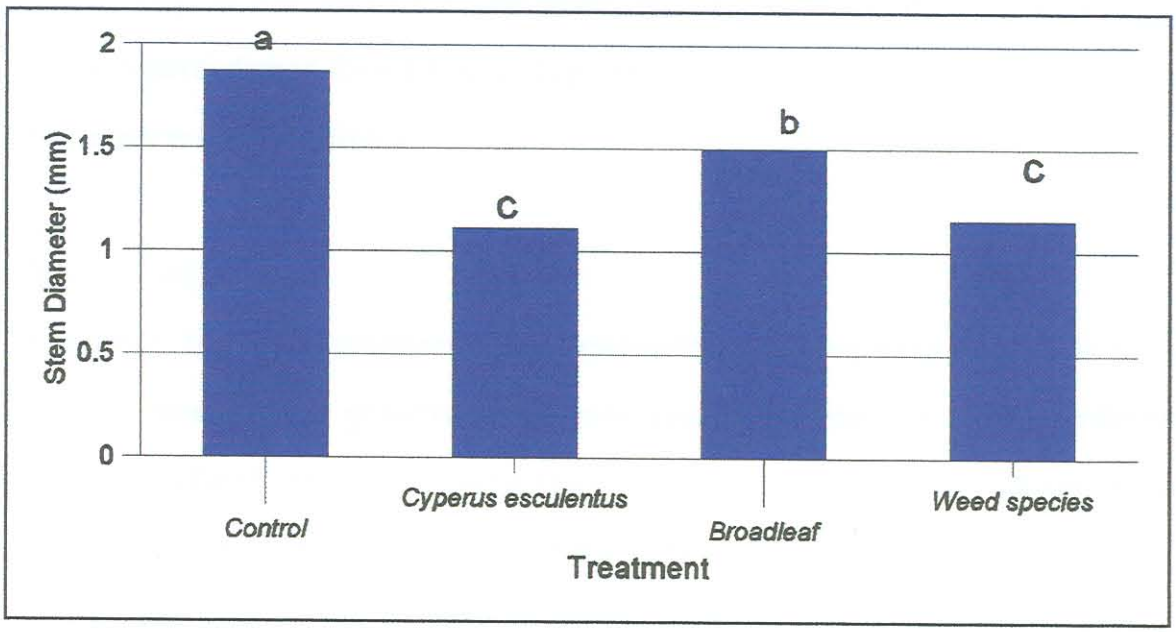

Figure 2 The influence of different weed treatments on the stem diameter growth of *Pinus patula* seedlings. Means followed by the same letter are not significantly different at P=0.05. (ANOVA appears in Table 2 in Appendix D)

Results obtained from the trial are in accordance with those from research done by Keeley & Thullen (1975), Stoller, Wax & Slife (1979), Drost & Doll (1980), Patterson, Buchanan, Street & Crowley (1980) and Keeley (1987). All of the above described the reduction in crop growth due to the interference effect of *C. esculentus*. Allelopathic interactions have been demonstrated to play a crucial role in natural and man made forests. Fisher & Adrian (1981) noticed a strong effect of *Paspalum notatum* (Bahia grass) on *P. elliottii* (slash pine). As the percentage of grass covered ground increased, the height growth decreased markedly. Gilmore (1985) noticed the erratic establishment of *P. taeda* (loblolly pine) on old fields covered with *Setaria faberii*

(giant foxtail). Water extracts inhibited germination and radicle elongation of seedlings in petri dishes. Dried foxtail tops were the most inhibitory while fresh tops and roots were less inhibitory.

5. Conclusions

It is evident that the presence of *C. esculentus* growing with *P. patula* can be deleterious towards the growth of the pine seedlings. However, the interference effect of broadleaf- and grass species with the pine seedlings also reduced growth significantly. It is concluded from the preliminary results that weeds, especially *C. esculentus*, are responsible for the poor growth and development of *P. patula* seedlings on oldland sites due to competition and allelopathy. When weed interference are minimized, seedlings should grow and develop normally on oldland sites.

