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AN EXAMINATION OF NORTH AND SOUTH AMERICAN ISOLATES OF PITHOMYCES CHARTARUM FOR PRODUCTION OF SPORIDESMIN AND SPORIDESMOLIDES[1]

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Two hundred and fifty two isolates of *Pithomyces chartarum* have been collected from pastures in Brazil (101), Colombia (30), Texas (5), and Nova Scotia (116) during the last 20 years. It has been possible to examine 57 of these, 2 from Texas, 3 from Nova Scotia, 20 from Colombia, and 32 from Brazil, in detail. Twenty-one of these isolates produced spores on rye-grain medium and all produced sporidesmolides I to V in similar proportions. Ten of the isolates also produced metabolites that inhibited the growth of *Micrococcus luteus* but none of the isolates produced sporidesmin or any of its known 9 congeners in amount greater than the detectable limits. Thus *P. chartarum* in these American countries appear to differ physiologically from isolates of the same species found in Australia, New Zealand, and South Africa.

Deux cent cinquante-deux échantillons isolés de *Pithomyces chartarum* ont été collectionés de pâturages du Brésil (101), de la Colombia (30), du Texas (5), et de la Nouvelle-Écosse pendant les 20 dernières années. On a pu examiner en détail 57 de ceux-ci, 2 du Texas, 3 de la Nouvelle-Écosse, 20 de la Colombie, et 32 du Brésil. Vingt-et-un de ces isolats ont produit des spores sur du milieu au seigle, et tous ont produit des sporidesmolides I à V en proportions egales. Des métabolites de dix isolats ont inhibé la croissance de *Micrococcus luteus*, mais nul isolat a produit du sporidesmin ou aucun de ses 9 congénères connus, en montant susceptible au repérage. *P. chartarum* des pays américains nommés ci-dessus semble alors se différencier du point physiologique des isolats de la même espèce découverts en Australie, en Nouvelle Zelande, et en Afrique du Sud.

Introduction

Photosensitivity is a common symptom of diseases of sheep and cattle that graze permanent pasture in the sub-tropics. It has been reported in Argentina (Regalado, 1981), Australia (Flynn, 1962), Brazil (Döbereiner *et al.*, 1976), Colombia (Aycardi *et al.*, 1982), New Zealand (Done *et al.*, 1960), South Africa (Kellerman *et al.*, Marasas *et al.*, 1972) and Texas (Halder *et al.*, 1981; Taber *et al.*, 1968). In all cases, the occurrence of photosensitivity was associated with dense populations of *Pithomyces chartarum* on the herbage debris. Isolates of this fungus from such areas in New Zealand, Australia and South Africa have been shown to produce a group of about 9 related metabolites known as sporidesmins (Fig. 1; Leigh and Taylor, 1976). Extensive toxicity studies of one of these metabolites have enabled veterinarians to reproduce many of the

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features of photosensitivity observed in the field (Taylor, 1971). However it has not been possible to demonstrate the production of sporidesmins by isolates of *P. chartarum* collected from pastures in Texas where cattle were photosensitive (Halder *et al.*, 1981; Ueno *et al.*, 1974).

During the last 25 years workers in several places in North and South America have sent to the Atlantic Research Laboratory (ARL), National Research Council of Canada, isolates of *P. chartarum* that they had obtained from pastures where sheep and cattle had been photosensitive. In many cases these workers had been unable to demonstrate the production of sporidesmin by these cultures. It seemed possible that an explanation of the difference in results in the Americas from those, e.g. in New Zealand might be due to misidentification because of the great morphological similarity between, e.g. *P. chartarum* and *P. maydicus* (Dingley, 1962) and/or that the American isolates produced a different toxin(s). We have therefore examined 54 of the 139 isolates sent to ARL in detail and have also subjected 3 isolates from the ARL Nova Scotian collection of 116, one of which had shown biological activity in a preliminary screen (Brewer *et al.*, 1974) to the same detailed investigation. The results of this work are reported.

