CHAPTER 4
CHEMICAL CHARACTERISTICS OF PHYTOTOXIC COMPOUNDS IN SILVERLEAF NIGHTSHADE FOLIAGE

4.1 Introduction

Phytochemical studies on *Solanum elaeagnifolium* have revealed several interesting secondary metabolites produced by this species. The main publications have dealt with alkaloids and sapogenins (Maiti & Mathew, 1967; Guerreiro *et al.*, 1971; Kavka *et al.*, 1973; Hanna *et al.*, 1996b), while the presence of flavonoids (Chiale *et al.*, 1991) and several phytosterol components (Hanna *et al.*, 1996a) have also been reported.

The glycoalkaloid, solasodine, found in the fruit of silverleaf nightshade, is an important steroidal alkaloid in the manufacture of pharmaceutical corticosteroidal drugs (Heap & Carter, 1999). No solasodine is present in leaves and stems of this plant (Bradley *et al.*, 1978). Other glycoalkaloids reported for silverleaf nightshade, include solamargine, found in the unripe fruits (Guerreiro et al, 1973), solasurine and solanelagnin, isolated from the stalks of this weed (Hanna *et al.*, 1996b). The saponin diosgenin, and 3-deoxy-Δ^3-diosgenin were obtained from silverleaf nightshade foliage (leaves and twigs) collected during flowering, as well as from samples collected after the fruit had ripened (Guerreiro *et al.*, 1971). These compounds were also encountered in unripe and ripe fruit of this species. The percentage of both compounds in the younger leaves and fruit doubled the amount revealed in leaves and fruit after fruit ripening (Guerreiro *et al.*, 1971).

Chiale *et al.* (1991) isolated the flavonoid kaempferol and kaempferol 3-glucoside, as well as a new monoacylated flavonoid glucoside characterised as kaempferol 3β-D-(6″-*O*-*cis*-cinnamoylglucoside), all from the aerial parts of silverleaf nightshade.

The types of secondary metabolites reported for silverleaf nightshade have all been implicated in allelopathic interactions (Rice, 1984). The steroidal saponins (glycoside derivatives of steroids) from silverleaf nightshade fruit have reportedly been involved in chemical interactions with other plant species (Curvetto *et al.*, 1976; Agüera & Boland, 1985). No publications could be located on research investigating the possible allelopathic or phytotoxic properties of the silverleaf nightshade foliage.

In Chapter 3 it was illustrated that foliar infusions and crude water-soluble foliar extracts of silverleaf nightshade inhibit germination and seedling root growth of cotton and lettuce. The objective of this investigation was to obtain preliminary information about the chemical nature of the compound(s) responsible for the phytotoxic activity exhibited by foliar extracts of silverleaf nightshade. It has to be emphasised that complete purification and identification of chemical constituents was not intended, as this falls outside the scope of this dissertation.

4.2 Materials and methods

Plant material:

The foliar material of *S. elaeagnifolium* was collected near Settlers in the Northern Province at anthesis, and left to dry in the dark at room temperature for three weeks.

Extraction of active compounds:

Dried plant material was homogenised in ethanol using an anti-explosion electrical blender and left one week for extraction on a stirrer. This process was repeated twice. The total ethanolic extract was combined after filtration and the solvent was evaporated under reduced pressure at 40°C. The total extract obtained was dissolved in 80% ethanol and partitioned with ethyl acetate to yield two fractions, an ethyl acetate fraction (A), and aqueous ethanolic fraction (B).

Isolation and preliminary detection of the nature of active compound(s):
It was decided to continue further fractionation only with the water-soluble fraction (B), as this probably contained the chemical constituents more likely to be available in a natural agricultural situation.

For preparative paper chromatography fraction B, dissolved in methanol, was applied to Whatman 3MMChr chromatography paper and developed in 15% acetic acid. After drying, the chromatogram was inspected under short wave and long wave ultraviolet (UV) light and bands were marked with pencil, cut out and bioassayed.

The most phytotoxic fraction was extracted from the paper and subjected to Sephadex LH-20 column chromatography using methanol as eluent. Phytotoxicity of the obtained fractions was evaluated with a lettuce germination bioassay. The active fractions were collected and subjected to preliminary chemical identification by preparing thin layer chromatography (TLC) plates of the active fractions in various solvents and spraying these with different chemical reagents specific for detecting different types of natural products.

For flavonoids, the following reagents were used: (a) Ammonia solution and (b) 5% $AlCl_3$ solution in methanol. In each case colours were recorded in day light and under UV (short and long wavelength), before and after spraying.

For alkaloids, the following reagents were used: (a) Ninhydrin solution (0.3% solution in BuOH), followed by heating the chromatogram to 100°C, and (b) Dragendorff reagents (Stahl, 1965). With the latter reagents alkaloids appear as stained orange spots on a yellow background. The sensitivity of the detection can be increased appreciably by subsequent spraying with 10% HCl.

For terpenoids vanillin in sulfuric acid was used as reagent.

Preliminary spectral analysis (^{1}H-NMR) of nearly pure fractions was done by the Chemistry Department at the University of Pretoria.

Lettuce germination bioassay:

Paper chromatography : Bands of different components on the dried chromatogram were cut into sections of approximately 32 cm². These sections were placed in sterile petri dishes, 20 lettuce seeds cv. Great Lakes and 2 ml of sterilised distilled water were added to each. A clean piece of identical paper, subjected to the same eluent, was used as a control. The chromatogram could not be sterilised in an autoclave due to the possibility of breakdown of the chemical constituents by exposure to high temperatures. Each treatment was replicated five times. Petri dishes were sealed with parafilm, whereafter they were placed in a growth chamber at 22°C in light for three days. The number of germinated seeds for each treatment was counted daily, and on the third day (72 hours) radicle lengths were measured. A radicle length of 2 mm or greater was considered as successful germination, and only root lengths of successfully germinated seeds were taken into account in statistical analysis.

