Allelopathic interference of silverleaf nightshade (*Solanum elaeagnifolium* Cav.) with the early growth of cotton (*Gossypium hirsutum* L.)

by

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LIST OF ABBREVIATIONS

rpm	:	revolutions per minute
µm	:	micrometer
PEG	:	polyethylene glycol
mOsm kg^{-1}	:	milliOsmol per kilogram
LSD	:	least significant difference
kg	:	kilogram
g	:	gram
m	:	meter
cm	:	centimeter
mm	:	millimeter
m^2	:	square meter
cm^2	:	square centimeter
ml	:	milliliter
L	:	liter

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ALLELOPATHIC INTERFERENCE OF SILVERLEAF NIGHTSHADE *(SOLANUM ELAEAGNIFOLIUM Cav.)* WITH THE EARLY GROWTH OF COTTON (*GOSSYPIUM HIRSUTUM* L.*)*

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ABSTRACT

Silverleaf nightshade *(Solanum elaeagnifolium),* a perennial broadleaf weed, has become a serious pest in many semi-arid areas of the world. Control of silverleaf nightshade is confounded by its ability to produce thousands of viable seeds from a single mother plant, a deep and well-developed root system and the ability to propagate vegetatively from creeping lateral roots, root fragments and from rhizomes. Exacerbating factors are longevity of its propagules in soil, particularly under adverse environmental conditions. Currently, in South Africa, it is one of the more serious weeds in cotton *(Gossypium hirsutum* L.) production, where its interference results in remarkable loss of product quantity and quality. Research has been done on the competition aspect of silverleaf nightshade with cotton but there is a serious lack of information on the allelopathic aspect. In this study, a series of experiments that included laboratory and glasshouse experiments were conducted to evaluate the allelopathic interference of silverleaf nightshade on early growth of cotton. Cotton seeds were exposed to silverleaf nightshade extract solutions or planted in soil into which silverleaf nightshade leaf material or ripe berries were incorporated. In all the experiments attempts were made to avoid or to reduce, at least, the influence of factors that could be potentially confounding. In this regard, PEG-6000 was used to evaluate the sensitivity of crop parameters to the osmolality of test solutions in order to ensure that

osmotic inhibition was not a confounding factor in the bioassays where the biological activity (phytotoxicity) of plant extracts were assessed. Competition for growth factors was the other major potentially confounding factor that was considered throughout, and steps were taken to negate its influence. Germination and early seedling growth of cotton cultivars Sicala, CA 223, Siokra V15, Tetra and Delta Opal were inhibited by test solutions (silverleaf nightshade extracts) and by soil-incorporated residues of silverleaf nightshade. Inhibitory effects of silverleaf nightshade solutions were observed when either a layer of filter paper or a thin layer of soil or quartz sand was used as substrate. In both laboratory and pot experiments, it appeared that cotton cultivar Sicala was the most sensitive to allelochemicals contained in extracts and residues of silverleaf nightshade. This finding of differential tolerance of crop cultivars towards allelochemicals contained in a weed is a rare occurrence in allelopathy research. Berries of silverleaf nightshade were generally more inhibitory to cotton than leaf material. For both types of plant material used, residues lost their inhibitory effect over time, probably as decomposition of allelopathic compounds in soil progressed. Information obtained from this study can be viewed as knowledge that contributes to the bridging of the gap between identification and isolation of allelochemicals from silverleaf nightshade, and confirmation of silverleaf nightshade allelopathy under natural conditions. Experiments involving soil as growth medium, in particular fieldwork, are needed to verify the validity of these findings under natural conditions.

INTRODUCTION

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) also known as "satansbos" is a serious perennial broadleaf weed in many semi-arid areas of the world including South Africa, Australia, Algeria, Egypt, Greece, India, Israel, Zimbabwe, Sicily and Spain (Hawkes & Edmonds, 1972; D'Arcy, 1974; Boyd *et al.,* 1984; Henderson *et al*., 1987; Bromilow, 1995). It has been declared a noxious weed everywhere it occurs as an invader (Robinson *et al.,* 1978; Boyd & Murray, 1982; Stubblefield & Sosebee, 1984). Strategies for control of silverleaf nightshade are confounded by its deep and well-developed root system as well as its ability to propagate from creeping lateral roots, root fragments and seeds (Cuthbertson, 1976; Boyd & Murray, 1982; Westerman & Murray, 1994; Olckers *et al.,* 1999). Cooley & Smith (1973) contend that silverleaf nightshade initially was a weed of minor importance and that its establishment as a serious weed has been encouraged by the extensive use of soil-incorporated pre-emergence herbicides. Abernathy (1975) reasoned that extensive herbicide use created conditions that are favourable for encroachment by this weed through reduced competition from annual weeds, and encouragement of bigger propagules as a result of the subsequent reduced reliance on tillage.

Silverleaf nightshade is one of the most serious, problematic weeds in cotton *(Gossypium hirsutum)* (Molnar & McKenzie, 1976; Abernathy & Keeling, 1979; Cilliers, 1999). Its interference with cotton results in significant reduction of plant height, boll size, lint quantity and quality (Green *et al.*, 1988; Smith *et al*., 1990). Competition for growth factors in the field is the only documented cause of adverse effects on the crop (Green *et al*., 1987; Green *et al.,* 1988; Jacobson *et al.*, 1994). By definition, however, interference of weeds with crops involves both competition and allelopathy (Rice, 1984). Currently, information on the allelopathic interference of silverleaf nightshade is very scanty (Boyd *et al.,* 1984; Bothma, 2002), but the presence of secondary metabolites that have pharmaceutical value has been reported in twigs and berries of this weed (Guerreiro *et al.,* 1971; Chiale *et al.,* 1991). Inhibition of crop growth in the laboratory by fractions of chemical extracts prepared from

silverleaf nightshade has been reported (Curvetto *et al*., 1976; Agüero & Boland, 1985; Bothma, 2002).

Apparently, growth inhibition of crops by silverleaf nightshade has not been reported beyond the laboratory environment. According to Heisey (1990) and Lewis (1986), identification of allelochemicals in a plant is not enough to conclude the occurrence of allelopathic interference. Huang *et al.*, (1999) further stated that demonstration of allelopathy in soilless laboratory bioassays does not prove that allelopathy will be observed in the natural plant-soil system. Numerous allelopathy authors, however, agree that though this may be the case, laboratory bioassays will remain a useful tool in allelopathy research as they can be used to experiment under controlled and precise conditions (Leather & Einhellig, 1986).

Chemical compounds that are toxic to livestock (Dollahite & Allen, 1960; Molnar & McKenzie, 1976) as well as compounds that have medicinal value, have also been reported in silverleaf nightshade (Maiti & Mathew, 1967; Khanna & Singh, 1987; Chiale *et al.*, 1991). The ripe berries of silverleaf nightshade, for example, are a rich source of the glycoalkaloid solasodine (Maiti *et al*., 1979). Solasodine is used in the pharmacochemical industry in the manufacture of corticosteroidal drugs. According to Nigra *et al*. (1989), solasodine is the second most important glycoalkaloid after diosgenin in the manufacture of corticosteroids. This makes silverleaf nightshade an important plant in some countries, such that domestication and multiplication schemes are strong considerations (Khanna & Singh, 1987; Heap & Carter, 1999).

When considering techniques and bioassays used in allelopathy, there has been concern that in most research bioassays, soil is not involved as a growing medium (Inderjit & Dakshini, 1995). This can be justifiably viewed as a shortfall of such bioassays because involvement of soil in allelopathy research as opposed to soil-less bioassays would help to bridge laboratory and natural field conditions (Reinhardt *et al*., 1999).

The aims of this study were to: (a) investigate the allelopathic influence of silverleaf nightshade extract solutions and residues on germination and the early growth of cotton; (b) investigate the influence of soil on the allelopathic activity of secondary metabolites produced by this weed species.

CHAPTER 1
LITERATURE REVIEW

1.1 Introduction

Silverleaf nightshade is a perennial broadleaf weed originating from the Americas (Goeden, 1971; Hawkes & Edmonds, 1972; D'Arcy, 1974; Boyd *et al*., 1984; Henderson *et al*., 1987; Bromilow, 1995). It is, however, unclear whether it originates from northern or southern America (Zimmerman, 1974; Morton, 1976; Symon, 1980). Because of its adaptability to a wide range of habitats and a high reproductive potential, silverleaf nightshade has spread and become an established weed in many parts of the world (Boyd *et al*., 1984).

Silverleaf nightshade is a very competitive and aggressive weed species (Boyd *et al.*, 1984; Trione & Cony, 1990). According to Roe (1971), a single plant can produce thousands of viable seeds. A dense population of silverleaf nightshade is capable of producing millions of viable seed (Cooley & Smith, 1973). The seriousness of silverleaf nightshade as a weed is enhanced by a growth habit where vegetative growth appears early in the spring followed by rapid growth from a well-developed root system (Boyd *et al.,* 1984). This characteristic gives silverleaf nightshade a competitive advantage over many agricultural crops. Currently, there is no known means of eradication of this weed (Henderson, *et. al.,* 1987; Bromilow, 1995). Even the best-known broadleaf herbicides such as phenoxy-acetic acid and phenoxy-propionic acid compounds do not control silverleaf nightshade effectively (Leys & Cuthbertson, 1977). This is mainly due to its exceptionally well-developed root system and its ability to propagate vegetatively and from seeds.

In addition to its remarkable reproduction potential and competition for growth factors, silverleaf nightshade serves as a host for several pests (Wene & Sheets, 1964; Goeden, 1971) and diseases (Index of Plant Disease in the United States, 1960). *Rhizoctinia solani* Kuehn (root rot), *Cercospora atromarginalis* Atk. (leafspot) and *Verticillium albo-atrum* Reinke and Berth

(wilt) are examples of pathogens which have been isolated from silverleaf nightshade. The Lygus bug *(Lygus hesperus),* an important pest of numerous crops, uses silverleaf nightshade as a host where it lays its eggs. Large numbers of nymphs and adults of the Lygus bug have been observed in silverleaf nightshade stands (Wene & Sheets, 1964).

In South Africa, silverleaf nightshade is known as “satansbos”, its Afrikaans common name that reflects its troublesomeness, which means “devil’s bush” when directly translated to English (Wasserman *et al*., 1988). Other vernacular names used to refer to silverleaf nightshade are “meloncillo del campo”, tomatillo (Pilar, 1937), white horsenettle (Bellue, 1946), bullnettle, silver horsenettle (Kearny & Peebles, 1960), tomato weed (Tideman, 1960), sandbrier, trompillo (Robbins, Ball & Bellue, 1970), silverleaf nettle (Hardin *et al*., 1972), purple nightshade, whiteweed (Cooley & Smith, 1973), western horsenettle (Gunn & Gaffney, 1974), silverleaf horsenettle (Munz, 1974), desert nightshade (Crittenden & Teller, 1975) and silverleaf bitter apple (Siebert, 1975).

Silverleaf nightshade was officially first recorded in South Africa in 1952 (Henderson & Anderson, 1966). It was then declared a weed under Proclamation No. 154 of 1966. Since then, the distribution of material contaminated with seed of silverleaf nightshade was banned in 1970 in this country (Wasserman *et al*., 1988). The Conservation of Natural Resources Act (Act No. 43 of 1983) declares that it is illegal to distribute any agricultural products contaminated with silverleaf nightshade (Wasserman *et al*., 1988).

Negative impacts of silverleaf nightshade have been reported worldwide on cotton, peanuts *(Arachis hypogaea),* grain sorghum (*Sorghum bicolor),* alfalfa *(Medicago sativa),* cereal grains and cultivated pastures (Boyd *et al.,* 1984). The main documented cause of the interference of silverleaf nightshade with crops is competition for growth factors (Green *et al.,* 1987; 1988; Jacobson *et al.,* 1994). Apart from the sparse reports where the allelopathic potential of silverleaf nightshade was observed in the laboratory (Bothma, 2002), no literature reporting allelopathic interference of this weed could be located. At

this stage, therefore, it is not clear whether allelopathy is involved in the interference of this weed with crops in the field.

1.2 Interference of silverleaf nightshade with cotton

According to Wasserman *et al*. (1988) heavy infestations of silverleaf nightshade affect the value and use efficiency of agricultural land, in that, production on such a land will be limited only to a few crops and lower returns will be experienced owing to the capital laid out for the control of this weed. In a study conducted specifically on the soil-water relationships between silverleaf nightshade and cotton, Green *et al.* (1987; 1988) reported that silverleaf nightshade competes with cotton for soil water. According to Davis *et al.* (1945), the root system of silverleaf nightshade can extend to a depth below 3 m, whereas the root system of cotton rarely exceeds a depth of 1.5 m (Stockton *et al*., 1967). Because of its deep and well-developed root system, silverleaf nightshade therefore competes more effectively for soil moisture and plant nutrients than cotton. Abernathy & Keeling (1979) reported that yield losses due to silverleaf nightshade interference reached 75 % in cotton grown under semi-arid conditions. Robinson *et al.* (1978) reported up to 15 % losses in cotton yield due to the presence of the weed in the absence of water shortage. Green *et al.* (1987) found that an increase from 0 to 32 silverleaf nightshade plants per 10 m of cotton row decreased lint yield linearly and that each additional weed per 10 m row further reduced yield by 1.5 %. The studies showed that the effect of competition was more pronounced under dry land conditions as compared to when the cotton was irrigated. All the above studies were conducted to investigate the competition aspect. At this stage it is not known if allelopathy also plays a role in the interference of silverleaf nightshade with cotton in the field.

1.3 Phytochemicals found in silverleaf nightshade

Plants are known to metabolize and produce a tremendous variety of secondary metabolites that act as chemical toxins (Rice, 1974; Fay & Duke, 1977; Leather, 1983; Lehle & Putnam, 1983; Ortega *et al*., 1988). The main function of these so-called chemical toxins is to promote the chances of survival of the plant. The same compound may simultaneously function against pathogens, insects, mammals, and against competing plants.

Generally, the family Solanaceae includes food plants, medicinal and poisonous species, ornamentals and various noxious weeds (Heiser, 1969). Phytochemical studies conducted on various solanaceous varieties revealed the presence of different chemical compounds in different concentrations in plant material of *S. elaeagnifolium, S. nigrum, S. tuberosum* (potato), *S. khasiunum, S. auricalatum, S. giganteum, S. sarrachoides, S. villosum* (hairy nightshade), *S. melongena* (eggplant) and *S. dulcamara* (European bittersweet nightshade) (Khanna *et al*., 1978; Maiti *et al.,* 1979; Keeler *et al*., 1990; Grosso *et al*., 1991; Zygadlo, 1994; Hanna *et al*., 1996 a&b).

In a similar study that was conducted in India, Maiti & Mathew (1967) concluded that out of 28 Solanaceae species tested, silverleaf nightshade contained the highest amount of the alkaloid solasodine. They reported that the ripe berries contained 3.2 % of their dry weight as solasodine. Kaul & Zutshi (1973) reported 1.8 % solasodine in berries. A concentration of 1.6 % was reported for green berries and 1.7% in ripe berries by Bradley *et al.* (1978). Other alkaloids that have been isolated from silverleaf nightshade are hyoscyamine and scopolamine (Boyd *et al.,* 1984). These compounds are well-known alkaloids in the manufacture of steroidal drugs in the field of pharmacology.

Alkaloids that are toxic to animals have also been isolated from silverleaf nightshade (Burrows *et al*., 1981). An example is atropine. When the alkaloids are ingested by animals, and combine with sugars in the gastrointestinal tract, they are transformed to glycoalkaloids such as solanine and solasonine.

These glycoalkaloids are similar to saponins and have an irritating effect on the gastrointestinal tract of animals. Moreover, once in the gastrointestinal tract, they can be hydrolyzed to release toxic alkalids or alkamines that are nerve poisons. According to Burrows *et al.* (1981), although the green berries of silverleaf nightshade have a toxic effect on animals, the ripe berries are even more toxic. This statement is in agreement with the findings of Guerreiro *et al*. (1971) who reported higher concentrations of toxins in ripe berries of silverleaf nightshade compared to leaves. Other phytochemicals reported in the different plant parts of silverleaf nightshade include squalene, the saponin 3-deoxy-Δ^3-diosgenin, steroidal glycoalkaloids, solamargine (Guerreiro *et al.,* 1971); the flavanoid kaempferol 3-beta-D-(6"–*cis*- cinnamoylglucoside) (Chiale *et al.,* 1991) as well as solasurine and solanelagnin (Hanna *et al*., 1996a). More recently, in a South African population of silverleaf nightshade, an unidentified flavonoid, alkaloid and saponin were reported by Bothma (2002).

1.4 Control of silverleaf nightshade on agricultural lands

Smith *et al*. (1990) reported that a single silverleaf nightshade plant left uncontrolled can increase 10-fold in a year and up to 40 times after two years. According to Cooley & Smith (1973), a dense stand of silverleaf nightshade plants is capable of producing 250 million seeds ha^{-1}. Up to 4000 seeds m^{-2} have been recovered from the soil in silverleaf nightshade infested fields (Molnar & McKenzie, 1976). Silverleaf nightshade seeds can be dispersed over long distances and can remain viable for up to ten years (Bellue, 1946). Seeds therefore undoubtedly play a very important role in the spread of silverleaf nightshade. Prevention of seed formation and dispersal is therefore very crucial in the control of this weed.

