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 cinnamoni* 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.

5. References

ALDRICH, J.D., 1984. Weed-crop ecology: Principles and practices. Breton Publishers. p. 215-241.

ALEXANDER, I.J. & FAIRLEY, R.I., 1983. Effects of nitrogen fertilization on populations of pine roots and mycorrhizae in spruce humus. *Plant and Soil* 71, 49-53.

BARNARD, E.L. & BLAKESLEE, G.M., 1980. Pitch canker of slash pine seedlings: A new disease in forest tree nurseries. *Plant Disease* 64, 695-696.

BELL, D.T. & KOEPPE, D.E., 1972. Non-competitive effects of giant foxtail on the growth of corn. *Agronomy Journal* 64, 321-325.

BHOWMIK, P.C. & DOLL, J.D., 1984. Allelopathic effects of annual weed residues on growth and nutrient uptake of corn and soyabeans. *Agronomy Journal* 76, 383-388.

BLOOMBERG, W.J., 1971. Disease of Douglas fir seedlings caused by *Fusarium oxysporum*. *Phytopathology* 61, 467-470.

BLOOMBERG, W.J., 1981. Disease caused by *Fusarium* in forest nurseries. In: P.E. Nelson, T.A. Tousson, & R.J. Cook (eds). *Fusarium:* Disease biology and taxonomy. Pennsylvania State University Press. University Park. p. 178-187.

BRANDSÆTER, L.A. & HAUGLAND, E., 1999. Bioassay sensitivity in the study of allelopathy. In A. Mallik, B. Barnes, D. Berube, J. Graham, K. Price & L. Luxton (eds). Program & Abstracts, Second World Congress on Allelopathy, Lakeview University, Canada.

CARLSON, C.A., 1994. The influence of fertilization on ectomycorrhizal colonization of *Pinus patula* roots. *South African Forestry Journal* 171, 1-6.

CHENG, H.H., 1992. A conceptual framework for assessing allelochemicals in the soil environment. In: S.J.H Rizvi & V. Rizvi (eds). Allelopathy: Basic and applied aspects. Chapmann & Hall Publishers. p.21-29.

CHU-CHOU, M., 1978. Mycorrhizal fungi of *Pinus radiata* in New Zealand. *Soil Biology and Biochemistry* 11, 557-562.

CONNELL, J.H., 1990. Apparent versus "real" competition in plants. In J.B. Grace & D. Tilman (eds). Perspectives on Plant Competition. Academic Press, San Diego. p. 9-25.

DAKSHINI, K.M.M. & INDERJIT., 1999. Multifaceted approach in the study of allelopathy. In A. Mallik, B. Barnes, D. Berube, J. Graham, K. Price & L. Luxton (eds). Program & Abstracts, Second World Congress on Allelopathy, Lakeview University, Canada.

DREW, A.P., 1988. Interference of black cherry by ground flora of the Allegheny uplands. *Canadian Journal of Forestry Research* 18, 652-656.

DROST, D.C. & DOLL, J.D., 1980. The allelopathic effect of yellow nutsedge (*Cyperus esculentus*) on corn (*Zea mays*) and soyabeans (*Glycine max*). *Weed Science* 28, 229-233.

EINHELLIG, F.A., 1987. Interactions among allelochemicals and other stress factors of the plant environment. In: G.R Waller (ed). Allelochemicals: Role In agriculture and forestry. American Chemical Society Washington DC. p. 343-357.

EINHELLIG, F.A. & RASMUSSEN, J.A., 1979. Effects of three phenolic acids on chlorophyll content and growth of soybean and grain sorghum seedlings. *Journal of Chemical Ecology* 5, 815.

ELLIOTT, L.F. & CHENG, H.H., 1987. Assessment of allelopathy among microbes and plants. In: G.R. Waller (ed). Allelochemicals: Role in agriculture and forestry. ACS Symposium Series 330. American Chemical Society, Washington DC. p. 505-515.

ERLAND, S. & SÖNDERSTRÖM, B., 1990. Effects of liming on ectomycorrhizal fungi infecting *Pinus sylvestris* L. *New Phytologist* 118, 675-688.

FISHER, R.F., 1987. Allelopathy: A potential cause of forest regeneration failure. In: G.R. Waller (ed). Allelochemicals: Role in agriculture and forestry. ACS Symposium Series 330, American Chemical Society, Washington DC. p. 176-184.

