CHAPTER 1
LITERATURE REVIEW

1.1 Introduction

Solanum elaeagnifolium Cav., commonly known as silverleaf nightshade, is native to the Americas (Boyd *et al.*, 1984), but has spread to many other semi-arid regions over the world. It has become a major problem weed in Australia, Argentina, Greece, India, Morocco, North America and South Africa (Heap & Carter, 1999). Silverleaf nightshade is known by many other vernacular names including meloncillo del campo, tomatillo, white horsenettle, bullnettle, silverleaf horsenettle, tomato weed, sand brier, trompillo, meloncillo, revienta caballo, silverleaf nettle, purple nightshade, whiteweed, western horsenettle, desert nightshade, morelle jaune and silverleaf bitter apple (Boyd *et al.*, 1984; Heap & Carter, 1999). It is also a declared weed in South Africa (Conservation of Agricultural Resources Act no. 43, 1983), where it is best known by its Afrikaans name, "satansbos" (Wasserman *et al.*, 1988).

Crops in which silverleaf nightshade have been reported a problem weed, were summarised by Boyd *et al.* (1984). These include alfalfa (*Medicago sativa* L.), cantaloupes (*Cucumis melo* L. var. *cantalupensis* Naud.), cotton (*Gossypium hirsutum* L.), grain sorghum (*Sorghum bicolor* (L.) Moench), peanuts (*Arachis hypogaea* L.), ragi (*Eleusine coracana* Gaertn.), rice (*Oryza sativa* L.), watermelons (*Citrullus lanatus* (Thunb.) Mansf.) and wheat (*Triticum aestivum* L.).

Research on silverleaf nightshade seems to have focused on chemical and biological control. Few studies have been dedicated to understanding ways in which this weed interferes with crop species. Interference comprises both competition and allelopathic interaction between plants (Putnam, 1985). Interference studies concerning

silverleaf nightshade, however, have primarily explored the competition aspect.

This literature survey will provide an overview of the research that has already been done on interference of silverleaf nightshade with crop species, in order to determine voids in current knowledge. The weed will also be described morphologically and anatomically as background for better insight into the mechanisms of interference. Finally, to establish how effective the battle against silverleaf nightshade is fought, a brief overview of control strategies employed against this weed species will be presented.

1.2 Morphology and biology

Following is a description of silverleaf nightshade as adapted from Cuthbertson *et al.* (1976), Symon (1981) and Boyd *et al.* (1984):

Silverleaf nightshade is an erect, clonal, herbaceous perennial up to 1 m, but more often 40 to 60 cm high with a silvery green appearance. The extensive underground root system is up to 3 m deep, producing usually annual vegetative growth in spring and summer. It has erect stems, branching towards the top. Acicular prickles or spines that are 2 to 5 mm long, straight, fine and often reddish, are usually present on stems, petioles and midribs of leaves. Older plants are sometimes nearly free of prickles. The 1 to 10 cm long leaves are oblong-lanceolate, distinctly sinuate-undulate and lower leaves are approximately 10 by 4 cm in size. The leaves and stems are covered with a close, dense, tomentum of sessile or shortly multiseriate-stalked, porrect-stellate trichomes with a medium or long central ray. The general aspect of these stellate trichomes is silvery-green, rarely rusty and slightly discolourous. According to Roe (1971) the hairs of *S. elaeagnifolium* are rather intermediate between porrect-stellate and peltate. These trichomes are responsible for the plant's dusky silvery appearance. Flowerbuds emerge singly or in small clusters at or near the tips of branches. The sepals may

also have prickles. The flowers are 2 to 3 cm in diameter and blue to violet of colour, rarely pale blue, white, deep purple, or pinkish colours are encountered. The fruit are smooth globular berries of 10 to 15 mm in diameter, each containing approximately 50 seeds. The average plant carries up to 60 berries, producing about 3 000 seeds per plant. The seeds are 3 by 2 mm in diameter, flat or biconvex, light to mid-brown in colour and smooth.

