

## HEPATOGENOUS PHOTOSENSITIVITY DISEASES IN SOUTH AFRICA

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### ABSTRACT

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Various hepatogenous photosensitivity diseases of ruminants in South Africa, caused by plants, fungi and an alga, are described. Information is given on botanical, mycological, toxicological, clinical and pathological aspects of the diseases. The intoxications were grouped according to the primary site of involvement and type of lesions in the liver. The aetiology, pathogenesis, and diagnosis of these conditions received special attention and the most important features are illustrated in colour.

### INTRODUCTION

The most significant contribution made by members of this Institute in the field of toxicology must surely be the store of knowledge on plant poisoning which they have built up over the years. This knowledge was not easily acquired since the toxicoses produced by many indigenous plants such as *Pachystigma pygmaeum* were quite different from anything known overseas. Other plants again, such as the fungus *Diplodia maydis*, despite their cosmopolitan nature, caused problems only in Southern Africa. South African investigators, therefore, more often than not, having no precedent to work on, could rely only on their own resources to find solutions to these unique toxicological problems. This paper on hepatogenous photosensitivity is a small tribute to those chemists, botanists, mycologists and veterinarians who accepted the challenge.

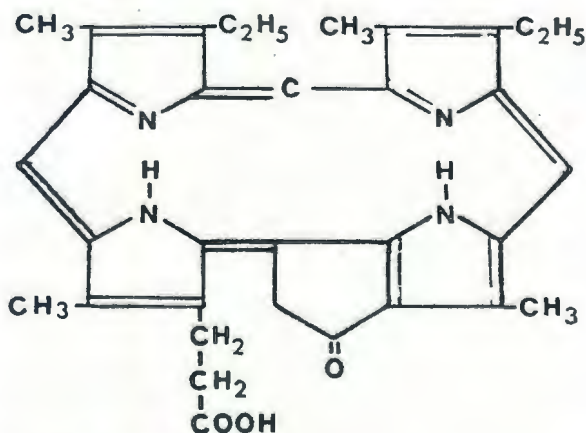


FIG. 1 Pheophorbtyrin

Hepatogenous photosensitivity of ruminants in South Africa can be induced by several hepatotoxic plants, 2 fungi and an alga (Steyn, 1949; Kellerman, Basson, Naudé, Van Rensburg & Welman, 1973). Generally speaking, these syndromes can be divided into 2 groups depending on whether the parenchyma or biliary system is the one primarily affected. In both instances the liver damage is of a type which results in the retention of pheophorbtyrin (Fig. 1), a photodynamic porphyrin derived from the degradation of chlorophyll by micro-

organisms in the gut (Rimington & Quin, 1934; Quin, Rimington & Roets, 1935). Where pheophorbtyrin comes into contact with sunlight, for instance, in the exposed, unpigmented parts of the body, such as the face of a Merino sheep, it reacts with rays of a certain wavelength to cause severe photosensitivity (Clare, 1952, 1955).



FIG. 2 Oedema of face, immobilization of lips by necrotic skin, icterus and coronitis

Photosensitivity (Fig. 2) is clinically characterized by a tendency on the part of the sheep to avoid sunlight, coupled with pruritis, erythema and swelling of the affected parts. Eventually the skin becomes leathery or parchment-like, often immobilizing the lips or eyelids before it sloughs off. Since pheophorbtyrin, like bilirubin, is excreted in the bile, hepatogenous photosensitivity is almost invariably accompanied by some degree of icterus. In all but the most acute cases coronitis is present (Theiler, 1918; Steyn, 1928; Van Tonder, Basson & Van Rensburg, 1972).

Some of the most important livestock diseases in South Africa are photosensitizations of this type.

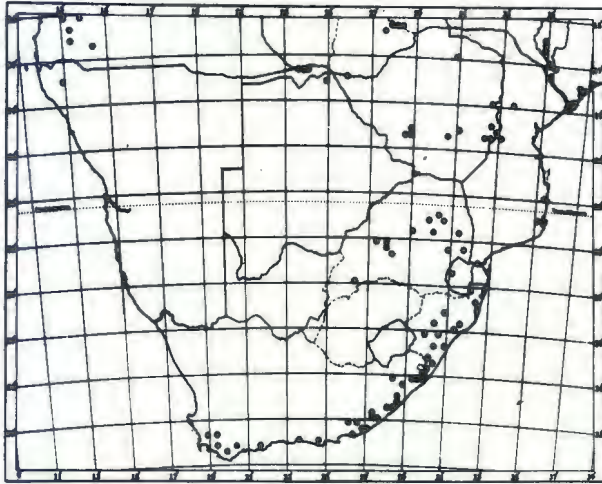


PHOTOSENSITIVITY RESULTING FROM DAMAGE  
PRIMARILY TO THE LIVER PARENCHYMA

The majority of photosensitivity diseases belong to this group, the principal of them being *Lantana* poisoning.

*Lantana camara* (L.) (Verbenaceae)

'Lantana'

FIG. 3 Distribution of *L. camara* (Vahrmeijer, 1981)FIG. 4 *L. camara*

*Lantana* is an exotic ornamental shrub that has become a noxious weed, particularly in the moist eastern parts of the country (Fig. 3). The small, trumpet-shaped, yellow to orange, red and mauve to white, flowers are borne in dense terminal clusters usually with flowers of 2 different colours occurring in one cluster. The fruits are small black berries, much relished by the birds that spread the seeds (Fig. 4) (Vahrmeijer, 1981).

*Lantana camara* and 2 related members of the Verbenaceae, *Lippia rehmannii* and *L. pretoriensis*, cause photosensitivity when dosed to sheep (Quin, 1933a; Steyn & Van der Walt, 1941). The active principle of *L. camara*, lantadene A, was isolated by Louw (1943, 1948, 1949), and those from *L. rehmannii*, icterogenins A, B and C, by Rimington and co-workers (Rimington & Quin, 1935; Rimington, Quin & Roets, 1937). In addition, Barton & De Mayo (1954) extracted rehmannic acid from the roots of *L. rehmannii*. All the aforementioned compounds are pentacyclic triterpene acids. The structures of the icterogenic agents, i.e. icterogenin (Fig. 5), rehmannic acid and 22- $\beta$ -angeloyloxy oleanolic acid, were determined by Barton & De Mayo (1954) and An-

derson, De Kock & Enslin (1961). A 22 $\beta$ -angeloyloxy side chain in ring E and a hydroxyl group, preferably in a 3 $\beta$  position on the A ring, were shown to be essential for icterogenic activity (Brown, Rimington & Sawyer, 1963; Brown & Rimington, 1964). The toxicity of *Lippia* plants can be influenced by factors such as pruning and weather, supposedly by affecting the synthesis and/or translocation of toxin between the leaves and roots (Roets, 1937).

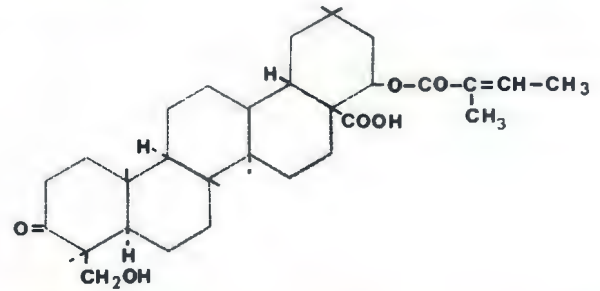
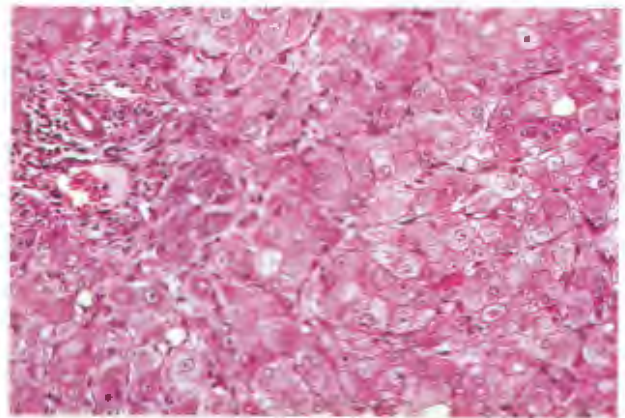


FIG. 5 Icterogenin

FIG. 6 Well-delineated swollen hepatocytes showing degenerative changes: HE  $\times$  450

Under natural conditions *L. camara* poisoning occurs almost exclusively in cattle. Other *Lantana* spp. are not of practical importance, nor are *L. rehmannii* and *L. pretoriensis*, which, despite containing pentacyclic triterpenes, seldom cause poisoning.

The macroscopical lesions are typical of hepatogenous photosensitivity, namely, icterus, photodermatitis, hepatitis, impaction of the ceacum and colon, and nephrosis. The gall bladders may be distended and oedematous (Seawright & Allen, 1972).

The histopathological changes (Seawright, 1963, 1964, 1965; Gonipath & Ford, 1969; Seawright & Allen, 1972; Seawright & Hrdlicka, 1977), involving the hepatocytes include swelling, distinct delineation of cell membranes, diffuse cloudy swelling and hydropic degeneration, and sometimes also mild to moderate fatty changes. Intracytoplasmic eosinophilic globules often accompany the above changes (Fig. 6). Individual hepatocytes can be necrotic, while, in more advanced cases, foci of coagulative necrosis may be distributed throughout the parenchyma. The nuclei of some hepatocytes may be enlarged and may show mitosis; other cells may be multinucleated, and nuclei sometimes contain pseudoinclusions or have prominent nucleoli. A mild portal reaction consisting of bile duct proliferation, fibrosis and mononuclear cell infiltration as well as bile stasis, is not uncommon in this poisoning.



Kidney lesions, which include vacuolation and degeneration of convoluted tubules, dilatation of tubules and the presence of various casts, have been described. In more chronic cases focal interstitial mononuclear cell infiltration and fibrosis may be seen.

*Asaemia axillaris* (Thunb.) Harv. ex Jackson  
(Asteraceae)

'Vuursiektebossie'



FIG. 7 *A. axillaris*



FIG. 8 *A. axillaris* growing near water

This is a many-branched, indigenous woody shrublet, growing up to 50 cm in height (Fig. 7 & 8). Small narrow leaves are arranged opposite each other on pale yellowish branches that end in spikes. The small yellowish to white flower heads are sessile (Fig. 7) (Kellerman *et al.*, 1973; Vahrmeijer, 1981).

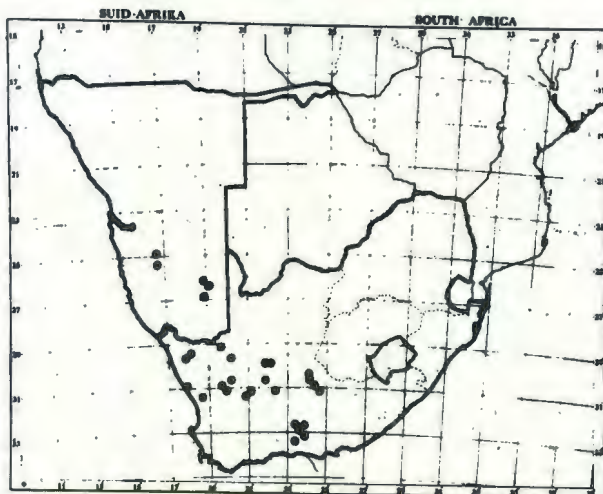


FIG. 9 Distribution of *A. axillaris*

Acocks (1953) described the "Vuursiektebossie" as a species typical of the Upper or Typical form of the Western Mountain Karoo extending into the Central Lower and False Arid Karoo (Fig. 9). Although an inhabitant of arid regions, it always grows near water, e.g. in the vicinity of dams, pans, vleis and rivers (Fig. 8).

*A. axillaris* has long been known from field observations to cause ovine hepatogenous photosensitivity (Steyn, 1949; D. G. Steyn, personal communication, 1973). Van der Walt & Steyn (1939) described a case of experimental *A. axillaris* poisoning where a sheep developed liver damage and icterus. Later it was confirmed that photosensitivity was part of the syndrome (Kellerman *et al.*, 1973).

