THIN LAYER CHROMATOGRAPHY PATTERNS OF *RHIZOPOGON* SPECIES AND THEIR POSSIBLE USE AS A TAXONOMIC CRITERION

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ABSTRACT. Thin layer chromatography patterns of *Rhizopogon* species and their possible use as a taxonomic criterion The possibility of characterizing *Rhizopogon* by thin-layer chromatography was investigated. Fifty-eight fruitbodies were sampled. The most detailed chromatograms were obtained from ethanol extracts and plates running with n-butanol-water-acetic acid (4:1:1), examined with ultra-violet light and marking out fluorescent spots. This preliminary study showed 17 chromatogram patterns, ten of which were clearly different and characteristic of only one current species (*R. evadens, R. occidentalis, R. pachydermus, R. pannosus, R. rocabrunae, R. roseolus, R. subsalmonius, R. verii, R. villosulus* and *R. vinicolor*). The chromatogram patterns may be a great aid in differentiating *Rhizopogon* species.

Key words: *Basidiomycotina*, hypogeous, *Rhizopogon*, thin layer chromatography.

RESUMEN. Modelos cromatográficos de especies del género Rhizopogon obtenidos por cromatografía en capa fina y su posible uso como criterio taxonómico. Se investigó la posibilidad de caracterizar las especies de Rhizopogon mediante cromatografía en capa fina. Se utilizaron 58 muestras de herbario. Los cromatogramas que mostraron mejor resolución fueron los obtenidos a partir de extractos en etanol utilizando n-butanol-agua-ácido acético (4:1:1) como eluente, examinando las placas con luz ultravioleta. Este estudio preliminar mostró 17 modelos cromatográficos, de los cuales 10 fueron claramente diferentes y característicos para una única especie *(R. evadens, R. occidentalis, R. pachydermus, R. pannosus, R. rocabrunae, R. roseolus, R. subsalmonius, R. verii, R. villosulus* y *R. vinicolor*). Los modelos cromatográficos pueden ser pues, de gran ayuda para diferenciar especies de *Rhizopogon*.

Palabras clave: *Basidiomycotina*, hipogeos, *Rhizopogon*, cromatografía en capa fina.

INTRODUCTION

Colours of fungi have traditionally been used as valuable taxonomic features, including colour reactions of fruitbodies after the application of chemical reagents. SINGER (1962) reviews the use of macrochemical reactions for taxonomic purposes by other authors (MÜLLER, HARLAY, BOURQUELOT, BERTRAND, ARNOULD, GORIS, BATAILLE, MAIRE, BARLOT, KÜHNER, SCHAEFFER, MÖLLER, SINGER), on Basidiomycetes. Many of the fungal compounds involved in these reactions are unknown; however, their taxonomic value is considerable.

The species of *Rhizopogon* Fr., when fresh, show a great range of peridium colours and some species show colour changes with chemical reagents, such as potassium hydroxide and ferrous sulphate (SMITH, 1964). However, herbarium samples show colours less bright than when fresh,

and few *Rhizopogon* species have the same chemical reactions on dried collections as when fresh (e.g. *R. subolivascens* A.H. Smith).

The purpose of this study was to develop a chemotaxonomic method, simple and easy to apply to *Rhizopogon*, to look for specific secondary chemicals that would be useful taxonomic markers, as a supplement to classical taxonomy.

FRIES (1958) made a preliminary chemotaxonomic study (paper chromatography) involving ethanolic extracts from 95 species (*Amanita*, *Tricholoma* and *Boletus*). BENEDICT et al. (1968) examined the distribution patterns of sugars and sugar alcohols (arabitol, mannitol, glucose, fructose, trehalose, heptulose) among some species of Boletales, concluding that their results may have chemotaxonomic significance at the species level. BENEDICT (1970) reported the presence of tetronic acids (blue pigment forming in contact with the air catalyzed by an oxidase), in *Boletus* and related species; the first isolated was variegatic acid, from *Suillus variegatus* (Swartz: Fr.) O. Kuntze. These acids are related to pulvinic acid of lichens. EDWARDS (1976) studied 21 species of *Suillus* from California.