Silverleaf nightshade's high seed production and the reported 60% germination after 10 years of storage at room temperature, illustrate that seed of silverleaf nightshade are important propagules of this species (Boyd & Murray, 1982). However, despite high viability and longevity of seeds, high numbers of seedlings are observed only occasionally (Heap & Carter, 1999). Vegetative reproduction is extremely effective since all parts of the root system can regenerate if cut off or damaged by cultivation (Cuthbertson et al., 1976). Regeneration from roots can occur from as deep as 50 cm in cultivated soils (Monaghan & Brownlee, 1979), and root fragments as short as 10 mm are able to regenerate (Richardson & McKenzie, 1981). Some root fragments can survive for 15 months with a capacity for regeneration (Fernandez & Brevedan, 1972).

Silverleaf nightshade is adapted to a wide range of habitats, a characteristic that contributes to its weediness in diverse regions around the world (Heap & Carter, 1999). The heaviest infestations occur on sandy soils with low organic matter content (Leys & Cuthbertson, 1977). Cool summers and high annual rainfall are important factors which may limit silverleaf nightshade distribution (Panetta & Mitchell, 1991). The plant appears to be susceptible to water-logging (Heap & Carter, 1999) and frost (aerial parts), and is highly resistant to drought (Wasserman et al., 1988).

Detailed descriptions of phenology, seedling development, and seed morphology have been given by Economidou & Yannitsaros (1975).

1.3 Leaf anatomy

Literature discussing the leaf anatomy of silverleaf nightshade is extremely scarce and difficult to obtain.

The leaves of *S. elaeagnifolium* have been described as isobilateral, with an abundance of glandular and non-glandular trichomes on both sides of the leaf (Cosa *et al.*, 1998). Crystal-bearing idioblasts are encountered inside the mesophyll (Pilar, 1937; Bruno *et al.*, 1999).

According to Bruno *et al.* (1999) glandular trichomes consist of a bicellular stalk with a spherical "uni-pluricellular" head, and non-glandular trichomes can be simple or stellate. Stellate trichomes have two variants: one with a stalk emerging from the epidermis surface, and one with an intrusive base growing into the mesophyll. Epidermal stellate trichomes have been described for several *Solanum* species (Seithe, 1979), while the variant with intrusive base has only been described for *S. elaeagnifolium* (Pilar, 1937; Cosa *et al.*, 1998; Dottori *et al.*, 1998; Bruno *et al.*, 1999). Both variants have 8 to 16 horizontal unicellular radii and a prominent vertical apical cell. Apparently, early stages of development are similar in both variants (Bruno *et al.*, 1999). The intrusive variant's stalk cells start to grow, in general without dividing, causing the stalk to intrude aggressively between mesophyll cells, probably as a result of enzymatic disorganization of the middle lamella. Bruno *et al.* (1999) further states that the stalks cells of mature trichomes reach the vascular bundles, even surrounding them. At adult stage, the cell wall of both variants are uniformly thick, with the emerging part strongly lignified, whereas there is little or no lignification in the intrusive portion of the trichome.

Stellate hairs show the greatest range of topographical variants of the trichome types, especially between upper and lower leaf surfaces

(Seithe, 1979). Upper surface trichomes may have a longer central thorn, and sometimes fewer, shorter rays with shorter stalks.

Thus far, the function of these intrusive stellate trichomes has not been elucidated. Bruno *et al.* (1999) speculated that its morphology might be related to the water economy of the plant, as the cells of the stalk have very thick walls, which seem to penetrate to the point of making contact with the xylem. These authors also considered it likely that it has an important structural function, reducing the risk of collapse when the plant is stepped upon.