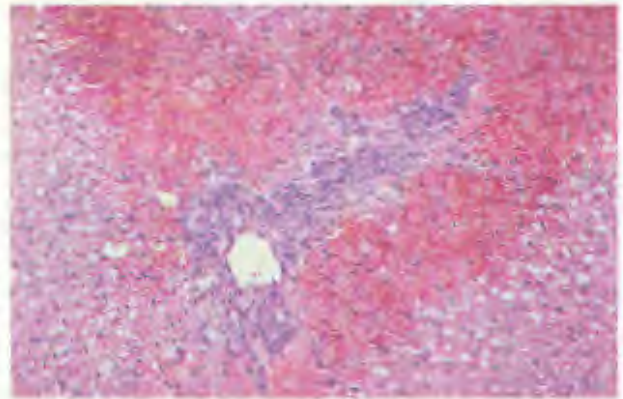


FIG. 10 Periportal necrosis and haemorrhage: HE  $\times$  200

Centrilobular necrosis, involving at least two thirds of the lobules, was observed in the liver of a sheep peracutely poisoned with 10 g/kg of the dried plant. Two sheep, dosed at lower levels (2.5 g/kg and 5.0 g/kg), died actually after becoming mildly photosensitive.

The principal lesions observed were prominent periportal fatty degeneration, midzonal coagulative necrosis and milder centrilobular changes (Kellerman *et al.*, 1973).

In a subsequent experiment, Coetzer & Bergh (1983) studied the hepatic lesions of 3 sheep forced to graze in a camp heavily infested with *A. axillaris*. They became sick 3–7 days after being introduced into the camp, were apathetic, had mildly swollen lips and produced intensely yellow urine. Marked peripheral coagulative necrosis and haemorrhage were evident in the liver lobules of one of the sheep. Examination of another sheep revealed marked ductular proliferation, parenchymal degeneration and scattered single cell necrosis. Hepatocellular unrest (anisocytosis, anisonulceosis, increased mitosis and binucleation) fatty metamorphosis and bile stasis completed the histopathological picture.

The results obtained by Kellerman *et al.* (1973) and Coetzer & Bergh (1983) showed that the liver lesions in *A. axillaris* poisoning (Fig. 10) ranged from distinct zonal necrosis (peripheral, midzonal and centrilobular) to diffuse degeneration and hepatocellular unrest. These changes were often accompanied by ductular proliferation and cholestasis.

Since the only documented, histopathologically confirmed outbreak of *A. axillaris* poisoning occurred in October, when the veld was bare (Coetzer & Bergh, 1983), it would appear that the plant is eaten only when sheep are driven by necessity to do so.



***Lasiospermum bipinnatum* (Thunb.) Druce**  
(Asteraceae)  
'Ganskweek'



FIG. 11 *L. bipinnatum*



FIG. 12 *L. bipinnatum* flower heads

*L. bipinnatum* is a soft-leaved, decumbent to erect herbaceous plant, standing about 40 cm high (Fig. 11). The capitulae of diameter *c.* 3 cm are borne terminally on long (up to 30 cm), seldom branched peduncles. The ray florets are white to purplish-pink and the disc florets are yellow (Fig. 12) (Adelaar, Terblanche, Smit, Naudé & Codd, 1964; Kellerman *et al.*, 1973).

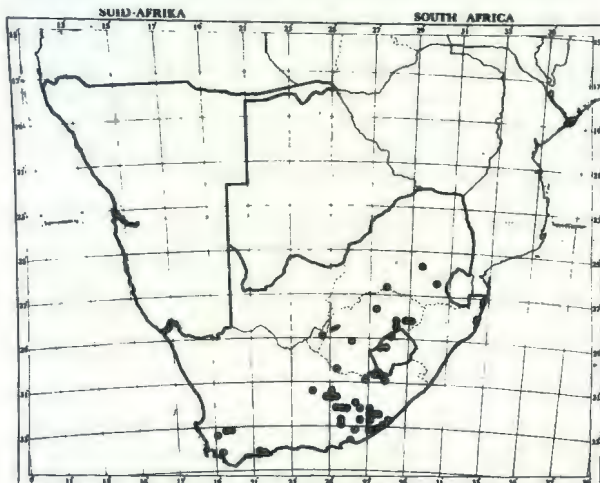


FIG. 13 Distribution of *L. bipinnatum*

Although the plant is widely distributed (Fig. 13), *L. bipinnatum* has not been recorded in Natal. It seems to prefer vleis, but can grow almost anywhere (Adelaar *et al.*, 1964).

Adelaar *et al.* (1964) demonstrated that it was hepatotoxic to sheep. They gave the acute minimum lethal dose of milled, dried material as approximately 7.0–9.7 g/kg. Doses of 6.7 g/kg per day were lethal in 5 days. Kellerman *et al.* (1973) confirmed experimentally that the plant could induce ovine hepatogenous photosensitivity.

Two outbreaks of bovine *L. bipinnatum* poisoning have been reported in the literature, one near Bethlehem in the Orange Free State (Fair, Tustin & Adelaar, 1970) and the other in the vicinity of Graaff-Reinet (Thornton, 1977). Thornton concluded that poisoning with this plant was one of the most important intoxications of sheep and cattle in the Graaff-Reinet district, especially in winter. The plant, apparently being more resistant to frost than grasses, can be abundant in late winter or spring when grazing is sparse (Adelaar *et al.*, 1964).

The clinical signs, apart from icterus and photosensitivity, may include colic (kicking at abdomen etc.), ruminal stasis, constipation (hard, blood-covered faeces in rectum), tenesmus and high temperature (41 °C) (Adelaar *et al.*, 1964; Fair *et al.*, 1970; Kellerman *et al.*, 1973; Thornton, 1977). Signs can occur within 3 days of the animal's being introduced into a toxic camp (Thornton, 1977).

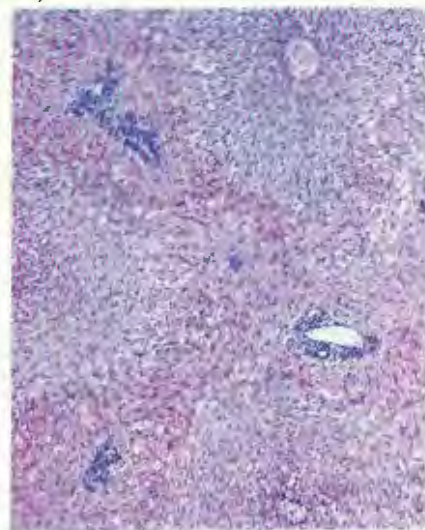


FIG. 14 Periportal necrosis and haemorrhage: HE × 200

The principal histopathological lesions in the liver lobules of the 1 sheep poisoned by Kellerman *et al.* (1973) consisted of a peripheral zone of fatty degeneration, a midzonal area of coagulative necrosis and a centrilobular area that was mildly affected. Adelaar *et al.* (1964) observed that in less acute cases there was a constant tendency for the lesions to be confined to the periphery of the lobules. Marked peripheral necrosis was reported by Fair *et al.* (1970). P. Jordaan & J. A. W. Coetzer (unpublished data, 1983) regarded this lesion as the most characteristic one of acute *L. bipinnatum* poisoning (Fig. 14). In subacute poisoning, instead of zonal necrosis, a range of changes, including marked bile duct proliferation, portal fibroplasia, anisonucleosis and diffuse parenchymal degeneration, was seen.

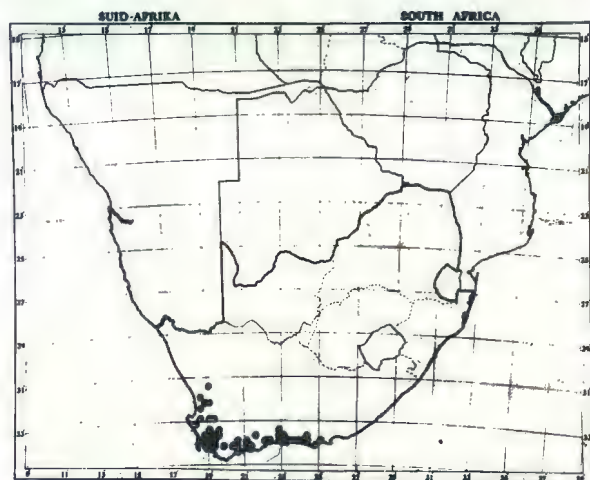


***Athanasia trifurcata* L. (Asteraceae)**

'Klaaslouwbos'

FIG. 15 *A. trifurcata*FIG. 16 *A. trifurcata* flower head

*A. trifurcata* is a perennial, erect, relatively unpalatable, aromatic shrub, growing about 1,3 m high, with dark or bright-green to grey-green leaves borne alternately. The leaves, up to 40 mm long, vary in size and are 3-5 lobed in the upper part. The flower heads are bright yellow (Fig. 15 & 16) (Kellerman, Coetzer, Schneider & Welman, 1983).

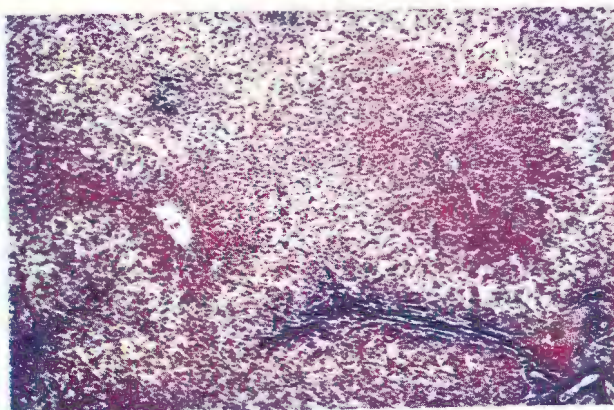
FIG. 17 Distribution of *A. trifurcata*

The plant is common in the south-western and south-eastern Cape Province (Fig. 17), where it grows, preferably on sandy soils, near the coast. Acocks (1975) lists *A. trifurcata* as one of the important species in the Coastal Renosterbosveld. It can be a troublesome weed on lands and overgrazed veld (Kellerman *et al.*, 1983).

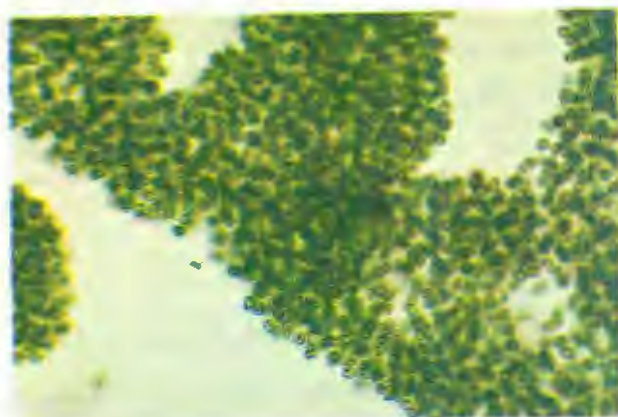
A sheep died after being given 10 g/kg of dried, milled plant from a toxic pasture (*vide infra*) on 2 successive days. Another became photosensitive after receiving 7 doses of 2,5 g/kg in 10 days (Kellerman *et al.*, 1983).

The only published account of an outbreak of *A. trifurcata* poisoning was of one investigated near Grabouw (Cape Province) by Dr D. J. Schneider. A number of ewes died and a few became photosensitive on a paddock heavily infested with the plant. Clinical signs appeared within 2 days after the introduction of a large flock into the camp to non-selectively graze down the weeds (Kellerman *et al.*, 1983).

The most notable clinical signs were apathy, anorexia, ruminal stasis, icterus and photosensitivity (Kellerman *et al.*, 1983).

FIG. 18 Centrizonal coagulative necrosis bordered by a zone of lytic necrosis and degenerated hepatocytes around triads: HE  $\times$  75

Depending on the levels and intervals of dosing different patterns of zonal necrosis (peripheral, midzonal, centrizonal) were seen in the various animals (Fig. 18). In the sheep that received the lowest dose of toxic material, the necrosis was not zonally distributed, but consisted mostly of multifocal areas of necrosis, accompanied by cloudy swelling or hydropic degeneration and unrest of hepatocytes. This sheep was the only one to become photosensitive (Kellerman *et al.*, 1983).

***Microcystis aeruginosa* forma *aeruginosa***FIG. 19 *M. aeruginosa*

Toxic specimens of this blue-green alga were identified by Stephens (1949) as *M. toxica* sp. nov. The pin-head-sized colonies of this alga are composed of nume-



rous minute (3,5–7 $\mu$ m) cells embedded in a mucilage (Fig. 19). On calm, hot, fine days the algae rise to the surface to form a lettuce-green scum that is blown by the wind against the leeward banks (Fig. 20) of dams, pans, etc.



FIG. 20 *M. aeruginosa* scum on eutrophied dam

Here the accumulated mass of algae may die to release a beautifully hued blue to purple chromoprotein, phycocyanin. The stench arising from the decomposing algae can be unpleasant (Steyn 1945a, 1945b, 1949; Stephens, 1949; Louw, 1950).