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Appendix D

1. Height

Table 1 Analysis of variance of the percentage height increase of *Pinus patula* seedlings at different weed treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	8	1532.081	191.510	5.58	0.0021
Error	15	515.239	34.349		
Total	23	2047.320			

$R^2 = 0.0600$

2. Diameter

Table 2 Analysis of variance of the percentage stem diameter increase of *Pinus patula* seedlings at different *Cyperus esculentus* treatments

Source	DF	Sum of Squares	Mean Square	F	Pr > F
Treatment	8	2.363	0.295	19.37	0.001
Error	15	0.229	0.015		
Total	23	2.592			

$R^2 = 0.1679$

Summary

The forestry industry in South Africa is dependent on acquiring new land to meet the growing demand for timber. Mondi Forests (Pty) Ltd. in KwaZulu-Natal , bought land that was previously used for agronomic production (hereafter referred to as oldlands). After establishing *Pinus patula* (patula pine) seedlings on oldlands, seedlings exhibited abnormal growth symptoms. These include an inadequately developed root system, stunting, lack of apical dominance, chlorosis and necrosis of the fascicles and necrosis of the growth tips. These symptoms were noticed only on seedlings planted on oldlands. Seedlings established on sites previously used for grazing only did not show these symptoms.

Various soil and pathogen studies were conducted, with no conclusive answers to what is known as the "oldland syndrome". Certain pathogens, *Fusarium subglutinans* f.sp. *pini, F. oxysporum* and *Pythium irregulare* were identified on seedling roots in the nursery. It was concluded that infected seedlings were transplanted to oldland sites. Soil studies in the north eastern Cape revealed ploughpans and poor drainage, which limited the root development of pine seedlings. Soil analysis of problematic oldland sites at the Giant's Castle Estate in KwaZulu-Natal did not reveal the foregoing characteristics. Soils were well drained with adequate nutrition. Weed control on the oldland sites was however judged to be inadequate. The dominant weed species at a site selected for study were *Bidens pilosa* (common blackjack), *Conyza albida* (tall fleabane), *Cyperus esculentus* (yellow nutsedge), *Helichrysum* spp. and *Tagetes minuta* (khaki bos). Of these, *C. esculentus* was the most dominant, especially early in the growing season. New tubers are formed each season thereby enlarging the weed population. The weed residues are ploughed in

when seedbed preparation is done for new pine seedlings, or left on the soil surface. The rodent *Tatera* spp. (gerbilles) line their nests with the *C. esculentus* residues, thereby creating favourable conditions decomposition and release of allelochemicals in the root sone of the pine seedlings. Without adequate weed control methods, pine seedlings are exposed to competition and possible allelochemicals excreted by the weeds.

Prior to the present study, no research had been done on the influence of herbaceous weeds on the growth of the *P. patula* seedlings. Applying agronomic principles for crop-weed interactions, could probably aid in resolving most of the pine seedling establishment problems. These interactions involved both competition for natural resources and the allelopathic potential of the weeds present. Therefore, symptoms of seedling establishment failure could be the manifestation of both competition and allelopathy.

The first experiment initiated, investigated the effect of incorporated residues of *B. pilosa, C. albida* and *C. esculentus* on the height and stem diameter growth of *P. patula* seedlings, as these species were the most prominent on the field trial site at the Giant's Castle Estate of Mondi Forest. Results from incorporated *B. pilosa* and *C. albida* were inconclusive, and therefore, it was decided to continue working with only *C. esculentus*.