Materials and Methods

Organisms. Pithomyces chartarum (Berk. & Curt.) M.B. Ellis, HLX 133 was obtained in New Zealand from a high-sporing sector of a wild isolate (Dingley *et al.*, 1962). Isolates HLX 975 and 976 were obtained from Dr. W.A. Taber, Texas A&M University, College Station, Texas. Isolates HLX 1533 to HLX 1581 and HLX 1605 to 1607 were from Brazil. The isolates HLX 1582 to 1604 were from Colombia. Isolates HLX 1159, 1160 and 1635 were collected with a spore-trap at Nappan, Nova Scotia. *Micrococcus luteus* HLX 701 was maintained, cultivated and used as an assay organism as previously described (Liss *et al.*, 1985).

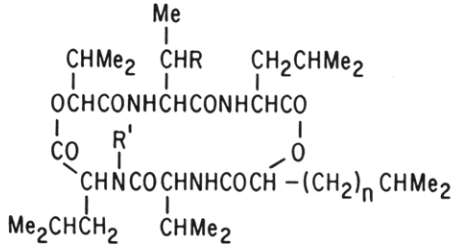
Cultivation of Organisms. All of the isolates were grown on rye grain (*Secale cereale*, Done *et al.*, 1961). Those isolates that sporulated feebly on rye (HLX 1159, 1160, 1557, 1574 and 1635) were also cultivated on the following medium (g L^{-1}): agar (Difco), 20; maltose, 30; Casitone (Difco), 3.5; yeast extract (Difco), 1; KH_2PO_4, 1; KCl, 0.5; $MgSO_47H_2O$, 0.5; $FeSO_4xH_2O$, 0.01; $CaCl_22H_20$, 0.037; and trace metal solution (Ross, 1960) 10 mL. All fermentations were carried out on lots of 4 or 5 isolates and in addition, as a positive control, a fermentation of HLX 133 on the same scale was included.

Harvesting and Extraction. The rye-grain cultures (usually 10) were mixed with 80% (v/v) methyl alcohol-water (*ca* 200 mL), were combined and the mixture was stirred for 24 h, was filtered and the filtrate evaporated. The residue was taken up in methyl alcochol (300mL), petroleum ether (b.p. 30-60°C, 300 mL) and water (15 mL) were added, the phases separated (some required centrifugation at 1500 r min^{-1}) and the lower phase was re-extracted (x 3) with petroleum ether (300 mL). The methanolic phase was evaporated the residue treated with water (50 mL), and the mixture either extracted continuously for 18 h with diethyl ether or, in about half of the fermentations, extracted with ether (x 3) in a tap funnel. The ethereal extract was evaporated and the residue used for biological assay and chemical analysis.

Isolation of Sporidesmolides (Fig 1). The residue from the rye-grain cultures was suspended in chloroform (2 L), the mixture filtered and the residue washed twice with chloroform. The cultures on agar were treated similarly, but with smaller, appropriate volumes of chloroform. The extract and washings were combined and evaporated and the residue was triturated with diethyl ether. The precipitated solid was purified to give the total sporidesmolide fraction as described previously (Russell *et al.*, 1962).

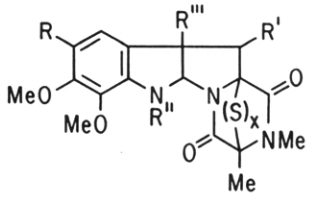
Analysis. Thin layer chromatography (t.l.c.) was carried out on Merck SiO_2 plates (20 x 20 x 0.01 cm) and benzene-ethyl acetate (4:1) was used as the developing solvent. Metabolites were detected on the plates by their ultraviolet absorption and by spraying the plates with neutral aqueous silver nitrate solution (1%, w/v) (Rahman *et al.*, 1970). Extracts were dissolved in methyl alcohol (*ca* 10 mg mL^{-1}) and two samples (0.5 mL) taken, both treated with 0.01*N* iodine solution (5 mL) and one with sodium azide (1 mL, 1% w/v). The 2 solutions were titrated with standard sodium arsenite and the concentrations of disulphides calculated as described (Brewer and Taylor, 1967). Similar solutions (0.05 mL) of the extracts were used for the disc bioassay. All bioassays were duplicated and all incorporated sporidesmin as a positive control.