Sephadex fractions : The solvent of fractions from the Sephadex column was evaporated under reduced pressure at 40°C. Fractions were weighed and dissolved in methanol and diluted in such a way that 1 ml contained 10% of the amount eluted from the column. Of these dilutions 0.8 ml was applied to one disc of 90 mm Whatman no. 1 filter paper, pre-sterilised by autoclaving for 30 minutes at 121°C. Therefore, the fractions were applied in the ratio in which it occurred in the plant. For the control treatment the same volume of pure solvent was applied to the filter paper. The solvent was allowed to evaporate from the treated filter paper. After solvent evaporation, the filter papers were placed in sterile 90 mm petri dishes, 10 lettuce seeds cv. Great Lakes and 2 ml of sterilised distilled water were added to each. Treatments were replicated

three times. Petri dishes were sealed with parafilm®, placed in a growth chamber and evaluated in a similar way as paper chromatography bioassays.

All bioassay experiments were performed inside a laminar flow cabinet.

Statistical analysis:

Analysis of variance (ANOVA) was performed using the statistical program GenStat (2000). A completely randomised design was used in all experiments. Analysis of variance (ANOVA) was used to test for differences between treatments. The root data was acceptably normal with homogeneous treatment variances. In the case of germination percentages, angular transformation was used to stabilise variances. Treatment means were separated using Tukey's studentised range test least significant difference (LSD) at the 5 % level of significance.

4.3 Results and discussion

Bioassays on the paper chromatogram of the water-soluble fraction indicated that the most polar fraction (5) was the most active, with pronounced inhibition of germination and root length of seedlings when compared to the control treatment (Table 4.1). Reddish colours obtained by the Dragendorff spraying reagents suggested that this fraction contained an alkaloid.

Table 4.1 Effect of paper chromatography fractions on germination of lettuce over three days and mean root length of resulting seedlings (ANOVA in Tables A11 and A12, Appendix A)

Fraction	Percentage germination			Root length
	24 h	48 h	72 h	(mm)
Control	69 a	85 a	92 a	11.87 ab
1	66 a	91 a	96 a	12.27 a
2	72 a	92 a	99 a	8.79 c
3	82 a	96 a	98 a	9.20 c
4	86 a	94 a	98 a	10.06 bc
5	0 b	3 b	8 b	1.33 d

Means in each column followed by different letters are significantly different according to Tukey's studentised range test LSD ($P < 0.05$)
Fractions are arranged in order of increasing polarity

Fractions 2, 3 and 4 also inhibited root growth of lettuce seedlings significantly, although germination was not affected. These compounds were probably flavonoidic constituents judging by the colours that were observed under UV light.

Of the fractions obtained from the Sephadex column, fraction 4 significantly inhibited germination of lettuce seeds (Table 4.2). However, no inhibition of root growth occurred. This fraction gave all the characteristics of an alkaloidal compound in both ninhydrin and Dragendorff reagents (Fig. 4.1). The concentration at which this fraction

was applied, however, is approximately five times the recommended concentration as suggested by Rimando *et al.* (2001). Fraction 6 significantly inhibited root growth of lettuce seedlings, although germination was not affected (Table 4.2). Preliminary ^{1}H-NMR analysis confirms the presence of a saponin in this fraction (Fig. 4.2). The concentration of this fraction applied, falls into an accepted range (Rimando *et al.*, 2001).

Table 4.2 Effect of Sephadex fractions on germination of lettuce over three days and mean root length of resulting seedlings (ANOVA in Tables A13 and A14, Appendix A)

Treatment	Concentration (mg/ml)	Percentage germination						Root length (mm)	
		24 h		48 h		72 h			
Control	-	100	a	100	a	100	a	11.03	a
1	2.00	96.7	a	96.7	a	96.7	a	9.19	ab
2	1.40	100	a	100	a	100	a	8.10	ab
4	5.60	10	c	16.7	c	26.7	b	8.07	ab
5	1.00	53.3	b	76.7	b	90	a	8.65	ab
6	0.72	100	a	100	a	100	a	6.43	b

Means in each column followed by different letters are significantly different according to Tukey's studentised range test LSD ($P < 0.05$)

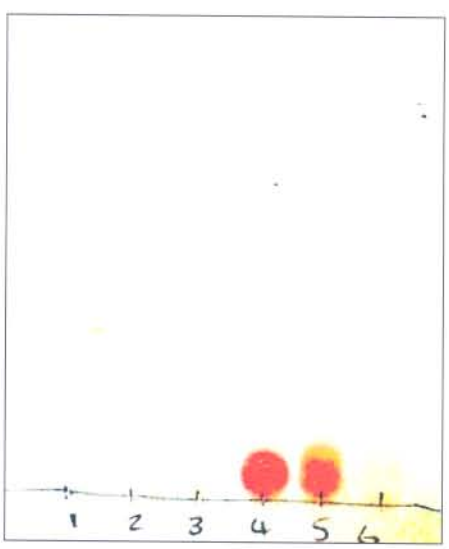

Fig. 4.1 TLC plates sprayed with Dragendorff reagents showing the presence of an alkaloid in fraction 4 from the silverleaf nightshade foliar extract Sephadex column