Apart from propagating through seed, the root fragments and rhizomes of silverleaf nightshade are capable of growing into new plants (Cuthbertson, 1976). Roots of silverleaf nightshade grow up to a depth of 3 m or more in the soil and may spread up to 2 m away from the plant. As the roots spread, new shoots grow from adventitious roots and sprout into new plants (Green *et al.,* 1988; Bromilow, 1995). According to Fernandez & Brevedan (1972) and

Molnar & McKenzie (1976), root fragments of silverleaf nightshade can remain viable in the soil for 15 months and a 1-cm long root fragment is capable of coppicing into a new mother plant. These characteristics reflect the aggressiveness of this weed, and therefore, the difficulty to control it.

Various methods of control are currently used for silverleaf nightshade, and due to the very little success achieved through these methods, more alternatives are still being sought (Olckers *et al.,* 1999). According to these authors, research on the biological control of silverleaf nightshade in the form of gall-forming moths and defoliating beetles is promising as a biological means of control. The defoliating beetles *Leptonotarsa texana* and *L. defecta* are host-specific and they spend their entire life cycle feeding and reproducing on silverleaf nightshade plants. These beetles, however, feed only on the foliage, flowers and buds; they do not damage the roots and fruits of silverleaf nightshade. Thus the control they provide is through weakening the plant, thereby affecting its competitiveness.

Concerning mechanical control, the obvious problem is the cutting of the underground plant parts into many small pieces that are capable of growing into new mother plants. The use of chemicals for control of silverleaf nightshade gives only temporary relief by killing the shoots of the weed. This is not a solution, however, because in the subsequent year new plants regenerate from the roots that had been left undamaged underground (Lemerle & Leys, 1991).

The use of the systemic, non-selective herbicide glyphosate also has its limitations because the weed grows in between and within rows so that prevention of crop injury is difficult, resulting in crop injury and reduced cotton lint yield (Westerman & Murray, 1994). The recent development of transgenic 'Roundup-ready' cotton cultivars (Bailey *et al*., 2003), perhaps will be very useful in this regard, because it means glyphosate can be used in such cotton cultivars with a reduced chance of crop injury. Transgenic glyphosate-resistant cotton cultivars contain a tolerant form of 5-enolpyruvylshikimate-3-phoshate synthase (E.C.2.5.1.19), the enzyme inhibited by glyphosate (Klee

et al., 1987; Thompson *et al*., 1987; Suh *et al.,* 1993). According to Bailey *et al.* (2003), glyphosate can be applied to glyphosate-resistant cotton up to the four-leaf stage of growth with minimal risk of injury. When applied after this stage, glyphosate should be applied directly on weeds avoiding contact with cotton stems as much as possible (Anonymous, 2001).

Shading has been investigated as another means of control of silverleaf nightshade. Boyd & Murray (1982) subjected silverleaf nightshade plants to shade levels of 0, 47, 63 and 92 % of full sunlight and then determined its vegetative, reproductive and physiological responses. They discovered that dry matter production declined markedly with increasing shade levels. They observed that the plants could not form fruits under 92 % shade, and that taproots of plants grown in full sunlight contained 16 % more total non-structural carbohydrates (TNC) per total gram dry weight than plants grown under 92 % shade. This suggests that shading can be used to control silverleaf nightshade. In agricultural situations, shade could be provided by tall-growing crops, or dense stands of shorter species such as grasses.

1.5 Allelopathy: A background

1.5.1 Introduction

The earliest reference to phytotoxicity of one plant on another dates back to ancient agriculture when Theophrastus (300 B.C.) observed that some plants inhibit the growth of other plants. At that time various assumptions without proper experimentation were made concerning problems in crop production that could not be rectified through nutrient amendments. De Candolle (1832) was the first to assume that chemicals secreted by crops caused ‘soil sickness’ and suggested that crop rotation was the only solution to this problem. Schreiner & Reed (1908) were the first to conduct proper research on this subject. They isolated chemical compounds from plants and from the soil. Since then, studies and research on allelopathy have been improving. The term allelopathy, however, was coined by Hans Molisch, a German scientist, in 1937 from two Greek words, *‘allelon’*, meaning to each other, and

'pathos', meaning to suffer (Molisch, 1937; Rizvi *et al*., 1992). Allelopathy refers to the direct or indirect effect of a plant on another plant through the production and release of chemical compounds into the environment (Rice, 1984). The effect may be inhibitory or stimulatory depending on the amount of the chemical reaching the receiving plant (Putnam & Tang, 1986; Rice, 1995).

The discovery of chemical interactions amongst plants provided new knowledge that, apart from competition for growth factors, plants can affect the growth of neighboring plants by secreting chemicals into the environment. A clear distinction between allelopathy and competition is that, in the former case, something is released into the environment (allelochemicals), whilst in the latter case, something is removed from the environment (nutrients, water, etc.). Allelopathic interactions can involve plants of the same species (intraspecific or autotoxicity) or species that are taxonomically different (interspecific or heterotoxicity or teletoxicity) (Kumar, 1991; Kohli *et al.*, 1998; Kushal, 1987).

Identification of allelochemicals and the explanation of the concept of allelopathy have advanced greatly in the last three decades and had been encouraged by the development of research techniques that did not exist in the past. These modern techniques allow for the identification and isolation of the different plant chemicals. Many different compounds released from plants and from microbes are now known to affect the growth or aspects of function of the receiving species (Einheillig, 1995). Whittaker & Feeny (1971) classified these phytochemicals into five groups: phenyl propanes, acetogenins, terpenoids, steroids and alkaloids.

1.5.2 Modes of release of allelochemicals into the environment

The synthesis of allelochemical compounds in plants is believed to have evolved through heritable mutations to enhance their protection and survival in the environment as a result of biological and physical evolutionary pressures on the plant species (Stone & Williams, 1992; Seigler, 1996; Wink, 1999). Allelochemicals are synthesized in plants as secondary metabolites (Whittaker

& Feeny, 1971; Wink, 1999) and are present as complex mixtures in virtually all plant parts and tissues including leaves, stems, roots, flowers and seeds (Rice, 1974; 1984; Putnam & Tang, 1986). They are called secondary metabolites because they accumulate in plants but they often lack an obvious purpose in plant metabolism (Rice, 1974).

For allelochemicals to be effective in their function, they need to be released from the plant and transferred to the target plant species in sufficient amounts that would cause the effect. Hence, Muller (1974) states that for allelopathy to occur a chemical should be (1) synthesized and produced by a plant, (2) transported from the producing organism to the target plant, and (3) the target plant should be exposed to the chemical at a concentration sufficient to cause an effect. Plants may release allelochemicals into the environment through volatilization (Muller, 1965), leaching (Del Moral & Muller, 1970), decomposition of plant residues (Einhellig, 1995), or as root exudates (Neill & Rice, 1971; Tang & Young, 1982).

1.5.2.1 Volatilization

Numerous plant species have been observed to release allelochemicals through their surfaces in the form of gases into the atmosphere (Muller, 1965; del Moral & Muller, 1970; Neill & Rice, 1971). This mechanism of release of allelochemicals tends to be of more significance in arid and semi-arid conditions (Rice, 1974). After release from the plant, volatiles are dispersed in the air following mass flow or diffusion, and can be intercepted by the receiver species in the form of a gas, or can be dissolved in dew or in water and are transferred into the soil where they will be taken up by plant roots (Muller, 1965; Einhellig, 1995). Amongst genera well known to secrete volatile allelochemicals are *Artemisia, Eucalyptus* and *Salvia* (Rice, 1984). Volatiles include cineole, pinene and camphor (Muller, 1966). Amongst numerous other examples of allelopathic inhibition by plant-released volatiles, Kim & Kil (2001) reported that volatile compounds released from leaves of tomato *(Lycopersicon esculentus)* are allelopathic to some crop species in a closed system.

1.5.2.2 Decomposition of plant residues

The greatest amounts of allelochemicals released from plants into the environment are released through plant material decomposition and through leaching from plant material (Putnam & Duke, 1974). It is always difficult however, to determine whether the toxic substance is contained in the plant material and simply released upon decomposition, or if the toxin is produced through modifications by microbes involved in decomposition (Rice, 1974). For example, the isoflavonoids produced by red clover are decomposed to even more toxic phenolic compounds (Chang *et al.,* 1969).

Yakle & Cruse (1984) reported that the positioning of plant residues in soil might affect allelopathy. They observed that maize residues were allelopathic to maize plants, and that the effect was stronger when residues were placed below the seed compared to when they were placed above the seed. The difference in allelopathic effect was attributed to the roots having direct contact with the decomposing plant material placed below the seed, compared to placement above the seed.

Effects of allelopathy have been reported also for tree species. For example, Patrick (1955) studied the cause of the replant problem experienced in Ontario peach orchards, and discovered that following microbial decomposition of peach root residues, toxic compounds that inhibit root respiration of excised peach roots are produced. The toxic compounds were produced as a result of the transformation of amygdalin released from peach roots to hydrogen cyanide and benzaldehyde.

The practical implication of the production of allelochemicals from decomposing plant material is that some herbicide-killed plant material or weeds that have been ploughed under, can inhibit the growth of crops even when the weed no longer grows in the field. In the case where plant material is used for mulching, the plant and material types used for mulch should be selected wisely so that the growth of the crop is not inhibited.

1.5.2.3 Leaching

Allelopathic compounds may be leached with rainwater or with irrigation water from aerial plant parts into the soil or onto plant surfaces (Lovett, 1982). Some allelochemicals are leached from decomposing residues in the soil (Muller, 1966). Allelopathic compounds released through leaching include organic acids, terpenoids, alkaloids, peptic substances, gibberillic acids, sugars, amino acids and phenolic compounds (Tukey, 1966).

1.5.2.4 Root exudation

Roots secrete chemical compounds either as exudates from live roots or from the cells that are sloughed off as they age (Rice, 1974; Putnam, 1985). These chemical compounds may be dissolved in the soil solution or taken up directly by the receiving plant. Rice (1984) hypothesized that in some cases allelochemicals may not be released but are exuded directly from the donor plant into the receiving plant through mycorrhizae and root grafts. Wheat, oats, maize and cowpeas are examples of plants that produce toxic inhibitory allelochemicals from their roots (Schreiner & Sullivan, 1909). Gaidamak (1971) reported the same findings for tomato and cucumber.

1.5.3 Factors that affect allelopathy

Allelopathy is a complex phenomenon involving a variety of interrelationships among plants (Kohli *et al.,* 1998). In order to understand the influence of the different factors that may be involved in allelopathy, it is essential to first understand the series of processes involved for the expression of allelopathy by a receiving plant and the factors that influence its response. The effects of allelopathy observed on the receiving plant are influenced by the concentration of the chemical reaching the sensitive receptor within the receiving plant, and its sensitivity (Muller, 1974). The amount of chemical absorbed by the receiving species is influenced by the amount of the phytochemical synthesized in the donor plant and the factors that are involved in the transit of the chemical between the donor and the receiving plant

(Cheng, 1989; 1995). The amount of chemicals produced in the donor plant is a result of the interaction of the plant's genetic factors and those of the environment (Wink, 1999). Allelopathy, therefore, is a very complex process influenced by a myriad of interacting climatic, soil and plant factors.

After allelochemicals are released from the plant into the environment, they are exposed to transfer, retention and transformation processes (Rizvi & Rizvi, 1992). Collectively, these processes are termed dissipation. Transfer refers to all the processes that lead to a change in the location of the chemical in the environment. Transfer of allelochemicals can take place in the air or in the soil, and in water through mass flow or diffusion following a concentration gradient in the medium. The rate of transfer of organic compounds in the soil depends on the chemo-physical character of the compound, the soil and on environmental factors (Reinhardt *et al.,* 1999). Retention or adsorption of phytochemicals refers to the attraction of the chemical onto soil components. The result of retention is transient reduction of the chemical's availability in the soil solution. According to Blum *et al*., (1987), adsorption of allelochemicals in the soil is usually reversible. In that case, adsorption will result only in the temporary loss of the allelochemical from the soil solution. Transformation refers to the modification of the original, released compound and can occur through photochemical, biological and chemical means (Rice, 1974). It is the resultant effect of the interaction of all the factors that are involved in dissipation that determines the fate of plant chemicals in the environment, and hence, their actual effectiveness in allelopathy. Thus, the expression of allelopathy is influenced by all factors involved from synthesis, to release from the plant, to dissipation in the environment, until they reach the sensitive receptor in the receiving plant. Just some of the governing factors are climate, plant and soil factors, stress factors, and treatments such as application of fungicides, herbicides, insecticides and plant growth regulators.

1.5.3.1 Climatic Factors

Climatic factors have a great influence on allelopathy during the production of allelochemicals by plants and also during their dissipation in the environment. Plants tend to produce higher amounts of allelochemicals when they are under stress than when they grow under optimal conditions. According to Wink (1999), the production of higher concentrations of allelochemicals by plants under stress conditions is quite logical because they are responding to their threatened survival. Del Moral (1972) reported that a combination of stress factors affects the amounts of allelochemicals produced in plants more than either factor acting on its own. In nature, a combination of stress factors usually affects plants at the same time (Rice, 1974).

Light

Light is one of the factors that reportedly affect the amounts of phytochemicals produced by plants. Nigra *et al*., (1989) found that the concentration of the alkaloid solasodine synthesized by *S. elaeagnifolium* was higher when the plants were exposed to a photoperiod of 16 hours than when they were grown in the dark. Chandler & Dodds (1983) reported similar findings for *S. nigrum*. Rice (1974) found that the concentration of allelochemicals synthesized by plants grown in the glasshouse was reduced compared to when the plants grew in direct sunlight. Cooner (1987) reasoned that the enhanced concentration of allelochemicals in the presence of light could be attributed to the promotion effect of photosynthesizing chloroplasts. Light may also be involved in the degradation (photo-degradation) of some allelochemicals in the environment.

Water

Water plays a very important role in allelopathy, because it serves as a solvent and carrier of allelochemicals and leachates from aerial plant parts and in the soil (Guenzi *et al*., 1967; Reinhardt *et al*., 1999). Water may also dilute allelochemicals in the soil and in the plant. The activity of soil microorganisms is sensitive to soil moisture levels, therefore, the water content of the soil will affect allelopathy in cases where soil microorganisms

are involved in the activation of an allelochemical (Reinhardt *et al.,* 1999). The activity of microorganisms also affects the rate at which allelochemicals decompose in the soil (Chou, 1989a). Therefore, water is an important factor affecting the allelopathic potential of a chemical because the resulting effect on the target species is a function of the concentration of the chemical at the receptor site and the duration of the exposure (Gershenzon, 1984; Rizvi & Rizvi, 1992). From the above explanation of the role of water in allelopathy, it is clear that water is involved in the concentration aspect and also influences the duration of exposure.

Temperature

Temperature also influences allelopathy. Martin (1957) reported increased amounts of allelochemicals from plants that were exposed to high ambient temperatures compared to those at lower temperatures. Koeppe *et al.* (1970) reported the same effect after exposing plants to chilling temperatures. These observations are in agreement with the reports stating synthesis and production of larger amounts of allelochemicals by plants under stress. Apart from the direct influence temperature has on allelopathy, it may indirectly affect allelopathy by affecting microbial activity in the soil (Reinhardt *et al*., 1999).

1.5.3.2 Plant Factors

Composition and concentration of allelochemicals produced by plants differ with age of plants, between plant parts, and amongst plant species (Putnam & Duke, 1974; Putnam & Tang, 1986; Wink, 1999; Qasem & Foy, 2001). According to Putnam & Duke (1974) differences may exist even within the same plant species amongst different genotypes.

Plant part

Higher concentrations of allelochemicals have been reported mostly in leaves but also in roots or seeds in some cases (Rice, 1974). Generally, leaves are the common source of allelochemicals and roots usually either contain low amounts of a particular compound, or chemicals of low toxicity (Rice, 1974).

Plant age

Differences in the amounts of allelochemicals produced by the same species at different growth stages have been reported. Fresh, green plant material tends to be more allelopathic than mature plant material (Kimber, 1973). Koeppe *et al.* (1969) reported that in tobacco and sunflower leaves the amount of phenolic compounds is higher in the younger leaves. However, because of the increase in size of the foliage with plant age, the total amount of toxins produced by older leaves becomes higher. According to Inderjit & Dakshini (1995), in some plants, a certain stage of growth has to be reached before allelochemicals are synthesized. Schumacher *et al*. (1983) reported that wild oats becomes allelopathic to spring wheat at the four-leaf stage and not before.