Chemotaxonomic studies involving *Rhizopogon* are relatively absent from the literature. GIL & STEGLICH (1987) report the presence, in *Rhizopogon*, of hydroxylated pulvinic acids responsible for the yellow and red colours of most boletales (variegatic acid and xerocomic acid), providing strong evidence for the inclusion of this genus in Boletales. BRESINSKY & STEGLICH (1989) reported the presence of ansaquinone rhizopogone in *R. pumilionum* (Ade) Bataille [under *R. roseolus* (Corda) Th. M. Fr. in MARTÍN (1995)]. The peridium of many *Rhizopogon* species react with potassium hydroxide, and becomes red (e.g. *R. occidentalis* Zeller et Dodge, *R. roseolus*); this reaction is correlated to the presence of tannins, oxiflavones or anthraquinones (LOCQUIN, 1984).

In these first steps toward the chemotaxonomy of *Rhizopogon*, we have chosen thin-layer chromatography (TLC), because it is a technique routinely used with success by lichenologists. Moreover, TLC permits comparisons among species, even when the chemical nature of the extracted substances is unknown.

Thin-Layer Chromatography (TLC)

TLC is a sensitive system that uses a thin layer of silica gel over a glass, plastic or aluminium support (stationary phase) where an extract of one or more samples (mobile phase) is spotted and, then, developed in standard solvent systems (CULBERSON & KRISTINSSON, 1970). The spots on the chromatograms are visible in normal light or have to be detected in short or long wavelength ultraviolet (UV) light. Some secondary metabolites are visualized by spraying with different solutions, such as 10 % sulphuric acid, and heating 10-15' at 100ºC or 110ºC. Identification is made by means the colour of the spots, the relative position on the sheet (Rf) or the comparison with a known control run at the same time (GALUN & SHOMER-ILAN, 1988; HALE, 1983).

MATERIAL AND METHODS

Material.- Ten specimens, belonging to five species of *Rhizopogon*, well characterized by their macro and microscopic features, were used in preliminary tests. All specimens were dried herbarium samples, collected in Spain between 1988 and 1993, and located in BCC herbarium: *R. luteolus* Fr. et Nordholm, BCC-MPM 1533, BCC-MPM 1545; *R. pannosus* Zeller et Dodge, BCC-MPM 1690; *R. roseolus,* BCC-MPM 1512, BCC-MPM 1513; *R. subsalmonius* A.H. Smith, BCC-MPM 1652;

R. villosulus Zeller, BCC-MPM 1643_1, 1643_2, 1643_3, 1643_4, four fruitbodies with different degrees of maturation.

A more extensive study was carried out with 58 collections, including the type of 25 species. Table 1 shows the list of material used in this study, indicating our register number, the label name (our previous identifications following early authors, or name written in label of loan), the current name [name that in our opinion is correct, MARTÍN (1995)], geographical area and year of the collection.

Extraction procedures.- In the preliminary tests, two extraction procedures were used, each one from 30 mg dry fruitbody. In the extensive study, the extraction of type material was made from 1 mg.

Acetone extract protocol (PÉREZ-VALCÁRCEL, 1994)

1.- Cut sample in tiny pieces and place in 1.5 ml eppendorf tube.
2.- Pour acetone to cover the sample.
3.- Soak 30'.
4.- Centrifugate 6000 rpm for 5' at room temperature.
5.- Transfer the liquid phase to a new eppendorf tube.
6.- Store at +4ºC until used.

Alcohol extract protocol (FRIES, 1958)

1.- Cut sample in tiny pieces and place in 1.5 ml eppendorf tube.
2.- Add 85 % ethanol (1 ml ethanol/ 30 mg sample).
3.- Mix for 10'.
4.- Boil for 2'.
5.- Mix for 2'.
6.- Centrifugate 6000 rpm for 5' at room temperature.
7.- Transfer the liquid phase to a new eppendorf tube.
8.- Store at +4ºC until used.

Chromatographic material.- Chromatograms were developed in standard Brinkman tanks having an height of 22 or 27 cm, on 20 x 20 cm Merck pre-coated Silica Gel F_{254} plates (layer thickness 0.2 mm). The starting line is 2 cm from the bottom edge of the plate. No more that one plate was run in a tank at the same time. In preliminary tests acetone and ethanol extracts were spotted onto the same chromatographic plate.