Sporidesmolides (Fig 1) were analysed by high pressure liquid chromatography (h.p.l.c.). A Zorbax ODS column (DuPont, 25 x 0.46 cm) was used. The mobile phase was methyl alcohol-water (41:9), the flow rate 0.7 mL min^{-1} and the

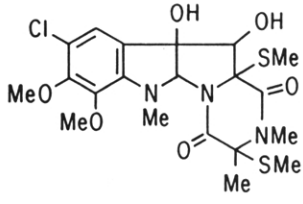


I R = R' = Me, n = 0
II R = Et, R' = Me, n = 0
III R' = H, R = Me, n = 0
IV R = R' = Me, n = 1
V R = Et, R' = Me, n = 1

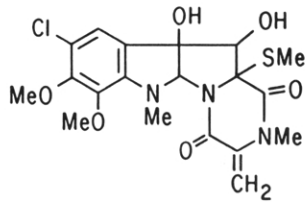
Sporidesmolides



Sporidesmin	R = Cl, R' = R''' = OH, R'' = Me, x = 2
B	R = Cl, R''' = OH, R' = H, R'' = Me, x = 2
E	R = Cl, R' = R''' = OH, R'' = Me, x = 3
G	R = Cl, R' = R''' = OH, R'' = Me, x = 4
H	R = H, R''' = Cl, R' = H, R'' = Me, x = 2
J	R = Cl, R' = R''' = OH, R'' = H, x = 2



Sporidesmin D



Sporidesmin F

Fig 1 Chemical formulae of sporidesmolides and sporidesmins.

separation was conducted at 50°. Sporidesmolides (2-20 mg mL^{-1}) in chloroform (or dichloromethane)-ethyl alcohol (3:1) were injected onto the column using a Waters injection block and detected by refractometry (Waters 410 refractometer). Calculation of the percentage composition of the eluted sporidesmolides was done by a Waters data module (Model 730).

Results

Experimentally, the production of a metabolite by a fungus can only be demonstrated when the metabolite accummulates in the culture medium in a concentration greater than the detection limit of a chemical or biological assay. In the case of sporidesmin(s) (Fig 1) the most convenient biological assay, to our knowledge, is that using *M. luteus* as the assay organism. In Fig 2 the relationship of zones of inhibition of this organism with respect to concentration of sporidesmin is plotted and it can be seen from this relationship that the minimum quantity of sporidesmin detectable is 1.5 μg.