FISHER, R.F. & ADRIAN, F., 1981. Bahia grass impairs slash pine seedling growth. *Tree Planter's Notes* 32, 19-21.

FUERST, E.R. & PUTNAM, A.R., 1983. Separating the competitive and allelopathic components of interference: Theoretical principles. *Journal of Chemical Ecology* 9, 937-944.

GILLMORE, A.R., 1985. Allelopathic effects of giant foxtail on germination and radicle elongation of loblolly pine seed. *Journal of Chemical Ecology* 11, 583-592.

GRAHAM, J.H., 1988. Interactions of mycorrhizal fungi with soilborne plant pathogens and other organisms: An introduction. *Phytopathology* 78 365-366.

HARPER, J.L., 1977. Population biology of plants. Academic Press, New York.

HARPER, J.R. & BALKE, N.E., 1981. Characterization of the inhibition of K^+ absorption in oat roots by salicylic acid. *Plant Physiology* 68, 1349.

HOLLIS, C.A., SMITH, J.E. & FISHER, R.F., 1982. Allelopathic effects of common under-storey species on germination and growth of Southern pines. *Forest Science* 28, 509-515.

HOLM, L.G., PLUCKNETT, D.L., PANCHO, J.V. & HERBERGER, J.P., 1977. The world's worst weeds. Distribution and Biology. University Press, Hawaii, Honolulu. 125-133.

HOROWITZ, M. & FRIEDMAN, J., 1971. Biological activity of subterranean residues of *Cynodon dactylon* L., *Sorghum halapense* L. and *Cyperus rotundus* L. *Weed Research* 11,88-93.

HUANG, J.W. & KUHLMAN, E.G., 1990. Fungi associated with damping off of slash pine seedlings in Georgia. *Plant Disease* 74, 27-30.

INDERJIT & WESTON, L., 1999. Are laboratory bioassays for allelopathy suitable for prediction of field responses? In A. Mallik, B. Barnes, D. Berube, J. Graham, K. Price & L. Luxton (eds). Program & Abstracts, Second World Congress on Allelopathy, Lakeview University, Canada.

JOBIDON, R., THIBAULT, J.R. & FORTIN, J.A., 1989. Effect of straw residues on black spruce seedling growth and mineral nutrition under greenhouse conditions. *Canadian Journal of Forestry Research* 19, 1291-1293.

KIL, B.S., 1989. Allelopathic effects of five pine species in Korea. In: C.H., Chou, & G.R. Waller (eds). Phytochemical Ecology: Allelochemicals, Mycotoxins and Insect Pheromones and Allomones. Institute of Botany. Academia Sinica Monographs Series No 9. Taipei, ROC. p. 81-99.

KIL, B.S. & YIM, Y.J., 1983. Allelopathic effects of *Pinus densiflora* on undergrowth of red pine forest. *Journal of Chemical Ecology* 9, 1135-1151.

KORF, H., KHALIL, S., LABUSCHAGNE,N. & REINHARDT, C.F., 1997. Role of mycorrhizae, pathogens and weeds in sustainable pine forest management. *Personal communication.*

LINDE, C., KEMP, G.H.J. & WINGFIELD, M.J., 1994. *Pythium irregulare* associated with *Pinus* seedling death on previously cultivated lands. *Plant Disease* 78, 1002-1005.

LINDERMAN, R.G., 1988. Mycorrhizal interactions with the rhizosphere microflora: The mycorrhizosphere effect. *Phytopathology* 78, 366-371.

LYNCH, J.M., 1987. Allelopathy involving micro-organisms. Case histories from the United Kingdom. In: G.R. Waller (ed). Allelochemicals: Role in agriculture and forestry. ACS Symposium Series 330. American Society, Washington DC. p. 44-52.

MARAIS, L.J., 1974. Mycorrhizal effects on *Pinus patula* Schlecht. & Cam. in South Africa. M.Sc. Thesis. University of Pretoria, Pretoria.

MARX, D.H., 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogen infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* 59, 153-163.

MARX, D.H., 1973. Mycorrhizae and feeder root disease. In: G.S. Marks & T.T. Kozlowski (eds). Ectomycorrhizae. Academic Press, London. p. 351-382.