Plant population density

Population density of both the target and the donor species influences the response of plants to allelopathic compounds. When the stand of the receiving species is dense, the phytotoxic effects of the received allelochemicals are not as severe as when the stand is sparse (Weidenhamer *et al*., 1989; Thijs *et al*., 1994). This is because the amount of chemical available for each plant is reduced through dilution. In contrast, when the donor species population is high, more toxins are produced, and hence, phytotoxicity could be high, especially if there are relatively fewer plants of the receptor species. Wu *et al.* (2000) for example, reported that the allelopathic inhibition of ryegrass by wheat residues was higher under a highly populated stand of wheat plants.

1.5.3.3 Soil Factors

A large fraction of the allelochemicals released by plants probably reaches the soil (Reinhardt *et al*., 1999; Inderjit, 2001). Therefore, soil factors that influence the fate of allelochemicals that enter the soil should have a great influence on allelopathy. Soil factors that affect the fate of allelochemicals are of physico-chemical and biological nature.

Soil texture

Soil texture significantly influences the expression of allelopathy in natural systems (Muller & Del Moral, 1966, Del Moral & Muller, 1969; Del Moral & Cates, 1971; Rice, 1984; Oleszek & Juryzista, 1987). Lehmann *et al*. (1987) observed that after introducing the same amount of a particular allelochemical to different types of soil, the amounts recovered were not the same. Kuiters & Denneman (1987) reported similar findings for phenolic compounds in sand and clay soils. They discovered that higher amounts of allelochemicals were extractable from the sandy soils than from the clayey soils. Oleszek & Juryzista (1987) concluded that heavy clay soils adsorb allelochemicals more than sandy soils. As a result, on sandy soils, allelopathic effects may be more pronounced than on heavy soils.

Soil pH, organic matter and micro-organisms

According to Dalton *et al*. (1983); Blum *et al.* (1987) and Dalton (1989), pH and organic carbon content influence the activity of allelochemicals in the soil. The tendency was that microbial activity was favoured more by high pH than by acidic conditions. Therefore, if an allelochemical requires microbial transformation to become either toxic or to be degraded, its effect is likely to be influenced by soil pH. Soil pH therefore indirectly affects the immobilization and uptake of allelochemicals through its influence on microbial activities (Brand *et al.,* 1986). Organic matter content of the soil also indirectly influences allelopathy through its effect on microbe populations. Amino acids and carbohydrates released from the rhizosheath, as well as from the decortication of the root, result in higher microbial activity in the region of the rhizosphere (Cunningham *et al.*, 1996). Degradation of organic compounds therefore tends to be higher in this region as compared to the rest of the soil around the root zone.

1.5.3.4 Herbicides, fungicides, insecticides and plant growth regulators

By causing stress on plants, herbicides affect the amounts of secondary metabolites synthesized by plants (Lydon & Duke, 1993). Application of pesticides and growth regulators has also been observed to affect allelopathy.

Herbicides are explicitly designed to interfere with the normal functioning of plant metabolism, thus they are likely to have significant effects also on secondary metabolism of both sensitive target and non-target plants. The effects of herbicides on allelopathy can be direct on the target species or indirect on the non-target plant through the elimination of competitors.

Different classes of herbicides affect different processes of plant metabolism. For example, the herbicide glyphosate is a specific inhibitor of the shikimate pathway by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP). The shikimate pathway in plants is a crucial process because it is the pathway through which several essential amino acids and plant secondary metabolites are synthesized. Some allelopathic compounds are also synthesized through the same pathway (Lydon & Duke, 1993). Laanest (1987) reported that through the inhibition of the shikimate pathway, interference occurs with other chemicals in the plant as well. Brammall & Higgins (1988) reported that glyphosate reduced the transport of phenolic compounds to tomato roots. Weston & Putnam (1985) reported that glyphosate-treated quackgrass was less allelopathic to several legumes compared to non-treated quackgrass. Johal & Rahe (1984); Levesque *et al*. (1987); Rahe *et al*. (1990) reported an increased susceptibility to pathogens in glyphosate-treated plants. Brown & Sharma (1984) observed that residues of glyphosate-treated plants are readily colonized by fungi.

According to Wender (1970) the effect of 2,4-D, an auxin-type herbicide, on allelochemic compounds is not constant but the tendency is an increase of phenolic compound production in treated plants. A similar effect on the production of scopolin by tobacco following a treatment with the auxin-type herbicide picloram was reported (Wender, 1970). A tendency of reduced flavonoid production in alachlor-treated plants has been reported (Molin *et al.,* 1986). According to Schwarz (1983) the induced production of the secondary metabolite oleoresin in paraquat-treated conifers is well known. Schwarz (1983) reported increased insect attacks in conifers following paraquat-induced oleoresin production. However, Beal *et al*. (1979) observed the opposite effect. The observed effects of paraquat on insect resistance suggest

that apart from its effect on oleoresin, paraquat affects other chemicals in unpredictable ways. Duke *et al*. (1991) reported enhanced production of gossypiol and hemi-gossypiol by cotton following treatment with the herbicide clomazone. These observations suggest that herbicide treatments on plants may affect various processes in plants including allelopathy in unpredictable ways.

Effects of fungicides on allelochemical production of some plant species have also been reported (Reilly & Klarman, 1972; Venkatramesh & Croteau, 1989; Gottstein & Gross, 1990), as well as effects from insecticides (Devlin *et al*., 1969; Parrott *et al*., 1983; Mitra & Purkayastha, 1986). The effects of plant growth regulators on the amounts of allelochemicals produced has been observed in both tissue culture and in whole plants (Furuya *et al*., 1971; Roper *et al.,* 1985; El-Keltawi & Croteau, 1986 a&b). Nigra *et al.* (1989), for example, observed an increase in the amount of solasodine produced by *S. elaeagnifolium* calli that was treated with plant growth regulators; however, the values obtained for the alkaloid solasodine in the calli were lower than those observed to occur naturally in fruits of this plant.

1.5.4 Influence of allelochemicals on physiological, biochemical and molecular processes of target plants

Very little is known currently regarding mechanisms of action of allelopathic compounds in host plants. This lack of information can be attributed to the following factors: (1) allelochemicals involved in allelopathy are diverse in nature, (2) a complex of compounds is usually involved, (3) allelochemicals are transformed in the soil leading to the production of new unidentified compounds, and (4) allelochemicals are retained in the soil (Putnam, 1985). However, despite the complexity of these challenges, some crucial life processes of plant functioning that are affected by allelochemicals have been identified (Rice, 1974).

For an allelochemical to inflict its effect on a receiver plant it has to be absorbed and translocated to the site where it is capable of affecting

metabolism (Fisher & Adrian, 1981). Therefore, it is only when the allelochemical is absorbed, translocated and is not detoxified by the plant, that it will affect the ontogeny or metabolism of the receiving plant. Tolerant or resistant plants, however, have an ability to detoxify absorbed allelochemicals or to block their translocation. Also, it has been discovered that some plants have an ability to select and not to absorb some allelopathic chemicals at all. When absorption and translocation occur readily in the plant, the toxin then inflicts its effect at cellular level at the sensitive site. Wink (1999) contends that the symptoms of allelopathy observed in plants are secondary effects, and that the primary effects occur at a cellular level.

Interference of allelochemicals on essential life processes of plants is not limited to only those mentioned here. Interference with mineral ion uptake, membrane associated processes, protein and nucleic acid metabolism have been implicated (Rice, 1974; Inderjit & Keating, 1999). Discussions on the mechanisms of action of allelochemicals have been done by Rice (1984); Muller (1986); Einhellig (1986; 1995) and Waller (1989).

1.5.4.1 Interference with cell elongation

In many allelopathy bioassays, seed germination, seedling length or seedling fresh mass are the parameters usually tested to quantify the effects of allelochemicals. Inhibitory effects and, in some cases, stimulatory effects on seedling length due to allelochemicals are well documented (Muller, 1965; Jankay & Muller, 1976; Rice, 1984; Ortega *et al.,* 1988). Wink & Latz-Brunig (1995) reported reduced hypocotyl elongation and root growth of garden cress *(Lepidium sativum)* in the presence of some phenolic compounds, organic acids, terpenoids and alkaloids. Aliotta *et al.* (1993) reported the same effects for coumarins and phenylpropanes on the germination and growth of radish roots.

1.5.4.2 Interference with photosynthesis

Reduced photosynthesis in intact plants due to various individual allelopathic compounds has been observed (Patterson, 1981; Stiles *et al.,* 1994). One such compound is sorgoleone (2-hydroxy-5-methoxy-3-[(8′z,11′z)-8′,11′,14′-pentadecatriene]-*p*-benzoquinone) (Netzyl *et al.,* 1988; Einhellig & Souza, 1992; Einhellig *et al.*, 1993). Sorgoleone has been observed to inhibit the evolution of CO_2 during photosynthesis in potato (Nimbal *et al.,* 1996). Gonzalez *et al.* (1997) found that sorgoleone inhibited Photosystem II electron transport reactions. Sorgoleone thus has potential as a natural herbicide because of ability to inhibit electron transfer between Q_A and Q_B at the reducing site of Photosystem II. The influence of allelochemicals on photosynthesis in host plants can also be indirect through interference with stomatal opening (Shimshi, 1963a; 1963b; Zelitch, 1967; Einhellig *et al.,* 1970; Einhellig & Kuan, 1971).

1.5.4.3 Interference with respiration

Allelopathic compounds have been observed to interfere with O_2 uptake, ATP production, electron transport and CO_2 production in host plants (Muller, 1969; Stenlid, 1970; Lang & Racker, 1974; Ortega *et al.,* 1988; Li *et al*., 1993; Stiles *et al*., 1994). In some cases, stimulation of respiration in host plants has also been observed (Dedonder & van Sumere, 1971; Lodhi & Nickell, 1973).

CHAPTER 2
ALLELOPATHIC INFLUENCE OF SILVERLEAF NIGHTSHADE EXTRACT SOLUTIONS ON GERMINATION OF COTTON AND EARLY GROWTH OF THE SEEDLINGS

2.1 Introduction

Interference of the weed silverleaf nightshade with cotton results in significant losses in yield and quality (Cooley & Smith, 1973; Green *et al.,* 1987). Silverleaf nightshade is a very difficult weed to control, and is regarded as one of the serious weeds in cotton (Cooley & Smith, 1973; Abernathy & Keeling, 1979; Green *et al*., 1987). Research on the interference of silverleaf nightshade with cotton has focused mainly on the competition aspect (Green *et al.*, 1987; 1988; Smith *et al.,* 1990; Westerman & Murray, 1994) whilst the allelopathic interference aspect has been poorly explored. By definition, weed interference refers to the negative effects of weeds on crops as a result of both competition and allelopathy (Harper, 1960). The main difference between these two phenomena is that in the case of competition there is removal of something (e.g. water, nutrients, light) from the environment, whilst allelopathy involves the introduction of something (allelochemicals) into the environment.

Chemical compounds, which are known to possess toxic, allelopathic and/or medicinal properties have been reported in silverleaf nightshade (Maiti & Mathew, 1967; Kaul & Zutshi, 1973; Burrows *et al.,* 1981). For example, high concentrations of the glycoalkaloid solasodine that are extracted from ripe berries of silverleaf nightshade is used in the manufacture of steroidal drugs (Chiale *et al*., 1991). Many studies have focussed on the phytochemistry of silverleaf nightshade, in particular on its medicinal properties (Kaul & Zutshi, 1973; Maiti *et al.,* 1979; Keeler *et al.,* 1990; Chiale *et al.,* 1991; Grosso *et al.,* 1991; Zygadlo, 1994; Hanna *et al.,* 1996a; 1996b). More recently, a study on the phytochemistry of silverleaf nightshade focusing specifically on its allelopathic potential has been conducted by Bothma (2002). This study, which included preliminary chemical analysis, using both bioassay and paper

chromatography techniques, indicated the presence of an alkaloid, a flavonoid and a saponin in silverleaf nightshade extracts. It was suggested that allelochemicals such as these act together in a synergistic manner to cause inhibition of crop seedling growth (Bothma, 2002).

Apart from reports of allelIochemicals in silverleaf nightshade, information on its actual allelopathic interference is very sparse (Bothma, 2002). The only report that could be located in this regard was the findings of Curvetto *et al.* (1976) that germination of cucumber was inhibited by a saponin extracted from berries of silverleaf nightshade. They also found that the germination of some crops and weed species was inhibited in the laboratory when they were grown on soil into which silverleaf nightshade berries, with pericaps removed, had been mixed. Another abstracted report on the allelopathic interference of silverleaf nightshade with cotton mentioned the inhibition of germination of a cotton cultivar GSA-7.1 by silverleaf nightshade infusions (Munger *et al*., 1984). However, the full report stating the techniques and methodologies used in this study could not be located.

In allelopathy research, bioassays are the main tool used to investigate biological activity of allelochemicals (Leather & Einhellig, 1985, 1986). When reviewing the reliability of results obtained from such bioassays, Leather & Einhellig (1985) contended that no single type of bioassay would meet all the requirements for detecting bioactivity of allelochemicals. For this reason it was proposed that a combination of several types of bioassays should be used in sequence in allelopathy investigations (Leather & Einhellig 1985; Reinhardt *et al.* 1999; Wu *et al.,* 2000). For example, Reinhardt *et al.* (1999) explained that in some bioassays the substrate containing the allelochemical(s) tested might affect the availability of the allelochemicals for uptake by plants or seeds. Reinhardt *et al*. (1999) further advised that methodologies and techniques used in allelopathy research should be selected to suit the type of compound being investigated as well as the test species. Techniques used in research that involves volatile compounds, for example, differ from techniques used in experiments that deal with non-volatile compounds (Smith, 1989). Examples of techniques available for allelopathy research include the Petri dish, sponge,

stairstep, paper roll, chromatographic methods, volatile bioassay, and pot experiments (Chou, 1989b).

Research has proven that apart from the type of bioassay used, the procedures and techniques used in allelopathy research can greatly affect the reliability of results obtained (Reinhardt *et al.,* 1999). Results from allelopathy experiments, however, can only be regarded as reliable when procedures and techniques used are appropriate and correct, and when all possible interfering factors have been excluded as much as possible. One of the possible confounding factors in allelopathy bioassays, specifically where extracts are tested on seeds and seedlings, is the effect of osmotic potential of test solutions. High osmotic potential of a test solution may result in the inhibition of germination of seeds in most plant species (Anderson & Loucks, 1966; Bradbeer 1988). It is for this reason that tests for osmotic inhibition are crucial in allelopathy research, so as to ascertain that the inhibitory effects observed are as a result of allelochemicals and not because of osmotic effects. Reinhardt *et al*. (1999) contended that the effect of osmotic potential had been overlooked in numerous allelopathy investigations.

The primary objective of this study was to investigate the allelopathic effects of silverleaf nightshade extract solutions on the early growth of five cotton cultivars by excluding all possible confounding factors as much as possible. Selection of cotton as a test species constituted a practical approach because silverleaf nightshade is considered to be a highly problematic weed in cotton fields both locally and internationally (Abernathy & Keeling, 1979; Green *et al.,* 1987; 1988; Bromilow, 1995).

2.2 Materials and methods

Tests for osmotic inhibition

The main objective of this initial experiment was to evaluate the sensitivity of germination, shoot and radicle growth of cotton to osmotic potential of a test solution. Therefore, this experiment was conducted to ascertain that allelopathic effects will not be confused with osmotic effects in the subsequent

experiments, where seeds of cotton would be exposed to extract solutions prepared from weed material. For this purpose, polyethylglycol (PEG-6000) was used to establish a range of osmotic potentials. PEG-6000 affects seed germination only by altering the osmolality of water, such that any effect observed on the germinating seeds is a result of osmotic potential of the solution.

This experiment was conducted under aseptic conditions, with preparations done under a laminar flow cabinet, where all apparatus were sterilized prior to being used. A range of PEG-6000 solutions of increasing osmolality of 3, 8, 24 and 55 mOsm kg^{-1} was prepared by dissolving 12.5, 25, 50 and 75 g PEG-6000 flakes, respectively, in 1 L of sterilized, distilled water. The solutions were then sterilized by passing them through 0.2 μm pore size Whatman Puradisc polyethersulfone (PES) membrane filters[1] (Fig. 2.1). Osmolalities of the solutions were then measured using a Roebling digital micro-osmometer[2]. The Roebling osmometer measures the freezing point of an aqueous solution. It operates by the principle that water freezes at 0 ^{O}C, and that an aqueous solution with an osmolality of 1 Osmol kg^{-1} of water freezes at –1.858 ^{O}C. The freezing point reduction below that of pure water is a direct measure of the osmotic concentration of the solution, because a linear correlation exists between the freezing point and the osmolality of an aqueous solution. The freezing point of the solution therefore determines the osmolality of the solution. The readout from the Roebling osmometer is given in mOsm kg^{-1}.