*M. aeruginosa* is a cosmopolitan alga (Ashworth & Mason, 1946; Gorham, 1964; McBarron & May, 1966; Heany, 1971) widely distributed in South Africa (Scott, 1974) where it causes stock losses principally in the south-eastern Transvaal and north-eastern Orange Free State (Steyn 1945b, 1949). As far as is known, outbreaks have not been recorded outside these 2 provinces. Periodic incidents of poisoning occur around the Vaal-dam (Steyn, 1945a) and Hartbeespoort Dam (T. S. Kellerman & J. A. W. Coetzer, unpublished data, 1974).

According to Steyn (1944, 1945a, 1945b), the alga produces 2 poisons; one a photosensitizing agent, phycocyanin, and the other a hepatotoxin. He suggested as an alternative that, if phycocyanin was not responsible for the photosensitivity, then it was caused by phylloerythrin (Steyn 1945b). In the light of Clare's (1955) doubts as to the photodynamic properties of phycocyanin and the recent demonstration of phylloerythrin in the blood of a sheep poisoned with *M. aeruginosa* (T. S. Kellerman & J. A. W. Coetzer, unpublished data, 1983), the latter supposition appears to be correct.

Louw (1950) isolated an alkaloid from *M. toxica* which is now regarded as not being the primary toxin. Recent work has shown that the hepatotoxin(s) of *M. aeruginosa* (Steyn, 1945a, 1945b; Ashworth & Mason, 1945) are, in fact, peptides (Gorham, 1964; Toerien, Scott & Pitout, 1976; Elleman, Falconer, Jackson & Runnegar, 1978). The sequence of the amino acids in a peptide isolated from a South African strain of *M. aeruginosa* has been determined (Santikarn, Williams, Smith, Hammond, Botes, Tuinman, Wessels, Viljoen & Kruger, 1983).

The toxin is released by the dead algae into the water where, despite being relatively heat stable (Steyn, 1944), it fairly rapidly breaks down (Stephens, 1949). Conventional purification processes may not completely detoxify water (Hoffmann, 1976).

Not all strains of *M. aeruginosa* are toxic (Hughes, Gorham & Zehnder, 1958). In addition to the relative toxicogenicity of a particular algal strain, toxicity is depen-

dent on the concentration of algae in the water. For this reason most poisoning occurs on the leeward verges of dams where algal concentrations are highest. Low temperatures (causing the algae to sink) and water turbulence (that disperses the algae) may also conceivably play a role. Calm, shallow, eutrophic water and fine, hot, sunny weather promote, algal growth (Steyn 1945a, 1945b).

Horses, cattle, sheep, dogs, turkeys, ducks and fish purportedly have been poisoned by algae in South Africa, but ruminants are the species most commonly affected (Steyn, 1949). In a recent dosing trial, dried alga was still toxic after storage for c. 10 years at 4 °C. The material was obtained by evaporating to dryness in the sun, algal suspensions collected during an outbreak at Hartbeespoort Dam. An oral dose of 2 g/kg killed a sheep in less than 24 h, while 1 out of 2 sheep receiving 1 g/kg died within 48 h. At these doses, death occurred before photosensitivity could set in. Photosensitivity, however, was induced within 4 days by dosing as little as 0,5 g/kg to a lamb on 3 successive days (T. S. Kellerman & J. A. W. Coetzer, unpublished data, 1983).

Steyn (1945a, 1945b, 1949) lists the principal signs of acute poisoning as paralysis and convulsions, but nervous signs were not seen in the recent investigation. Less acutely affected animals display constipation (faeces hard and covered with blood), weakness, a drop in milk yield and photosensitivity. Apathy, icterus, inappetence and ruminal stasis complete the clinical picture (Steyn, 1945a, 1945b, 1949; T. S. Kellerman & J. A. W. Coetzer, unpublished data, 1983).



FIG. 21 Yellow, friable liver

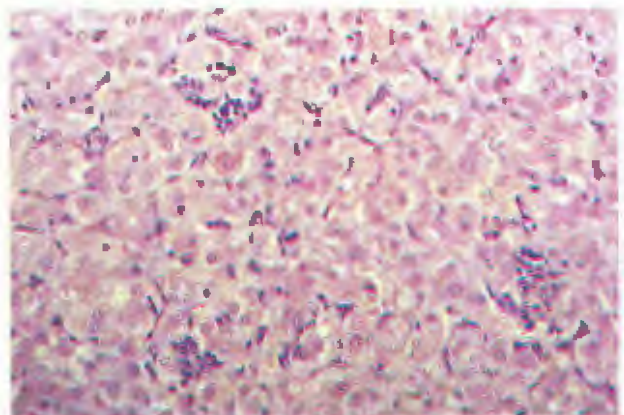


FIG. 22 Diffuse fatty metamorphosis caused by *M. aeruginosa* intoxication



Massive hepatic necrosis was evident in the sheep peracutely intoxicated as described above. The lesions in subacutely affected sheep that became photosensitive were moderate to severe fatty degeneration of hepatocytes, necrosis of individual or small foci of hepatocytes, formation of intracytoplasmic eosinophilic globules in some hepatocytes and pigmentation of Kupffer cells, especially in the centrilobular areas (Fig. 21, 22). In addition, mild centrilobular fibrosis was observed in the more chronically affected sheep (T. S. Kellerman & J. A. W. Coetzer, unpublished data, 1983).

Copper sulphate can control the bloom (Steyn, 1945b, 1949).

#### *Phomopsis leptostromiformis* (Kühn) Bubák

*P. leptostromiformis* is a phytopathogen of certain *Lupinus* spp. (Fig. 23) and can also grow saprophytically on its dead host. In the living plant it forms yellowish to brown, sunken lesions, containing black stromatic pycnidia, mostly on the lower stems. Dark-brown, water-soaked spots, often with the pycnidia concentrically arranged, appear on the pods. A coarse white mycelium, which may be visible on the pods, eventually turns them a dark-brown, colour (Fig. 24). The infected seeds are brown and shrivelled (Marasas 1977, 1978).



FIG. 23 Field of lupins



FIG. 24 Pods infected by *P. leptostromiformis*

The fungus causes lupinosis (Van Warmelo, Marasas, Adelaar, Kellerman, Van Rensburg & Minné, 1970), a hepatotoxicosis primarily of sheep, but also of cattle and more rarely of horses and swine (Gardiner, 1967; Van Rensburg, Marasas & Kellerman, 1975, Marasas 1978).

It has been reported in several countries, including Germany (Kühn, 1880), New Zealand (Brash, 1943), South Africa (Van Warmelo *et al.*, 1970) and Australia (Gardiner, 1967). In South Africa, the disease occurs in the Winter Rainfall Area of the Cape Province where lupins are widely cultivated as a green and forage crop (Groenewald, Smit & Adelaar, 1954, Van Warmelo *et al.*, 1970).

During October 1969, 530 ewes out of 850 died of lupinosis on a field of white lupins (*L. albus*) in the Hermon district. Examination of the field revealed that the sheep had grazed principally on pods, and many of them had typical signs of *P. leptostromiformis* infection. Rainfall and temperature in the area had been above normal for the month (Van Warmelo *et al.*, 1970).

*P. leptostromiformis*, isolated from plant material collected in the toxic field and cultured on sterile lupin seeds, caused typical signs of the disease in sheep (Van Warmelo *et al.*, 1970). This was the first experimental induction of lupinosis by dosing pure cultures of a fungus to animals.

Van Warmelo *et al.* (1970) reported that an oral dose of 2.5 g/kg of culture administered daily for 5 days caused the death of a sheep in 10 days. A higher dose of 7.5 g/kg per day was fatal in 5 days. The LD<sub>50</sub> of another culture was estimated to be approximately 4 g/kg (T. S. Kellerman & W. F. O. Marasas, unpublished data, 1971).

Crystalline phomopsin A, the principal mycotoxin responsible for lupinosis (Culvenor *et al.*, 1977), has now been characterized as a cyclic hexapeptide (Culvenor *et al.*, 1983).

Sheep usually become sick within 2–3 days of receiving a lethal dose, and die about 4–8 days later. The clinical signs include anorexia, apathy, weakness, ruminal stasis, constipation and icterus, occasionally accompanied by photosensitivity (Gardiner, 1967; Van Warmelo *et al.*, 1970). Photosensitivity, however, is not a very prominent feature of the syndrome in South Africa (Kellerman *et al.*, 1973). In more chronic intoxication, the course of the disease is prolonged and, in addition to the usual signs, there is a reduction in body mass (Gardiner, 1967).



FIG. 25 Yellow liver showing marked fatty degeneration

The most conspicuous gross lesions in sheep that die from acute lupinosis are hepatosis (Fig. 25), icterus, gastrointestinal stasis and haemorrhages. In subacute to chronic cases, the liver may be markedly atrophic (particularly on the left side) and fibrotic, with nodular regeneration giving rise to the so-called "boxing glove" liver (Gardiner, 1967; Van Warmelo *et al.*, 1970).



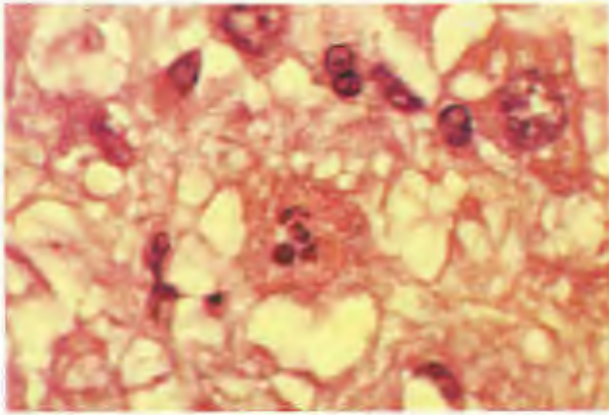


FIG. 26 Diffuse fatty degeneration of hepatocytes. Note mitotic figure: HE  $\times$  200

The histopathological lesions in the liver of acute cases include hepatocellular fatty degeneration (Fig. 26), the formation of eosinophilic globules in their cytoplasm and anisonucleosis, megalocytosis, multinucleation and vesiculation. Some hepatocytes and Kupffer cells may be pigmented. In chronic ovine intoxication, prominent centrilobular fibrosis is evident (Gardiner, 1967; Van Warmelo *et al.*, 1970).

The disease can occur in sheep grazing both sweet and bitter varieties of *L. angustifolius*, *L. albus* and *L. luteus*, the 3 species commonly cultivated in South Africa (Groenewald *et al.*, 1954; Van Warmelo *et al.*, 1970; Marasas, 1978). Control of the disease would ideally be achieved by breeding varieties of lupins resistant to the fungus. *L. angustifolius* is more resistant to infection by the fungus than *L. albus* or *L. luteus*, but this resistance breaks down when the plants become senescent, with the result that the saprophytic growth on the dead plants is similar for all species (Van Jaarsveld & Knox-Davies, 1974; Marasas, 1978). *L. mutabilis* has reportedly the highest resistance to both infection and subsequent saprophytic colonization by *P. leptostromiformis*, a property that might be useful in future lupin breeding programmes (Van Jaarsveld & Knox-Davies, 1974; Marasas, 1978).

Until resistant varieties become available, control should aim at preventing the ingestion of toxic plants, but this would be difficult to apply in practice, as the factors required by the fungus for growth and toxin production are not yet well enough understood for the toxicity of pastures to be forecast. Nevertheless, mature pastures should be avoided after heavy, prolonged rains accompanied by above normal ambient temperatures (Gardiner, 1967; Marasas, 1978).

#### Stellenbosch photosensitivity

This is an ovine hepatogenous photosensitivity syndrome of unknown aetiology encountered in the Winter Rainfall Area, usually in spring or early summer. On some farms the disease is almost an annual occurrence with a morbidity of about 1% to 50% and a mortality of up to c. 25%. The mortality, however, is usually low. Sheep, especially lambs, may become photosensitive within 48 h of being introduced into a 'toxic' pasture. The pastures on which outbreaks occur vary widely in kind (clovers, oats, lucerne, ryegrass, etc.). No unifying or common factor has been observed between outbreaks that might point to a possible causal agent. Although, amongst others, sporidesmin-producing strains of *Pithomyces chartarum* have been isolated from these pastures during outbreaks, it is not thought that sporidesmin plays a role in the aetiology of the disease. Militating against

its involvement is the short latent period and unusual hepatic lesions. The histopathological lesions vary from necrosis of individual hepatocytes or haphazardly scattered foci of hepatocellular necrosis to diffuse necrosis or fatty degeneration of the hepatocytes. A portal reaction consisting of mild to severe bile duct proliferation may be evident. At present the most likely causal agent is thought to be an unidentified toxic fungus (T. S. Kellerman, J. A. W. Coetzer, G. C. A. van der Westhuizen, W. F. O. Marasas & D. J. Schneider, unpublished data, 1975).