1.4 Interference of *S. elaeagnifolium* with crop species

Several studies have been conducted on the interference of silverleaf nightshade with crop species, mostly focusing on cotton (Green *et al.*, 1987; Green *et al.*, 1988; Smith *et al.*, 1990, Jacobsen *et al.*, 1994; Westerman & Murray, 1994). Silverleaf nightshade has also been considered one of the most troublesome perennial weeds in cotton (Green *et al.*, 1987; Cilliers, 1999), with reported lint yield reductions of up to 75% under semi-arid conditions with moderate silverleaf nightshade infestations (Abernathy & Keeling, 1979). Striking cereal yield reductions of 4 to 77% (mean 41%) have also been reported (Heap & Carter, 1999). Cuthbertson *et al.* (1976) reported a 12% wheat yield loss in southern New South Wales at silverleaf nightshade densities of 9 shoots m^{-2}, while greater reductions were recorded in Southern Australia where 3 to 5 shoots m^{-2} reduced wheat yield by up to 60% (Lemerle & Leys, 1991). Hackett & Murray (1982) reported a 47 to 65% yield reduction in peanuts. Interference and yield losses appear to be most severe on sandy soils and in seasons with low rainfall (Heap & Carter, 1999).

Green *et al.* (1987) determined the effect that silverleaf nightshade densities might exert on cotton height, lint yield, boll size and fiber

quality. Silverleaf nightshade plants used in the experiment were grown from seed, transplanted to the field at four- to six-true-leaf stage and left to grow for six weeks until a height of approximately 30 cm was reached. All silverleaf nightshade plants were then clipped near the soil surface immediately following cotton planting. Experiments were conducted under dryland and irrigation conditions. Cotton height was significantly reduced at silverleaf nightshade densities as low as four plants per 10 m of cotton row. At the highest weed density of 32 plants per 10 m of row, cotton height was reduced by 25% compared to plants without weed interference. Lint yield decreased as weed density increased, with dryland cotton showing significant decreases at lower weed densities than irrigated cotton. Therefore, it was concluded that irrigated cotton competed more effectively with the weed than dryland cotton, suggesting that soil water was a primary competition factor between silverleaf nightshade and cotton. Linear regression predicted a 1.54% lint yield reduction for each silverleaf nightshade plant per 10 m of cotton row. Increasing silverleaf nightshade density, starting at densities as low as two weeds per 10 m of row, progressively reduced boll size. Fiber properties were not affected.

Smith *et al.* (1990) continued research on the silverleaf nightshade plantings established by Green *et al.* (1987), to assess the influence of more mature silverleaf nightshade stands on cotton yield. The number of stems of one silverleaf nightshade plant per 10 m of cotton row, increased 10 fold after one year and up to 40 fold after two years of uncontrolled growth. As could be expected, yield reductions increased with increasing silverleaf nightshade stand maturity. Linear regression predicted a 9% yield loss for each additional 1 kg dry weight of silverleaf nightshade per 10 m of row for one-year-old stands. From two-year-old stands, a 21% yield loss was predicted for each additional 1 kg dry weight of silverleaf nightshade per 10 m of row. The competitive advantage of irrigated cotton over dryland cotton mentioned in initial experiments (Green *et al.*, 1987), was no longer evident.

8

Water is one of the primary growth factors that plants compete for. Competition between two plants usually begins when their root systems overlap in their exploration for water and nutrients, and is intensified under dryland and semi-arid conditions (Pavlychenko & Harrington, 1935). The competitive ability of weeds can be influenced by soil moisture conditions and, as mentioned above, young silverleaf nightshade plants apparently compete more effectively with dryland than with irrigated cotton (Green *et al.*, 1987). The ability of plants to extract soil moisture is partly dependent on their root distribution in the soil profile (Davis *et al.*, 1965). Silverleaf nightshade roots have been reported at depths of up to 3 m or more (Wasserman *et al.*, 1988), while the rooting depth of cotton seldom exceeds 1.5 m, with the upper 1 m of the soil profile being the principle soil moisture extraction region (Green *et al.*, 1988). Therefore, silverleaf nightshade has the potential to extract moisture from much greater depths in the soil profile than cotton.