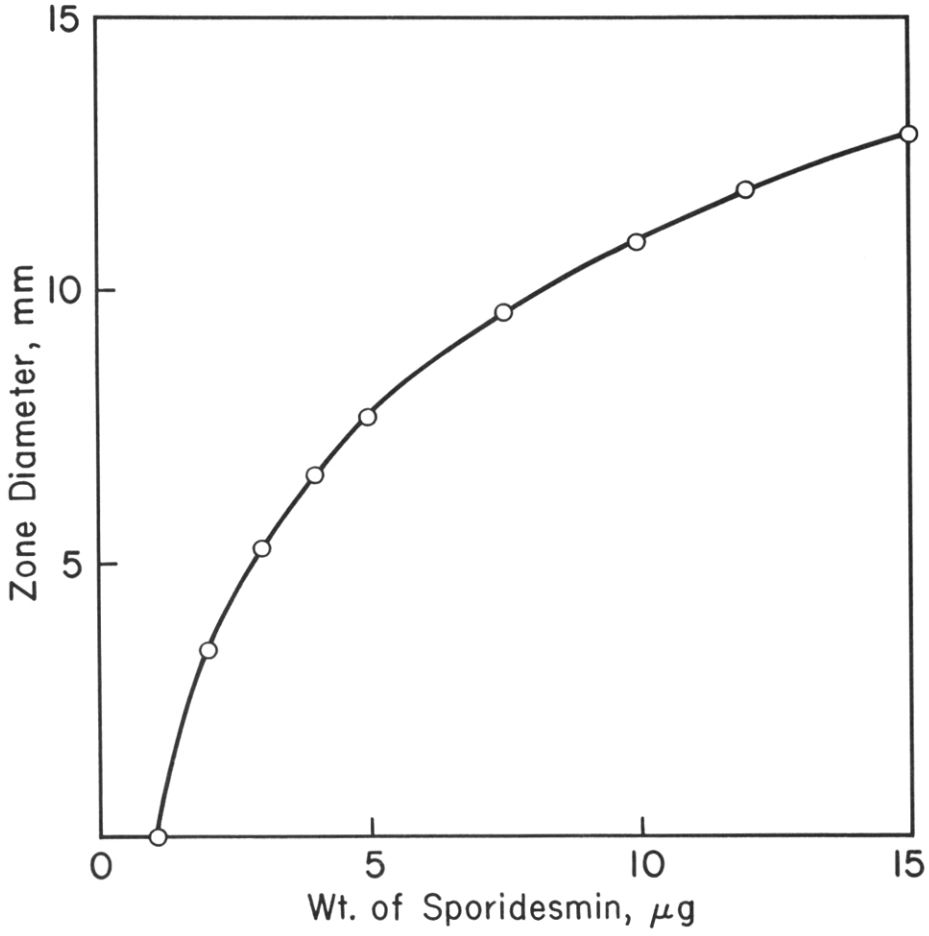


Fig 2 Growth inhibitory activity of sporidesmin against *Micrococcus luteus*.

In the case of chemical analyses of sporidesmin, 0.1 - 0.5 μg can be detected on thin layer chromatograms by reflectance ultraviolet light or by spraying the plates with neutral aqueous silver nitrate. Thus, if 750 μg of an extract suspected of containing sporidesmin is chromatographed, sporidesmin present in about 0.03% can be detected. Volumetrically, (Brewer and Taylor, 1967) sporidesmin concentrations of about 0.01% can be reliably estimated.

The weights of extracts grown on 750 g of rye grain lay in the range 0.3 - 1.2 g. A maximum of 5 mg of this material could be analysed by any of the above methods before materials other than sporidesmin interfered with the analysis. Thus 90-360 μg of sporidesmin in 750 g of nutrients could be detected.

The results reported here are in no way a thorough survey of *Pithomyces* in different countries, but rather an examination of different isolates that were available. In all cases where no activity was observed, this result is indicated in Table I by a blank.

The isolates were grown in groups of 4 or 5 and each group included an HLX 133 fermentation on the same scale. Sporidesmin was produced in all 12 HLX 133 fermentations, the yield from 10 flasks of the rye-grain medium being 226 ± 61 mg, and 40 μg of the extracts gave inhibition zone diameters in the range 9-12 mm. By comparison the biological activities given in Table I are low, with the possible exception of HLX 1604. The extract from this isolate contained a component(s) that has the same R_F as sporidesmin in the chromatographic system used, but the material did not react with silver nitrate.

In only 6 cases (HLX 1533, 1537, 1557, 1569, 1582, and 1598) were extracts obtained that were biologically active and also catalysed the decomposition of azide in the presence of iodine. Chromatography of these extracts showed that no sporidesmin or known sporidesmin derivative was present in concentrations above the minimum detectable limit. In 2 cases, HLX 1533 and HLX 1537, spots on chromatograms were obtained that reacted with silver nitrate, but their R_F corresponded to no known sporidesmin congener.

These results suggested to us that the organisms isolated in North and South America might belong to a different species of *Pithomyces*. *Pithomyces maydicus* (IMI 46232, 98084) is morphologically very similar to *P. chartarum* (Dingley, 1962) and the two fungi are most easily distinguished by the different sporidesmolides found in the spicules of their spore coats (Bertraud *et al.*, 1963; Bishop *et al.*, 1965). Recently it has been shown* that the sporidesmolides found on the spores of HLX 133 are a more complex mixture than hitherto supposed (Taylor, 1970). This increased complexity, however, probably serves to characterise the spicules on a particular isolate more rigorously. The isolates examined, that produced spores prolifically, were therefore analysed for the composition of the sporidesmolides in their spicular coats. These organisms (21 of 57) are indicated in Table I by the appearance of analytical results in the sporidesmolide column. Sporidesmolides were isolated and purified from 5 (HLX 1548, 1585, 1590, 1598 and 1607) of these 21 cultures. The melting points of these samples varied in the range 246-251° and their specific optical rotations ($[\alpha]_D^{24}$, c 0.5 $CHCl_3$) lay in the range -200° to -207°. All gave leucine, allo-isoleucine, N-methyl-leucine and valine after acid hydrolysis (Russell, 1962). The mean value for the sporidesmolide I (Fig 1) component of the mixture in the 21 samples was 63 ± 6%. It may be concluded that none of these isolates can be classified as *P. maydicus* and that their identification as *P. chartarum* is correct.