Developing solvent systems.- Six solvent systems were tested: **(A)** benzene-dioxane-acetic acid (190:25:4); **(B)** hexane-ethyl ether-formic acid (130:80:20); **(C)** toluene-acetic acid (85:15); **(D)** n-butanol-water-acetic acid (4:1:1): **(E)** benzene-ethyl ether-methanol (85: 10: 5); **(F)** cyclohexane-ethyl acetate (3:1). The developing times were 35-45' in solvents A, B and C; 3 h 30' in D and 1 h 30' in E and F.

Chromatographic procedure.- The extracts were spotted onto the plates on the starting line using capillary tubes. In preliminary tests, four, six, eight and ten applications were made, waiting for each spot to dry before the next application. The maximum diameter of the spots was 5 mm. The chromatograms were allowed to develop to a height of 18 cm from the origin of the applied extract.

After removal from the tanks, the plates were air dried and examined: **(a)** immediately with visible light, **(b)** under UV-lamp (cromato-vue cabinet CC-10: 245 nm and 366 nm) and **(c)** after treatment with 10 % H_2SO_4 and heated to 105º for 15'. Before this treatment, permanent records of the chromatograms were made using tracing paper; indicating the Rf value (PATERSON & RUTHEFORD, 1991), colour and approximate size of each spot.

Register number	Label Name	Current name	Geographical area	Collection year	Patterns C	D
739	*R. abietis*	*R. abietis*	Spain	1985	F	G
2346	*R. abietis**	*R. abietis*	USA	1964	F	G
2357	*R. angustisepta**	*R. angustisepta*	Germany	-	A	K
751	*R. aurantiacus*	*R. aurantiacus*	Sweden	1992	B	P
439	*R. briardii**	*R. roseolus* (C)	France	1962	H	I
451	*R. briardii*	*R. roseolus* (C)	Spain	1990	G	I
464	*R. briardii*	*R. roseolus* (C)	Spain	1985	G	I
2320	*R. colossus**	*R. villosulus*	USA	1954	A	A
2321	*R. colossus**	*R. villosulus*	USA	1956	B	A
758	*R. corsicus*	*R. corsicus*	Belgium	1989	E	C
759	*R. corsicus*	*R. corsicus*	Spain	1988	E	C
2161	*R. corsicus*	*R. corsicus*	Spain	1972	E	C
2434	*R. corsicus**	*R. corsicus*	France	1972	E	C
2151_1	*R. ellenae*	*R. ellenae*	Spain	1991	C	B
2151_2	*R. ellenae*	*R. ellenae*	Spain	1991	C	B
2352	*R. evadens**	*R. evadens*	USA	1964	A	M
769	*R. fuscorubens*	*R. fuscorubens*	USA	1983	I	P
2312	*R. fuscorubens**	*R. fuscorubens*	USA	1964	A	L
2363	*R. gigasporus**	*R. roseolus* (E)	Tunisia	1982	G	I
38	*R. luteolus*	*R. luteolus*	Spain	1989	E	C
804	*R. marchii**	*R. marchii*	Italy	1897	F	G
828	*R. marchii*	*R. marchii*	Italy	1988	F	G
823	*R. marchii*	*R. marchii*	Germany	1950	F	G
2285	*R. nigrescens*	*R. nigrescens*	USA	1970	B	L
2358	*R. occidentalis**	*R. occidentalis*	USA	-	F	H
2467	*R. occidentalis*	*R. occidentalis*	Spain	1985	F	H
920	*R. ochraceorubens*	*R. ochraceorubens*	Sweden	1992	A	Q
924	*R. ochraceorubens*	*R. ochraceorubens*	USA	1967	I	Q
1448	*R. ochraceorubens*	*R. ochraceorubens*	USA	1969	I	Q
2375	*R. ochroleucus**	*R. ochroleucus*	USA	1956	F	G
2353	*R. olivaceofuscus**	*R. olivaceofuscus*	USA	1964	D	C
2356	*R. pachydermus**	*R. pachydermus*	USA	1967	A	N
951	*R. pannosus*	*R. pannosus*	Spain	1988	F	F
2315	*R. pannosus**	*R. pannosus*	USA	1916	F	F
2323	*R. parksii**	*R. villosulus*	USA	-	A	A
1700	*R. pumilionum**	*R. roseolus* (B)	Germany	1919	H	I
2215	*R. pseudoroseolus**	*R. roseolus* (C)	USA	1962	H	I
951	*R. reticulatus**	*R. villosulus*	U.K.	1953	A	A
2435	*R. rocabrunae*	*R. rocabrunae*	Spain	1994	H	J
1090	*R. roseolus*	*R. roseolus* (D)	Spain	1991	G	I
2371	*R. roseolus*	*R. roseolus* (B)	USA	1962	G	I
2364	*R. sardous*	*R. roseolus* (C)	Italy	1981	G	I
2314	*R. separabilis**	*R. separabilis*	USA	1935	A	K
2374	*R. subalpinus**	*R. subalpinus*	USA	1963	D	C
2312	*R. subolivascens**	*R. subolivascens*	USA	1962	D	C
2373	*R. subsalmonius**	*R. subsalmonius*	USA	1962	F	E
980	*R. subsalmonius*	*R. subsalmonius*	Spain	1993	F	E
1498	*R. subsalmonius*	*R. subsalmonius*	Spain	1993	F	E
2372	*R. ventricisporus**	*R. roseolus* (E)	USA	1964	G	I
767	*R. verii*	*R. verii*	Spain	1989	B	O
2365	*R. verii**	*R. verii*	Tunisia	1982	B	O
2368	*R. verii*	*R. verii*	Italy	1984	B	O
2324	*R. villosulus**	*R. villosulus*	USA	1939	A	A
2331	*R. villosulus*	*R. villosulus*	Spain	1993	B	A
2340	*R. villosulus*	*R. villosulus*	Spain	1994	A	A
999	*R. vinicolor*	*R. vinicolor*	France	1986	D	D
1283	*R. vulgaris*	*R. roseolus* (B)	Spain	1990	H	I
1793	*R. vulgaris*	*R. roseolus* (B)	France	1972	H	I