MARX, D.H. & BRYAN, W.C., 1971. Influence of ectomycorrhizae on survival and growth of aseptic seedlings of loblolly pine at high temperatures. *Forest Science* 17, 37-41.

McCOOL, P.M., 1988. Effects of air pollutants on mycorrhizae. In: S. Schulter-Hostede, N.M. Darrel, L.W. Blank & A.R. Wellburn (eds). Air pollution and plant metabolism. Elsvier Applied Science. London. p. 356-365.

MEISSNER, R., NEL, P.C. & SMIT, N.S.H., 1979. Influence of red nutgrass (*Cyperus rotundus*) on growth and development of some crop plants. *Proceedings of the 3rd National. Weed Conference of South Africa.* p.39-52.

MITCHELL, R.J., RUNION, G.B., KELLY, W.D., GJERSTAD, D.H. & BREWER, C.H., 1991. Factors associated with loblolly pine mortality on former agricultural sites in the conservation reserve program. *Journal of Soil and Water Conservation* 46, 306-311.

MULLER, C.H., 1969. Allelopathy as a factor in ecological process. *Vegetatio* 18, 348-357.

MULLER, C.H., MULLER, W.H. & HAINES, B.L., 1964. Volatile growth inhibitors produced by shrubs. *Science* 143, 471.

NEWTON, A.C. & PIGOTT, C.D., 1991. Mineral nutrition and mycorrhizal infection of seedling oak and Birch II. The effect of fertilizers on growth nutrient uptake and ectomycorrhizal infection. *New Phytologist* 117, 45-52.

NOBLE, A.D. & SCHUMANN, A.W., 1992. A survey of pine establishment problems on ex-agricultural conservation reserve programme (CRP) sites in the South Eastern United States. *ICFR Bulletin Series* 15, 1-10.

NOBLE, A.D. & SCHUMANN, A.W., 1993. The amelioration of *Pinus patula* mortality on former agricultural sites through fertilization: A bioassay and greenhouse study. *South African Forestry Journal* 164, 35-41.

PARKE, J.L. LINDERMAN, R.G. & BLACK, C.H., 1983. The role of ectomycorrhizas in drought tolerance of Douglas-fir seedlings. *New Phytologist* 95, 83-95.

PATTERSON, D.T., 1981. Effects of allelochemicals on growth and physiological responses of soybeans (*Glycine max*). *Weed Science* 29, 53.

PELLISSIER, F., 1993. Allelopathic effect of phenolic acids from humic solutions of two spruce mycorrhizal fungi: *Cenococum graniforme* and *Laccaria laccata. Journal of Chemical Ecology* 19, 2105.

PERRY, D.A. & CHOQUETTE, C., 1987. Allelopathic effects on mycorrhizae: Influence on structure and dynamics of forest ecosysytems. In: G.R. Waller (ed). Allelochemicals: Role in agriculture and forestry. American Chemical Society Washington DC. p. 185-194.

PUTNAM, A.R., 1985. Weed allelopathy. In: S.O. Duke (ed). Weed physiology. Volume 1: Reproduction and Ecophysiology. CRC Press. p. 131-155.

PUTNAM, A.R. & DUKE, W.B., 1974. Biological suppression of weeds: evidence for allelopathy in assessing of cucumber. *Science* 185, 370-372.

PUTNAM, A.R. & TANG, C.S., 1986. Allelopathy: State of the science. In: A.R. Putnam, & C.S. Tang (eds). The science of allelopathy. Wiley, New York. p. 1-19.

RADOSEVICH S.R. & HOLT, J.S., 1984. Weed-ecology: Implications for vegetation management. Wiley-Interscience Publications. p. 93-138.

RAMBELLI, A., 1973. The rhizosphere of mycorrhizae. In: G.L. Marks, & T.T Koslowski, (eds). Ectomycorrhizae. Academic Press, New York. p. 299-343.

REINHARDT, C.F., KHALIL, S., LABUSCHAGNE, N., CLAASSENS, A. & BEZUIDENHOUT, S.R., 1996. Bioassay techniques in assessing the allelopathic effect of weeds in crops and plantation species. In: A. Torres, R.M. Oliva, D. Castellana & P. Cross (eds). First world congress on allelopathy. Cadiz Spain 16-20 September. p. 133-140.

RICE E.L., 1974. Allelopathy. Academic Press.