Green *et al.* (1988) found that less water was available lower in the soil profile when silverleaf nightshade was grown with cotton. The largest differences in soil moisture depletion between cotton with and without silverleaf nightshade occurred during the flowering and early fruiting stages, a critical time for water demand and utilisation by cotton. Soil water was depleted more rapidly in plots with silverleaf nightshade during this period, except in irrigated, high rainfall conditions. Jacobson *et al.* (1994) confirmed that silverleaf nightshade began extracting soil water earlier in the growing season. In a wet year, soil water loss did not differ between cotton plots with and without silverleaf nightshade (Green *et al.*, 1988). However, even under these conditions cotton lint yield was reduced by 30% at the highest weed density of 32 plants per 10 m of cotton row. It was concluded that competition for available soil moisture is an important factor in silverleaf nightshade interference with cotton. However, there are other important factors that are not accounted for in current research.

1.5 Secondary metabolites of silverleaf nightshade

The Solanaceae contain many groups of secondary metabolites including flavonoids (Harborne & Swain, 1979), a number of triterpenes, tropane alkaloids, steroidal alkaloids, saponins and nicotine types (Seigler, 1981).

Phytochemical studies on silverleaf nightshade 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 most publicised secondary metabolite of silverleaf nightshade is certainly the steroidal glycoalkaloid, solasodine, which is used to produce diosgenin, an important steroidal sapogenin in the manufacture of pharmaceutical corticosteroidal drugs (Heap & Carter, 1999). Maiti and Mathew (1967) reported yields of 3.2% of solasodine from berries (on a dry weight basis) in India, while lower amounts of 1.5% to 1.85% for green and ripe berries respectively, were reported in Egypt by Hanna *et al.* (1996b). Bradley *et al.* (1978) reported that green fruit of field-collected Australian silverleaf nightshade plants contained 1.6% and ripe fruit 1.7% solasodine, while no solasodine was present in leaves and stems. The saponin, diosgenin, and 3-deoxy-Δ^3-diosgenin were obtained as final products by processing *Solanum elaeagnifolium* foliage (leaves and twigs) collected during flowering, as well as from samples collected after the fruit had ripened (Guerreiro *et al.*, 1971). The percentage of both compounds in the first sample doubled the amount shown in the second sample. Unripe and seasoned fruit yielded both mentioned compounds as well as solasodine, and the alkaloid percentage in unripe fruit doubled the amount obtained from seasoned fruit.

Studies also revealed the presence of other steroidal glycoalkaloids, including solamargine, found in the unripe fruits of silverleaf nightshade (Guerreiro et al, 1973), solasurine, and solanelagnin, isolated from the stalks of this weed (Hanna *et al.*, 1996b). The presence of these compounds renders the plant even more valuable as a potential source of raw material for the steroid industry (Hanna *et al.*, 1996b).

There has been considerable research and interest in the domestication of silverleaf nightshade for commercial production of steroidal drugs in India and Argentina (Kanna & Singh, 1987; Heap & Carter, 1999). Maiti & Mathew (1967) considered silverleaf nightshade to be the most promising source of solasodine of 28 *Solanum* species studied, due to its high yield and few thorns. Research has even explored breeding qualities, as well as multiplication techniques and cultivation potential for this species (Khanna & Singh, 1987; Trione & Cony, 1988).

Chiale *et al.* (1991) first reported flavonoids from this species. These authors isolated the flavonoid kaempferol, and kaempferol 3-glucoside, as well as a new monoacylated flavonoid glucoside characterized as kaempferol 3β-D-(6"-O-*cis*-cinnamoylglucoside), all from the aerial parts of silverleaf nightshade.

Hanna *et al.* (1996a) found that the sterol content of the seeds and stems of S. *elaeagnifolium* composed mainly from squalene, campa-sterol, stigmasterol and β-sitosterol. A little amount of cholesterol (5.16%) was present only in the stems.

