Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 9, Number 2, October, 1937.

Printed in the Union of South Africa by the Government Printer, Pretoria.

The Distribution and Possible Translocation of Icterogenin in *Lippia rehmanni* (Pears).

By G. C. S. ROETS, Section of Toxicological Chemistry, Onderstepoort.

IT has been definitely established for a number of plants that the concentrations of their active toxic principles in their various parts differ to a remarkable degree.

By drenching 500 gm. amounts of dried powdered leaves of *Lippia rehmanni* (Pears) mixed with water to sheep through a stomach tube, Quin (1933) found that the plant causes geeldikkop symptoms. The active principle has been isolated in crystalline form by Rimington and Quin (1935). On account of the marked icterus this substance produces, it was proposed to call it " Icterogenin ". A method was devised by Rimington, Quin and Roets (1937), which enabled the author to carry out with a fair degree of accuracy quantitative determinations of Icterogenin in the various organs of the plant.

When, on one occasion, *Lippia rehmanni* (Pears) material was being collected on a plot established at Onderstepoort, the characteristic root system aroused attention. Roots were found up to 12 feet in length and ¾-inch in diameter. The relatively thick cortex could easily be removed from the stele by hand, when fresh. A sample of these roots was taken on the 6th April, 1936. It was air-dried and powdered and a quantitative determination of Icterogenin was carried out. It was found that 350 gm. yielded 2·5 gm. of the sodium salt of Icterogenin, equivalent to 0·71 per cent.

This was a very high figure, since from previous batches of dried leaves 0·25 per cent, had been considered an excellent yield. The available plants, which were in the post-seeding stage by that time, were dug up, divided into leaves, stems, root cortex and root steles, and dried in the laboratory by spreading on large sheets of paper. Quantitative determinations were carried out separately on a powdered sample of each fraction, and the yields expressed as the percentage sodium salt of Icterogenin, were as follows:—

Leaves	0·096
Stems	a trace only.
Root cortex	2·04
Root steles	a trace only.

These figures were so suggestive that it was decided to carry out further investigations on *Lippia rehmanni* (Pears). An evenly distributed patch of plants, growing under natural conditions on the farm Derdepoort near Pretoria, was selected for this purpose, and material gathered from time to time. Determinations were made on the root cortex and leaves, collected from the same plants, representing respectively the underground and above ground localisation of the toxic substance. The inflorescences were included with the leaves because the amounts of the former were relatively very small.

The data obtained during the period from the 6th May, 1936 to the 27th April, 1937, are tabulated below. The rainfall figures quoted were recorded at Onderstepoort approximately 4 miles from the spot where the plant material was collected, the rainfall in both places being approximately the same.

The figures for the leaves and root cortex are expressed as the percentage of sodium salt of Icterogenin yielded by air dried materials. The percentage of sodium in the sodium salt is about 4·1. Only traces of Icterogenin could be detected in the stems and steles of the roots.

	Date of sampling.	Percentage sodium salt of Icterogenin.		Rainfall in inches.	Remarks.
		leaves.	Root cortex.		
1936.					
March........	—	—	—	8·50	—
April.........	—	—	—	0·51	—
May..........	6	0·17	1·59	4·26	Post seeding stage.
June.........	—	—	—	—	—
July..........	—	—	—	—	—
August........	—	—	—	—	—
September....	28	0·28	1·01	0·46	Budding stage.
October.......	—	—	—	2·68	—
November....	5	0·12	1·76	6·53	—
December.....	10	0·10	3·38	3·37	—
1937.					
January......	—	—	—	4·51	—
February.....	—	—	—	10·64	Rain nearly all in first half of month.
March.........	11	0·32	2·54	1·38	Post-seeding stage; some plants pruned.
April..........	27	0·26	1·92	2·20	Post-seeding stage; from unpruned plants.
April..........	27	0·96	4·40	—	Tender young leaves from pruned plants.

Appreciation of the significance of the above data will be facilitated by recalling certain aspects of plant poisoning as it occurs in the field.