#### PHOTSENSITIVITY RESULTING FROM DAMAGE PRIMARILY TO THE BILIARY SYSTEM

Although only a minority of photosensitivity diseases belong to this group, they are of major economic importance.

#### *Pithomyces chartarum* (Berk & Curt.) M. B. Ellis



FIG. 27 Conidia of *P. chartarum*

The fungus produces rough, barrel-shaped, dark-brown conidia (Fig. 27), borne singly on short simple conidiophores. They can be distinguished from like spores by normally having 3 transverse septa and 1–2 longitudinal septa dividing the middle cells (Marasas & Schumann, 1972; Di Menna, Mortimer & White, 1977).

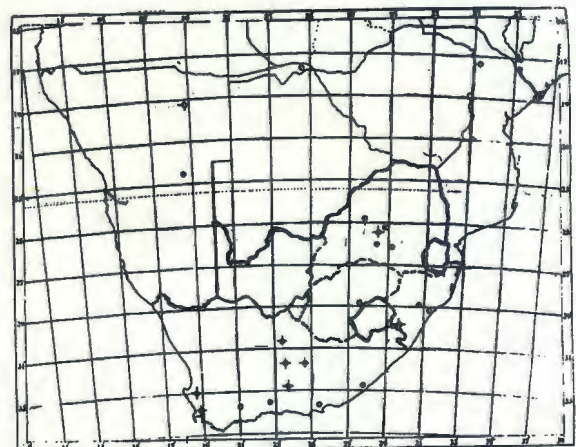


FIG. 28 Distribution of *P. chartarum* in South Africa (Roux, 1977)



*P. chartarum* is a cosmopolitan fungus (Marasas & Schumann, 1972) known to cause hepatogenous photosensitivity of livestock (facial eczema) in New Zealand (Clare, 1955; Thornton & Percival, 1959; Mortimer, White & Di Menna, 1978b), Australia (Hore, 1960) and South Africa (Marasas, Adelaar, Kellerman, Minné, Van Rensburg & Burroughs, 1972).

In South Africa, the fungus has been found in a wide range of conditions of temperature, moisture and substrate (Fig. 28), e.g. in the cool, relatively moist coastal belt, in the hot, semi-arid Karoo and in the warm, temperate grasslands of the Transvaal Highveld (Marasas & Schumann, 1972; Roux, 1977; Van der Merwe, Eicker, Marasas & Kellerman, 1979; Kellerman, Van der Westhuizen, Coetzer, Roux, Marasas, Minné, Bath & Basson, 1980). Facial eczema has been diagnosed in sheep on ryegrass/clover pastures (Fig. 29) near Humansdorp (Marasas *et al.*, 1972) and in cattle (Fig. 30) grazing similar pastures on a sewage farm near Johannesburg. In the latter instance, subacute facial eczema, complicated by purulent nephritis and cystitis, was induced by dosing cultures of *P. chartarum* from the toxic pasture to a young bull (G. C. A. van der Westhuizen, Cecilia Roux, T. S. Kellerman & J. G. Pienaar, unpublished data, 1977). The disease has also been reported to occur in the Winter Rainfall Area of the Cape Province (D. J. Schneider, personal communication, 1982).



FIG. 29 Ryegrass/Clover pasture



FIG. 30 Cow with facial eczema

*P. chartarum* lives saprophytically on dead plant material littering pastures. During favourable weather conditions, numerous conidia are produced (Thornton & Sinclair, 1960; Brook, 1963) that contain a potent hepa-

toxic epipolythiadioxopiperazine, sporidesmin (Syngé & White, 1959; White, Mortimer & Di Menna, 1978) (Fig. 31). The toxic conidia are transported by wind and water from the litter onto the surfaces of the surrounding ryegrass/clover leaves, to which they adhere. Should sufficient conidia-polluted leaves be ingested by stock, facial eczema will result (Crawley, Mortimer & Smith, 1961; Smith, Crawley & Lees, 1962; Di Menna & Parlé, 1970). Even during fairly severe outbreaks of facial eczema both in New Zealand (Di Menna & Parlé, 1970) and South Africa (G. C. A. van der Westhuizen & Cecilia Roux, unpublished data, 1977), *P. chartarum* may not be a major component of the pasture mycoflora. Contrary to the findings in New Zealand, however, both toxigenic and non-toxic strains in South Africa are frequently isolated from the same material, and non-toxic strains predominate (Roux, 1977; Marasas & Kellerman, 1978; Kellerman *et al.*, 1980).

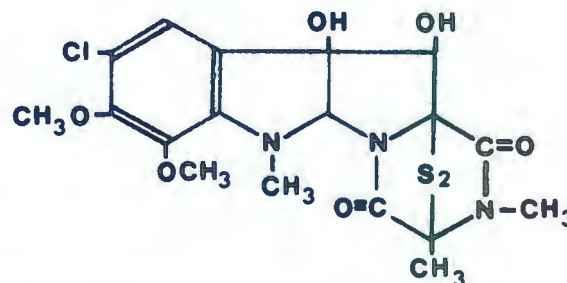


FIG. 31 Sporidesmin

Mortimer & Taylor (1962) found that 1.0 mg/kg sporidesmin, administered orally, to sheep induced over 80% photosensitivity and c. 70% mortality. At 0.5 mg/kg two thirds of the animals became photosensitive, and 8% died. Mild liver lesions were apparent at 0.3 mg/kg, but photosensitivity did not ensue. High oral doses of 3.0 mg/kg cause death within 4 days, often without photosensitivity.

The pathological changes have been thoroughly investigated (Mortimer, 1963; Marasas *et al.*, 1972; Mortimer, White & Di Menna, 1978b; Kellerman, *et al.*, 1980; Coetzer, Kellerman, Sadler & Bath, 1983). Reportedly the liver often had a mottled appearance and the morphology of the liver could be distorted by areas of nodular regeneration. The walls of the larger intra- and extrahepatic bile ducts and *ductus cysticus* were often conspicuously thickened and oedematous (Fig. 32).

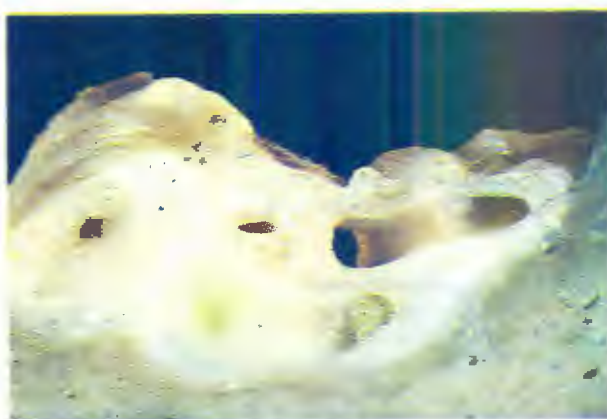


FIG. 32 Thickened extrahepatic bile ducts



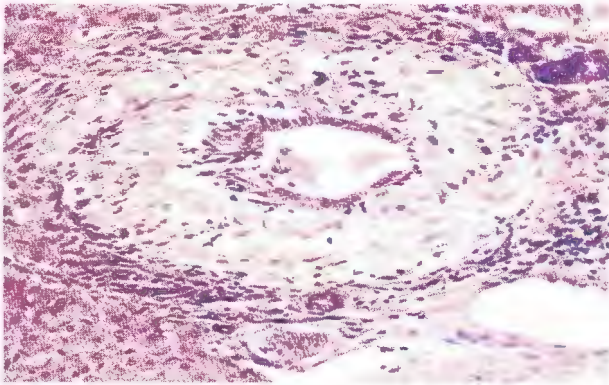


FIG. 33 Lamellar periductal fibrosis and oedema: HE × 500

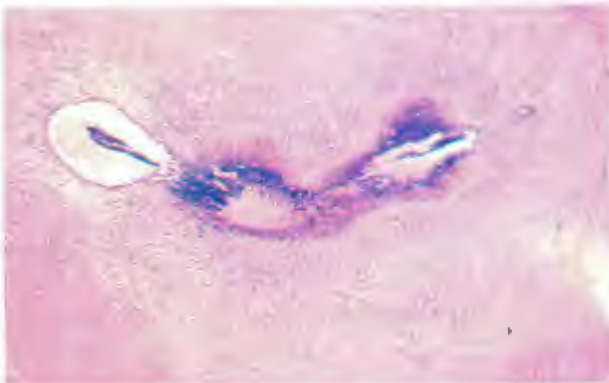


FIG. 34 Necrotic intrahepatic bile duct with granulation tissue proliferation: HE × 30

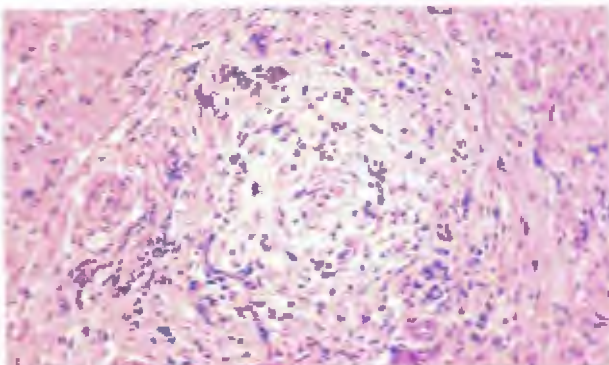


FIG. 35 Obliterated bile duct: HE × 500

A moderate to severe portal fibroplasia and bile duct proliferation were almost constantly present, the fibrosis being mostly concentrically arranged around the bile ducts in the portal triads and the larger ducts traversing the parenchyma (Fig. 33).

Generally speaking, the mucosae of the major intra- and extrahepatic bile ducts and *d. cysticus* were more consistently affected by degeneration and/or necrosis than the smaller ducts or ductules in the portal triads. The ductal necrosis was often associated with marked periductal granulation tissue proliferation and fibrosis (Fig. 34). In some instances the lumina were entirely replaced by scar tissue (Fig. 35). The above changes, together with the accumulation of necrotic debris and inspissated bile, culminate in the partial or complete occlusion of the affected ducts. Vascular lesions, which include fibrinoid degeneration and necrosis or subintimal

thickening by fibrosis of the blood vessel wall in juxtaposition to the affected duct, sometimes accompanied the changes in the biliary system.

A cystic nephrosis, sometimes accompanied by an ulcerative to haemorrhagic cystitis (Fig. 36) and/or cholecystitis (Fig. 37), was another common feature at necropsy.

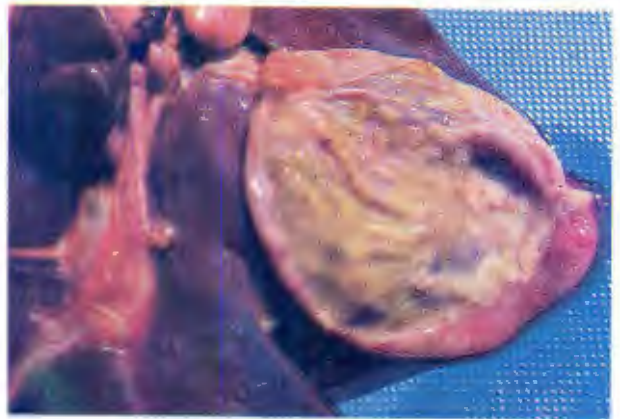
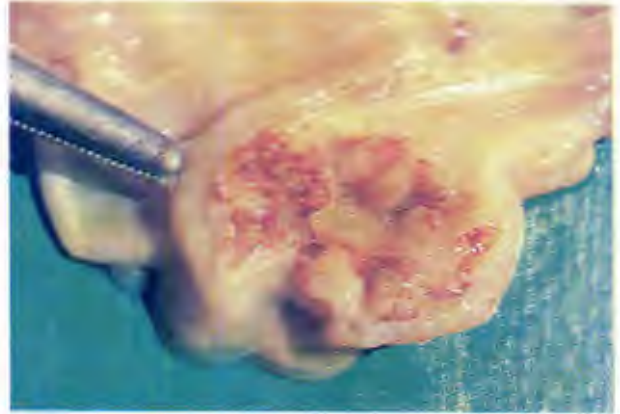


FIG. 36 & 37 Ulcerative cystitis and cholecystitis

One of the characteristic features of facial eczema is that it has a latent period or a period of apparent clinical normality between ingestion of sporidesmin and manifestation of photosensitivity. This interval corresponds to the time taken for the obliterative cholangitis and subsequent retention of phylloerythrin to take place. In experiments in South Africa the latent period was about 10–14 days, with an approximate range of 9–24 days (Kellerman *et al.*, 1980).