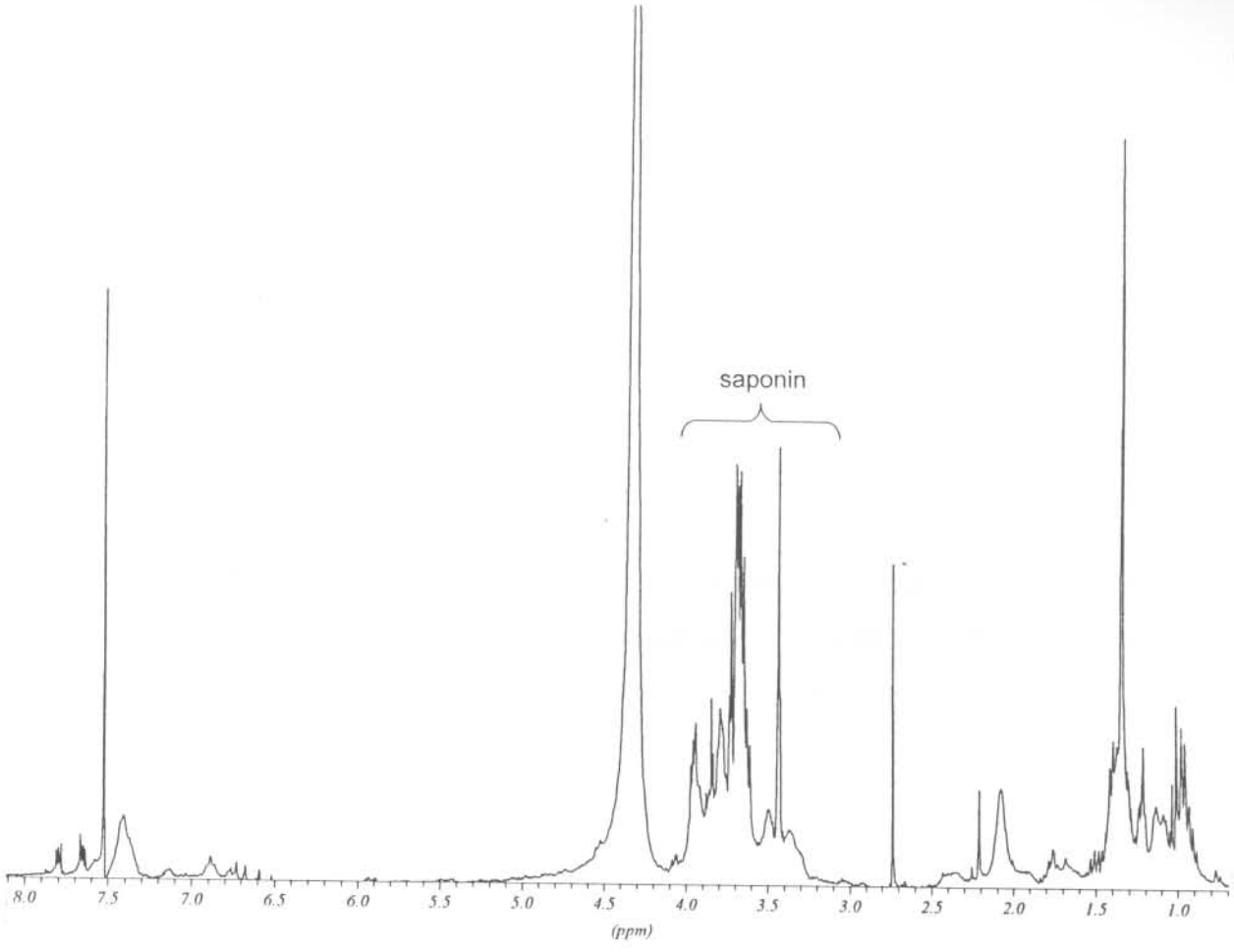

Figure 4.2 Preliminary ^{1}H-NMR spectral analysis of fraction 6 indicating the presence of a relatively pure saponin

The phytotoxic activity observed with the fractions from the Sephadex column, was much less pronounced than activity obtained with the crude water-soluble extract at very low concentrations. Significant phytotoxic activity was observed with at least two of the fractions from the Sephadex column, as well as with possible flavonoidic compounds from the paper chromatogram. This suggests a synergistic effect, where not one specific compound is responsible for inhibition of lettuce germination and growth, but a combination of these various constituents in the crude extract. However, further investigation is necessary to confirm this hypothesis. Furthermore, purification and complete spectral analysis for these compounds are still required for their identification.

CHAPTER 5
POTENTIAL LINK BETWEEN LEAF ANATOMY AND ALLELOPAHTIC POTENTIAL OF SILVERLEAF NIGHTSHADE

5.1 Introduction

Anatomical studies on silverleaf nightshade were first performed by Pilar (1937) and only in the late 1990's the leaf anatomy of this plant was further investigated by Cosa *et al.* (1998), Dottori *et al.* (1998) and Bruno *et al.* (1999). Interesting anatomical features described for the leaves, include two variants of stellate non-glandular trichomes. One variant has a stalk emerging from the epidermis surface, and the other has an intrusive base growing into the mesophyll. Glandular trichomes are plentiful on both leaf surfaces, and there is an abundance of crystals in the leaves.