Five cotton cultivars, namely: Tetra, Delta Opal, CA 223, Siokra V15 and Sicala were used in this experiment. These were the same cultivars that would be exposed to the silverleaf nightshade extract solutions in the subsequent experiments. For sterilization of distilled water it was autoclaved for 30 minutes at 121^{O}C. Fungicide-coated cotton seeds were surface-sterilized in a 10 % commercial bleach solution, for 10 minutes, then rinsed three times with sterilized, distilled water. The seeds were then placed under the laminar flow cabinet to dry. Petri dishes and filter paper, wrapped with

[1] Whatman®Puradisc™ 25AS 0.2μm Sterilizing Grade Filters. Website http://.www.whatman.com

aluminum foil, were autoclaved at 121^{O}C for 30 minutes before use. All metal apparatus used were sterilized with 70 % ethanol solution and flaming. For each of the five cotton cultivars, five seeds were placed in a 9-cm diameter Petri dish lined with a single layer of 9-cm diameter Whatman No. 1 filter paper. For each PEG-6000 concentration, 5 ml aliquots were then introduced into each Petri dish. Distilled water was used as a control treatment. There were ten replications for each treatment. Each replication was composed of two Petri dishes with five seeds each (five seeds X two Petri dishes = 10 seeds per replication) totalling 2500 seeds. The Petri dishes were then wrapped with Parafilm® and incubated in the dark at 25^{O}C. Germination percentage, shoot and radicle length were determined after five days. A seedling with a radicle length of 2 mm or more was considered successfully germinated.

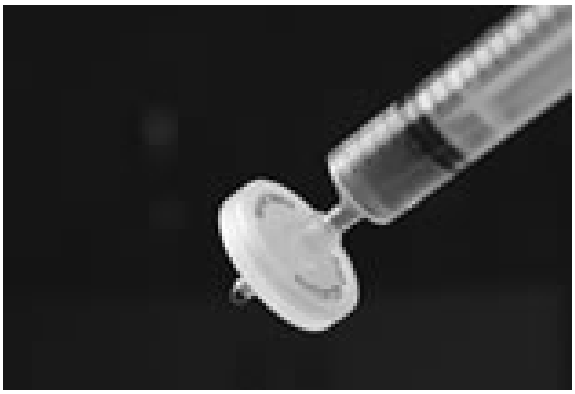

Fig 2.1 Whatman® Puradisc polyethersulferone (PES) filter used for filtering the test solutions (with a syringe attachment)

Allelopathic potential of silverleaf nightshade extract solutions

Preparation of extract solutions

Silverleaf nightshade plants were picked from the field at the University of Pretoria experimental farm when they were growing actively. The plants were picked at this stage because of the possibility that the leaves of actively growing plants might have a higher concentration of allelochemicals than of

[2] Herman Roebling, Meβtech, Katleweg 32, 1000 Berlin 38. Telephone 030 803 5671

mature or senescing plants. Immediately after collection the silverleaf nightshade plants were frozen and stored in this state until they were needed for preparation of the test solutions. A silverleaf nightshade stock solution was prepared by soaking 100 g of the frozen silverleaf nightshade leaves in 1 L of distilled water at room temperature in the dark for 24 hours. The solution was sieved through cheesecloth to remove the solid particles and then passed through a single layer of Whatman No. 1 filter paper. A 50 % concentration solution of the stock solution was prepared by mixing the solution with sterilized distilled water in a 1:1 ratio. Osmolalities of the solutions were then measured using a Roebling digital micro-osmometer. The two extract solutions (50 and 100 %) were then used in the bioassays; first using Petri dishes, and subsequently, paper rolls.

Petri dish technique

Five cotton cultivars, namely: Tetra, Siokra V15, Delta Opal, CA 223 and Sicala were used in the experiment. For each cultivar, five fungicide-coated seeds were placed in each 9-cm diameter Petri dish lined with a single layer of Whatman No. 1 filter paper. A volume of 5 ml of the 100 % or the 50 % silverleaf nightshade solution was then added into each Petri dish. Distilled water was used as the control treatment. There were five replications for each treatment. The Petri dishes were wrapped with parafilm® and kept in a germination chamber in the dark at 25^{O}C. Germination percentage, shoot and radicle length were determined after five days. Only seedlings with a radicle length of more than 2 mm were considered to have germinated successfully.

Paper roll technique

Four cultivars, namely: Tetra, Sicala, Siokra V15 and Delta Opal were used in the experiment. A volume of 100 ml of the 100 % or of the 50 % solution was used to wet a double layer of germination paper. For each cultivar forty seeds were arranged in two rows of 20 seeds each on the germination paper. The seeds were placed with the radicle end of the seed pointing downwards so as to allow the radicle to grow straight down. Another double layer of germination paper was carefully placed on top of the seeds. The germination papers were then rolled up lightly and placed into transparent plastic bags to avoid

excessive evaporation of water. Only one roll was placed inside each plastic bag. The plastic bags were tied lightly with a rubber band at the top leaving enough space for gas exchange. Distilled water was used as control treatment. There were four replications for each treatment. This gave a total of 1920 seeds. The germination paper rolls were placed upright in the germination chamber, in the dark at 25^{O}C. Germination percentage, shoot and radicle length were determined after five days. Only seeds with a radicle length of more than 2 mm were considered to have germinated successfully.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the statistical program SAS®. A completely randomized design was used for all experiments. Analysis of variance was used to test for significant differences between treatment means expressed as percentage of the control. The error components for data of all parameters of cotton exposed to PEG-6000 solutions were normally distributed. For seeds exposed to the silverleaf nightshade solutions in Petri dishes, the arcsine (angular) transformation was performed on data for the shoot length parameter, in order to meet the requirements for standard analysis procedures. Data for the parameters germination and radicle length were acceptably normally distributed. For seeds exposed to the silverleaf nightshade solutions in paper rolls, the arcsine transformation was performed for the shoot and radicle length parameters. Data for germination percentage were acceptably normally distributed. Treatment means were separated using Tukey's studentised range for testing least significant differences at the 5 % level of significance.

2.3 Results and discussion

Tests for osmotic inhibition

From a practical viewpoint, for all the parameters measured, those osmotic potentials that caused a reduction (inhibition) of 20 % or less from the control treatment were regarded as having had negligible effect on the growth and development of cotton. Focus will only be on the PEG-6000 solutions with osmolalities of 24 and 55 mOsm kg^{-1} as these were the osmolalities closest or

similar to osmolalities of the 50 % (29 mOsm kg^{-1}) and the 100 % (55 mOsm kg^{-1}) silverleaf nightshade solutions, respectively, which were tested in subsequent experiments.

Germination of cultivar Tetra was significantly inhibited beyond the set threshold of 20 % reduction from the control only at 55 mOsm kg^{-1} (Table 2.1). Germination of cultivars Delta Opal and Sicala was already inhibited beyond 20 % at 24 mOsm kg^{-1}. Germination of cultivars CA 223 and Siokra V15, however, was not affected significantly at all osmolalities of the PEG-6000 solutions used.

Table 2.1 The effect of increasing osmolality of PEG-6000 solution on germination of cotton cultivars (Data expressed as percentage of control) (ANOVA in Table A1, Appendix A)

	Osmolality of PEG-6000 solution (mOsm kg^{-1})				
Cultivar	0	3	8	24	55
Tetra	100 ab	102.9 ab	128.6 a	105.7 a	74.3 ab
Delta Opal	100 ab	89.7 ab	106.9 ab	44.8 b	41.3 b
CA 223	100 ab	141.7 a	133.3 a	125 a	95.8 ab
Siokra V15	100 ab	148 a	140 a	112 ab	84 ab
Sicala	100 ab	110.3 ab	80 ab	58.6 b	34.5 b

Means in the table followed by the same letter are not significantly different according to Tukey's studentised range LSD ($P< 0.05$)

Shoot length was the most sensitive parameter to osmolality amongst the parameters tested (Table 2.2). Shoot growth of cultivars Tetra, Delta Opal, Siokra V15 and Sicala was already inhibited beyond 20 % from the control at 24 mOsmkg^{-1}. For CA 223, shoot length was inhibited beyond the set threshold of 20 % only at 55 mOsm kg^{-1}

Table 2.2 The effect of increasing osmolality of PEG-6000 solution on shoot length of cotton cultivars (Data expressed as percentage of control) (ANOVA in Table A2, Appendix A)

	Osmolality of PEG-6000 solution (mOsm kg^{-1})				
Cultivar	0	3	8	24	55
Tetra	100 a	80 a	80.9 a	58.4 ab	62 ab
Delta Opal	100 a	82.9 a	72.9 a	55.3 ab	41.3 b
CA 223	100 a	110.9 a	100.3 a	97.9 a	65.1 ab
Siokra V15	100 a	92.7 a	59.1 ab	63.2 ab	73.7 ab
Sicala	100 a	95.1 a	101.5 a	67.4 ab	20.3 b

Means in the table followed by the same letter are not significantly different according to Tukey's studentised range LSD ($P<0.05$)

Radicle growth was the least responsive parameter to osmolality changes compared to the germination and the shoot length parameters (Table 2.3). Radicle growth of cultivars Tetra, CA 223 and Siokra V15 showed no significant response to the PEG-6000 solutions at all osmolalities. For cultivars Delta Opal and Sicala, radicle growth was appreciably inhibited, although not significantly, at 55 mOsm kg^{-1}, the highest osmolality tested. Based on germination, shoot and radicle length responses at both the 24 and 55 mOsm kg^{-1} levels, Delta Opal and Sicala appeared to be the most sensitive to osmotic effects, and CA 223 the most tolerant.

Table 2.3 The effect of increasing osmolality of PEG-6000 solution on radicle length of cotton cultivars (Data expressed as percentage of control) (ANOVA in Table A3, Appendix A)

Cultivar	Osmolality of PEG-6000 solution (mOsm kg^{-1})				
	0	3	8	24	55
Tetra	100 ab	103.5 ab	102.4 ab	97.6 ab	88.9 ab
Delta Opal	100 ab	72.3 b	78.9 ab	82.6 ab	72.8 ab
CA 223	100 ab	137.9 a	133.7 a	151.9 a	88.2 ab
Siokra V15	100 ab	122.1 a	88.2 ab	82.9 ab	149.1a
Sicala	100 ab	82.0 ab	85.7ab	84.3 ab	57.0 b

Means in the table followed by the same letter are not significantly different according to Tukey's studentised range LSD ($P<0.05$)

Allelopathic potential of silverleaf nightshade solutions

Petri dish technique

The main effect of cultivar was significant for parameters shoot length and radicle length. The main effect of solution concentration was significant for all three parameters tested. Except for CA 223, no inferences can be made using the data for the shoot length parameter, since the osmolalities of the test solutions exceeded 24 mOsm kg^{-1} that was found to be inhibitive (>20 % reduction from the control treatment) towards shoot growth for the other cultivars (Table 2.2). In order to avoid possible confounding osmotic effects, thereby ensuring that only allelopathic effects are measured, only the data for the radicle length parameter will be considered for making inferences about the allelopathic potential of the infusions.

Data in Table 2.4 show a greater magnitude of inhibition of radicle length for the cultivars Tetra, CA 223, Siokra V15 and Sicala when they were exposed to the silverleaf nightshade solutions (29 and 55 mOsm kg^{-1}) as compared to the inhibition that was observed when they were exposed to similar osmotic potentials (24 and 55 mOsm kg^{-1}) of the PEG-6000 solutions (Table 2.3). Because osmolalities of the weed solutions were similar to osmolalities of the

PEG-6000 solutions this could mean that the inhibition caused by the weed solutions is a result of osmotic inhibition plus an additional inhibitory factor present in the silverleaf nightshade solutions. It is proposed that this additional factor represents allelochemicals. Radicle growth of cultivar Delta Opal was relatively tolerant to the silverleaf nightshade solutions, as there was no real difference in response irrespective of whether it was exposed to the two extract solutions (Table 2.4) or the two PEG-6000 solutions of 24 and 55 mOsm kg^{-1} (Table 2.3). In this experiment, except for Delta Opal, the growth reductions from the controls were substantially greater than those observed in the experiment for assessing sensitivity to osmotic effects. However in the case of Sicala it is risky to infer that the observed inhibition of radicle growth (Table 2.4) is a result of allelopathic interference, because at 55 mOsm kg^{-1} PEG-6000 solution this cultivar was more strongly affected than all other cultivars (Table 2.3).

Table 2.4 Shoot and radicle length of cotton cultivars exposed to silverleaf nightshade solutions in Petri dishes (Data averaged across solution concentration and expressed as percentage of the control) (ANOVA in Tables A4 and A5, Appendix A)

Cultivar	Shoot length (%)	Radicle length (%)
Tetra	51.4 ab	62.6 a
Delta Opal	70.5 a	73.6 a
CA 223	64.1 ab	64.7 a
Siokra V15	66.5 a	70.9 a
Sicala	45.7 b	41.1 b

Means in the same column followed by the same letter are not significantly different according to Tukey's studentised range LSD ($P<0.05$)

For all cultivars, germination, shoot length and radicle length of the seedlings were significantly inhibited by an increase in the concentration of extract solutions (Table 2.5). Therefore, the inhibitory effect of the solutions was

concentration-dependent. However, the osmolalities of both test solutions exceeded the value of 24 mOsm kg^{-1} that was found to be inhibitive towards shoot length and also germination of some cultivars. Therefore, it was considered prudent not to make inferences concerning the effect of solution concentration based on the data for germination or shoot length. However, in the case of radicle length, which was found to be relatively tolerant to osmotic inhibition, allelopathic activity increased significantly as the solution concentration increased. Shoots and radicles of the seedlings in both the 50 and 100 % solution concentrations showed a brown discoloration of tissues. The browning was not observed in seedlings of the control treatment. Yellowing of leaflets on most of the seedlings in the 100 % solution was also observed.

Table 2.5 The effect of silverleaf nightshade extract solution concentration on the germination, shoot and radicle length of cotton in Petri dishes (Data averaged across cultivars and expressed as percentage of the control) (ANOVA in Tables A4, A5 and A6, Appendix A)

Solution concentration (%)	Osmolality (mOsm kg^{-1})	Germination (%)	Shoot length (%)	Radicle length (%)
0	0	100 a	100 a	100 a
50	29	51.8 b	45.7 b	57.9 b
100	55	24.2 c	25.3 c	32.8 c

Means in the same column followed by the same letter are not significantly different according to Tukey's studentised range LSD ($P<0.05$)

Paper roll technique

The main effects of cultivar and solution concentration were significant for the parameters germination and radicle length. As in the case of the Petri dish technique, however, it was considered wise to restrict inferences on allelopathic effects to those for the radicle length parameter, since it proved to be relatively tolerant to osmolality.

Similar to the Petri dish experiment results, inhibition of cultivars Siokra V15, Tetra and Sicala was greater when solution extracts of the weed were used than when the PEG-6000 solutions of similar osmolality were used. As was the case in the Petri dish experiment, Delta Opal was relatively less affected by solution extracts. Data in Table 2.6 show that cultivars Delta Opal and Siokra V15, with a reduction from their respective controls of 13.3% and 11.0%, respectively, were significantly more tolerant to the solutions than cultivars Sicala and Tetra with reductions of 27.5 % and 25.1 %, respectively.

Table 2.6 Germination and radicle length of cotton cultivars exposed to silverleaf nightshade solutions in germination paper rolls (Data averaged across solution concentrations and expressed as percentage of the control) (ANOVA in Tables A7 and A8, Appendix A)

Cultivar	Germination (%)	Radicle length (%)
Siokra V15	93.6 b	89.0 a
Delta Opal	98.3 ab	86.7 a
Sicala	98.9 a	72.5 b
Tetra	101.7 a	74.9 b

Means in the same column followed by the same letter are not significantly different according to Tukey's studentised range LSD ($P<0.05$)

Considering the effect of solution concentration, results show radicle length inhibition increased significantly with an increase in solution concentration (Table 2.7). Radicle growth was significantly inhibited already at 50 % concentration. This result confirmed that of the Petri dish experiment, even though inhibitory effects were generally lower in the paper roll experiment.

Table 2.7 The effect of silverleaf nightshade extract solution concentration on the germination and radicle length of cotton in germination paper rolls (Data averaged across cultivars and expressed as percentage of control) (ANOVA in Table A7 and A8, Appendix A)

Solution conc. (%)	Osmolality (mOsm kg^{-1})	Germination (%)	Radicle length (%)
0	0	100 a	100 a
50	29	95.7 b	83.29 b
100	55	98.7 ab	59.06 c

Means in the same column followed by the same letter are not significantly different according to Tukey's studentised range LSD ($P<0.05$)

2.4 Conclusions

Results obtained from both types of bioassays used in this study showed inhibition of early growth of cotton exposed to silverleaf nightshade extract solutions. Osmotic inhibition could be excluded as a possible cause of the inhibition in most cases for the radicle length parameter. This leaves allelopathic interference as the probable cause of the significant reductions observed in radicle growth. However in the case of germination and shoot length, the inhibition observed in the presence of weed extract solutions could not conclusively be associated with allelopathy because these parameters were significantly sensitive to osmotic potential for some cultivars. Therefore, for the germination and shoot length parameters we contend that it would not be possible to separate the effects of allelopathy from osmotic interference. In both bioassays the radicle growth of cultivars Sicala and Tetra appeared to be the most sensitive to the silverleaf nightshade extracts, and cultivars Delta Opal and Siokra V15 more tolerant. This differential tolerance of cultivars, however, could not be conclusively confirmed due to sensitivity of radicle length of cultivar Sicala to osmotic potential at 55 mOsm kg^{-1}, which was equivalent to that of the 100 % weed extract solution. Exclusion of the evaluation of sensitivity of parameters to osmotic potential in these

experiments therefore would have led to incorrect conclusions that the inhibition was as a result of allelopathic inhibition.