Little is known about the control of facial eczema in South Africa. In New Zealand, outbreaks are forecast by monitoring conditions favouring sporulation. Rainfall greater than just a few points, followed by 2 or more successive nights of grass minimum temperatures of 12 °C or over, constitutes a danger period (Mitchell, Walshe & Robertson, 1959), the occurrence and frequency of which are used for predicting outbreaks. Spore counts, however, are now regarded as being a better gauge of the toxicity of pastures (Di Menna & Bailey, 1973). The build-up of *P. chartarum* populations can be controlled by the application of substituted benzimidazole fungicides (Parlé & Di Menna, 1972; Mortimer, Di Menna & White, 1978a) and, as far as therapy is concerned, some progress has been made in the use of zinc salts in the prophylaxis of facial eczema (Smith, Embling, Towers, Wright & Payne, 1977; Towers & Smith, 1978; Mortimer *et al.*, 1978a).



*Tribulus terrestris*

'Dubbeltjie'



FIG. 38 *T. terrestris*

*T. terrestris* is a prostrate, creeping plant with a semi-perennial underground stem and root-system. Each year a mat of aerial branches emerges bearing 5-8 pairs of hairy leaflets. The small flowers are yellow (Fig. 38). Devil's thorn, the popular English name for the plant, is derived from the sharp, spiky excrescences on the woody fruits (Vahrmeijer, 1981).

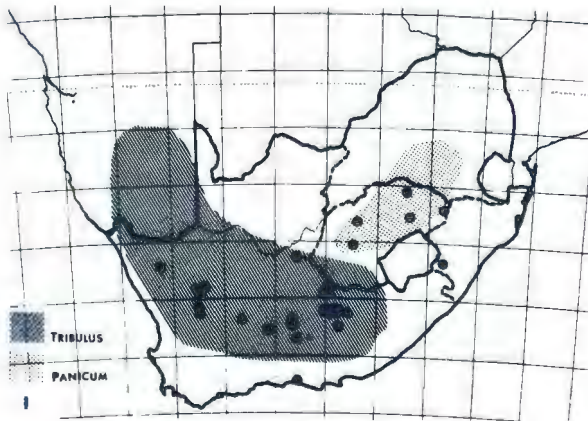


FIG. 39 Distribution of outbreaks of geeldikkop and dikoor

The plant has a country-wide distribution, but is extensively grazed only in the semi-arid Karoo areas (Fig. 39 & 40) where it is particularly common on overgrazed or run-down veld.

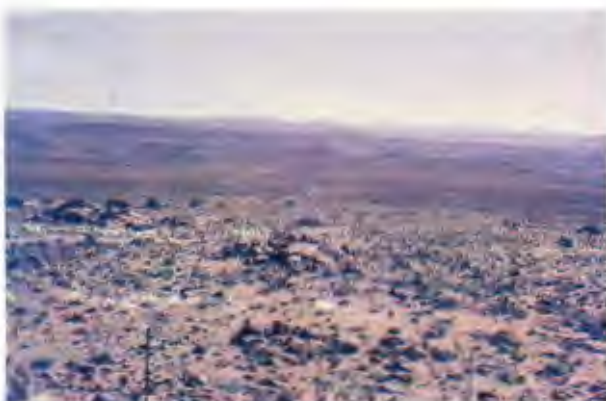


FIG. 40 Karoo panorama



FIG. 41 Wilted *T. terrestris* seedlings in a toxic camp. Notice how inconspicuous the plants are at this stage

The plant has long been known to cause geeldikkop (yellow thick head), a major hepatogenous photosensitivity disease of sheep in the Karoo (Theiler, 1918). Typically, outbreaks of geeldikkop occur when *T. terrestris* is wilted (Fig. 41) during hot dry spells following summer rains (Quin, 1928). In severe outbreaks hundreds of thousands of sheep may be affected (Steyn, 1949).

*T. terrestris* is not invariably toxic (Quin, 1930). In fact, considering the vast amount of this highly nutritious plant grazed with impunity by sheep in the Karoo each year, it must be very rarely so. The ability of *T. terrestris* to become sporadically toxic under certain weather conditions led to speculation that the wilted plant could sometimes produce a hepatotoxin (Theiler, 1918; Quin, 1928; Van Tonder *et al.*, 1972; Bath, Van Tonder & Basson, 1978). Recently, G. F. Bath, (unpublished data, 1979), induced geeldikkop with lyophilized alcoholic extracts of *T. terrestris*, the toxic principle of which has not yet been identified.

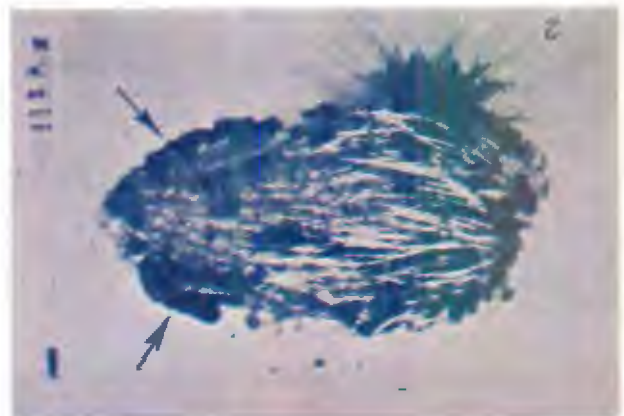


FIG. 42 *P. chartarum* colonies on an incubated *T. terrestris* leaflet

Another theory put forward was that during outbreaks *T. terrestris* was contaminated by hepatotoxic fungal spores (Brown, 1959; Van Tonder *et al.*, 1972; Kellerman *et al.*, 1980). The presence of such spores on *T. terrestris*, it was argued, would at once explain the sporadic nature of the outbreaks, the difficulty encountered in reproducing geeldikkop with *T. terrestris* and the occurrence of an apparently identical disease (dikoor) on *Panicum* grass pastures (*vide infra*) where *T. terrestris* was absent.



A wide-ranging mycotoxicological survey of pastures during outbreaks of geeldikkop/dikoor was consequently launched to try to identify this hypothetical, toxic fungus (Brown, 1964; Gouws, 1965; Kellerman *et al.*, 1980). The only fungus (Fig. 42) capable of causing hepatogenous photosensitivity to be isolated, however, was *Pithomyces chartarum* (Kellerman *et al.*, 1980). Much has already been said in this communication about the toxicity and distribution of *P. chartarum* in South Africa.

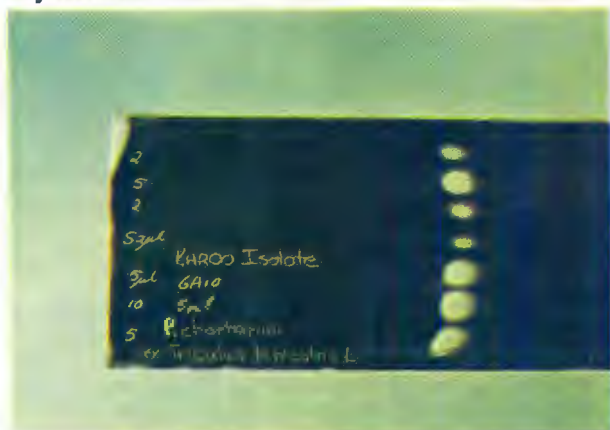


FIG. 43 Thin layer chromatograph of sporidesmin: Karoo isolate of *P. chartarum*

Hepatogenous photosensitivity was induced in sheep by dosing them with cultures of a *P. chartarum* isolate obtained from *T. terrestris* collected in a toxic camp during an outbreak of geeldikkop in the Karoo (Fig. 43). At the Veterinary Research Institute, Onderstepoort, these trials, with sheep on a lucerne diet, led to the induction of only facial eczema. However, when culture material, equivalent to the estimated amounts of sporidesmin (c.0,4–0,7 mg/kg), ingested during outbreaks of facial eczema in New Zealand, was dosed to sheep on wilted *T. terrestris* in the Karoo, interesting results were obtained: Firstly, more dosed than undosed sheep became photosensitive and, secondly, they all had typical lesions of geeldikkop. Similarly dosed sheep on natural Karoo veld with little *T. terrestris* developed facial eczema (Kellerman *et al.*, 1980). This, together with the fact that some sheep grazing on wilted *T. terrestris* became photosensitive at supposedly subclinical doses, indicated that sporidesmin, even at doses as low as c. 0,25 mg/kg, could trigger geeldikkop in sheep grazing on wilted *T. terrestris* in the Karoo.

The absence of ulcerative lesions in the gall and urinary bladders, the presence of a nephrosis (akin to that of facial eczema), and the hepatitis in geeldikkop have been described in detail elsewhere (Theiler, 1918; Van Tonder *et al.*, 1972; Kellerman *et al.*, 1980).

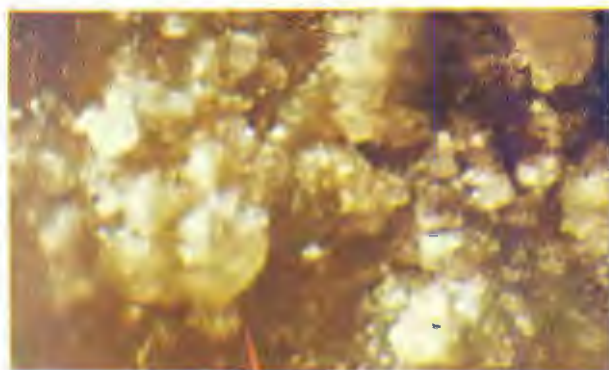


FIG. 44 Crystalloid material in extra hepatic bile duct

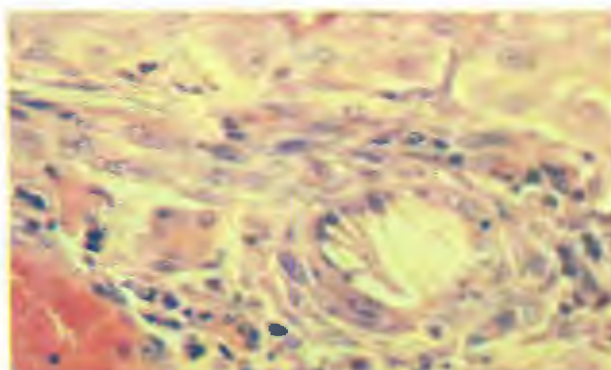


FIG. 45 Occlusion of bile duct by crystalloid material (microlith): HE × 500

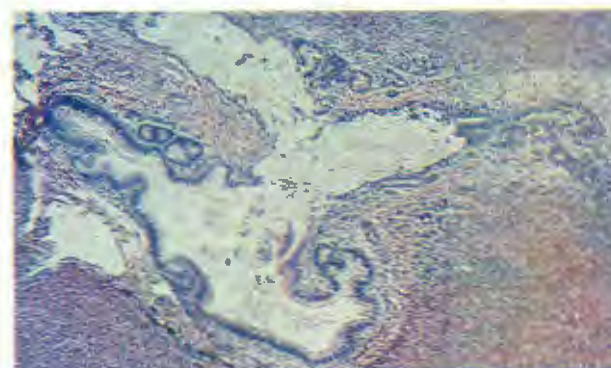


FIG. 46 Accumulation of crystalloid material in a large intrahepatic duct: HE × 75

The most conspicuous histopathological lesions of geeldikkop in the livers of the experimental animals (*vide supra*) was the accumulation of crystalloid material in the portal bile ducts, in the larger intra- and extrahepatic bile ducts and in the *d. cysticus* (Fig. 44–46). The lumina of some of these ducts, especially those of the larger bile ducts and *d. cysticus*, were often partially or almost entirely occluded by crystalloid material (Fig. 45 & 46). A mild to moderate periductal lamellar fibrosis was associated with these occlusions, and the ducts appeared to be somewhat dilated. At sites where crystalloid material had accumulated, the lining epithelium revealed degenerative and necrotic changes or were atrophic. The morphology of the bile ducts and ductules in the portal triads was often distorted by the presence of the material and by uneven proliferation of epithelium (Coetzer *et al.*, 1983). Crystalloid material may also be found outside of the bile ducts, for instance, in hepatocytes, between hepatocytes and in Kupffer cells (Van Tonder *et al.*, 1972).