It was established in Chapters 3 and 4 that silverleaf nightshade foliage may be phytotoxic to crop species and that the allelopathic activity shown by foliar extracts are caused by a synergistic effect of several compounds. The active compounds in the most phytotoxic fraction were characterised as an unidentified alkaloid and a saponin, while less pronounced growth inhibition of the test species also occurred as a result of three flavonoidic fractions. In plants the highest content of phytotoxic compounds is found in leaves and these plant inhibiting substances may be contained in specialised structures (Roshchina & Roshchina, 1993). A number of plants have specific idioblasts that contain tannins, alkaloids or glucosinolates (Wink, 1999). More often, secondary metabolites are concentrated in trichomes or glandular hairs, stinging hairs or the epidermis itself (Wink, 1999). Kanchan & Jayachandra (1980) found that growth inhibitors contained in soft, fine trichomes of *Parthenium hysterophorus* L. resulted in allelopathic growth inhibition of 10-day-old wheat (*Triticum aestivum* L.) seedlings. Also, several sesquiterpene lactones from the glandular trichomes on sunflower leaves (*Helianthus*

annuus L.) cv. VYP® influenced root and shoot growth of lettuce (*Lactuca sativa* L. var. *nigra*), tomato (*Lycopersicon esculentum* Mill.), barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) seedlings (Macías *et al.*, 1996). However, it has also been shown that significant relationships between the number of glandular trichomes on leaves and either the release or phytotoxic activity of allelochemicals do not necessarily exist (Nilsson *et al.*, 1998).

In this study the leaf anatomy of silverleaf nightshade was examined in an attempt to relate specific cells or structures to the allelopathic potential exhibited by foliar infusions and extracts. The study was also conducted to confirm and contribute to previous anatomical studies on the leaves of this plant. The focus was on the various trichome types, but other anatomical features of the leaves will also be discussed briefly.

5.2 Materials and methods

Plant material:

Leaves of *Solanum elaeagnifolium* were collected from plants at anthesis on the Hatfield experimental farm of the University of Pretoria. Leaves were selected from four different size classes – 1 cm, 2 cm and 5 cm in length, as well as mature leaves (approximately 7 to 8 cm in length). Plant material was collected early in the morning and kept in water until preservation.

Scanning electron microscopy (SEM):

Sections of 3mm by 3mm, excised from the middle of the lamina between the mid rib and leaf margin, were fixed in 2.5% glutaraldehyde in a 0.1M $NaPO_4$ buffer (pH 7.4) for two hours. Glutaraldehyde fixation was followed by three rinses (10 minutes each) in the same buffer. Post-fixation was done with 1% aqueous OsO_4 for two hours. OsO_4 was removed with three rinses (10 minutes each) of distilled water. Dehydration was done as described for root tips in Chapter 3. This was followed by critical point drying in a Polaron critical point drier. The dried

samples were mounted on aluminium stubs and made conductive by exposing them to vapour from a 0.5% RuO_4 solution (Van der Merwe & Peacock, 1999). Specimens were viewed with a JOEL 840 scanning electron microscope operated at 5 kV and images were recorded digitally.

Light transmitting microscopy (LTM):

Sections of 1mm by 1mm, excised from the middle of the lamina between the mid rib and leaf margin, were fixed in glutaraldehyde, dehydrated and embedded in LR White medium grade resin as for LTM in Chapter 3. Transverse sections were made, stained and images recorded as described for root tips in Chapter 3.

5.3 Results and discussion

General:

The leaves of silverleaf nightshade are isobilateral and amphistomatic, with a uniseriate adaxial and abaxial epidermis covered by a variaty of numerous trichomes (Fig. 5.1). This corroborates findings of Cosa *et al.* (1998).

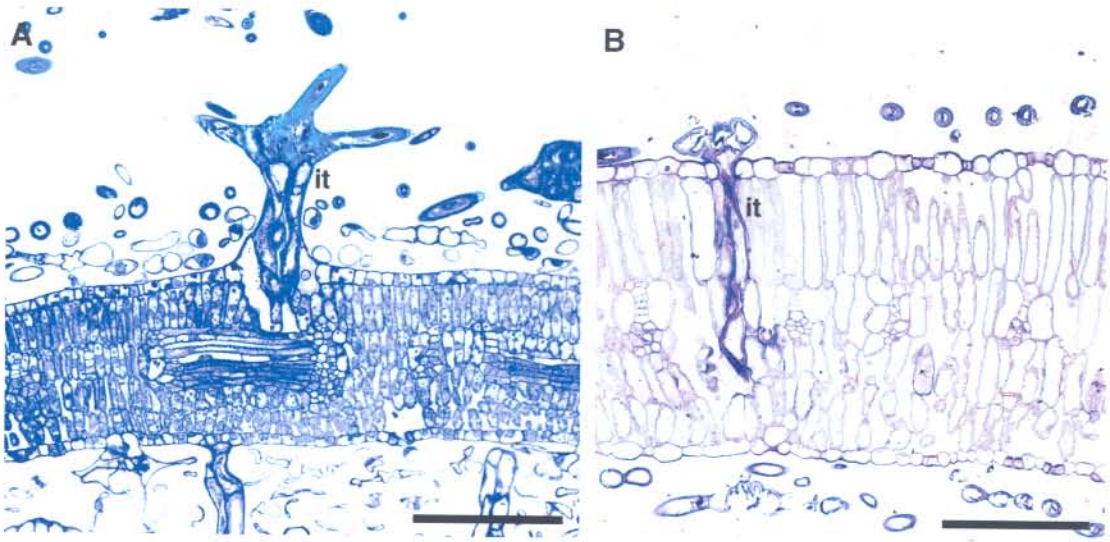

Figure 5.1 Transverse section of a young (A) and mature (B) silverleaf nightshade leaf, showing its isobilateral and amphistomatic nature and intrusive stellate trichomes (it); Scale bars = 100 μm

Crystals:

There is an abundance of crystals contained in idioblasts in the leaves, especially close to the vascular bundles (Fig. 5.2). Bruno *et al.* (1999) also noted the presence of these crystals in the leaves of silverleaf nightshade. The crystalline inclusions observed in silverleaf nightshade leaves are most probably small pyramidal crystals of calcium oxalate monohydrate known as "crystalline sand" (Cody & Horner, 1985). The most frequent crystal mineral salt deposition in idioblasts is calcium oxalate, often present as crystalline sand (Roshchina & Roshchina, 1993). According to Cody & Horner (1985) crystal sand is found in 36 angiosperm families and is most prevalent in the Amaranthaceae, Rubiaceae, and Solanaceae.