Generally, inhibition observed in the paper roll technique was not as obvious as in the Petri dish technique. Similar findings were reported by Bothma (2002). The moisture differences in the two bioassays could be the reason for the inconsistency of results obtained from the two types of bioassays. In the paper roll experiment, the seeds absorbed water from the moist paper rolls, whilst in the Petri dish experiment there was a greater amount of free water available. Another possible cause for the inconsistency may be dispersion or even adsorption of solutes onto the germination paper because it had a larger surface area as compared to the filter paper. The result of adsorption would have caused the allelochemical solutes to occur in lower concentrations in the immediately available solution, and therefore, would be less available at a given point in time for uptake by the seeds in the germination paper bioassay. Clearly, if based purely on the question of which technique was most likely to yield significant growth responses, the Petri dish bioassay gave the expected results. If the intention would have been to compare the effectiveness of the two techniques, which was not the case here, special care would have had to be taken to ensure equal solute concentrations and equal solute uptake potential in the two assays.

Findings of the present study, combined with the information reported by Bothma (2002) of isolation of allelochemicals from silverleaf nightshade foliage, provide strong evidence to suggest that silverleaf nightshade has the potential for allelopathic interference. The differences observed in results obtained from the two bioassay types in this study confirmed the relevance of the use of a series of bioassay types in allelopathy research. From these results, however, it cannot be concluded that the same findings could be expected in natural field conditions. For practical relevance of these results, therefore, the investigations need to be advanced further, towards evaluating the effect of soil, because in nature interactions between plants occur in soil. In the following chapter soil will be used as substrate for growing cotton in the presence of silverleaf nightshade extract solutions.

CHAPTER 3
INFLUENCE OF SOIL ON THE ALLELOPATHIC EFFECTS OF SILVERLEAF NIGHTSHADE EXTRACT SOLUTIONS ON COTTON

3.1 Introduction

For allelochemicals to elicit allelopathy they need to be released from the producing plant and transferred to the receiving plant in sufficient concentrations to trigger an effect at the sensitive site of the receiving plant (Muller, 1974). Obviously, any factor that affects the quantity or quality of allelochemical(s) that reach the receiving plant will have an influence on the potency of the allelochemical(s). According to Lovett (1982), a large fraction of the allelochemicals released from the producing plant reaches the soil and are taken up by other plants from the soil. When allelochemicals reach the soil they become exposed to and interact with various biotic and physicochemical soil components. It is the resultant effect of these interactions that determines the fate of allelochemicals in the soil. Soil is a living biological system providing habitat for micro-organisms such as fungi, bacteria, actinomycetes, algae and protozoa (Wild, 1996) which play a profound role in the expression of allelopathy due to their ability to modify its effects (Blum *et al.,* 1987). Micro-organisms can influence allelopathy directly by affecting the amounts of allelochemicals in the soil solution through their ability to influence release of chemical compounds bound to soil particles, or indirectly, by affecting the availability of soil nutrients for uptake by plants. The influence of soil micro-organisms on allelopathy through transformation of allelochemicals will be dealt with in depth in the next chapter.

Apart from the interactions with micro-organisms, allelochemicals may become bound (adsorbed) to soil colloids such as organic matter and clay minerals (Inderjit & Dakshini, 1995). According to Fisher & Adrian (1981) soil colloids are capable of adsorbing most allelochemicals. The result of adsorption of allelochemicals is reduced concentration of the immediately

available allelochemical in the soil solution. When the reaction resulting in adsorption is a simple reaction with the humic acids, part or all of the chemical will purportedly become available for uptake by a receiver plant after desorption (Blum *et al.,* 1987). However, if the allelochemical is adsorbed onto humic acids, it may get precipitated and will then be permanently inactivated. These dynamics in turn will have an influence on allelopathy by affecting the amount of allelochemical(s) available for uptake by plants.

Because in nature plants receive allelochemicals mainly from the soil (Waller & Einhellig, 1999) it means logically for allelopathy to receive due recognition in plant ecosystems it must be proved to occur in the soil environment (Reinhardt *et al.,* 1999). Confirmation of allelopathy in laboratory soil-less media is therefore not sufficient to assume that the phenomenon will occur under similar conditions for plants growing in the soil (Reinhardt *et al*., 1999; Inderjit, 2001). Numerous authors and critics of allelopathy have pointed out that very little attention is paid to soil ecology in laboratory and field experiments in allelopathy research. In fact, many studies on allelopathy do not involve soil (Blum *et al.,* 1987; Inderjit & Dakshini, 1995). The uncertainties and criticism surrounding the phenomenon of allelopathy will continue to exist until a holistic and objective approach is used in allelopathy research and that investigations are carried out in environments closely resembling those that can be expected in nature (Reinhardt *et al*., 1999).

In the previous chapter inhibition of cotton by silverleaf nightshade extracts during its early development was observed in a soil-less environment. The aim of the present study, which was also conducted under controlled conditions in the laboratory, was to investigate whether the presence of soil will have an influence on the phytotoxicity of allelochemicals contained in silverleaf nightshade, and also to investigate the effect of the presence of microbes.

3.2 Materials and methods

Experiment 1: Allelopathic effect of extract solutions added to a natural soil or an inert quartz sand

Preparation and sterilization of the test solution

Silverleaf nightshade plants were picked from the field at the University of Pretoria experimental farm when they were at the vegetative stage of growth. Immediately after collection, the silverleaf nightshade plants were frozen, until they were needed for preparation of the extract solutions. A silverleaf nightshade solution was prepared by soaking 100 g of the frozen silverleaf nightshade leaves in 1 L of distilled water at room temperature in the dark for 24 hours. The solution was sieved through cheesecloth to remove the solid particles and then passed through a single layer of Whatman No. 1 filter paper using a vacuum pump. The solution was then centrifuged at a speed of 4200 rpm for ten minutes and sterilized by passing it through 0.2 μm pore size Whatman Puradisc polyethersulfone membrane millipore filters. This solution represented the 100 % test solution. A 50 % solution was prepared by mixing one part of the stock solution with one part of sterilized distilled water. Osmolalities of the solutions were then measured using a Roebling digital micro-osmometer.

Sterilization of growth media, seeds and apparatus

The soil used in the experiment was collected from the University of Pretoria experimental farm. Properties of the soil used in this experiment are provided in Appendix B. Prior to preparation of bioassays, soil was passed through a 2-mm sieve. The soil was then autoclaved at 121^{O}C for 90 minutes in transparent glass jars of about 6 cm diameter, which were covered with aluminium foil, and left to cool to room temperature in a laminar flow cabinet. The other soil treatment, pure quartz sand, was washed under running tap water in a mesh sieve, then oven-dried to constant mass before it was autoclaved and allowed to cool in a similar manner as described above for the natural soil. Distilled water was autoclaved at 121^{O}C for 30 minutes. Petri dishes and filter paper, sealed and covered with aluminium foil were

autoclaved for 30 minutes at 121^{O}C. Fungicide-coated seeds of cotton variety Sicala were surface-sterilized by soaking in a 10 % sodium hypochlorite commercial bleach solution for ten minutes. The seeds were then rinsed three times with sterilized distilled water and air-dried under a laminar flow cabinet.

Preparation of bioassays

All experiments were prepared in a laminar flow cabinet where all surfaces were cleaned with a 70 % ethanol solution. All metallic instruments used were sprayed with the ethanol solution and flamed before use. In each 9-cm diameter Petri dish, a substrate was prepared by introducing the silverleaf nightshade solution onto a thin layer of sand or soil. The substrate was prepared in such a way that it was neither too wet nor too dry for seed germination, and also so that an equal volume of the solution could be used for both types of media. Previous experiments had shown that germination of cotton seed is very sensitive to available moisture and temperature levels. After trying different solution volumes and growing media mass, a satisfactory substrate was obtained when 18 ml of the sterilized test solution was added into each 9-cm diameter Petri dish containing either 44 g of sterilized soil or 60 g of sterilized quartz sand. Ten surface-sterilized, fungicide-coated cotton seeds of cv Sicala were embedded on the substrate in each Petri dish. Sterilized distilled water was used as the control treatment and there were ten replications for each treatment. This totalled 600 seeds. The Petri dishes were wrapped with Parafilm® to avoid contamination of the bioassays, and incubated in a germination chamber in the dark at 25^{O}C for five days.

Experiment 2: Allelopathic effect of leaf material incorporated into a natural soil or an inert quartz sand

Silverleaf nightshade plants were collected from the field at the University of Pretoria experimental farm when they were at the vegetative stage. The plants were then air-dried in full light for two weeks inside a glasshouse before the foliage was crushed with a mortar and pestle into a coarse, powdery consistency. A mixture of leaf material and quartz sand (pre-washed and oven-dried) or soil (sieved through a 2-mm mesh sieve) was prepared by mixing weed material with growing medium to a concentration of 1 % (m/m) in

each 9-cm diameter Petri dish. Ten fungicide-coated cotton seeds were then embedded on the mixture in each Petri dish. A volume of 22 ml of distilled water was then added to each Petri dish. No plant material was used for the control treatments and there were ten replications for each treatment. This totalled 600 seeds. Petri dishes were incubated in the dark in a growth chamber at 25^{O}C for five days. Germination percentage, shoot and radicle length measurements were taken five days after planting. Only seedlings with a radicle length of more than 2 mm were considered successfully germinated.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the statistical program SAS®. A completely randomized design was used for all experiments. Analysis of variance was used to test for significant differences between treatment means expressed as percentage of the control. For the experiment where seeds were exposed to extracts, error components of data for the germination parameter were normally distributed. The arcsine (angular) transformation was performed on data for the shoot length and radicle length parameters, in order to meet the requirements for standard analysis procedures. Arcsine transformation was performed for the germination and shoot length parameters in the experiment where plant material was used. Treatment means were separated using Tukey's studentised range for testing least significant differences at the 5 % level of significance.

3.3 Results and discussion

Experiment 1: Allelopathic effect of extract solutions added to either a natural soil or an inert quartz sand

For both the germination and the shoot length parameters, the interaction for growth medium X solution concentration was significant. In the case of radicle length, the main effects of solution concentration and growth medium were significant.

Germination of Sicala was significantly inhibited by both the 50 and 100 % test solutions in both the quartz sand and the soil media (Figure 3.1). However, the germination inhibition effect of the 100 % test solution was significantly greater in the quartz sand than in the natural soil.

After centrifugation of the silverleaf nightshade extract solutions, osmolalities of the 50 % and the 100 % solutions were measured to be 24 mOsm kg^{-1} and 52 mOsm kg^{-1}, respectively. The lower osmolalities of the extract solutions observed after centrifugation than before the process (Chapter 2) were probably because some solutes were removed by centrifugation. In the previous chapter (Table 2.1), it was observed that germination of Sicala is already inhibited by a solution with an osmolality of 24 mOsm kg^{-1}. Therefore, because of the possible clouding effect of osmotic inhibition it is considered prudent not to accept that inhibition of germination caused by the silverleaf nightshade solutions in the present experiment was a result of solely allelopathy. We contend that omission of tests for osmotic inhibition in this experiment would have led to misleading and incorrect inferences being made regarding the germination response of cv Sicala to extract solutions of silverleaf nightshade.

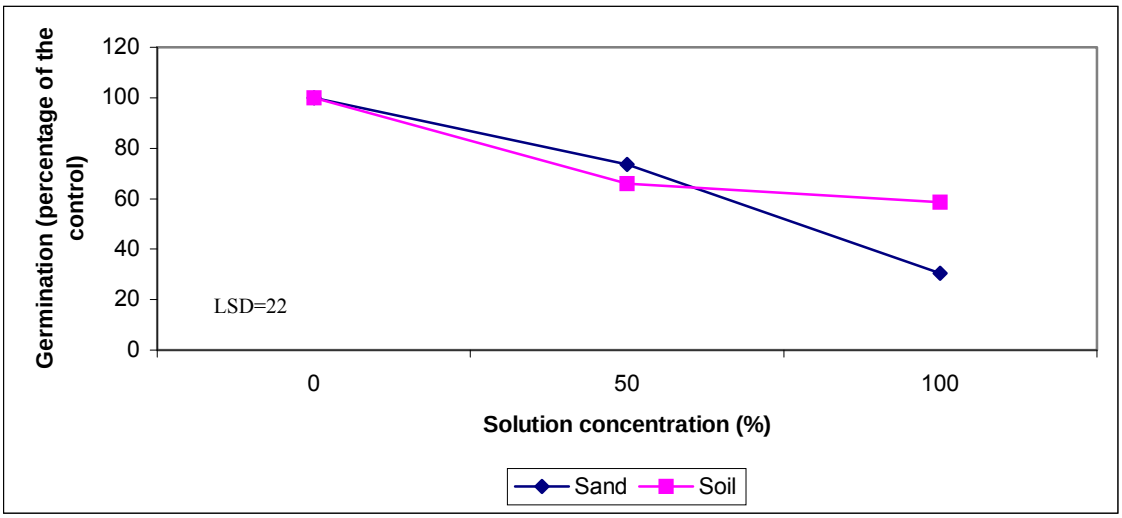

Fig 3.1 Influence of silverleaf nightshade extract concentration on germination of cv Sicala when quartz sand or soil were used as growth medium (ANOVA in Table A9, Appendix A)

The response of shoot length to extract solutions was also concentration-dependent. The tendency was increased inhibition as the solution concentration increased (Fig 3.2). In contrast to the germination response, inhibition of shoot length observed at 50 and 100 % solution concentrations were similar in the quartz sand medium. For shoot length, in the natural soil, inhibition caused by the 100 % solution was significantly greater than that observed at 50 % concentration. Inhibition differences observed for natural soil or quartz sand media were not significantly different at a particular concentration. As in the case of germination, however, due to the possibility of osmotic inhibition, inhibitory effects observed on shoot length can not be attributed solely to allelochemicals in the test solutions. However, inhibition of shoot length observed in the current experiment at the 50 % solution was of greater magnitude than that observed in earlier experiments (Table 2.2) where PEG-6000 solutions of similar osmotic potential were used. The additional inhibitory effect observed in the present experiment is suspected to be a result of allelochemicals. No inferences could be made using data for the 100 % solution due to the possible confounding effect of osmolality at this concentration.

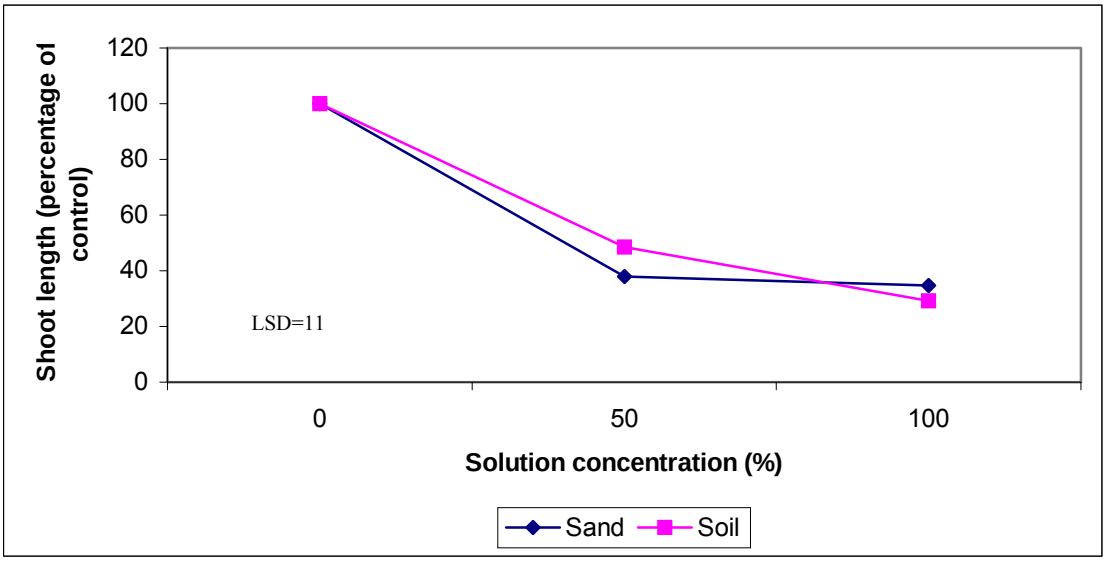

Fig 3.2 Influence of silverleaf nightshade extract concentration on shoot length of cv Sicala when sand or soil were used as growth medium (ANOVA in Table A 10, Appendix A)

In the previous chapter, for the radicle length parameter, osmotic potential was excluded as a possible clouding effect at the 50 % concentration, and hence, inferences made with regard to the allelopathic potential of the extract solution of similar osmotic potential would be regarded as reliable. In the present experiment, radicle length was significantly reduced in the quartz sand (53.1 %) compared to the soil medium (48.4 %) (Table 3.1). In practical terms, however, this difference is arguably not noteworthy. In this experiment therefore, the influence of natural soil on allelopathic potential was variable and not conclusive. The differential response of the different parameters to extract solutions in the quartz sand and natural soil media can not be explained at this stage. Obviously, findings could have been different for another soil, or for the same soil if it had not been sterilized. The presence of micro-organisms could have a strong influence on the dissipation rate of allelochemicals (Blum *et al.*, 1987; Cheng, 1995).