1.6 Biological activity of silverleaf nightshade

1.6.1 Antimicrobial activity

Hanna *et al.* (1996a) examined the antimicrobial activity of ethanolic extracts of S. *elaeagnifolium* seeds and stems. The test

11

organisms included gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, gram-negative bacteria, *Escherichia coli*, one species of pathogenic yeast, *Candida albicans*, and the pathogenic fungus *Aspergilus niger*. The seed extract exhibited very high activity against *E. coli*, while it inhibited *S. aeraus* moderately. The stem extract was highly active against both *E.coli* and *S. aureus*. Both seed and stem extracts showed moderate inhibition against *B. subtilis*, while no inhibition of *C. albicans* or *A. niger* was obtained by either extract.

1.6.2 Phytotoxic activity

The steroidal saponins, glycoside derivatives of steroids, of silverleaf nightshade have been reported to be involved in chemical interactions with other plant species.

The only report on allelopathic effects of silverleaf nightshade on other plant species was by Curvetto *et al.* (1976). They reported that an aqueous solution of the saponins extracted from silverleaf nightshade fruits, gradually reduced cucumber (*Cucumis sativa* L.) growth. They further discovered that fruit from which the pericarp had been removed, and were placed in petri dishes with soil, interfered with germination and seedling development of several crop and weed species. These saponins were not chemically identified.

Agüera & Boland (1985) obtained evidence suggesting that unidentified saponins from *S. elaeagnifolium* fruit extracts act directly on clover root membranes, altering their properties. After treatment with the saponin extract from silverleaf nightshade berries, Ca^{2+}-ATPase activity and ATP-dependent calcium uptake was inhibited *in vitro* in clover root fragments. During *in vivo* experiments pretreatment of clover seedlings with the steroidal saponin extracts, at concentrations equivalent to 15 µM diosgenin,

stimulated calcium uptake without significantly altering potassium and phosphate uptake. However, concentrations 10 times lower resulted in a selective inhibition of calcium transport.

1.7 Overview of control strategies

Over the decades a wide array of herbicides have been screened for efficacy in controlling silverleaf nightshade. The possibility of chemical control of silverleaf nightshade was investigated in the USA as early as the late thirties, while the first documented trials in South Africa started in 1952 (Wasserman *et al.*, 1988). Although instances of success have been recorded, there are few weeds which have withstood the onslaught of chemical research as effectively as silverleaf nightshade (Heap & Carter, 1999). Herbicides have been effective in killing shoots, however regeneration from roots occur during the following year (Lemerle & Leys, 1991). There is still no registered herbicide for control of silverleaf nightshade.

Because of the extensive root system, plants recover from conventional slashing and cultivation methods for weed control, and cultivation methods might even aid in spreading the weed (Cuthbertson *et al.*, 1976). The inability of cultural or chemical methods to control silverleaf nightshade has made it a major candidate for biological control in many countries including the USA, South Africa and Australia (Heap & Carter, 1999). Many research efforts have been dedicated to identifying and evaluating natural enemies of silverleaf nightshade as possible biocontrol agents.

Neither mechanical, chemical nor biological control methods have achieved wide-spread success in controlling silverleaf nightshade. Wasserman *et al.* (1988) identified the major reason for failed silverleaf nightshade eradication campaigns, to be inadequate knowledge of the

13

weed itself. Priorities in actions against the silverleaf nightshade problem according to these authors, include:

- Country-wide surveys on the present incidence of silverleaf nightshade and the views of farmers in infested areas.
- Registration of suitable herbicides.
- Efficient dissemination of existing knowledge on the control of silverleaf nightshade among producers.
- In-depth ecological and physiological studies of the plant and its seed.
- Intensification of efforts aimed at the biological control of this weed.

Currently integrated programmes of various control methods are recommended to limit the spread of this weed (Heap & Carter, 1999).