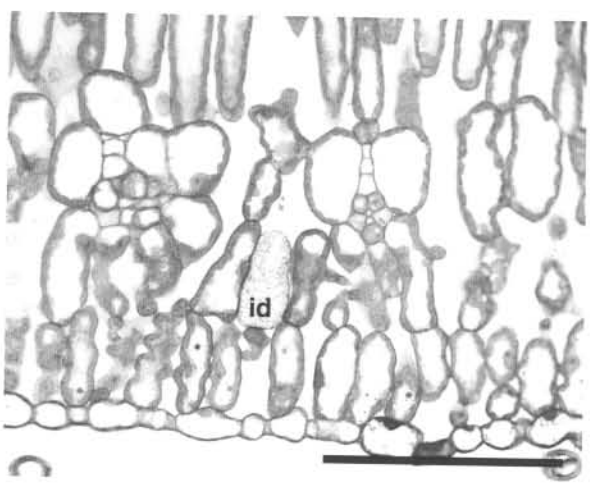

Figure 5.2 Transverse section of a silverleaf nightshade leaf showing crystals, most likely calcium oxalate, contained in an idioblast (id); Scale bar = 100 μm

Trichomes:

Seithe (1979) listed eight recognisable trichome types that occur on mature *Solanum* plants. Of these, it appears that silverleaf nightshade leaves have three types, namely stellate hairs, multicellular glands and multicellular prickles.

- Glandular trichomes:

  Multicellular glands are present on the adaxial and abaxial leaf surfaces, and seem to be more prevalent on younger leaves. Bruno

et al. (1999) described the glandular trichomes as consisting of a bicellular stalk with a spherical "uni-pluricellular" head. In this study, however, only a single stalk cell could be observed for glandular trichomes (Fig. 5.3), which is in agreement with Seithe (1979) who stated that the stalk of glandular trichomes in the genus *Solanum* always remains unicellular. According to the categorisation of Seithe (1979), the glandular trichomes on silverleaf nightshade leaves can be classified as storied glands, with two or three storeys of cells, each with two or more glandular cells.

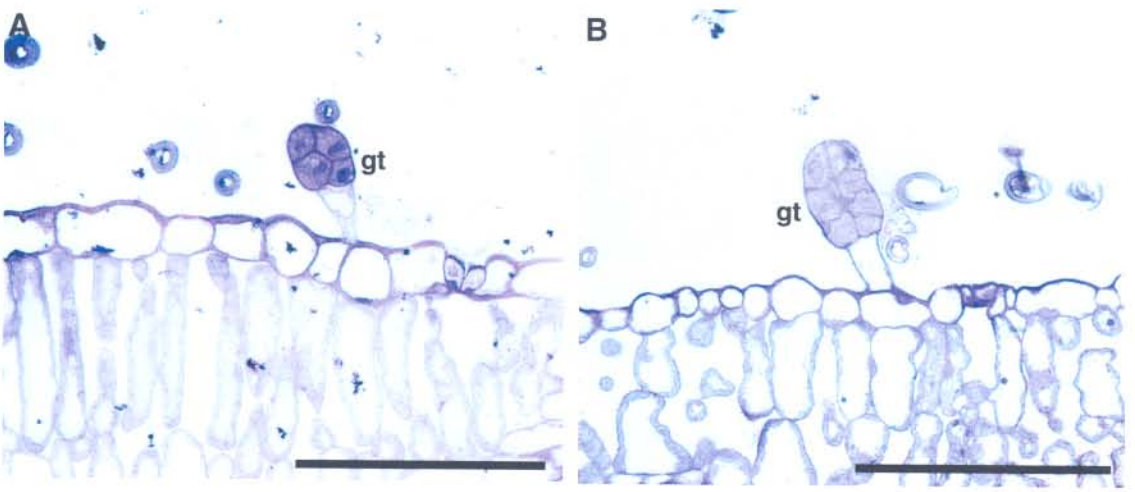

Figure 5.3 Transverse sections through glandular trichomes (gt) on the silverleaf nightshade leaf surface showing the storied glandular trichome head and one stalk cell; Scale bars = 100 μm

- Non-glandular trichomes:

  As also found by Bruno *et al.* (1999), two variants of non-glandular porrect-stellate trichomes could be distinguished on both sides of the silverleaf nightshade leaf. One has a multicellular stalk emerging from the epidermis surface, while the other variant has fewer but much larger intrusive stalk cells growing into the mesophyll (Fig. 5.4). Epidermal stellate trichomes have been described for several *Solanum* species (Seithe, 1979), while the variant with the intrusive base has only been described for *S. elaeagnifolium* (Pilar, 1937; Cosa *et al.*, 1998; Dottori *et al.*, 1998; Bruno *et al.*, 1999). In both variants the stellate head consists of 8 to 16 horizontal unicellular

radii and one prominent vertical apical spine cell (Fig. 5.5) with a large basal lumen (Fig. 5.4B), borne on a biseriate stalk. These silverleaf nightshade trichomes were described by Roe (1971) as being rather intermediate between porrect-stellate and peltate. From a surface view it is not possible to distinguish between intrusive and non-intrusive variants (Fig. 5.5)