Table 3.1 The effect of growth medium on radicle length of cv Sicala exposed to silverleaf nightshade extract solutions added to a thin layer of soil or sand (Data averaged across solution concentration and expressed as percentage of control) (ANOVA in Table A11, Appendix A)

Growing medium	Radicle length (%)
Sand	46.9 c
Soil	51.6 b
Control	100 a

Means in the same column followed by the same letter are not significantly different according to Tukey's studentised range LSD (P<0.05)

In the case of the solution concentration main effect, radicle length response was similar to that of germination and shoot length in that inhibition increased with increasing concentration (Table 3.2). Significant inhibition of 69.9 % and 82.3 % was observed from the 50 % and 100 % solution, respectively. Unlike germination and shoot length, the possibility of osmotic inhibition as a cause of the observed radicle length inhibition can be excluded for the 50 % solution

concentration because the radicle length of Sicala was not significantly inhibited at a similar osmolality of the PEG-6000 solution (Chapter 2). As in the case of shoot length, a smaller magnitude (57%) of inhibition was observed for radicle growth in a PEG-6000 solution with a similar osmolality as the 100% extract solution. The greater magnitude (82.3%) observed in this experiment in the 100% extract solution is probably due to an additional, negative allelopathic effect.

Table 3.2 The effect of silverleaf nightshade extract solutions on radicle length of cv Sicala when grown on either sand or soil medium in Petri dishes (Data averaged across media, and expressed as percentage of control) (ANOVA in Table A11, Appendix A)

Infusion concentration	Radicle length (%)
0	100 a
50	30.1 b
100	17.7 c

Means in the same column followed by the same letter are not significantly different according to Tukey's studentised range LSD (P<0.05)

Due to the variable and inconclusive effect of natural soil, as well as the unexplained variable response of parameters in this experiment, it was deemed necessary to conduct a similar experiment but using weed debris instead of weed extracts. In the following experiment plant material will be incorporated into soil or quartz sand in Petri dishes. The environment in the Petri dishes would thus be more representative of that into which allelochemicals are released in nature. Such an approach will exclude possible clouding of results by osmotic potential, and will include the influence of soil micro-organisms.

Experiment 2: Allelopathic effect of plant material incorporated into a natural soil or quartz sand

For the parameters germination and radicle length, only the main effect of plant material was significant. The interaction growth medium X plant material was significant in the case of shoot length.

Germination and radicle length were both inhibited significantly in the media containing silverleaf nightshade material compared to the control treatment where no plant material was added (Fig 3.3). Germination and radicle length were inhibited 62.5 % and 53.14 %, respectively, from the respective controls.

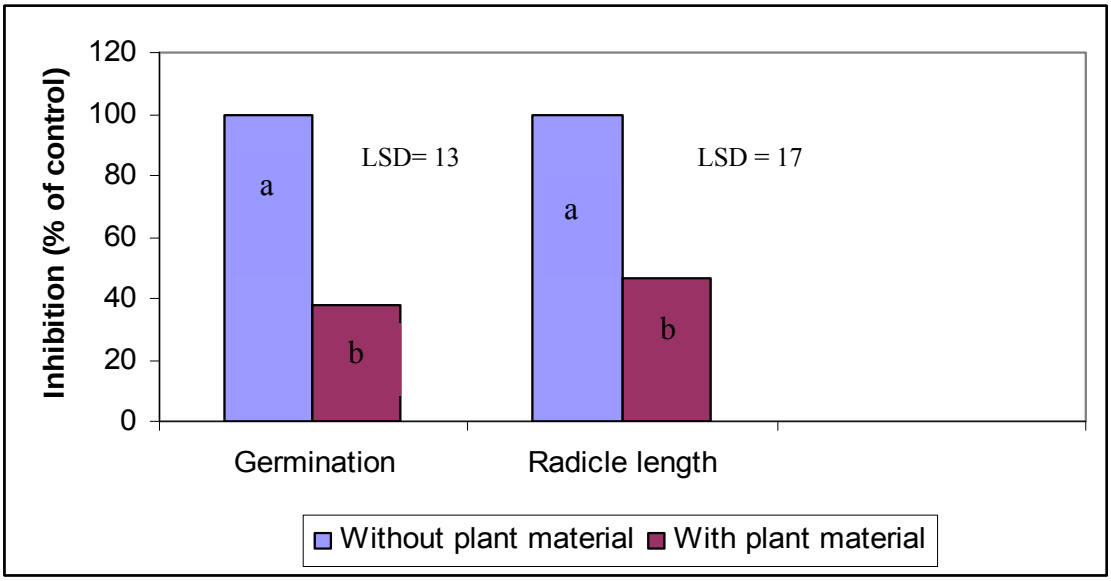

Fig 3.3 Germination and radicle length of cv Sicala grown on soil or quartz sand mixed with fresh silverleaf nightshade leaf material (Data averaged across media and expressed as percentage of control) (ANOVA in Tables A12 and A14, Appendix A)

In the case of shoot length, inhibition was higher in the quartz sand, with a significantly higher reduction of 61.3 % compared to 20.8 % in the soil medium (Table 3.3). In this experiment the difference in allelopathic activity between sand and soil was distinct, possibly because the microbial component of the natural soil was not neutralized through sterilization, as was

the case in the previous experiment. Furthermore, higher growth inhibition in the quartz sand can be attributed to the expected inherently low to zero microbial activity in it, which would have been conducive to maximum biological activity of allelochemicals present. Another possible cause of the observed higher growth inhibition in quartz sand than in natural soil could be the adsorption of allelochemicals in the soil, but not in the inert sand. Because quartz has no capacity for adsorbing allelochemicals the result would be higher availability of allelochemicals in this medium for absorption by seeds and seedlings.

Table 3.3 The effect of growth medium and silverleaf nightshade leaf material on the shoot length of the cotton cultivar Sicala (Data expressed as percentage of control)

Growth medium	Growth medium + plant material
Sand	38.7 c
Soil	79.2 b
Control	100 a

Means in the same column followed by the same letter are not significantly different according to Tukey's studentised range LSD ($P<0.05$) (ANOVA in Table A13, Appendix A)

3.4 Conclusions

In this study the allelopathic (phytotoxic) effects of silverleaf nightshade leaf extracts and material was investigated by using soil as growth medium, and then in the absence (Exp 1) as well as in the presence (Exp 2) of micro-organisms. Results showed significant inhibition of early growth and development of cotton by silverleaf nightshade solution extracts in sterile conditions, as well as by its residues in the presence of micro-organisms. These findings suggest that the response of various growth parameters of

cotton to the presence of allelochemicals was not affected by the microbial status of the growth medium.

It should be noted that results probably would have been different if another soil type was used. This is because the reactions that an allelochemical undergoes in the soil are largely controlled by edaphic factors such as moisture, nutrient status and organic matter content (Fisher & Adrian, 1981). For example, the nature and amount of soil organic matter determine whether simple adsorption or complexing by humic substances takes place. Soil moisture determines whether aerobic or anaerobic decomposition takes place, which in turn determines the nature of decomposition products. Soil nutrient status and soil temperature determine the rate of microbial activity. Microbial degradation is also controlled by the spectrum and activity of micro-organisms present in the soil. The roles of various factors in the fate of an allelochemical causes its effects to be variable in different soils, and even in a particular soil under varying environmental conditions.

It has been argued that aseptic, laboratory bioassays in allelopathy research represent an environment that is different from natural conditions, and hence, findings may not reflect well allelopathic effects occurring in nature (Putnam, 1985). However, this fact should no detract from the value of laboratory bioassays for investigating particular mechanisms and the influence that various environmental factors has on them. Even though results of the present study can be expected to differ under different soil conditions, the fact that inhibition was observed in the presence of soil, could be an indication that similar effects can be expected under natural conditions. The role of decomposition of plant material on its allelopathic potential is reported in the next chapter.

CHAPTER 4

EFFECT OF DECOMPOSITION OF SILVERLEAF NIGHTSHADE DEBRIS IN SOIL ON ITS ALLELOPATHIC EFFECTS ON COTTON

4.1 Introduction

Accumulation of allelochemicals at phytotoxic levels, coupled with their persistence and availability in the environment, are determining factors for the expression of allelopathic interference in plant-to-plant interactions (Inderjit, 2001). The volume and concentration of allelochemicals in the soil fluctuate due to structural alterations that phytochemicals undergo during the decomposition process (Rietveldt, 1983). The result of the structural changes of allelochemicals could be reduced phytotoxicity (Chou 1989a; Schmidt & Ley, 1999) or enhanced toxicity of the original chemical (Kaminsky, 1981; Novak, *et al*., 1995; Huang *et al.,* 1999; Inderjit, 2001; Schmidt & Ley, 1999). The latter scenario occurs when the toxic substances responsible for allelopathy are products of degradation of the original phytochemical released from a plant (Rietveld, 1983; Inderjit, 2001). This means that the allelopathic potential of plant residues of the same plant may differ at different stages of decomposition in the soil. Amongst other examples, where this has been observed, is the oxidation of hydrojuglone to juglone, a highly potent quinone that is inhibitory to some species (Rietveld, 1983).

With these different possible effects of transfer and transformation processes on allelochemicals in soil it is unlikely that the same compounds extracted or released from plants are those that reach the receiving plant. Moreover, apart from the direct allelopathic inhibition caused by allelochemicals on plants, there is also a possibility of indirect inhibition of the receiving plant through effects on essential relationships with other sensitive organisms in the soil (Reinhardt *et al*., 1999). Reinhardt *et al*. (1999), for example, provided strong evidence to suggest that the inhibition of pine *(Pinus patula)* seedling growth by the weed *Cyperus esculentus* is a secondary effect as a result of primary inhibition of essential ectomycorrhizae by allelochemicals released from the

weed. It is impossible to evaluate the influence of such relationships when soil is not used as a substrate in allelopathy studies.

Amongst growth media and substrates used in allelopathy bioassays, soil is the most difficult medium to work with in allelopathy research (Reinhardt *et al.,* 1999). However, although this may be the case, the exclusion of soil in allelopathy bioassays will only contribute to uncertainties and criticism associated with the allelopathy phenomenon. Amongst challenges involved in soil allelopathy bioassays, especially where plant material (organic matter) is incorporated into the soil, is the possibility of nitrogen immobilization due to a high demand of nitrogen by microbial decomposers when the C:N ratio of decomposing material is higher than that of the microbes (Handreck & Black, 1994; Inderjit & Dakshini, 1994; Reinhardt *et al*., 1999). Nitrogen immobilization would result in a reduced amount of nitrogen being available for plants, thereby resulting in confusing nutrient deficiency with allelopathy. Addition of organic matter into soil may also result in depletion of phosphorus due to enhanced microbial activity. For reliability of bioassays, where plant material is incorporated into soil, therefore, measures should be taken to ensure provision of adequate nutrition in the growth medium (Reinhardt *et al*., 1999).

In previous chapters, allelopathic inhibition of cotton by silverleaf nightshade was observed when either extract solutions of silverleaf nightshade was added to soil-less growth media, or to soil in the presence or absence of micro-organisms, or when fresh, undecomposed silverleaf nightshade leaf material was incorporated in soil. The objective of the present experiment is to evaluate the effect of decomposition on the phytotoxicity emanating from silverleaf nightshade residues occurring in the soil.

4.2 Materials and methods

Silverleaf nightshade plants were collected from the field at the University of Pretoria experimental farm when they were at the fruiting stage, with the berries yellow and ripe. Immediately after collection, the silverleaf nightshade plants were stored in the freezer until they were needed for preparation of treatments in the pot experiment. The soil used is in this pot experiment was the same as that reported in Chapter 3.

The experiment was conducted in a glasshouse at the phytotron at the University of Pretoria experimental farm. Two cotton cultivars were used, namely: Delta Opal and Sicala. These two cultivars were chosen because results from the previous experiments suggested they differed in sensitivity, with Delta Opal apparently more tolerant than Sicala to the allelochemicals produced by silverleaf nightshade. Plastic pots of 26-cm diameter were used in the experiment. The plastic pots were lined with a single layer of plastic bag to prevent water and nutrients from leaching out of the soil. Treatments used in the experiment were 2 kg of natural soil mixed with either 20 g silverleaf nightshade leaves (cut into pieces of approximately 1 cm^2) or 20 g of ripe berries of silverleaf nightshade (cut into halves).

Soil was watered to field capacity, the mass of the pots was noted and the bags tied at the top to prevent water loss. Subsequently, the pots were incubated at 20/30ºC (12/12h) in the dark in order that the plant material could decompose for 0, 2, 4 or 6 weeks. During these incubation periods the pots were weighed and watered regularly to maintain moisture content at field capacity. Eight fungicide-coated cotton seeds of the cultivars Sicala or Delta Opal were planted at a depth of 5 cm in each pot after the respective periods of decomposition had elapsed. Soil with no plant material added was used as the control treatment. After sowing of seeds, the pots were again watered to field capacity, and were then weighed daily and watered accordingly to maintain water content at field capacity until harvesting. At each watering event a fixed volume of complete nutrient solution (e.g. 50, 100 or 200 ml) was first applied to all the pots, and then distilled water was used to make up

any water deficit in relation to the field capacity level. Properties of the nutrient solution are given in Appendix B.

There were four replications for each treatment. Seedlings were thinned to six seedlings per pot 10 DAS. Each treatment was harvested six weeks after planting. The entire duration of the experiment was from July to end of September 2002. Glasshouse temperature recordings during this period ranged from a minimum of 13^{O}C at night to a maximum of 34^{O}C during the day.

Parameters measured were emergence percentage, seedling height, leaf surface area, leaf fresh mass, leaf dry mass and root dry mass. Emergence percentage was recorded 8 DAS. Seedling height was recorded weekly until harvesting. Leaf surface area, fresh mass and dry mass were recorded at harvesting. Leaf surface area was measured using a scanning leaf area meter.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the statistical program SAS®. A completely randomized design was used for all experiments. Analysis of variance was used to test for significant differences between treatment means expressed as percentage of the control. The error components for data for all parameters were subjected to arcsine transformation in order to meet the requirements for standard analysis procedures. Treatment means were separated using Tukey's studentised range for testing least significant differences at the 5 % level of significance.

4.3 Results and discussion

The second-order interaction cultivar X incubation period X plant material was significant for leaf dry mass. The first-order interaction plant material X incubation period was significant for the parameters emergence, leaf surface area and leaf fresh mass. For the plant height parameter, the first-order interaction incubation period X cultivar was significant. The main effect of

cultivar was significant for the parameters emergence, leaf surface area and root dry mass. The main effect for plant material was significant for the parameter seedling height.

Leaf dry mass of both cotton cultivars showed the largest reductions where they were grown on soil containing silverleaf nightshade berries, in particular freshly incorporated material (Table 4.1). The cultivar Delta Opal was not affected by the incorporated leaf material irrespective of the decomposition period. Cultivar Sicala showed significant reductions in leaf dry mass in response to fresh (incubation 0) weed leaf material, and to the same material that had been incorporated for two weeks. Where the weed leaf material had been incorporated for periods of four and six weeks, the leaf dry mass of cv Sicala was not reduced significantly.

Table 4.1 Leaf dry mass of cotton seedlings exposed to fresh or decomposing residues of silverleaf nightshade leaves or berries (Data expressed as percentage of control) (ANOVA in Table A19, Appendix A)

	Cultivar			
	Sicala		Delta Opal	
Incubation period	Leaf material	Ripe Berries	Leaf material	Ripe Berries
0	51.6 b	19.4 c	96.8 a	48.4 b
2	50.0 b	26.5 b	98.2 a	35.9 b
4	90.7 a	66.7 b	123.5 a	48.3 b
6	80.0 a	45.6 b	90.8 a	83.6 a
Control	100 a	100 a	100 a	100 a

Means in the table followed by the sane letter are not significantly different according to Tukey's studentised range LSD ($P<0.05$)

As observed for seedling dry matter (Table 4.1), cotton emergence was inhibited more by the berry material of the weed than by its leaf material, and the effect was more pronounced the fresher the weed material was (Table

4.2). Some of the seedlings in soil ameliorated with the silverleaf nightshade berries turned yellow and died off after emergence. This injury symptom, however, was not observed on seedlings exposed to silverleaf nightshade leaves. Fresh leaf debris was less inhibitory and lost its influence more rapidly than the berries. The explanation for this can be that the amount of allelochemicals in, or produced from, the silverleaf nightshade berries was higher than that in the leaves. Alternatively, different allelochemicals could have been released from the two types of plant material. Progressive reduction of inhibitory effects with increased decomposition of the plant debris suggests that the biological activity of the allelochemicals involved was negatively affected by residue decomposition. This is an indication that in this case the biologically active allelochemicals involved were likely released directly from the silverleaf nightshade residues rather than being a product of microbial transformation in the soil. This confirmed results obtained in the previous chapters where the early growth of cotton was inhibited by fresh residues or extracts of silverleaf nightshade leaves.