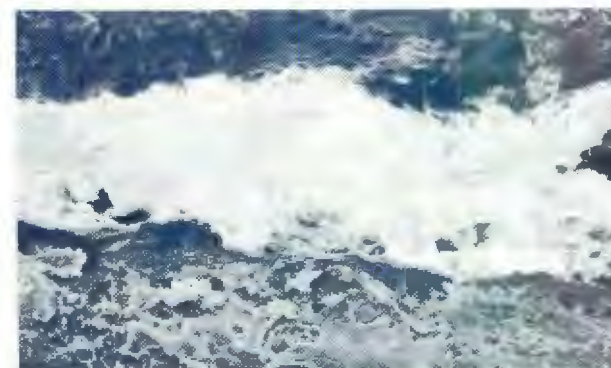


FIG. 47 Extrahepatic bile duct occluded by crystalloid material: × 160



FIG. 48 Microlith composed of plate-like structures:  $\times 1200$ 

Scanning electron microscopical studies revealed that the amount of crystalloid material in the extrahepatic bile ducts and *d. cysticus* varied at different locations along their lengths and also between animals. While the lumina in some segments could be almost completely obliterated by crystalloid material (Fig. 47), at other points along the same ducts they may be sparsely distributed or even absent. The morphology of the microliths varied considerably, but they were mostly composed of plate-like structures (Fig. 48) (Coetzer *et al.*, 1983).

The mucosal surfaces were affected to varying degrees along the lengths of the ducts, the lining epithelium being most severely affected where the microliths aggregated. Here swelling and partial or complete loss of microvilli, sometimes accompanied by separation and sloughing of isolated groups of epithelial cells, were evident (Coetzer *et al.*, 1983).

The microliths are now believed to be the principal biliary occluding mechanism responsible for the retention of phylloerythrin in geeldikkop (Kellerman *et al.*, 1980; Coetzer *et al.*, 1983). Little is known about their chemical composition, but they do not appear to be made up of common bile salts such as cholesterol, cholic acid, sodium glycocholate or sodium taurocholate (L. A. P. Anderson, personal communication, 1978).

The factor(s) responsible for the formation of microliths in ovine livers are contained by *T. terrestris*. The plant apparently constitutes a lithogenic diet for sheep that can either saturate or supersaturate the bile with lithogenic factor(s) or their products. The lithogenic factors could conceivably precipitate spontaneously (thus enabling *T. terrestris* alone to cause geeldikkop) or, as the experimental results indicate, the formation of microliths can be triggered by low levels of sporidesmin. In the latter instance, the ability of either the plant or the mycotoxin to cause photosensitivity would be enhanced. If the role of microliths in the pathogenesis of geeldikkop is correctly understood, fluctuations in the lithogenic status of *T. terrestris* as a result of locality, growth stage or physiological condition would have a profound effect on the incidence of the disease (Kellerman *et al.*, 1980; Coetzer *et al.*, 1983).

Geeldikkop, like facial eczema, has a latent period, but in the case of the geeldikkop this can be as short as 3–5 days (Van Tonder *et al.*, 1972) or even 2 days (G. F. Bath & T. S. Kellerman, unpublished data, 1982). The short latent periods sometimes seen in geeldikkop is now believed to arise from the deposition of biliary-occluding microliths early on in the intoxication (Kellerman *et al.*, 1980; Coetzer *et al.*, 1983).

In a limited experiment, designed to test the specificity of the triggering mechanism for the precipitation of microliths, sheep on wilted *T. terrestris* were dosed with 4 different hepatotoxins. The sheep that were poisoned with *Senecio retrorsus* or made photosensitive with the alga *M. aeruginosa* or the fungus *P. leptostromiformis* all developed typical lesions of their respective intoxications. Only those that received *P. chartarum* got geeldikkop. The ability to trigger geeldikkop, therefore, appears limited (Coetzer & Kellerman, unpublished data, 1979).

The most important objective of research on geeldikkop must now be to identify the microlith-promoting factor in *T. terrestris*.

### *Panicum* spp.

Outbreaks of photosensitivity with icterus sporadically occur in sheep grazing on *Panicum* spp. (*P. maximum*, *P. laevifolium*, *P. coloratum*) in summer (Steyn, 1928; Rimmington & Quin, 1937; Marasas & Kellerman, 1978; Kellerman *et al.*, 1980). The grasses grow (Fig. 49) profusely in parts of the Highveld of the Transvaal, Natal and Orange Free State, usually where the soil has been disturbed by cultivation (Fig. 50). As in geeldikkop, outbreaks occur when the pastures are wilted during hot dry spells following summer rains (Steyn, 1928).

FIG. 49 *P. laevifolium*FIG. 50 A wilted toxic *P. laevifolium* pasture



The mycoflora of *Panicum* pastures has been studied during outbreaks of photosensitivity (dikoor) and between outbreaks (Kellerman *et al.*, 1980). The only fungus so far isolated capable of causing ovine hepatogenous photosensitivity was *P. chartarum*. This fungus was identified in the aerospora above an *Eragrostis curvula* pasture adjacent to a *P. coloratum* field where an outbreak of dikoor had occurred (Van der Merwe *et al.*, 1979), and was repeatedly isolated from *Panicum* grass during outbreaks of the disease (Kellerman *et al.*, 1980; G. C. A. van der Westhuizen, Cecilia Roux, T. S. Kellerman, unpublished data, 1983). The possible role of *P. chartarum* in the aetiology of dikoor is now being investigated.

The remarkable similarity between geeldikkop and dikoor has been well documented (Steyn, 1928; Quin, 1928, 1930, 1933b), but dikoor was generally regarded as being a milder disease (Quin, 1928; Van Tonder *et al.*, 1972). Recent investigations have not confirmed this observation, however, as neither the liver lesions nor the photosensitivity were milder than those of geeldikkop. A lower mortality would probably be due to the more clement environment of the Highveld or to the fact that affected sheep are more easily noticed on small intensively farmed units.

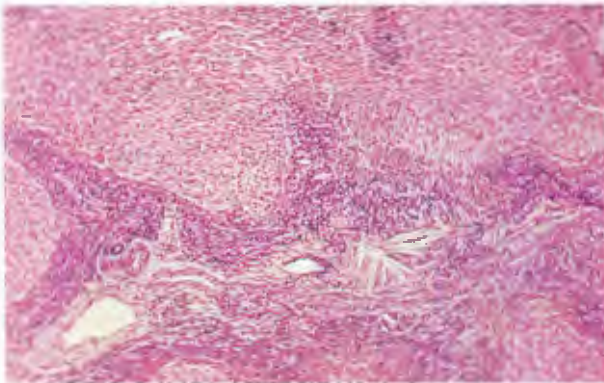


FIG. 51 Dikoor: Notice the crystalloid material and other changes consistent with geeldikkop, HE  $\times$  200

The pathological lesions are identical with those of geeldikkop (Fig. 51) and, barring the nature of the pasture on which the outbreaks occur, the 2 diseases cannot be distinguished from each other.

A similar disease has been described in sheep grazing on *P. mileaceum* in New Zealand, where apparently the quantity of birefringent crystals in the bile ducts roughly correlated with the degree of symptoms seen (Clare 1952).

#### DISCUSSION

Poisonings that induce photosensitivity by primarily affecting the hepatocytes can be roughly divided into 3 groups according to their histopathological lesions. The degenerative changes provoked by *L. camara*, for instance, are quite different from either the zonally distributed necrosis of *A. axillaris*, *L. bipinnatum* and *A. trifurcata* poisoning or the diffuse fatty changes induced by *M. aeruginosa* and *P. leptostromiformis*. The spectra of lesions produced by the various poisonings can overlap, however, sometimes making these distinctions less obvious (e.g. in both *M. aeruginosa* and *L. bipinnatum* poisonings diffuse fatty degeneration may be present). Nevertheless, it is interesting to note that all the plants that produce zonally distributed hepatic lesions belong to the Asteraceae and that the fungus and alga responsible for fatty changes both contain toxic peptides.

Adams (1974) pointed out that plant poisons and mycotoxins could damage the liver at different sites, including the hepatocytes, biliary system, intrahepatic blood vessels and, probably, the stroma. Cytotoxins exert their effect mainly on the hepatocytes, causing various combinations of degeneration and necrosis (Zimmerman, 1978). In general, hepatic necrosis may be focal, massive, or zonally (i.e. centrilobularly, midzonally or peripherally) distributed in the lobules. Although much has been published on the possible pathogenesis, diagnostic significance and sequelae of these lesions, many questions regarding them remain unanswered (Zimmerman, 1978). It would seem, however, that hepatotoxins that produce zonal necrosis seldom cause the massive type, and vice versa (Klatskin, 1975).

In the recent study of ovine *A. trifurcata* poisoning, periportal or peripheral zonal necrosis could be shifted through the midzonal area to the centrilobular zone by varying the dosing regimen (Kellerman *et al.*, 1983). In *A. axillaris* poisoning, the liver lesions were found to range from distinct zonal necrosis to diffuse degeneration and hepatocellular unrest, often accompanied by ductular proliferation and cholestasis (Kellerman *et al.*, 1973; Coetzer & Bergh, 1983). Similarly, although a range of changes has been seen in the livers of sheep intoxicated by *L. bipinnatum* (Adelaar *et al.*, 1964; Kellerman *et al.*, 1973), periportal coagulative necrosis and haemorrhage seem to be the most characteristic lesion of acute poisoning by this plant. In the light of these findings it would appear that, in addition to the particular effects of specific hepatotoxins on certain areas within a lobule, the level and intervals between exposures to a toxin may also be important in determining the final nature, distribution and extent of the lesions in the liver. Extreme care should be taken not to overestimate the diagnostic significance of this type of lesion (Coetzer & Bergh, 1983).

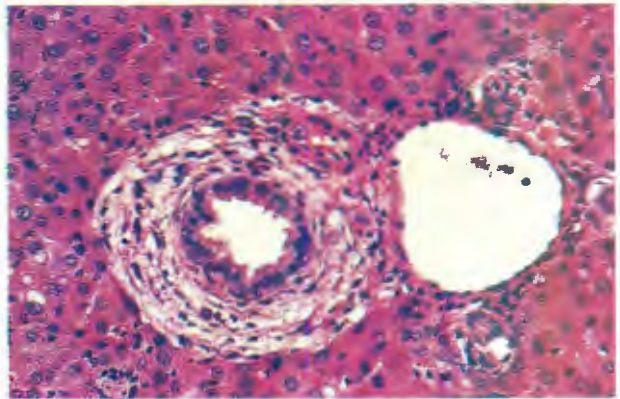


FIG. 52 Concentric lamellar fibrosis and oedema around bile duct: HE  $\times$  200

Apart from the occluding mechanisms operating in facial eczema and geeldikkop (the 2 most important photosensitizations resulting from biliary obstruction), sheep can be made photosensitive by surgical ligation of the common bile duct (Quin, 1933c; Ford & Gopinath, 1976; Coetzer *et al.*, 1983). The most conspicuous changes arising from ligation were mild to marked lamellar fibrosis and oedema around many bile ducts in the portal triads (Fig. 52) and larger bile ducts traversing the liver. From this it is clear that obstruction of bile ducts by whatever mechanism, whether by necrosis and scarring, as in facial eczema, by microliths as in geeldikkop/dikoor or by ligation of the common bile duct, leads to very similar hepatic lesions. The most important of these is concentric, lamellar fibrosis around the bile ducts,



which is indicative of obstructive cholangitis. Other changes include bile duct proliferation, oedema and fibroplasia in the portal triads, foci of periportal hepatocellular necrosis (bile infarcts) and diffuse parenchymal degeneration. In general, the severity of the biliary lesions probably depends on the degree of occlusion and whether major or minor bile ducts are affected (Coetzer *et al.*, 1983).

The degree of portal reaction does not always vary significantly amongst sheep affected with facial eczema and geeldikkop/dikoor. Despite such similarities, the 2 diseases are distinct entities that can be distinguished on histopathological grounds. The mechanical damage to larger bile ducts (such as pressure atrophy and/or spotty degeneration of the epithelium), caused by microliths in geeldikkop, never progresses to the advanced stage seen in facial eczema. The severe thickening of the bile ducts especially characteristic of facial eczema (where the entire walls of the major ducts may also be necrotic and be surrounded by proliferating granulation tissue) is absent. Absent, too, is the thickening of blood vessel walls in juxtaposition to affected bile ducts and the urinary (Fig. 36) and gall bladder (Fig. 37) lesions sometimes seen in facial eczema. Many of these changes in facial eczema are, of course, macroscopically visible.