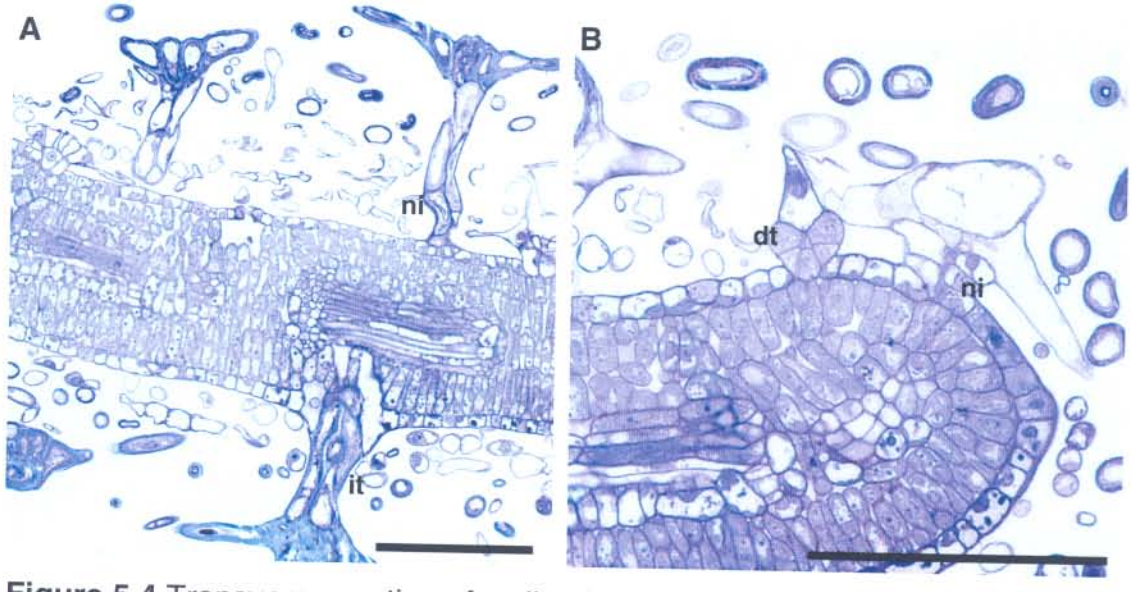

Figure 5.4 Transverse section of a silverleaf nightshade leaf showing intrusive (it) and non-intrusive (ni) variants of stellate trichomes and a stellate trichome in early stages of development (dt); Scale bars = 100 μm

Stellate trichomes are extremely closely spaced on young leaves, however, density of trichomes decreases dramatically as leaves mature. On fully mature leaves the stellate trichomes are markedly denser on the abaxial side of the leaf than on the adaxial side (Fig. 5.5).

Bruno *et al.* (1999) illustrated that the early stages of development are similar in both stellate trichome variants. Basal cells start to grow, in general without dividing, and cause the stalk to intrude aggressively between mesophyll cells, probably as a result of enzymatic disorganization of the middle lamella. When mature, the stalks seem to reach the vascular bundles. At adult stage, the cell

walls of both variants are uniformly thick; the emerging part is strongly lignified whereas there is little or no lignification in the intrusive portion of the trichome. By differential staining with Toluidine Blue O (0.5% in a sodium carbonate buffer) the same could be observed in this study.