$$\mathbf{Rf} = \frac{\text{Distance from the origin to the front part of a metabolite on a TLC plate}}{\text{Distance moved by the solvent from on a TLC plate}} \times 100$$

In preliminary tests, we used two control substances currently used by lichenologists: atranorin and norstictic acid (extracted from *Parmotrema hypoleucinum* (Steiner) Hale, BCC 4850), to check the chromatographic procedure 1

RESULTS AND DISCUSSION

Preliminary tests.- **(a)** Under visible light no spots were seen. **(b)** Under UV light, in all six systems, acetone extracts showed fewer spots than alcohol extracts. **(c)** Four and six applications gave few spots; but with 10 applications, a dark streak was obtained from the mature fruitbody (with a gelatinized gleba) of *R. villosulus* (BCC-MPM 1643_4), that masked any possible spots. **(d)** Young *R. villosulus* (BCC-MPM 1643_1, with white gleba), gave fewer spots than mature. **(e)** The best resolution and separation were obtained with solvents C and D, which gave different chromatographic patterns for each species [In solvent A the five species showed the same chromatogram (with only two bright green spots); in solvent B, *R. villosulus* and *R. luteolus* showed identical patterns and the separation of some spots of *R. subsalmonius* and *R. roseolus* was not clear; in solvent E, three patterns appeared: *R. villosulus-R. subsalmonius*, *R. pannosus-R. roseolus* and *R. luteolus*; in F, also three patterns appear, one of them shared by *R. villosulus-R. subsalmonius-R. luteolus*]. **(f)** After treatment with 10% H_2SO_4, any new spots were observed.

Extensive study.- Extractions were made with ethanol, samples were spotted eight times, solvents C and D were used as developing systems, and plates were not treated with H_2SO_4.

In Table 1, capital letters indicate the chromatographic patterns seen with solvents C and D (patterns **A-I**, solvent C; patterns **A-Q**, solvent D), which are diagrammed in Fig. 1. Fewer spots were observed using UV light at 245 nm than with 366 nm. In the diagrams, we have represented the spots seen with UV-366 as filled ovals, and the additional spots seen under UV-245 as dotted ovals. Colours given in the diagrams approximate those observed. Spots obtained with solvent C were smaller (median size 5 x 2 mm) than with solvent D (median size 5.5 x 4 mm).