*It is intended to publish full details of the hitherto undescribed metabolites of *P. chartarum* in the Canadian Journal of Chemistry as soon as possible.

Table I Geographical origin, plant substrates and production of sporidesmolides and toxic metabolites by isolates of *Pithomyces chartarum*.

Accession No. (HLX)	Origin	Substrate	Yield	Sporidesmolides % Composition					Bioassay (HLX 701)		
				I	II	III	IV	V	Wt. on disc mg	Inhibition Zone diameter mm	$AgNO_3$ + N_3^-/I_2^{**}
	U.S.A										
975	Texas	Herbage	23	73	15	3	7	2	4	10	
976	Texas	Herbage							3	1	
	Canada										
1159	Nova Scotia	Herbage	83*	69	28	1	+	1		8	
1160	Nova Scotia	Herbage	43*	62	35	1	+	1	7	14	
	Brazil										
1533	Sao Paulo	*Linum usitatissimum*							1	8	+
1535	Mina Gerais	*Pennisetum purpureum*							1	3	
1537	Sao Paulo	*Paspalum notatum*							1	5	+
1540	Sao Paulo	*Oryza sativa*									
1542	Mato Grasso	*Oryza sativa*									
1544	Sao Paulo	*Oryza sativa*									
1545	Sao Paulo	*Brachiaria mutica*	19	59	21	7	8	4			
1548	Sao Paulo	*Brachiaria decumbens*	63	64	24	2	7	3			
1549	Sao Paulo	*Brachiaria decumbens*									
1550	Sao Paulo	*Panicum maximum*									+
1551	Sao Paulo	*Pennisetum purpureum*									
1553	Sao Paulo	*Oryza sativa*									
1554	Sao Paulo	*Oryza sativa*	20	69	18	2	9	2			
1556	Sao Paulo	*Oryza sativa*									
1557	Sao Paulo	*Panicum maximum*	59*	54	39	2	+	4	1	3	+
1558	Sao Paulo	*Oryza sativa*									
1565	Rio de Janeiro	*Brachiaria decumbens*									
1566	Sao Paulo	*Brachiaria decumbens*							1.5	4	
1567	Goias	*Panicum maximum*									
1568	Goias	*Pennisetum purpureum*									
1569	Goias	*Brachiaria decumbens*							1	3	+
1571	Sao Paulo	*Brachiaria decumbens*	20	63	23	2	10	3			
1573	Brasilia	*Desmodium intortum*	29	68	22	2	5	2			
1574	Sao Paulo	*Paspalum plicatulum*	155*	60	35	3	+	2			

1577	Sao Paulo	*Stylosanthes sp.*							1	3	
1578	Goias	*Panicum maximum*	55	51	34	4	7	4			+
1579	Sao Paulo	*Panicum maximum*									
1580	Sao Paulo	*Setaria anceps*									
1581	Sao Paulo	*Oryza sativa*									
	Colombia										
1582	Santander	*Brachiaria sp.*							6	10	+
1583	Santander	*Brachiaria sp.*									+
1584	Limonar	*Panicum sp.*									
1585	Santander	*Andropogon sp.*	93	72	16	1	7	4			
1586	Santander	*Andropogon gayanus*	11	64	18	2	12	3			
1587	Limonar	*Andropogon sp.*									
1589	Santander	*Andropogon gayanus*									
1590	Santander	*Brachiaria sp.*	28	57	27	3	9	4	4	10	
1591	Pto. Gaitan	*Brachiaria sp.*									
1592	Pto. Gaitan	*Brachiaria sp.*									
1594	Santander	*Brachiaria sp.*									
1595	Limonar	*Andropogon sp.*	35	63	18	4	12	3	6	9	
1596	Carimagua	*Brachiaria sp.*							13	13	
1597	Santander	*Andropogon gayanus*	71	69	21	3	5	2	8	5	
1598	Santander	*Andropogon sp.*	20	60	24	2	10	3	9	19	+
1599	Limonar	*Brachiaria sp.*									
1600	Carimagua	*Brachiaria sp.*									
1602	Santander	*Brachiaria sp.*							7	15	
1603	Carimagua	*Brachiaria sp.*							13	13	
1604	Santander	*Brachiaria sp.*	80	74	16	3	6	1	8	31	
	Brazil										
1605	Goias	*Hyparrhenia rufa*	18	59	31	2	5	3			
1606	Goias	*Mellinis minutiflora*									+
1607	Sao Paulo	*Digitaria decumbens*	78	64	22	6	6	2	0.2	5	
	Canada										
1635	Nova Scotia	Herbage	2*	56	38	2	+	4			

Yields in the sporidesmolide columns are given in g/50 g of rye except for those marked with an asterisk where the yields are mgL^{-1} of agar medium.
A + in the sporidesmolide IV column indicates that this component was detected but not integrated.