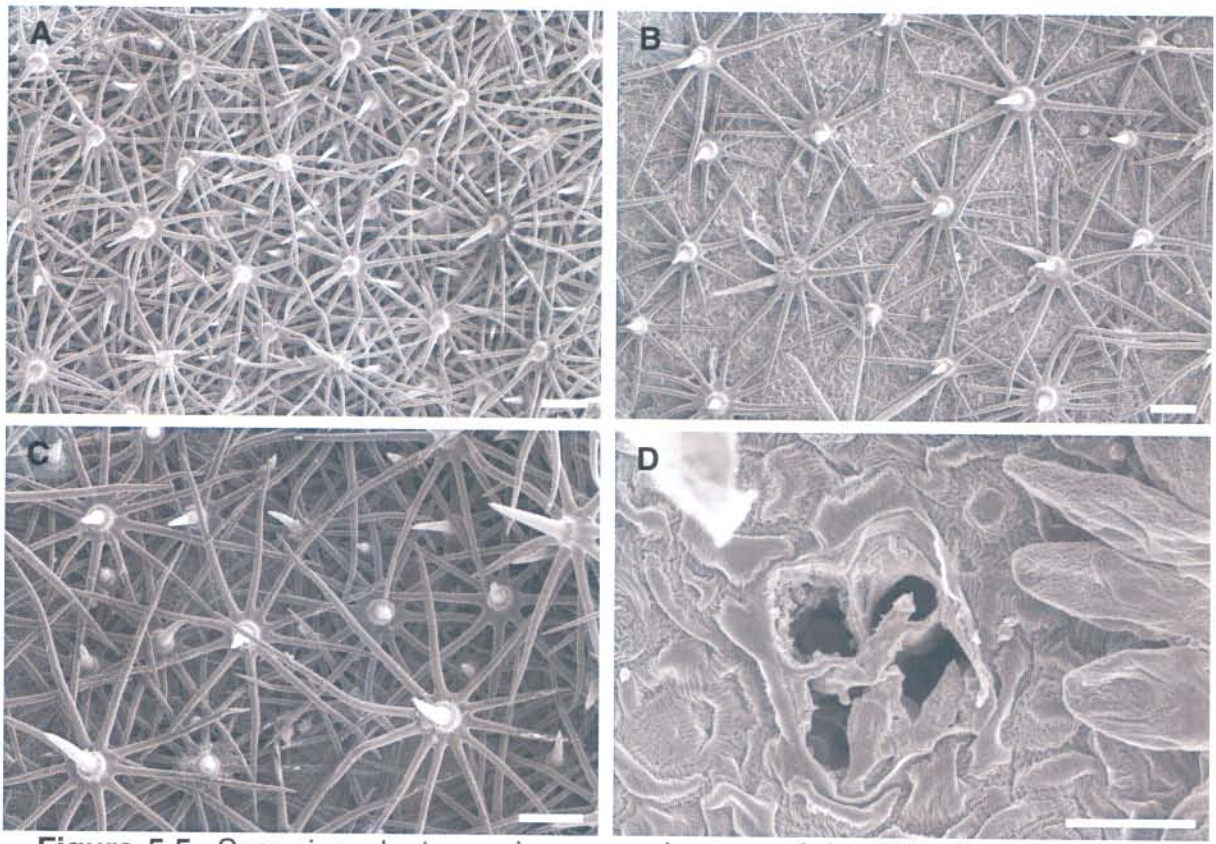

Figure 5.5 Scanning electron microscope images of the (A) adaxial surface of a 2 cm-leaf, (B) adaxial surface of a mature leaf and (C) abaxial surface of a mature leaf of silverleaf nightshade; (D) Removal of an intrusive trichome shows basal cells entering the leaf surface; Scale bars: A – C = 100 μm, D = 10 μm

According to evolutionary tendencies proposed by Seithe (1979) for stellate trichomes of *Solanum*, those of silverleaf nightshade would belong to the more evolved group, since they cumulate the characteristics of a short non-glandular apical cell, many horizontal non-glandular radii, and the presence of a biseriate stalk.

The tendency to develop either a stellate hair or a prickle seems to be determined according to the "pattern scheme" described by Bünning (1948), known previously for stomata (Seithe, 1979). In certain instances the stimulus for extending cells to form stellate hairs is replaced by another stimulus which promotes cell divisions and turns hairs into prickles. The same mechanism might possibly determine development of either epidermal or intrusive stellate hair types.

Currently, the function of these stellate trichomes with its intrusive bases is unclear. Bruno *et al.* (1999) speculated that its morphology might possibly be related to the water economy of the plant, since the stalk cells, with its very thick walls, seem to penetrate to the point of making contact with the xylem. However, before any conclusions of involvement in water transport can be made, the presence of pits and the nature thereof has to be established. Furthermore, Bruno *et al.* (1999) raised the possibility that, as observed with sclereids in the epidermis of other xerophytic species (Martinez, 1995), the thick-walled intrusive cells of these trichomes might have an important sustaining function, reducing the risk of collapse when the plant is stepped on.

The only other species in which intrusive non-glandular trichomes have been reported, is the rootless epiphytic terrestrial species of *Tillandsia* in the Bromeliaceae (Gibson, 1996). They have broad peltate trichomes, which are flexed upward under dry conditions, absorb water into the dead cells when wetted, and then flatten on the water film drawing water into live stalk cells intruding into the mesophyll. However, it is not likely that a relationship exists between these trichomes and those of silverleaf nightshade, as all cells of the intrusive stellate trichomes in the latter seem to be dead.

Bruno *et al.* (1999) also mentioned the presence of simple and non-glandular trichomes. In this study, however, no simple hairs were

detected by SEM or light microscopy on any of the samples examined.

Xeromorphic adaptations:

Except for its extensive underground root system, silverleaf nightshade has highly specialised leaves adapted to the xerophytic environment in which it normally grows. The amphistomatic leaves with isolateral palisade mesophyll are typical of non-succulent desert shrubs (Gibson 1996). The abundance of crystals in the leaves can also be considered of adaptative value to xerophytic conditions (Fahn & Cutler, 1992). Furthermore, the wealth of silvery stellate trichomes on the leaf surface contributes to silverleaf nightshade's drought resistance (Bruno *et al.*, 1999), as dead non-glandular trichomes may significantly reduce absorbed infrared radiation and thereby leaf temperature (Gibson, 1996). The presence of dense trichomes has also been explained as a way to reduce leaf transpiration (Gibson, 1996).

Structures possibly linked to allelopathic potential:

Although higher plants are capable of producing literally thousands of secondary metabolites, it is interesting to note that trichomes generally contain the most active constituents, even though it contains less amounts and kinds of secondary metabolites as compared to the whole leaf or other plant tissues (Wink, 1999). Therefore, when considering cells or structures that might play a role in the allelopathic potential of silverleaf nightshade leaves, there are two obvious features to consider. Firstly the glandular trichomes and secondly the non-glandular stellate trichomes with intrusive basal cells.

The secretion of alkaloids by glandular hairs of two Solanaceous species, potato (*Solanum tuberosum* L.) and tobacco (*Nicotiana tabacum* L.) have been described (Roshchina & Roshchina, 1993). It is likely that glandular trichomes on *S. elaeagnifolium* leaves may harbour the alkaloidal compound responsible for the allelopathic effects of silverleaf nightshade foliar extracts, and even the phytotoxic saponin or flavonoids. The basal

cells of the intrusive stellate trichome variant seem to reach the vascular bundles, and one of the main concentration sites of alkaloids in plants is the vascular bundle sheath (Roshchina & Roshchina, 1993). It can therefore be considered a possibility that the intrusive stellate trichomes might be involved in excreting alkaloidal compounds situated in the vascular bundle sheath of silverleaf nightshade leaves. These theories, however, still need to be confirmed by further chemical investigation of the exact contents of these trichomes.