With rare exceptions like *Cicuta occidentalis*, which occurs in parts of the United States of America and of which the old rootstocks when exposed through heavy rains are exceedingly poisonous throughout the year (Howes, 1933), plant poisoning in stock is, in nearly every instance, caused by the above ground parts of plants, which are ingested by the animals while grazing. Animals may eat the plants accidentally or be attracted by the fresh greenness of the leaves, whilst the remaining vegetation is still dry, as for example occurs in the case of "tulp" poisoning caused by species of *Homeria* and *Moraea* (Steyn, 1928) and "gifblaar" poisoning caused by *Dichapetalum cymosum*. Plants which have a high nutritive value and are eagerly eaten by stock may occasionally become poisonous and be responsible for heavy losses. As an example, certain species of *Geigeria* may be mentioned, normally good fodder plants, but at times becoming highly toxic. From these plants which are the cause of "vermeersiekte" (vomiting disease) in sheep, Rimington, Roets and Steyn (1936) have isolated the active principle "vermeeric acid". Hydrocyanic acid poisoning may frequently occur in animals grazing on natural pastures on which certain grasses (species of *Cynodon*, *Sorghum*, etc.), are present. These grasses under certain sets of conditions are liable to develop dangerous quantities of HCN. Geeldikkop may be caused in an analogous manner at certain times by species of *Tribulus* or *Panicum*.

Returning to the experimental findings quoted in Table I, it is clear that wide fluctuations may be encountered in the toxicity of *Lippia rehmanni* (Pears). The plants which are not disturbed by grazing or cutting show periodical variations between 0·10 and 0·32 per cent. in the concentration of the toxic substance. As the soil in the selected patch showed a high degree of uniformity, the variations in poison concentration may be due either to the stage of growth or to the metabolic activities of the tissues as influenced by climatic conditions.

Stage of growth, which has received due consideration from modern workers on the nutritive value of pasture plants, is of great importance also as far as the toxicity of plants is concerned. Thus *Echinopogon ovatus* Beauv., the "Noogoora Burr", is poisonous only in the young stage while still bearing its cotyledons. *Lotus australis* Andr., when immature, contains a cyanogenetic glucoside but none of this toxic substance when mature (Howes, 1933). The leaves and stems of *Cicuta occidentalis* are poisonous when young, but harmless in the later stages of development. At the period of reproduction the nicotine concentration in the leaves of the tobacco plant increases (Vickery, Pucher and Levenworth, 1933). In *Lippia rehmanni* there is a decrease from 0·28 in September to 0·10 in December in the Icterogenin content of the leaves. There is, however, a rise to 0·32 in March, 1937, followed by a decrease to 0·26 in April, a month later. Plants in the post-seeding stage on the 6th May, 1936, yielded 0·17, while the concentration in the sample almost a year later (27th April, 1937) was 0·26, the plants again being in the post-seeding stage.

From the above figures, it appears that the stage of growth does not determine the toxicity in the case of *Lippia rehmanni* (Pears).

The fact that many plants store poisonous substances in their underground parts was well known to the Natives in many countries before Civilisation. Thus bulbs of *Adenium lugardi* form one of the sources of Bushman arrow poisons. Also in *Glyceria spectabilis* the hydrocyanic compound is distributed in the panicles, leaves, and roots (Minssen, 1934). Guerin (1933) pointed out that all the various organs, with the exception of fruits, of *Glyceria aquatica* contain a cyanogenetic glucoside. Marais and Rimington (1934) determined quantitatively the hydrocyanic acid liberated by "Linamarin" in different parts of *Dimorphotheca cuneata* Less., and found it to be distributed in the flowers, leaves, stems and roots. In *Urginea Burkei* Baker the flower heads, leaves and bulbs are poisonous (Steyn, 1934). According to information which was kindly given to me by Dr. D. G. Steyn, of Onderstepoort the minimal lethal dose for rabbits of the fresh leaves of *Dichapetalum cymosum*, collected on the same day varied from 1·2 to 6 gm. per kilo body weight. A dose of 60 gm. of thte underground stems of the same plants proved harmless, whereas in previous experiments the cortex of the underground stems had been far more toxic than the leaves.