Even though good results were obtained in preliminary tests, the resolution and separation with solvent C, were not enough in the extensive study to be of practical use. All samples showed a strong fluorescent green spot (Rf=36.0) and one or more spots, except in group A, where only the strong fluorescent green spot was observed. A second spot, dark green (Rf=34.0), was observed in the rest of the samples (under 366 nm patterns B, D, E showed only the two green spots). The "ubiquitous" nature of these spots limits their chemotaxonomic significance. Nevertheless, their constancy makes them useful as reference spots for judging the correctness of the chromatographic development of each plate. In patterns F and I a blue-violet (Rf=27.0) spot was observed. Pattern A occurred in 11 specimens: *R. villosulus* (Reg. 2323, Reg. 2324, Reg. 2340, Reg. 2320 and Reg. 951) of the Sect. *Villosuli* (with duplex peridium) and *R. angustisepta* Zeller et Dodge, *R. separabilis* Zeller, *R. evadens* A. H. Smith, *R. pachydermus* K. A. Harrison et A.H. Smith, *R. fuscorubens* A.H. Smith (Reg. 2312) and *R. ochraceorubens* A.H. Smith (Reg. 920) of the Sect. *Rhizopogon*. The same was observed in pattern B, D, F and I. Moreover, extraction of the same species showed different

Table 1. *Rhizopogon* collections used in TLC.; indicating register number, label and current name, geographical area, year of the collection and the different patterns obtained in solvent C and D. (*: type material; letters between parenthesis after current name *R. roseolus* refer to the group of spore volume, according to GROSS et al. (1980)).

patterns, such as *R. villosulus* (A, B), *R. fuscorubens* (A, I), *R. ochraceorubens* (A,I) and *R. ochroleucus* A.H. Smith (D,F).

Patterns C, G and H showed spots that may be important as taxonomic markers. Thus, in pattern C there are two spots, one yellow (Rf=3.3) and another blue-violet (Rf=16.7); only the two fruitbodies of *R. ellenae* A.H. Smith (Reg. 2151_1 and 2151_2), showed these spots. In patterns G and H, different spots appeared, one light green (Rf=3.3) and one salmon colour (Rf=10.0), which were observed in extracts of *R. roseolus*, without a difference between fruibodies containing small spores (suffix B after current name *R. roseolus*) or those with abnormal spores (suffix E after current name *R. roseolus* in Table 1), and from *R. rocabrunae* M.P. Martín, ined.

With solvent system D, 17 chromatographic patterns where obtained, many of them monospecific. As in solvent C, a strong fluorescent green "ubiquitous" spot (Rf=88.0) appeared in all collections, attesting to a correct chromatographic development.

Chromatographic patterns A, B, D, E, F, H, I, J, M, N and O were neatly different, and they are characteristic of only one of the species, as defined here. Thus, pattern A (a blue violet spot, Rf=48.2) was shared by all samples under *R. villosulus*, which supports our morphological studies. All samples under *R. roseolus*, showed the same pattern (I), with two blue-violet spots (Rf=46.3, Rf=53.0) and one dark green (Rf=72.0), with no differences between fruitbodies with different spore volumes. The dark green spot of *R. roseolus* was not exclusive to this species, as a spot with the same colour and Rf was observed in other patterns (B, E, F, N, O, P and Q); but the combination of the two blue violet spots was observed only in *R. roseolus*. Pattern B (*R. ellenae*) had a blue-violet spot similar to that observed in *R. villosulus* (Rf=48.2), but no spot was seen under 245 nm. Patterns E, F, N, O and P showed one of the blue-violet spots seen in *R. roseolus* (Rf=53.0); but also in E, F, N and O some spots appeared that may be used as taxonomic markers: *R. subsalmonius* (pattern E) showed a salmon spot (Rf=58.6) and a yellow one (Rf=24.5); *R. pannosus* (pattern F) a croceus spot (Rf=41.0) and a yellow spot with the same Rf and fluorescence as that observed in *R. subsalmonius*. In pattern O (*R. verii* Pacioni) a red spot (Rf=62.0) appeared, not observed in any other taxa. *R. pachydermus* (pattern N) showed many different spots: yellow (Rf=76.2), red (Rf=69.7), blue-violet (Rf=59.7) and orange (Rf=49.5). The orange spot was also observed in *R. evadens* (pattern M).