Own experiments to evaluate allelopathic potential of silverleaf nightshade made use of foliar extracts where either frozen material was soaked or dried foliage was homogenised in organic solvents for extraction. Concurrent experiments at the University of Pretoria aimed at evaluating phytotoxicity of silverleaf nightshade foliage in soil, made use of ground, dry foliar material incorporated into potted soil in which the test species was sown (Mkula, unpublished). All these methods resulted in extraction or availability of most of the compounds in the entire leaf. Further studies should explore the phytotoxic activity of a leaf wash of fresh intact foliage with water or organic solvents (Duke *et al.*, 1994). Such a leaf wash would contain most, if not all, substances present on the leaf surface, enabling the assessment of whether allelochemicals in silverleaf nightshade are indeed located in structures on the leaf surface or are excreted onto the leaf surface.

CHAPTER 6
GENERAL DISCUSSION AND CONCLUSION

Research investigating interference of silverleaf nightshade with crop species has mainly explored the competition aspect, while literature concerning its possible allelopathic interference with crop species is extremely limited. Published research concentrate solely on phytotoxic activity of saponins in silverleaf nightshade fruit (Curvetto *et al.*, 1976; Agüero *et al.*, 1985). Due to its importance as a weed, it would be of great value to know and understand all mechanisms by which silverleaf nightshade interfere with crops, however significant voids still exist in knowledge concerning the allelopathic potential of this weed. The experiments conducted in this study were aimed at contributing to knowledge of the allelopathic potential of the foliage of silverleaf nightshade.

6.1 Bioassay technique

Numerous different approaches for the execution of germination bioassays in allelopathy, are encountered in literature. Some important conclusions were reached while experimenting to find an appropriate bioassay for evaluating the allelopathic potential of silverleaf nightshade foliage (Chapter 2):

- The use of organic solvents in extraction or fractionation of an extract necessitates evaluation of phytotoxicity of these solvents' residues after evaporation.
- The importance of choosing relevant test species in a bioassay was highlighted.
- It was established that the use of germination paper rolls, known as rag dolls, is not an effective method for evaluating allelopathic potential of extracts on seed germination and seedling growth.
- Maintaining aseptic working conditions are essential in germination bioassays with extracts in Petri dishes, as it prevents fungal growth which may distort results.

- Osmolalities of extracts used in bioassays need to be monitored to exclude the possibility of osmotic inhibition.

6.2 Allelopathic potential of silverleaf nightshade foliage

Previous studies showed the phytotoxicity of compounds in the fruit of silverleaf nightshade towards cucumber and clover (Curvetto et al., 1976; Agüera & Boland, 1985). This study revealed the presence of phytotoxic substances in the foliage of silverleaf nightshade as well. Germination and early root growth of cotton and lettuce were inhibited by crude foliar infusions and water-soluble extracts at low concentrations where osmotic inhibition was not present (Chapter 3). While this does not prove allelopathy, it indicates that foliage of silverleaf nightshade does have the potential to exert allelopathic inhibition on crop species. Roots of lettuce seedlings exposed to the phytotoxic extracts exhibited severe swelling, marking a rapid transition from the meristematic zone to differentiated cells, close to the root tip. Cell division and elongation were inhibited in the root tip and an apical shift of root hair differentiation occurred.

6.3 Preliminary identification of active compounds

In Chapter 4 it was established that more than one chemical fraction of the foliar silverleaf nightshade extract inhibited germination and/or early root growth of the test species. Three fractions, probably flavonoids, on the preparative paper chromatogram significantly inhibited root growth. More pronounced inhibition of root growth as well as germination were caused by the most polar fraction containing alkaloidal compound(s). This fraction proved to contain two phytotoxic compounds, an unidentified alkaloid and a saponin. All these phytotoxic compounds probably act together in the crude extract resulting in a synergistic effect where more pronounced inhibition of germination and growth occurs than is observed with more pure fractions. This theory, however, needs to be substantiated with further chemical investigation.

6.4 Leaf anatomy as linked to allelopathic potential

There are many reports on the occurrence of phytotoxic substances in glandular trichomes of plants (Roshchina & Roshchina, 1993; Duke *et al.*, 1994). It was considered likely that the glandular trichomes observed on the isobilateral, amphistomatic leaves of silverleaf nightshade might contain some of the phytotoxic compounds found in the foliar extract. It was furthermore speculated that the intrusive stellate trichomes, described in Chapter 5, might be involved in excreting alkaloids from the vascular bundle sheath, one of the main alkaloid concentration sites in plants, onto the leaf surface. These speculations, however, still needs to be confirmed by means of chemical analysis of the exact contents of the various trichomes.

6.5 Conclusions and recommendations

Although the results of this study are an indication of the allelopathic potential of silverleaf nightshade, it does not prove allelopathy. Analysis of world literature on allelopathy (Rice, 1984) indicates that there are few examples of verifiable allelopathic influence of intact plants on the growth of other plant species. Therefore, many questions have yet to be addressed in further investigations before more definite conclusions may be drawn. Follow up studies at the University of Pretoria have already established that phytotoxicity of foliage remains intact in the soil environment, resulting in growth inhibition of cotton seedlings (Mkula, unpublished). The next step would be to verify whether the same reaction occurs under field conditions. Proper purification and identification of compounds responsible need to be performed and it should be ascertained whether these compounds are present in soil where silverleaf nightshade interferes with crops.