Table 4.2 Emergence percentage of cotton exposed to soil ameliorated with fresh or decomposing silverleaf nightshade leaves or berries (Data averaged across cultivars and expressed as percentage of control) (ANOVA in Table A15, Appendix A)

	Type of plant material	
Incubation period (weeks)	Leaf material	Ripe berries
0	56.7 b	35.2 c
2	84.1 ab	65.5 b
4	86.4 ab	74.5 ab
6	101.9 a	71.1 ab
Control	100 a	100 a

Means in the same column followed by the same value are not significantly different according to Tukey's studentised range at LSD ($P< 0.05$)

For both cultivars, cotton seedlings in ameliorated soil were visibly shorter and smaller compared to seedlings in the unameliorated soil Moreover, the seedlings exposed to soil ameliorated with berries were visibly even shorter and smaller compared to those exposed to soil ameliorated with silverleaf nightshade leaves. These visual symptoms are reflected by data presented in Table 4.3. Leaf material of the weed had no significant effects on the leaf area of cotton (Table 4.3). Data in Table 4.3 show that leaf surface area of cotton seedlings exposed to silverleaf nightshade berries was significantly inhibited compared to the control treatment. These observations are in agreement with literature (Guerreiro *et al.*, 1971) reporting higher concentrations of allelochemicals in berries of silverleaf nightshade than in its foliage. The inhibitory effect of the weed residues, in particular the berry material, decreased as the decomposition period in the soil increased. The same effect was reported above for cotton emergence and leaf dry mass.

Similar to the parameter leaf dry mass (Table 4.1) and emergence (Table 4.2), seedling height was inhibited more where soil contained relatively fresh weed material (Table 4.4). This inhibitory effect was least pronounced at the maximum decomposition period. Delta Opal tended to be more tolerant than Sicala at the earliest (0 and 2 weeks) stages of weed material decomposition.

Table 4.3 Leaf surface area of cotton seedlings exposed to soil ameliorated with fresh or decomposing silverleaf nightshade leaves or berries (Data averaged across cultivars and expressed as percentage of the control) (ANOVA in Table A17, Appendix A)

Incubation period (weeks)	Type of plant material	
	Leaf material	Ripe berries
0	71.7 ab	32.2 c
2	88.3 ab	28.4 c
4	75.7 ab	35.1c
6	83.5 ab	71.0 ab
Control	100 a	100 a

Means in the same column followed by the same letter are not significantly different according to Tukey's studentised range LSD (P<0.05)

Table 4.4 Height of cotton seedlings exposed to fresh or decomposing silverleaf nightshade debris (Data averaged across type of plant residue and expressed as percentage of the control) (ANOVA in Table A16, Appendix A)

	Incubation period (weeks)			
Cultivar	0	2	4	6
Sicala	57.5 b	69.3 b	66.9 b	83.5ab
Delta Opal	79.1 b	89.4 ab	69.1 b	87.2 ab
Control	100 a	100 a	100 a	100 a

Means in the same column followed by the same letter are not significantly different according to Tukey's studentised range LSD (P<0.05)

Inhibition observed in leaf fresh mass (Table 4.5) followed the same trend as that observed in leaf dry mass, emergence, leaf surface area and seedling height. For all these parameters the inhibition was mostly significant when the seedlings were exposed to silverleaf nightshade berries, but then only at the earliest stages of decomposition, with a progressive reduction of the inhibitory effect as the duration of decomposition increased. Exceptions to these results

for the berry treatment, i.e. where weed leaf material caused significant growth reduction, were observed in Sicala leaf dry mass at 0 and 2 weeks incubation as well as in emergence at 0 weeks incubation.

Table 4.5 Fresh mass of cotton seedlings exposed to soil ameliorated with fresh or decomposing leaves or berries of silverleaf nightshade (Data averaged across cultivars and expressed as percentage of control) (ANOVA in Table A18, Appendix A)

Incubation period	Type of plant material	
(weeks)	Leaf material	Ripe berries
0	77.0 a	32.2 c
2	80.5 a	29.9 c
4	90.9 a	36.8 b
6	89.3 a	71.4 ab
Control	100 a	100 a

Means in the same column followed by the same letter are not significantly different according to Tukey's studentised range LSD ($P<0.05$)

In summary, cv Delta Opal generally tended to be more tolerant to the silverleaf nightshade residues than cv Sicala as regards the parameters emergence, leaf surface area, leaf fresh mass and root dry mass (Figure 4.1; Tables 4.1, 4.4). Inhibition observed on root dry mass confirmed results obtained in the Petri dish experiments and paper roll where radicle length was inhibited by solution extracts of silverleaf nightshade. In the case of seedling height, averaged across cultivar, results showed that the berries were significantly more inhibitory than the leaves (Figure 4.2).

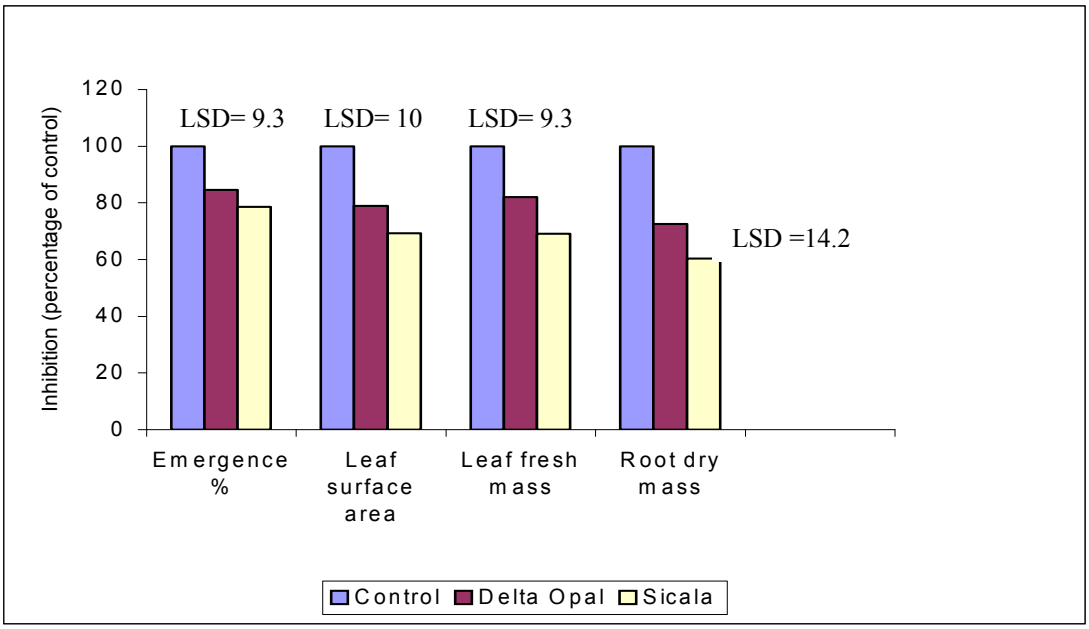

Fig. 4.1 Emergence percentage, leaf surface area, leaf fresh mass and root dry mass of cotton seedlings exposed to silverleaf nightshade residues (Data averaged across plant material and incubation period expressed as percentage of control) (ANOVA in Tables A15, A17, A18 and A20, Appendix A)

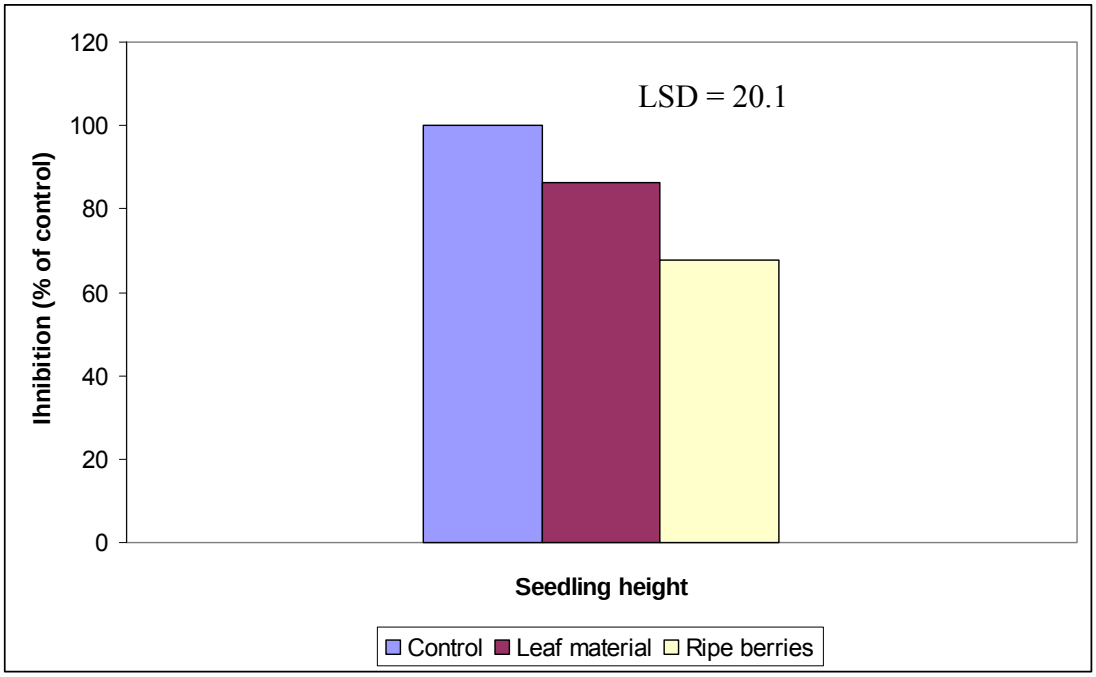

Fig 4.2 Height of cotton seedlings exposed to leaf material or ripe berries of silverleaf nightshade (Data averaged across cultivars and expressed as percentage of control) (ANOVA in Table A16, Appendix A)

4.4 Conclusions

Results showed inhibition of all parameters tested when cotton seeds were sown in soil ameliorated with weed residues. Generally, the level of inhibition was higher in the presence of berries of the weed than in the presence of leaf material. This suggests higher concentrations of allelochemicals, or a different type of allelochemical(s), in ripe berries of silverleaf nightshade than in its leaves. For both types of weed material the inhibitory effect decreased with the progression of decomposition in the soil. With time, and therefore, with increased decomposition of the weed material, the allelochemicals probably decomposed, hence the progressive loss of phytotoxic effect. This also indicates that the allelochemicals involved are released already in the toxic form and do not require transformation by microbes in order to become toxic. The greater tolerance to phytotoxic effects observed for Delta Opal compared to Sicala confirmed the tendency that was observed where the two cultivars

were exposed to extract solutions prepared from silverleaf nightshade leaves in paper rolls and in Petri dishes (Chapter 2).

Earlier it was argued that during extraction of weed material for allelopathy bioassays, chemicals that are not released under natural conditions may be evaluated. Therefore, the use of weed material and a natural soil in this experiment reduced the potential problems associated with chemical extraction. Factors such as osmotic potential were thus likely avoided or greatly reduced in this type of bioassay. Because results reported here were observed after all possible confounding factors were excluded or minimized as much as possible, the growth inhibition observed is attributed to allelochemicals that were released from the plant material tested.

Findings of this study provide strong evidence that silverleaf nightshade has an allelopathic influence on the early growth of cotton. However, due to the complexity of reactions that may occur in the field, field studies to verify these results under natural conditions need to be done in order that allelopathic interaction between weed and crop can be confirmed.

CHAPTER 5
GENERAL DISCUSSION AND CONCLUSION

Although the presence in leaves and berries of secondary metabolites (Khanna *et al.,* 1978; Maiti *et al.,* 1979; Bothma 2002), some of which could have allelopathic potential, has been reported for silverleaf nightshade, information on its allelopathic effects is very scanty. This investigation on the allelopathic potential of silverleaf nightshade was done to evaluate whether this highly effective alien invader has the capacity to interfere with other species in this way, in particular with cotton.

5.1 Allelopathic inhibition of early growth of cotton by silverleaf nightshade extract solutions

The study presented in Chapter 2 was aimed at investigating the allelopathic effects of silverleaf nightshade extract solutions on the early growth of cotton. Results showed that the early growth of cotton was inhibited by extracts prepared from silverleaf nightshade leaves that were collected at the vegetative growth stage. These findings were made after osmotic potential and other possible confounding factors had been excluded. Due to the sensitivity of germination and shoot length of some cultivars to osmotic potential, inferences about the allelopathic activity of the test solutions could not be made for these parameters. Radicle length proved to be more tolerant to osmotic inhibition and was thus the only parameter that could be used to base inferences on as regards the allelopathic activity of test solutions. Although the growth responses of cotton cultivars to the extract solutions tended to differ substantially, the confounding factor of osmotic effect with regard to some parameters precludes a clear finding on the differential tolerance of cultivars.

As has been argued by many authors writing about the phenomenon of allelopathy, laboratory bioassays are very useful in allelopathy research, but arguably they have their shortcomings (Leather & Einhellig, 1986). One

obvious shortcoming of many allelopathy studies that are based on bioassays is that soil is seldom used as growth medium. Furthermore, the allelopathic effects exerted by plant extracts probably does not mirror well the natural release of allelochemicals from plants or their residual matter. In subsequent experiments it was deemed important that soil should be used as a growing medium, and that fresh weed material be used instead of the aqueous extract solutions.

5.2 Influence of soil on the allelopathic effects of silverleaf nightshade

Although soil-less bioassays are useful for investigating the allelopathic potential of plant extracts, they are not enough to conclude that the same results will be obtained in the presence of soil. This experiment was conducted with the main objective of evaluating the influence of soil on the allelopathic activity of silverleaf nightshade extracts (Chapter 3). The tests were done both in aseptic conditions as well as in the presence of microorganisms so as to investigate the influence of microbes on the phytotoxicity of the weed extracts. Two types of growth media, a natural soil and inert quartz sand, were used with the main aim of evaluating the influence of adsorption by soil colloids. Results showed that the early growth of cotton (cv Sicala) was inhibited regardless of the microbial status of the growth medium, which suggests that microbial transformation was not a prerequisite for the activation of the allelochemicals involved. Response of the parameters on the two types of media was variable in the absence of microorganisms but there tended to be greater inhibition on the quartz sand compared to natural soil when microorganisms were not excluded from the medium. This finding suggests adsorption of allelochemicals to soil colloids, which might render them temporarily unavailable for uptake by the roots of plants.

Further experimentation needs to be done to verify the validity of these observations in environments more closely resembling those occurring in nature. Subsequent experiments allowed for the evaluation of the role of decomposition of plant material in the soil, in order to simulate more closely the natural release of allelochemicals from plant litter. This approach also

excluded the complications of osmotic effects, as well as the elimination of microorganisms, thereby presenting an environment that more closely resembled natural conditions.

5.3 Effect of decomposition of silverleaf nightshade material in soil on its allelopathic potential

Experiments reported in Chapter 4, where fresh weed material was incorporated into the soil, represented an environment that is closer to natural field conditions. The sequence and methodology of preceding experiments (Chapter 2 and 3) conform with the challenge that for validity and conclusiveness of allelopathy studies it is essential that laboratory bioassays should be followed by carefully designed experiments that employ live plants and/or plant residues under greenhouse and field conditions (Foy, 1999).

The possibility of confusing allelopathy with growth retardation of plants resulting from nitrogen immobilization caused by soil microorganisms following the introduction of fresh plant material into soil is another concern in allelopathy studies. In the present study a nutrient solution was used to supply sufficient nutrition for the seedlings. Also, a practical plant material: soil ratio of 1% (m/m) was used.

Results (Chapter 4) showed that emergence of cotton and early development of seedlings was inhibited in soil that was mixed with leaves or ripe berries of silverleaf nightshade and that inhibition was greater in the soil mixed with berries than where leaves were incorporated. The finding that growth inhibition of cotton was more severe on soil mixed with ripe berries than where leaves were incorporated, supports that of Guerreiro *et al.* (1971) who reported higher concentrations of allelochemicals in ripe berries than in leaves of silverleaf nightshade. For both types of plant material, the inhibitory effect on cotton seedlings decreased with progression of decomposition of the material in soil, suggesting that the allelochemicals involved were prone to decomposition. Based on these results it can be concluded that the allelochemicals involved were not activated by microbial transformation, but

rather, were probably released in the toxic form. The tendency of cv Sicala to be more sensitive and Delta Opal more tolerant which was observed in Chapter 1 was observed in this study as well. It should be noted that because biotic and abiotic soil factors, which could have a profound effect on allelopathy, differ from soil to soil, an allelochemical will probably behave differently in different soils.