** Chemical and chromatographic analysis of epipolythiodiketopiperazines (19,5).

Discussion

The results appear to suggest that isolates of *Pithomyces chartarum* from widely distributed pastoral sites in the Americas do not produce sporidesmins in laboratory culture despite the occurrence of photosensitivity in ruminants grazing the pastures. In Australia, New Zealand and South Africa the presence of isolates of this fungus that do not produce sporidesmins (Fig 1) is well-documented (Dingley *et al.*, 1962) and indeed, in South Africa, it was thought for many years that *P. chartarum* was not part of the etiology of geeldikkop or dikoor (Kellerman *et al.*,). However, the number of isolates examined in this work from Brazil certainly exceeds the numbers that had to be investigated in South Africa and New Zealand before an organism capable of producing sporidesmin was found, but the number of isolates from a particular location was small. Another difficulty in demonstrating the production of sporidesmins by wild isolates of this fungus lies in their rapid loss of this ability after a few subcultivations in the laboratory (Dingley *et al.*, 1962). Such a loss would have to be very rapid in the case of the Nova Scotia isolates, where the cultures obtained directly from the spore-trap were subcultivated only once to provide the giant colonies for the bioassay.

We have considered the possibility that tryptophan metabolism in American isolates of *P. chartarum* might be directed towards the production of sporidesmin-D and sporidesmin-F (Jamieson *et al.*, 1969). Apart from a low toxicity (Taylor, 1971) these metabolites do not catalyse the decomposition of azide by iodine as efficiently as the di- and higher poly-sulphides, and they react very slowly with silver nitrate. Some of the isolates, particularly those from Colombia gave ultraviolet-absorbing spots on t.l.c. with the same R_F as sporidesmin-D but they could be distinguished from the latter metabolite because they had an intense pale blue fluorescence when irradiated with long wave length ultraviolet light.

One may conclude either that the density of organisms in the *Pithomyces chartarum* population in the Americas capable of producing sporidesmins is lower than it is in South Africa, New Zealand and Australia, or that the agent(s) inducing photosensitivity are not associated with this fungus, or are not related to sporidesmin.

References

Aycardi, E.R., Garcia, O., Henaw, F.J. and **Torres, B.** 1982. Fotosensibilidad experimental en bovinos en un areas tropical utilizando hongo *Pithomyces chartarum*. *Revista acovez*, 6: 23-24.

Bertaud, W.S., Morice, I.M., Russell, D.W. and **Taylor, A.** 1963. The spore surface in *Pithomyces chartarum*. *J. gen. Microbiol.* 32: 385-395.

Bishop, E., Griffiths, H., Russell, D.W., Ward, V. and **Gartside, R.N.** 1965. Spore surface depsipiptides in some *Pithomyces* species. *J. gen. Microbiol.* 38: 289-297.

Brewer, D. and **Taylor, A.** 1967. The biological properties of 3,6-epidithia-diketopiperazines: degradation of gliotoxin-B by *Bacillus subtilis* (HLX 373). *Can. J. Microbiol.* 13: 1577-1589.

Brewer, D., Taylor, A. and **Hoehn, M.M.** 1974. The antibiotic activity of cultures from fungal spores collected by a spore-trap on permanent pasture. *Can. J. Microbiol.* 20: 721-729.

Dingley, J. 1962. *Pithomyces chartarum*, its occurrence, morphology and taxonomy. *N.Z. J. Agric. Res.* 5: 49-61.

Dingley, J.M., Done, J., Taylor, A. and **Russell, D.W.** 1962. The production of sporidesmins and sporidesmolides by wild isolates of *Pithomyces chartarum* in surface and in submerged culture. *J. gen. Microbiol.* 29: 127-135.