RICE, E.L., 1984. Allelopathy. 2nd edition. Academic Press.

RICE, E.L., 1995. Allelopathy in forestry. In: E.L. Rice (ed). Biological control of weeds and plant diseases: Advances in applied allelopathy. University of Oklahoma Press, Norman.

RIETVELD, W.J., 1975. Phytotoxic grass residues reduces germination and initial root growth of Ponderosa pine. *USDA Forest Service Research Paper*. RM 153. 4p.

RIZVI, S.J.H., HAQUE, H., SINGH, V.K. & RIZVI, V., 1992. A discipline called allelopathy. In: S.J.H. Rizvi, & V. Rizvi (eds). Allelopathy: Basic and applied aspects. Chapmann & Hall Publishers. p. 1-8.

SATIN, G.R., BOYER, J.S. & GERDEMANN, J.W., 1971. Nutrient status and mycorrhizal enhancement of water transport in soybeans. *Plant Physiology* 49, 700-703.

SCHENCK, N.C., 1981. Can mycorrhizae control root disease? *Plant Disease* 65, 230-234.

SCHUMANN, A.W. & NOBLE, A.D., 1993. Evidence of induced nutrient deficiency in pine plantings on previously cropped lands. *South African Forestry Journal* 165, 1-8.

SMITH C.W. & VAN HUYSSTEEN, L., 1992. The North-eastern Cape old lands syndrome: An initial investigation into soil physical problems and planting techniques. *ICFR Research Bulletin Series* 26, 1-10.

STOLLER, E.W., WAX, L.M. & SLIFE, F.W., 1979. Yellow nutsedge (*Cyperus esculentus*) competition and control in corn (*Zea mays*). *Weed Science* 27, 32-37.

STOWE, L.G., 1979. Allelopathy and its influence on the distribution of plants in an Illinios old field. *Journal of Ecology* 67, 1065-1068.

SZCZEPANSKI, A.J., 1977. Allelopathy as a means of biological control of water weeds. *Aquatic Botany* 3, 103.

TAMES, R.S., GETSO, M.D.V. & VIEITEZ, E., 1973. Growth substances isolated from tubers of *Cyperus esculentus* var *aureus*. *Physiology of Plants* 28, 195-200.

TANG, S., 1999. A chemical mini-rhizosthron for the study of allelopathy. In A. Mallik, B. Barnes, D. Berube, J. Graham, K. Price & L. Luxton (eds). Program & Abstracts, Second World Congress on Allelopathy, Lakeview University, Canada.

THOBIESSEN, P. & WERNER, M.B., 1980. Hardwood seedling survival under plantations of Scotch (*sic.*) pine and red pine in Central New York. *Ecology* 61, 25-29.

TINKER, P.B., 1984. The role of micro-organisms in mediating and facilitating the uptake of plant nutrients from soil. *Plant and Soil* 76, 77-91.

VILJOEN, A., WINGFIELD, M.J. & CROUS, P.W., 1992. Fungal pathogens in *Pinus* and *Eucalyptus* seedling nurseries in South Africa: A review. *South African Forestry Journal* 161, 46-51.

VILJOEN, A., WINGFIELD, M.J. & MARASAS, W.F.O., 1994. First report of *Fusarium subglutinans* f.sp.*pini* on pine seedlings in South Africa. *Plant Disease* 78, 309-312.

WEIDENHAMER, J.D., 1999. Separating resource competition from allelopathy is not only realistic , it is essential. In A. Mallik, B. Barnes, D. Berube, J. Graham, K. Price & L. Luxton (eds). Program & Abstracts, Second World Congress on Allelopathy, Lakeview University, Canada.

WHITTAKER, R.H. & FEENEY, P.P., 1971. Allelochemicals: chemical interaction between species. *Science* 171, 757-770.

WILLIAMSON, G.B., 1990. Allelopathy, Koch's postulates and the neck riddle. In J.B. Grace & D. Tilman (eds). Perspectives on Plant Competition. Academic Press, San Diego. p 143-161.

WILLIS, R.J., 1985. The historical bases of the concept of allelopathy. *Journal of the History of Biology* 18, 71-102.

WU, H., PRATLEY, J., LEMERLE, D. & HAIG, T., 1999. Crop cultivars with allelopathic capability. *Weed Research* 39, 171-180.