5.4 Conclusions and recommendations

It is suggested that this investigation provided strong evidence that silverleaf nightshade has significant allelopathic potential, and in this way inhibited the early growth and development of cotton. The differential tolerance of the cotton cultivars observed in this study ought to be researched in greater depth, especially if cultivar selection could be a practical solution in cases where the weed is especially problematic in this crop. Because allelopathic activity from the weed was retained in soil medium, allelopathic effects of silverleaf nightshade might well occur under field conditions and not only under controlled conditions. Typical follow-up experiments would be field experiments to verify current knowledge contributed by this study. Allelopathy field trials will pose further unique challenges, the main one probably being the separation of the two phenomena that govern plant-plant interference, namely: allelopathy and competition.

APPENDIX A

Abbreviated analysis of variance (ANOVA) tables

Table A1 Analysis of variance of germination percentage of cotton cultivars exposed to increasing concentration of PEG-6000 solutions (Table 2.1)

Source	DF	MS	F-value	Pr > F
Cultivar	4	1921.17	3.80	0.0064
Concentration	4	9253.26	18.32	< 0.0001
CV * Concentration	16	672.40	1.33	0.1934
Error	100	504.99		
Corrected total	124			
CV (%)	28.7			
R^2	0.53			

Table A2 Analysis of variance of shoot length of cotton cultivars exposed to increasing concentration of PEG-6000 solutions (Table 2.2)

Source	DF	MS	F-value	Pr > F
Cultivar	4	1603.12	2.94	0.0240
Concentration	4	13204.62	24.24	<0.0001
CV * Concentration	16	1211.48	2.22	0.0086
Error	100	544.71		
Corrected total	124			
CV (%)	29.5			
R^2	0.60			

Table A3 Analysis of variance of radicle length of cotton cultivars exposed to increasing concentration of PEG-6000 solutions (Table 2.3)

Source	DF	MS	F-value	Pr > F
Cultivar	4	1528.73	2.11	0.0846
Concentration	4	8031.00	11.10	< 0.0001
CV * Concentration	16	849.83	1.17	0.3011
Error	100	723.26		
Corrected total	124			
CV (%)	35			
R^2	0.43			

Table A4 Analysis of variance of shoot length of cotton cultivars exposed to increasing concentration of silverleaf nightshade extract solutions in Petri dishes (Table 2.4)

Source	DF	MS	F-value	Pr > F
Cultivar	4	1674.70	4.13	0.0050
Concentration	2	31468.48	77.67	<0.0001
CV * Concentration	8	440.39	1.09	0.3847
Error	60	405.15		
Corrected total	74			
CV (%)	34			
R^2	0.75			

Table A5 Analysis of variance of radicle length of cotton cultivars exposed to increasing concentration of silverleaf nightshade extract solutions in Petri dishes (Table 2.4)

Source	DF	MS	F-value	Pr > F
Cultivar	4	2505.08	5.29	0.0010
Concentration	2	27041.05	57.08	< 0.0001
CV * Concentration	8	846.80	1.79	0.0973
Error	60	473.71		
Corrected total	74			
CV (%)	34.5			
R^2	0.71			

Table A6 Analysis of variance of percentage germination of cotton cultivars exposed to increasing concentration of silverleaf nightshade extract solutions in Petri dishes (Table 2.5)

Source	DF	MS	F-value	Pr > F
Cultivar	4	145.49	0.43	0.7854
Concentration	2	36766.09	109.00	<0.0001
CV * Concentration	8	139.402	0.41	0.9086
Error	60	337.29		
Corrected total	74			
CV (%)	31.2			
R^2	0.79			

Table A7 Analysis of variance of germination percentage of cotton cultivars exposed to increasing concentration of silverleaf nightshade extract solutions in paper rolls (Table 2.6 & Table 2.7)

Source	DF	MS	F-value	Pr > F
Cultivar	3	134.61	6.89	0.0009
Concentration	2	75.68	3.87	0.0300
CV * Concentration	6	40.09	2.05	0.0839
Error	36	19.55		
Corrected total	47			
CV (%)	4.5			
R^2	0.53			

Table A8 Analysis of variance of radicle length of cotton cultivars exposed to increasing concentration of silverleaf nightshade extract solutions in paper rolls (Table 2.6 & Table 2.7)

Source	DF	MS	F-value	Pr > F
Cultivar	3	2857.09	23.04	<0.0001
Concentration	2	2596.65	20.94	< 0.0001
CV * Concentration	6	805.11	6.49	0.0001
Error	36	124.03		
Corrected total	47			
CV (%)	12.7			
R^2	0.81			

Table A9 Analysis of variance of germination percentage of cotton cultivars exposed to increasing concentration of silverleaf nightshade extract solutions in a thin layer of soil or sand in Petri dishes (Fig. 3.1)

Source	DF	MS	F-value	Pr > F
Medium	1	683.95	2.46	0.1223
Concentration	2	14530.64	55.61	<0.0001
Medium* Conc.	2	1765.09	6.36	0.0033
Error	54	277.49		
Corrected total	59			
CV (%)	23.3			
R^2	0.70			

Table A10 Analysis of variance of shoot length of cotton cultivars exposed to increasing concentration of silverleaf nightshade extract solutions in a thin layer of soil or sand in Petri dishes (Fig 3.2)

Source	DF	MS	F-value	Pr > F
Medium	1	38.98	4.33	0.0422
Concentration	2	26569.64	350.75	<0.0001
Medium * Conc.	2	333.16	4.61	0.0142
Error	54	75.68		
Corrected total	59			
CV (%)	14.89			
R^2	0.93			

Table A11 Analysis of variance of radicle length of cotton cultivars exposed to increasing concentration of silverleaf nightshade extract solutions in a thin layer of soil or sand in Petri dishes (Table 3.1 and Table 3.2)

Source	DF	MS	F-value	Pr > F
Medium	1	331.12	5.59	0.0217
Concentration	2	39358.17	664.12	<0.0001
Medium * Conc.	2	89.68	1.51	0.2294
Error	54	59.26		
Corrected total	59			
CV (%)	15.6			
R^2	0.96			

Table A12 Analysis of variance of germination of cotton cultivars exposed to soil or sand ameliorated with silverleaf nightshade leaf material in Petri dishes (Fig 3.3)

Source	DF	MS	F-value	Pr > F
Medium	1	86.81	0.47	0.5025
Concentration	1	19531.25	105.88	< 0.0001
Medium * Conc.	1	86.81	0.47	0.5025
Error	16	184.46		
Corrected total	19			
CV (%)	19.7			
R^2	0.87			

Table A13 Analysis of variance of shoot length of cotton cultivars exposed to soil or sand ameliorated with silverleaf nightshade leaf material in Petri dishes (Table 3.3)

Source	DF	MS	F-value	Pr > F
Medium	1	2055.77	7.33	0.0156
Concentration	1	8422.94	30.02	< 0.0001
Medium * Conc.	1	2046.53	7.29	0.0157
Error	16	280.56		
Corrected total	19			
CV (%)	21			
R^2	0.74			

Table A14 Analysis of variance of radicle length of cotton cultivars exposed to soil or sand ameliorated with silverleaf nightshade leaf material in Petri dishes (Fig. 3.3)

Source	DF	MS	F-value	Pr > F
Medium	1	147.60	0.46	0.5077
Concentration	1	14117.74	43.92	< 0.0001
Medium * Conc.	1	147.60	0.46	0.5077
Error	16	321.43		
Corrected total	19			
CV (%)	24.4			
R^2	0.74			

Table A15 Analysis of variance of emergence percentage of cotton cultivars exposed to soil ameliorated with silverleaf nightshade leaf material or berries in pots (Table 4.2 & Fig. 4.1)

Source	DF	MS	F-value	Pr > F
Cultivar	1	3295.89	9.60	0.0028
Plant material	2	10255.53	29.86	<0.0001
CV* Plant material	2	297.85	0.87	0.4244
Decomposition period	3	2206.48	6.42	0.0006
CV*DEC	3	236.00	0.69	0.5628
Plant material * DEC	6	689.56	2.01	0.0756
CV*plant mater*DEC	6	493.16	1.44	0.2129
Error	72	343.42		
Corrected total	95			
CV (%)	24.4			
R^2	0.61			

Table A16 Analysis of variance of seedling height of cotton cultivars exposed to soil ameliorated with silverleaf nightshade leaf material or berries in pots (Table 4.4 & Fig. 4.2)

Source	DF	MS	F-value	Pr > F
Cultivar	1	198.02	1.21	0.2747
Plant material	2	6946.63	42.49	<0.0001
CV* Plant material	2	1.195	0.01	0.9927
Decomposition period	3	1741.72	10.65	<0.0001
CV*DEC	3	537.96	3.29	0.0254
Plant material * DEC	6	547.65	3.35	0.0057
CV*plant mater*DEC	6	171.81	1.05	0.4000
Error	72	163.47		
Corrected total	95			
CV (%)	15.1			
R^2	0.68			

Table A17 Analysis of variance of leaf surface area of cotton cultivars exposed to soil ameliorated with silverleaf nightshade leaf material or berries in pots (Table 4.3 & Fig. 4.1)

Source	DF	MS	F-value	Pr > F
Cultivar	1	1973.33	5.21	0.0254
Plant material	2	26800.98	70.74	<0.0001
CV* Plant material	2	511.505	1.35	0.2657
Decomposition period	3	1680.277	4.44	0.0064
CV*DEC	3	325.401	0.86	0.4665
Plant material * DEC	6	852.96	2.25	0.0478
CV*plant mater*DEC	6	251.661	0.66	0.6787
Error	72	378.86		
Corrected total	95			
CV (%)	26.2			
R^2	0.72			

Table A18 Analysis of variance of leaf fresh mass of cotton cultivars exposed to soil ameliorated with silverleaf nightshade leaf material or berries in pots (Table 4.5 & Fig. 4.1)

Source	DF	MS	F-value	Pr > F
Cultivar	1	1494.43	4.33	0.0410
Plant material	2	27071.49	87.41	<0.0001
CV* Plant material	2	11910.01	3.45	0.0372
Decomposition period	3	3249.97	9.41	<0.0001
CV*DEC	3	44.487	0.13	0.9427
Plant material * DEC	6	783.017	2.27	0.0463
CV*plant mater*DEC	6	293.889	0.85	0.5349
Error	72	345.254		
Corrected total	95			
CV (%)	25.05			
R^2	0.75			

Table A19 Analysis of variance of leaf dry mass of cotton cultivars exposed to soil ameliorated with silverleaf nightshade leaf material or berries in pots (Table 4.1)

Source	DF	MS	F-value	Pr > F
Cultivar	1	5855.89	9.54	0.0029
Plant material	2	25866.99	42.16	<0.0001
CV* Plant material	2	2618.63	4.27	0.0177
Decomposition period	3	1717.475	2.80	0.0461
CV*DEC	3	403.376	0.66	0.5809
Plant material * DEC	6	1065.249	1.74	0.1249
CV*plant mater*DEC	6	754.275	1.23	0.3015
Error	72	613.585		
Corrected total	95			
CV (%)	31.2			
R^2	0.64			

Table A20 Analysis of variance of root dry mass of cotton cultivars exposed to soil ameliorated with silverleaf nightshade leaf material or berries in pots (Fig. 4.1)

Source	DF	MS	F-value	Pr > F
Cultivar	1	2212.205	2.74	0.1025
Plant material	2	32860.573	40.64	<0.0001
CV* Plant material	2	1048.449	1.30	0.2798
Decomposition period	3	694.507	0.86	0.4665
CV*DEC	3	916.269	1.13	0.3415
Plant material * DEC	6	2147.274	2.66	0.0220
CV*plant mater*DEC	6	823.050	1.02	0.4207
Error	72	808.654		
Corrected total	95			
CV (%)	41.9			
R^2	0.61			

APPENDIX B

Table 1 Properties of the soil used in the experiments

Ammonium Acetate Extractable						Organic carbon %	SO^{-2}_4 mg kg^{-1}	Coarse sand %	Silt %	Clay %	CEC cmol kg^{-1}
pH water	P Bray 1 mg kg^{-1}	Ca mg kg^{-1}	K mg kg^{-1}	Mg mg kg^{-1}	Na mg kg^{-1}	0.47	154.08	68.5	8.2	22.1	3.60
6.1	25.6	531	45	158	11						

Table 2 Composition of Nitsch (1972) nutrient solution used in the Petri dish experiment (Chapter 3) and in the pot experiment (Chapter 4)

Salt	Concentration (g L^{-1})
KNO_3	610
KH_2PO_4	310
$MgSO_4.7H_2O$	610
$(NH_4)_2\ SO_4$	310
$Ca\ (NO_3)_2.4H_2O$	2440
EDTA Na_2Fe	60
KCL	6.1
H_3BO_3	6.7
$MnSO_4.H_2O$	3.8
$ZnSO_4.7H_2O$	0.61
$(NH_4)_6\ MO_7\ 24.4H_20$	6.1
$CuSO_4.5H_2O$	0.31
H_2SO_4	0.31

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SUMMARY

ALLELOPATHIC INTERFERENCE OF SILVERLEAF NIGHTSHADE *(Solanum elaeagnifolium* Cav.*)* WITH THE EARLY GROWTH OF COTTON *(Gossypium hirsutum* L.*)*

1. This work was done to substantiate limited reports in literature on the presence of allelochemicals in silverleaf nightshade, and to determine whether this alien invader has the potential to inhibit the growth of other plants through allelopathy. Allelopathic interference of silverleaf nightshade with early growth of cotton was evaluated by exposing cotton seeds to silverleaf nightshade extract solutions, or by growing cotton on soil ameliorated with silverleaf nightshade plant material. Attempts were made to eliminate, or at least reduce, possible confounding factors in all bioassays conducted.

2. Crude extracts used for preparation of test solutions were obtained through cold-water extraction (infusion), by soaking silverleaf nightshade leaves in water. Prior to exposing the cotton seeds to silverleaf nightshade extract solutions, sensitivity of cotton germination, radicle and shoot length to osmolality of solution was evaluated by exposing the seeds to a range of PEG-6000 concentrations of varying osmolality. Seeds of five different cotton cultivars viz.- Sicala, Tetra, Siokra V15, Delta Opal and CA 223 were then exposed to silverleaf nightshade extract solutions in Petri dishes lined with a layer of filter paper, or in germination paper rolls moistened with the extract. In all the bioassays, growth inhibition of cotton was observed. Inhibition was more pronounced in the Petri dish experiments than in paper rolls. Amongst cultivars tested, cultivar Sicala tended to be more sensitive to silverleaf nightshade allelopathy, whilst cultivar Delta Opal tended to be more tolerant. This response could not be confirmed in the laboratory experiments (Chapters 3) but was observed again in the pot experiment (Chapter 4).

When cotton seeds were grown in a sterile environment in natural soil or inert quartz sand containing the extracts or when the media were mixed with silverleaf nightshade leaves in Petri dishes in the presence of microbes, results showed that germination and early growth of cotton cv Sicala was inhibited in both media regardless of the microbial status of the medium. Response of parameters was variable in the absence but inhibition tended to be greater in the sand bioassay when microbes had not been excluded. In pot experiments, cotton seeds were exposed to soil into which silverleaf nightshade leaf material or ripe berries was incorporated to attain a concentration of 1 % (m/m) plant material: soil. Plant material was allowed to decompose in the soil for 0, 2, 4 or 6 weeks before seeds were planted. Moisture levels in the pots were maintained at field capacity until harvesting. A complete nutrient solution was used for watering the seedlings until harvesting. Seedling emergence and general growth was reduced in the pots where residues were incorporated, compared to the control treatment, where no plant material was incorporated. Inhibition of emergence was greater in pots where ripe berries were incorporated. In pots containing ripe berries, seedlings were visibly shorter and less vigorous than those where leaf material was incorporated. Moreover, some of the seedlings that had emerged became chlorotic, misformed (twisted), and died off soon afterwards in the presence of berries. This was not observed for seedlings where leaf material was incorporated. Results also showed that for both types of plant material the inhibitive effects decreased with time. As it was observed in the Petri dish experiments when weed extracts had been used (Chapter 1), cv Sicala tended to be more susceptible whilst cv Delta Opal tended to be more tolerant to silverleaf nightshade residues.

3. The use of soil in the investigation narrowed the gap between conditions prevailing in the laboratory and those in the field. To the best of our knowledge, there has hitherto been no reports of silverleaf nightshade allelopathy outside the laboratory environment. Results reported here are thus the first from bioassays, which use soil as growth medium, to assess the allelopathic potential of silverleaf nightshade. Due to the complex nature of the

phenomenon of allelopathy, further experimentation to elucidate its role in plant-plant interactions in natural environments is needed.