The principal objective of research in biliary occlusive diseases must now be to isolate and characterize the microlith-promoting agent in *T. terrestris*.

Although the mechanisms responsible for the retention of phylloerythrin in biliary occlusive diseases are at least partially understood, little is known about the pathogenesis of photosensitization resulting from parenchymal injury. It is not clear why, in certain primarily hepatocytic toxicoses (such as *Hertia pallens*, *Pteronia pallens* and *Senecio* poisoning), icterus is manifested without photosensitivity, while in others (such as in *L. bipinnatum* poisoning) icterus is accompanied by retention of phylloerythrin. In these intoxications, unlike the biliary occlusive diseases, the nature of the pathological lesions do not shed much light on the manner of phylloerythrin retention. Perhaps a comparative study of the chemical pathological changes in photosensitizing and non-photosensitizing hepatic parenchymal intoxications would provide answers to some of these questions.

#### ACKNOWLEDGEMENTS

We are grateful to past and present members of the Mycological Section of the Plant Protection Research Institute and the Botanical Research Institute, without whom this work could not have been undertaken. In particular we are indebted to Drr G. C. A. van der Westhuizen and W. F. O. Marasas, and the Misses C. Roux and W. G. Welman for their outstanding contributions.

#### REFERENCES

- ACOCKS, J. P. H., 1953. Veld types of South Africa. *Botanical Survey Memoir*, No. 28.
- ACOCKS, J. P. H., 1975. Veld types of South Africa. 2nd ed. *Memoirs of the Botanical Survey of South Africa*, No. 40.
- ADAMS, S. E. I., 1974. Hepatotoxic activity of plant poisons and mycotoxins in domestic animals. *Veterinary Bulletin*, 44, 767-776.
- ADELAAR, T. F., TERBLANCHE, M., SMIT, J. D., NAUDÉ, T. W. & CODD, L. E., 1964. A hitherto unknown poisonous plant: *Lasiospermum bipinnatum* (Thunb.) Druce. Preliminary communication. *Journal of the South African Veterinary Medical Association*, 35, 11-16.
- ANDERSON, L. A. P., DE KOCK, W. T. & ENSLIN, P. R., 1961. The constitution of two physiologically active triterpenoids from *Lippia rehmannii*. *Journal of the South African Chemical Institute*, 14, 58-63.
- ASHWORTH, C. T. & MASON, M. F., 1946. Observations on the pathological changes produced by a toxic substance present in blue-green algae (*Microcystis aeruginosa*). *American Journal of Pathology*, 22, 369-383.
- BARTON, D. H. R. & DE MAYO, P., 1954. Part XV. The constitution of icterogenin, a physiologically active triterpenoid. *Journal of the Chemical Society for 1954*, Part 1, 887-900.
- BATH, G. F., VAN TONDER, E. M. & BASSON, P. A., 1978. Geeldikkop: Preservation of toxic material. *Journal of the South African Veterinary Association*, 49, 23-25.
- BRASH, A. G., 1943. Lupin poisoning of sheep. *New Zealand Journal of Agriculture*, 67, 83-84.
- BROOK, P. J., 1963. Ecology of the fungus *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis in pasture in relation to facial eczema disease in sheep. *New Zealand Journal of Agricultural Research*, 6, 147-228.
- BROWN, J. M. M., 1959. Advances in geeldikkop (*Tribulosis ovis*) research. 1. The history of geeldikkop research. *Journal of the South African Veterinary Medical Association*, 30, 97-111.
- BROWN, J. M. M., RIMINGTON, C. & SAWYER, B. C., 1963. Studies on biliary excretion in the rabbit. 2. The relationship between the chemical structure of certain natural or synthetic pentacyclic triterpenes and their icterogenic activity. Part 1: The substituents on carbon atoms 3, 17, 22 and 24. *Proceedings of the Royal Society, Series B*, 157, 473-491.
- BROWN, J. M. M., 1964. Advances in geeldikkop (*Tribulosis ovis*) research. 6. Studies on selected aspects of the biochemistry of geeldikkop and enzootic icterus. *Journal of the South African Veterinary Medical Association*, 35, 507-532.
- BROWN, J. M. M. & RIMINGTON, C., 1964. Studies on biliary excretion in the rabbit. 2. The relationship between the chemical structure of certain natural or synthetic pentacyclic triterpenes and their icterogenic activity. Part 2: The substituents on carbon atoms 17, 19, 20 and 22. *Proceedings of the Royal Society, Series B*, 160, 246-257.
- CLARE, N. T., 1952. Photosensitization in diseases of domestic animals. Review Series No. 3 of the Commonwealth Bureau of Animal Health. Farnham Royal, Bucks., England: Commonwealth Agricultural Bureau.
- CLARE, N. T., 1955. Photosensitization in animals. *Advances in Veterinary Science*, 2, 182-211.
- COETZER, J. A. W. & BERGH, T., 1983. Photosensitivity in South Africa. IV. Pathological changes in the liver in ovine photosensitivity caused by the plant *Asaemia axillaris* (Thunb.) Harv. Ex Jackson. *Onderstepoort Journal of Veterinary Research*, 50, 53-55.
- COETZER, J. A. W., KELLERMAN, T. S., SADLER, W. & BATH, G. F., 1983. Photosensitivity in South Africa. V. A comparative study of the pathology of the ovine hepatogenous photosensitivity diseases, facial eczema and geeldikkop (*Tribulosis ovis*), with special reference to their pathogenesis. *Onderstepoort Journal of Veterinary Research*, 50, 59-71.
- CRAWLEY, W. E., MORTIMER, P. H. & SMITH, J. DREW, 1961. Characteristic lesions of facial eczema produced in sheep by dosing material containing *Pithomyces chartarum* collected from pasture. *New Zealand Journal of Agricultural Research*, 4, 552-559.
- CULVENOR, C. C. J., BECK, A. B., CLARK, M., COCKRUM, P. A., EDGAR, J. A., FRAHN, J. L., JAGO, M. V., LANIGAN, G. W., PAYNE, A. L., PETERSON, J. E., PETERSON, D. S., SMITH, L. W. & WHITE, R. R., 1977. Isolation of toxic metabolites of *Phomopsis leptostromiformis* responsible for lupinosis. *Australian Journal of Biological Science*, 30, 269-277.
- CULVENOR, C. C. J., COCKRUM, P. A., EDGAR, J. A., FRAHN, J. L., GORST-ALLMAN, C. P., JONES, A. J., MARASAS, W. F. O., MURRAY, K. E., SMITH, L. W., STEYN, P. S., VLEGGAR, R. & WESSEL, P. L., 1983. Structure elucidation of Phomopsin A, a novel cyclic hexapeptide mycotoxin produced by *Phomopsis leptostromiformis*. *Journal of the Chemical Society, Chemical Communications* (in press).
- DI MENNA, MARGARET E. & PARLÉ, J. N., 1970. Moulds on perennial ryegrass and white clover. *New Zealand Journal of Agricultural Research*, 13, 51-68.
- DI MENNA, MARGARET E. & BAILEY, J. R., 1973. *Pithomyces chartarum* spore counts in pasture. *New Zealand Journal of Agricultural Research*, 16, 343-351.
- DI MENNA, MARGARET E., MORTIMER, P. H. & WHITE, E. P., 1977. The genus *Pithomyces*. In: WYLLIE, T. D. & MOREHOUSE, G. L. (eds). *Mycotoxic fungi, mycotoxins, mycotoxicoses*. An encyclopedic handbook. Vol. 1, 99-103. New York: Marcel Decker, Inc.
- ELLEMAN, T. C., FALCONER, I. R., JACKSON, A. R. B. & RUNNEGAR, M. T., 1978. Isolation, characterisation and pathology of the toxin from a *Microcystis aeruginosa* (= *Anacystis cyanea*) bloom. *Australian Journal of Biological Science*, 31, 209-218.
- FAIR, A. E., TUSTIN, R. C. & ADELAAR, T. F., 1970. Case report. Poisoning of cattle by ganskweek (*Lasiospermum bipinnatum*) (Thunb.) Druce. *Journal of the South African Veterinary Medical Association*, 41, 231-232.