Lippia rehmanni is no exception to the numerous examples recorded, where toxic substances are distributed both in the underground and above-ground parts of the plants. In comparing the figures tabulated above it is, however, of interest to note that a decrease in the Icterogenin content of the leaves is accompanied by a corresponding increase in that of the root cortex from September to December. Conversely in the March sample there is a decrease in the Icterogenin content of the root cortex and an increase in the leaves. In April the concentrations decreased both in the leaves and in the root cortex. The sample (27th April), which had been defoliated by cutting and pruning on the 11th March, so as to simulate the effect of trampling and close grazing, showed a markedly higher Icterogenin concentration, both in the leaves and in the root cortex, as compared with that of the undisturbed plants collected on the same day. These figures strongly suggest a translocation between the upper and underground parts of the plant. A consideration of the data on the pruned plants indicated a definite possibility that pruning, resulting in fresh developmental activity, stimulates the plants to synthesize an excessive amount of Icterogenin, which thereafter may be relatively rapidly transferred to the root cortex.

In this connection it may be mentioned that on Karoo pastures geeldikkop as caused by *Tribulus* may sometimes occur when the plants are well developed, although it is more frequently encountered on closely grazed and trampled pastures. The same applies to "geeldikkop" caused by veld grass. Steyn (1928) observed the disease on old lands. *Panicum maximum* was suspected of causing these outbreaks. Rimington and Quin also report cases of geeldikkop on closely cut grass pasture, on which *Panicum laevifolium* predominated. So far attempts to detect Icterogenin in these plants have failed, although work along these lines is still in progress.

Apart from pruning and cutting, climatic conditions may be responsible for the rate of Icterogenin synthesis and its possible translocation in the plant. Thus Henrici (1926) found that in certain grasses hydrocyanic acid appears suddenly, only to disappear again a few hours after a heavy rain, when the wilted tissues recover normal turgescence. Climatic conditions are probably responsible for the development of toxic amounts of hydrocyanic acid in *Cynodon incompletus* Nees (Finnemore and Jaffray, 1935). *Atalaya hemiglauca* F. Muell. causes the disease known as "walkabout" in Australia. In many cases the plants develop little or none of the responsible saponin under the less favourable Northern and Southern temperature conditions (Howes, 1933). Sunshine and temperature may influence the alkaloidal content of *Atropa belladonna*. In conditions of low temperature and deficient sunshine the plant was found to be lower in alkaloidal content. Moreover the type of light may influence the development of the toxic substance in *Datura stramonium* L. (Steyn, 1934). Ghosh and Krishna found the Ephedrin content of *Ephedra* lowest during the rainy season and highest under the drier climatic conditions in autumn. In *Lippia rehmanni*, taking into consideration the unpruned plants only, the highest figure for the root cortex and the lowest one for the leaves were both found in the sample collected on the 10th December after a fair, early summer rainfall. Root cortex gathered on the 28th September after only a few spring showers had followed the dry winter months, gave the lowest yields of Icterogenin so far recorded. The concentration was the highest in the leaves after a high followed by a moderately low rainfall. Further work may throw more light on this behaviour of the plant.

Icterogenin occurs in the plant as the free acid. Hand sections have been made through various tissues rich in Icterogenin but no crystalline material could be observed microscopically. It is evidently associated with other organic substances, which may influence its physical behaviour. The sodium salt of Icterogenin, for example, while still accompanied by other organic substances, is fairly soluble in water, as was evident during the process of isolation. It is, however, almost insoluble in water when pure.

SUMMARY.

(1) Icterogenin may be present in variable amounts in the leaves and inflorescence of *Lippia rehmanni* (Pears) whereas only traces are to be found in the stem and root stele.

(2) There is a possible translocation of the toxic substance to the root cortex from the leaves, where presumably it is formed. The further possibility exists that the Icterogenin so stored in the root cortex may under certain specific conditions be translocated to the leaves of the plant. This point is receiving special attention in further work on this problem.

(3) The stage of growth does not appear to be of great importance as far as the toxicity of *Lippia rehmanni* (Pears) is concerned.