Pine seedling height and stem diameter growth were more inhibited by leachate obtained from actively growing *C. esculentus* than from incorporation of mature *C. esculentus* leaf material. Allelochemicals released from the actively growing plants were continuously applied to pine seedlings, while no addition of new leaf residues

was made to the soil. The incorporated weed material therefore probably released only a limited quantity of allelochemicals. Associated mycorrhizae growth on the roots of the pine seedlings were inhibited by both applied root leachate and incorporated leaf residues. This finding suggests that *C. esculentus* could have an indirect influence on the growth of the trees. Mycorrhizae are critical for the health and growth of the pine seedlings. Any inhibition of these fungi will have an impact on the host. As competition for light, space, nutrients and water was limited, it is concluded that *C. esculentus* was potentially more allelopathic than competitive towards pine seedling growth.

To have confirmation of the foregoing allelopathy-related hypothesis, seed germination and ectomycorrhizae studies were initiated. *Lactuca sativa* (lettuce) and *Zea mays* (maize) were used as test species in the germination experiments. Lettuce is considered a very sensitive plant and is often used in herbicide bioassays. Maize was included as it is an important crop in South Africa. Aqueous extracts of *C. esculentus* tubers, immature plant parts and mature plant parts were prepared and tested against growth of the ectomycorrhizal fungus *Boletus maxamaria*, *L. sativa* germination, and seedling growth and emergence of *Z. mays*.

Results of the ectomycorrhizae experiment confirmed results previously obtained with the incorporation into soil of *C. esculentus* leaf material which inhibited growth of the ectomycorrhizal fungus *Boletus maxamaria*. The occurrence of growth inhibition by the weed on the pine seedlings, associated with the symbiont, could be pivotal in explaining the establishment problems of new pine seedlings on former crop sites infested with *C. esculentus*.

Although mature weed extract had a greater inhibitory effect on the mycorrhizal growth than the other extracts, it was not confirmed where lettuce was used as an indicator species. It could be that test species' responses to allelochemicals differ, and this possibility should therefore be considered in explaining results. With the elimination of soil as a factor in the hydroponics experiment, it was evident that allelochemicals released from *C. esculentus* had an inhibitory effect on the growth of *L. sativa*. Emergence of maize, planted to soil in which *C. esculentus* were grown for three weeks, was retarded and it is thus conceivable that, under conditions favouring the production and release of allelochemicals in high concentrations by *C. esculentus*, maize seedlings would be placed under chemical stress that might weaken their resistance to other environmental stress factors. Although anomalies were found in work done with the allelopathic compounds identified in growth media of *C. esculentus*, there were indications of germination inhibition. This confirms the results from the germination work done with the extracts.

The significant negative growth response of pine seedlings in the presence of *C. esculentus* confirmed earlier results and reports by other researchers of the allelopathic characteristics of the weed. Boron deficiency was eliminated as a possible cause for seedling establishment failure on oldland soils, as none of the symptoms of deficiency was observed. Addition of boron did not prevent poor growth of seedlings exposed to *C. esculentus*. As *C. esculentus* was actively growing during the first period of seedling growth, allelochemicals were probably continuously released. This phenomenon is reflected in the significant growth differences among seedlings growing in the presence of the weed and those not. Partial recovery from the inhibition effects of these compounds was observed

during the winter, when no weeds were present. When *C. esculentus* was removed or suppressed by a groundcover, the growth was significantly higher than seedlings still exposed to the weed.

Results obtained from experiments done in the greenhouse were confirmed by the field trial. Seedlings growing without weeds present had a significantly higher growth increase than those growing in the presence of weeds. *C. esculentus* had a more detrimental effect on growth than broadleaved species. Seedlings continuously exposed to *C. esculentus* had to be replanted twice.

In conclusion, *P. patula* seedlings exposed to *C. esculentus* show growth inhibition. As the mycorrhizae that are associated with *P. patula* are affected negatively also, seedlings are likely to be more vulnerable to pathogens and adverse environmental conditions. It is clear from the results that pine seedlings could be successfully established on oldlands, provided that an effective weed control programme is followed, in particular a strategy that prevents the establishment of *C. esculentus.*