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- FORD, E. J. H. & GOPINATH, C., 1976. The excretion of phylloerythrin and bilirubin by calves and sheep. *Research in Veterinary Science*, 21, 12-18.
- GARDINER, M. R., 1967. Lupinosis. *Advances in Veterinary Science*, 11, 85-138.
- GOPINATH, C. & FORD, E. J. H., 1969. The effect of *Lantana camara* on the liver of sheep. *Journal of Pathology*, 99, 75-85.
- GORHAM, P. R., 1964. Toxic algae. In: JACKSON, D. F. (ed.). *Algae and man*, 307-336. New York: Plenum Press.
- GOUWS, L., 1965. Toxigenic Dematiaceae. In: Symposium on mycotoxins in foodstuffs. 15-23. Publications S 15 of the CSIR, Pretoria.
- GROENEWALD, J. W., SMIT, J. D. & ADELAAR, T. F., 1954. Methionine deficiency, a possible cause of liver injury in sheep. *Journal of the South African Veterinary Medical Association*, 25, 29-33.
- HEANY, S. I., 1971. The toxicity of *Microcystis aeruginosa* Kutz from English reservoirs. *Water Treatment and Examination*, 20, 235-244.
- HORE, D. E., 1960. Facial eczema. *Australian Veterinary Journal*, 36, 172-176.
- HOFFMAN, J. R. H., 1976. Removal of *Microcystis* toxin in water purification processes. *Water S.A.*, 2, 58-61.
- HUGHES, E. O., GORHAM, P. R. & ZEHNDER, A., 1958. Toxicity of a unialgal culture of *Microcystis aeruginosa*. *Canadian Journal of Microbiology*, 4, 225-236.
- KELLERMAN, T. S., BASSON, P. A., NAUDÉ, T. W., VAN RENSBURG, I. B. J. & WELMAN, WILHELMINA G., 1973. Photosensitivity in South Africa. I. A comparative study of *Asaemia axillaris* (Thunb.) Harv. ex Jackson and *Lasiospermum bipinnatum* (Thunb.) Druce poisoning in sheep. *Onderstepoort Journal of Veterinary Research*, 40, 115-126.
- KELLERMAN, T. S., VAN DER WESTHUIZEN, G. C. A., COETZER, J. A. W., ROUX, CECILIA, MARASAS, W. F. O., MINNÉ, J. A., BATH, G. F. & BASSON, P. A., 1980. Photosensitivity in South Africa. II. The experimental production of the ovine hepatogenous photosensitivity disease geeldikkop (*Tribulosis ovis*) by the simultaneous ingestion of *Tribulus terrestris* plants and cultures of *Pithomyces chartarum* containing the mycotoxin sporidesmin. *Onderstepoort Journal of Veterinary Research*, 47, 231-261.
- KELLERMAN, T. S., COETZER, J. A. W., SCHNEIDER, D. J. & WELMAN, WILHELMINA G., 1983. Photosensitivity in South Africa. III. Ovine hepatogenous photosensitivity caused by the plant *Athanasia trifurcata* L. (Asteraceae). *Onderstepoort Journal of Veterinary Research*, 50, 47-52.
- KLATSKIN, G., 1975. Toxic and drug-induced hepatitis. In: SCHIFF, LEON, 1975 (ed.). *Diseases of the liver*. (4th ed.) Philadelphia: Lippincott.
- KÜHN, J., 1880. Die Schmarotzerpilze der Lupinenpflanze und die Bekämpfung der Lupinenkrankheit der Schafe. *Berichte aus dem Landwirtschaftlichen Institut der Universität Halle*, 2, 115-128.
- LOUW, P. G. J., 1943. Lantatin, the active principle of *Lantana camara* L. I. Isolation and preliminary results on the determination of its constitution. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 18, 197-202.
- LOUW, P. G. J., 1948. Lantadene A, the active principle of *Lantana camara* L. II. Isolation of lantadene B and oxygen functions of lantadene A and lantadene B. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 23, 233-238.
- LOUW, P. G. J., 1949. Lantadene A. The active principle of *Lantana camara* L. III. Pyrolytic distillation of lantadene A. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 22, 321-327. See also IV. Dehydrogenation of lantadene A and lantadene B. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 22, 329-334.
- LOUW, P. G. J., 1950. The active constituent of the poisonous alga, *Microcystis toxica* Stephens. *South African Industrial Chemist*, 4, 62-66.
- MARASAS, W. F. O., ADELAAR, T. F., KELLERMAN, T. S., MINNÉ, J. A., VAN RENSBURG, I. B. J. & BURROUGHS, G. W., 1972. First report of facial eczema in sheep in South Africa. *Onderstepoort Journal of Veterinary Research*, 39, 107-112.
- MARASAS, W. F. O., 1977. The genus *Phomopsis*. In: WYLLIE, T. D. & MOREHOUSE, L. G. (eds). *Mycotoxic fungi, mycotoxins, mycotoxicoses*. An encyclopedic handbook. Vol. 1, 111-118. New York: Marcel Decker Inc.
- MARASAS, W. F. O., 1978. Lupinosis in sheep. In: WYLLIE, T. D. & MOREHOUSE, L. G. (eds). *Mycotoxic fungi, mycotoxins, mycotoxicoses*. An encyclopedic handbook. Vol. II, 213-217. New York: Marcel Decker Inc.
- MARASAS, W. F. O. & KELLERMAN, T. S., 1978. Photosensitivity in cattle. In: WYLLIE, T. D. & MOREHOUSE, L. G. (eds). *Mycotoxic fungi, mycotoxins, mycotoxicoses*. An encyclopedic handbook. Vol. II, 73-84. New York: Marcel Decker Inc.
- MARASAS, W. F. O. & SCHUMANN, INGRID H., 1972. The genus *Pithomyces* in South Africa. *Bothalia*, 10, 509-516.
- McBARRON, E. J. & VALERIE, MAY, 1966. Poisoning of sheep in New South Wales by the blue-green alga *Anacystis cyanea* (Keutz.) DR. and Dail. *Australian Veterinary Journal*, 42, 449-453.
- MITCHELL, K. J., WALSH, T. O. & ROBERTSON, N. G., 1959. Weather conditions associated with outbreaks of facial eczema. *New Zealand Journal of Agricultural Research*, 2, 584-604.
- MORTIMER, P. H., 1963. The experimental intoxication of sheep with sporidesmin, a metabolic product of *Pithomyces chartarum*. IV. Histological examinations of orally-dosed sheep. *Research in Veterinary Science*, 4, 166-185.
- MORTIMER, P. H. & TAYLOR, A., 1962. The experimental intoxication of sheep with sporidesmin, a metabolic product of *Pithomyces chartarum*. I. Clinical observations and findings at post-mortem examination. *Research in Veterinary Science*, 3, 147-160.
- MORTIMER, P. H., DI MENNA, MARGARET E. & WHITE, E. P., 1978a. Pithomycotoxicosis. "Facial eczema" in cattle. In: WYLLIE, T. D. & MOREHOUSE, L. G. (eds). *Mycotoxic fungi, mycotoxins, mycotoxicoses*. An encyclopedic handbook. Vol. II, 63-72. New York: Marcel Decker Inc.
- MORTIMER, P. H., WHITE, E. P. & DI MENNA, MARGARET E., 1978b. Pithomycotoxicosis. "Facial eczema" in sheep. In: WYLLIE, T. D. & MOREHOUSE, L. G. (eds). *Mycotoxic fungi, mycotoxins, mycotoxicoses*. An encyclopedic handbook. Vol. II, 195-203. New York: Marcel Decker Inc.
- PARLÉ, J. N. & DI MENNA, MARGARET E., 1972. Fungicides and control of *Pithomyces chartarum*. II. Field trials. *New Zealand Journal of Agricultural Research*, 15, 54-63.
- QUINN, J. I., 1928. Recent investigations into geeldikkop affecting sheep and goats in the Cape Province. *Journal of the South African Veterinary Medical Association*, 1, 43-45.
- QUINN, J. I., 1930. Further investigations into the problem of geeldikkop (Tribulosis) in small stock. *Report on Veterinary Research, Union of South Africa*, 16, 413-416.
- QUINN, J. I., 1933a. Studies on the photosensitization of animals in South Africa. V. The toxicity of *Lippia rehmannii* Pears. and *Lippia pretoriensis* Pears. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 1, 501-504.
- QUINN, J. I., 1933b. Studies on the photosensitization of animals in South Africa. I. The action of various fluorescent dyestuffs. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 1, 459-468.
- QUINN, J. I., 1933c. Studies on the photosensitization of animals in South Africa. VI. The effect of surgical obstruction of the normal bile flow. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 1, 505-526.
- QUINN, J. I., RIMINGTON, C. & ROETS, G. C. S., 1935. Studies in the photosensitization of animals in South Africa. VIII. The biological formation of phylloerythrin in the digestive tracts of various domestic animals. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 4, 461-471.
- RIMINGTON, C. & QUIN, J. I., 1934. Studies on the photosensitization of animals in South Africa. VII. The nature of the photosensitizing agent in geeldikkop. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 3, 137-157.
- RIMINGTON, C. & QUIN, J. I., 1935. The isolation of an icterogenic principle from *Lippia rehmannii* (Pears). *South African Journal of Science*, 32, 142-151.
- RIMINGTON, C. & QUIN, J. I., 1937. Dikoor or geeldikkop on grassveld pastures. *Journal of the South African Veterinary Medical Association*, 8, 141-148.
- RIMINGTON, C., QUIN, J. I. & ROETS, G. C. S., 1937. Studies on the photosensitization of animals in South Africa. X. The icterogenic factor in geeldikkop. Isolation of active principles from *Lippia rehmannii* (Pears). *Onderstepoort Journal of Veterinary Science and Animal Industry*, 9, 225-255.
- ROETS, G. C. S., 1937. The distribution and possible translocation of icterogenin in *Lippia rehmannii* Pears. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 9, 583-588.
- ROUX, CECILIA, 1977. 'n Studie van die voorkoms, verspreiding en morfologie van *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis in Suid-Afrika en sommige aspekte van sy fisiologie. M.Sc. tesis, Randse Afrikaanse Universiteit.
- SANTIKARN, S., WILLIAMS, D. H., SMITH, R. J., HAMMOND, S. J., BOTES, D. P., TUINMAN, A., WESSELS, P. L., VILJOEN, C. C. & KRUGER, HELENE, 1983. A partial structure for the toxin BE-4 from the blue-green algae, *Microcystis aeruginosa*. *Journal of the Chemical Society, Chemical Communications*, 652-654.
- SCOTT, W. E., 1974. The isolation of *Microcystis*. *South African Journal of Science*, 70, 179.
- SEAWRIGHT, A. A., 1963. Studies on experimental intoxication of sheep with *Lantana camara*. *Australian Veterinary Journal*, 39, 340-344.



- SEAWRIGHT, A. A., 1964. Studies on the pathology of experimental lantana (*Lantana camara* L.) poisoning of sheep. *Pathologia Veterinaria* 1, 504-529.
- SEAWRIGHT, A. A., 1965. Electron microscopic observations of the hepatocytes of sheep in lantana poisoning. *Pathologia Veterinaria*, 2, 175-196.
- SEAWRIGHT, A. A. & ALLEN, J. G., 1972. Pathology of the liver and kidney in lantana poisoning of cattle. *Australian Veterinary Journal*, 48, 323-331.
- SEAWRIGHT, A. A. & HRDLICKA, JANE, 1977. The oral toxicity for sheep of triterpene acids isolated from *Lantana camara*. *Australian Veterinary Journal*, 53, 230-235.
- SMITH, J. DREW, CRAWLEY, W. E. & LEES, F. T., 1962. The spore load of *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis on green leaves of herbage in facial eczema studies. *New Zealand Journal of Agricultural Research*, 5, 22-35.
- SMITH, B. L., EMBLING, N. R., TOWERS, N. R., WRIGHT, D. E. & PAYNE, E., 1977. The protective effect of zinc sulphate in experimental sporidesmin poisoning of sheep. *New Zealand Veterinary Journal*, 25, 124-127.
- STEPHENS, E. L., 1949. *Microcystis toxica* sp. nova: A poisonous alga from the Transvaal and Orange Free State. *Transactions of the Royal Society of South Africa*, 32, 105-112.
- STEYN, D. G., 1928. Dikoor in sheep. *Journal of the South African Veterinary Medical Association*, 1, 47-50.
- STEYN, D. G. & VAN DER WALT, S. J., 1941. Recent investigations into the toxicity of known and unknown poisonous plants in the Union of South Africa XI. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 16, 121-147.
- STEYN, D. G., 1944. Vergiftiging deur slyk (algae) op damme en panne. *South African Medical Journal*, 18, 378-379.
- STEYN, D. G., 1945a. Poisoning of animals and human beings by algae. *South African Journal of Science*, 41, 243-244.
- STEYN, D. G., 1945b. Poisoning of animals with algae (scum of waterbloom) in dams and pans. *Bulletin of the Department of Agriculture and Forestry*, Pretoria.
- STEYN, D. G. 1949. Vergiftiging van mens en dier. Pretoria: Van Schaik.
- SYNGE, R. L. M. & WHITE, E. P., 1959. Sporidesmin: a substance from *Sporidesmium bakeri* causing lesions characteristic of facial eczema. *Chemistry and Industry*. UK Department of Agriculture, New Zealand. Animal Research Division publication No. 188, 1546-1547.
- THEILER, A., 1918. Geeldikkop in sheep (*Tribulosis ovis*). *Report on Veterinary Research, Union of South Africa*, 7 & 8, 1-56.
- THORNTON, D. J., 1977. Ganskweek (*Lasiospermum bipinnatum*) poisoning in cattle. *Journal of the South African Veterinary Association*, 48, 210-211.
- THORNTON, R. H. & PERCIVAL, J. C., 1959. A hepatotoxin from *Sporidesmium bakeri* capable of producing facial eczema disease in sheep. *Nature*, London, 183, 63.
- THORNTON, R. H. & SINCLAIR, D. P., 1960. Some observations on the occurrence of *Sporidesmium bakeri* Syd. and facial eczema disease in the field. *New Zealand Journal of Agricultural Research*, 3, 300-313.
- TOERIEN, D. F., SCOTT, W. E. & PITOUT, M. J., 1976. *Microcystis* toxins: Isolation, identification, implications. *Water S.A.*, 2, 160-162.
- TOWERS, N. R. & SMITH, B. L., 1978. The protective effect of zinc sulphate in experimental sporidesmin intoxication of lactating dairy cows. *New Zealand Veterinary Journal*, 26, 199-202.
- VAHRMEIJER, J., 1981. Poisonous plants of Southern Africa that cause stock losses. Cape Town: Tafelberg.
- VAN DER MERWE, W. J. J., EICKER, A., MARASAS, W. F. O. & KELLERMAN, T. S., 1979. Aerospora of an *Eragrostis curvula* pasture in South Africa. *Onderstepoort Journal of Veterinary Research*, 46, 19-25.
- VAN DER WALT, S. J. & STEYN, D. G., 1939. Recent investigations into the toxicity of known and unknown poisonous plants in the Union of South Africa. IX. *Onderstepoort Journal of Veterinary Science and Animal Industry*, 12, 335-366.
- VAN, JAARVELD, A. B. & KNOX-DAVIES, P. S., 1974. Resistance of lupins to *Phomopsis leptostromiformis*. *Phytophylactica*, 6, 55-60.
- VAN RENSBURG, I. B. J., MARASAS, W. F. O. & KELLERMAN, T. S. 1975. Experimental *Phomopsis leptostromiformis* mycotoxicosis of pigs. *Journal of the South African Veterinary Association*, 46, 197-204.
- VAN TONDER, E. M., BASSON, P. A. & VAN RENSBURG, I. B. J., 1972. "Geeldikkop": Experimental induction by feeding the plant *Tribulus terrestris* L. (Zygophyllaceae). *Journal of the South African Veterinary Medical Association*, 43, 363-375.
- VAN WARMELO, K. T., MARASAS, W. F. O., ADELAAR, T. F., KELLERMAN, T. S., VAN RENSBURG, I. B. J. & MINNÉ, J. A., 1970. Experimental evidence that lupinosis of sheep is a mycotoxicosis caused by the fungus, *Phomopsis leptostromiformis* (Kühn) Bubak. *Journal of the South African Veterinary Medical Association*, 41, 235-247.
- WHITE, E. P., MORTIMER, P. H. & DI MENNA, MARGARET E., 1978. Chemistry of sporidesmins. In: WYLLIE, T. D. & MOREHOUSE, G. L. (eds). *Mycotoxic fungi, mycotoxins, mycotoxicoses*. An encyclopedic handbook. Vol. 1. 427-447. New York: Merck Dekker, Inc.
- ZIMMERMAN, H. J., 1978. *Hepatotoxicity*. New York: Appleton Century Crofts.