(4) Climatic conditions may to a large extent influence the synthesis and translocation within the plant tissues.

(5) Pruning and cutting cause a marked increase in the concentration of Icterogenin in both the leaves and root cortex.

(6) From the work carried out on the plant *Lippia rehmanni* (Pears) as described in this paper, the suggestion presents itself that

the reciprocal translocation of toxic principles between the above-ground and the subterranean portions of a plant may be a factor of great significance in the determination of its toxicity. This point merits further investigation.

ACKNOWLEDGEMENTS.

I wish to thank Drs. Rimington, Quin and Steyn for their interest and encouragement in this work. My thanks are also due to Mr. J. Wolmarans for his kind permission to collect the necessary plant materials on his farm.

REFERENCES.

FINNEMORE, H., AND JAFFRAY, A. B. (1935). The amount of Hydrocyanic acid in a Blue couch grass, *Cynodon incompletus* Nees. *J. Counc. Sci. Ind. Res.*, Vol. 8, No. 2, pp. 136-138.

GHOSH, T. P., AND KRISHNA, S. (1930). Jahreszeitliche Veranderung des Alkaloidgehalts der indischen Ephedra Arten. *Berichte Deuts. Pharm. Gesellschaft*, Band 40, pp. 636-43.

GUERIN, P. (1933). Hydrocyanic acid in *Glyceria aquatica* Wahlb. (*G. spectabilis* M. et K.). *Herb. Abs.*, Vol. 3, No. 1, p. 10.

HENRICI, M. (1926). Preliminary report upon the occurrence of Hydrocyanic acid in the grasses of Bechuanaland. *11th and 12th Repts. of the Dir. of Vet. Serv. and An. Ind.*, pp. 495-8.

HOWES, F. N. (1933). Variability in Stock-poison plants. *Kew Bull.*, No. 7, pp. 305-21.

MARAIS, J. S. C., AND RIMINGTON, C. (1934). Isolation of the poisonous principle of *Dimorphotheca cuneata* Less, *Onderstepoort Jnl. Vet. Sci. and An. Ind.*, Vol. 3, No. 1, pp. 111-118.

QUIN, J. I. (1933). Studies on the photosensitisation of animals in South Africa. V. The toxicity of *Lippia rehmanni* (Pears) and *Lippia pretoriensis* (Pears). *Onderstepoort Jnl. Vet. Sci. and An. Ind.*, Vol. 1, No. 2, pp. 501-4.

RIMINGTON, C., ROETS, G. C. S., AND STEYN, D. G. (1936). Chemical studies upon the Vermeerbos, *Geigeria aspera* Harv. II. Isolation of the active principle, " Vermeeric acid ". *Onderstepoort Jnl. Vet. Sci. and An. Ind.*, Vol. 7, No. 2, pp. 507-520.

RIMINGTON, C., AND QUIN, J. I. (1935). The isolation of an Icterogenic principle from Lippia rehmanni Pears. *S.A. Jnl. of Sci.*, Vol. 32, pp. 142-151.

RIMINGTON, C., QUIN, J. I., AND ROETS, G. C. S. (1937). Studies upon the photosensitisation of animals in South Africa. X. The icterogenic factor in geel-dikkop. Isolation of active principles from *Lippia rehmmanni* Pears. *Onderstepoort Jnl. Vet. Sci. and An. Ind.*, Vol. 9, No. 1.

STEYN, D. G. (1929). Dikoor in sheep. *Rept. Proc. First Trienn. Dept. of Agric., Un. of South Africa*, 23-28th July, 1928, pp. 196-199.

STEYN, D. G. (1934). Tulp poisoning. *13th and 14th Repts. of the Dir. of Vet. Ser. and An. Ind.*, Part 1, p. 197.

STEYN, D. G. (1934). The Toxicology of Plants in South Africa. Central News Agency, South Africa. pp. 31-37 and 552.

VICKERY, H. B., PUCKER, G. W., LEVENWORTH, C. S., AND WAKEMAN, A. J. (1935). Chemical investigations of the Tobacco plant. *Conn. Agri. Expt. Sta. Bull.* 374.