Döbereiner, J., Tokarnia, C.H., Monteiro, M. de C da C, da Cruz L.C.M., de Carvalho, E.G. and **Primo, A.T.** 1976. Intoxicacao de bovinos e ovinos em pastos de *Brachiaria decumbens* contaminados por *Pithomyces chartarum*. *Pesq. agropec. bras. Ser. Vet.* 11: 87-94.

Done, J., Mortimer, P.H. and **Taylor, A.** 1960. Some observations on field cases of facial eczema: liver pathology and determinations of serum bilirubvin, cholesterol, transaminase and alkaline phosphatase. *Res. vet. Sci.* 1: 76-83.

Done, J., Mortimer, P.H., Taylor, A. and **Russell, D.W.** 1961. The production of sporidesmin and sporidesmolides by *Pithomyces chartarum*. *J. gen. Microbiol.* 26: 207-222.

Flynn, D.M. 1962. Facial eczema. Part I. History of the disease in Victoria. *J. Dept. Agric. Victoria (Australia)* 1962: 5-6.

Halder, C.A., Taber, R.A. and **Camp, B.J.** 1981. Absence of sporidesmin production by twelve Texas isolates of *Pithomyces* spp. *Appl. Environ. Microbiol.* 41: 212-215.

Jamieson, W.D., Rahman, R. and **Taylor, A.** 1969. Sporidesmins. Part VIII. Isolation and structure of sporidesmin-D and sporidesmin-F. *J. Chem. Soc. (C)* 1969: 1564-1567.

Kellerman, T.S., van der Westhuizen, G.C.A., Coetzer, J.A.W., Roux, C., Marasas, W.F.O., Minne, J.A., Bath, G.F. and **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 J. vet. Res.* 47: 231-261.

Leigh, C. and **Taylor, A.** 1976. The chemistry of the epipolythiopiperazine-3,6-diones. *In* "Mycotoxins and other fungal related food problems". Advances in Chemistry No. 149, American Chemical Society 1976; pp. 228-275.

Liss, S.N., Brewer, D., Taylor, A. and **Jones, G.A.** 1985. Antibiotic activity of an isocyanide metabolite of *Trichoderma hamatum* against rumen bacteria. *Can. J. Microbiol.* 31: 767-772.

Marasas, W.F.O., Adelaar, T.F., Kellerman, T.S., Minne, J.A., van Rensburg, I.B.J. and **Burroughs, G.W.** 1972. First report of facial eczema in sheep in South Africa. *Onderstepoort J. vet. Res.* 39: 107-112.

Rahman, R., Safe, S. and **Taylor, A.** 1970. Separation of polythiadioxopiperazine antibiotics by thin layer chromatography. *J. Chromatog.* 53: 592-594.

Regalado, A.M. 1981. Intoxicacion por *Pithomyces chartarum* en bovinos en el partido de daireaux, Buenos Aires. *Gaceta Veterinaria* 43: 471-473.

Ross, D.J. 1960. A study of the physiology of *Pithomyces chartarum* (Berk. & Curt.) M.B. Ellis. II. Some factors influencing sporulation in culture. *N.Z. J. Sci* 3: 441-452.

Russell, D.W. 1962. Depsipeptides of *Pithomyces chartarum:* the structure of sporidesmolide I. *J. Chem. Soc.* 1962: 767-772.

Russell, D.W., Synge, R.L.M., Taylor, A., and **White, E.P.** 1962. Similarity of depsipeptides from *Pithomyces chartarum* and from pasture samples from "facial eczema" areas. *J. Chem. Soc.* 1962: 554-555.

Taber, R.A., Pettit, R.E., Taber, W.A. and **Dollahite, J.W.** 1968. Isolation of *Pithomyces chartarum* in Texas. *Mycologia* 60: 727-730.

Taylor, A. 1970. The occurrence, chemistry and toxicology of the microbial peptidelactones. Advances in Applied Microbiology, Vol. 12, Academic Press, New York. pp. 189-276.

Taylor, A. 1971. The toxicity of sporidesmins and other epipolythiadioxopiperazines. *In* Microbial Toxins Vol. 7, Academic Press, New York. pp. 337-376.

Ueno, K., Giam, C.S. and **Taber, W.A.** 1974. Absence of sporidesmin in a Texas isolate of *Pithomyces chartarum*. *Mycologia* 66: 36-362.

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