Others patterns were specific. *R. vinicolor* A.H. Smith (pattern D) showed a red spot (Rf=69.7) similar to that observed in *R. pachydermus*, but no other significant spots were seen under 366 nm. *R. rocabrunae* (pattern J) that shared the same chromatographic pattern as *R. roseolus* in solvent C, gave a very different pattern in solvent D: no blue-violet spots were observed, but one yellow (Rf=22.2) and another pale blue (Rf=6.7). *R. occidentalis* (pattern H) showed two blue-violet spots with a different Rf from that of *R. roseolus* (Rf=56.5 and Rf=63.1).

However, not all species become separated with these chromatographic procedures. Thus, some patterns were shared by more than one species. For instance, *R. abietis* A.H. Smith and *R. marchii* (Bres.) Zeller et Dodge, which share very similar peridium type, but have different spore shapes, gave pattern G (a salmon spot, Rf=63.0). *R. angustisepta* and *R. separabilis* gave pattern K, without spots at 366 nm (except the strong fluorescent green Rf=88.0), even though in the peridium there are numerous orange-yellow deposits adhering at the exterior of the hyphae. *R. ochraceorubens* has a peridium of *corsicus*-type with numerous patches of ochre to vinaceous pigments distributed along all over the peridium; however, no marker spots were observed (pattern Q). *R. fuscorubens*, that has a peridium of *luteolus*-type, with numerous reddish-orange to reddish pigments along the hyphae, shown two different patterns: L (Reg. 2312) and P (Reg. 769), which were shared by *R. nigrescens* A.H. Smith and *R. aurantiacus* A.H. Smith respectively. The poor results with these specimens which are strongly pigmented under the light microscope indicates that

additional extracts and solvents must be used to clarify these species. Something similar was observed in pattern C, which was shared by five species quite different morphologically, and no spot was seen which could be used as taxonomic marker: *R. olivaceofuscus* A.H. Smith, *R. subolivascens* and *R. subalpinus* A.H. Smith have masses of pigment between the peridium hyphae, whereas in *R. corsicus* Demoulin et Moyersoen (ined.) and *R. luteolus* no pigments have been observed in the peridium, though reddish masses of pigments appear between the hyphae of the rhizomorphs.

The results indicate that chromatographic examination of the ethanol extracts of *Rhizopogon* fruitbodies can be useful in taxonomic analysis. However, we need more data to fully establish the useful taxonomic markers, and to integrate these characters in the delimitation and description of the taxa.

ACKNOWLEDGMENTS

Thanks are due to Dr. X. Llimona, Dr. F.D. Calonge, Dr. E. Gràcia and Dra. J. Palacio for their suggestions and comments during the preparation of this work, to Dr. J.A. Schmitt by sending bibliography, to the directors and curators of the different herbaria consulted (AQUI, E, FH, H, K, LG, M, MA-Fungi, MICH, NY, S) for the loan of specimens, and to Dr. M. Glenn for her suggestions and her kind English correction.

REFERENCES

BENEDICT, R.G. & V.E. TYLER (1968).- Occurrence of sugars and sugar alcohols in the *Boletaceae*. *Herba Hungarica* 7(2-3): 17-17-20.

BENEDICT, R.G. (1970).- Chemotaxonomic relationships among the *Basidiomycetes*. In Benedict, R.G.. *Advances in apl. microbiology* 13: 1-23.

BRESINSKY, A. & W. STEGLICH (1989).- *Rhizopogon pumilionus* als produzent des ansachinons *Rhizopogon*. *Z. Mykol.* 55(2): 169-174.

CULBERSON, C.F. & H. KRISTINSSON (1970).- A standardized method for the identification of lichen products. *J. Chromatog.* 49: 85-93.

EDWARDS, R.J. (1976).- *Chromatographic investigations of California species of the genus Suillus*. San Francisco State University. 90 pp. (unpublished thesis).

FRIES, N. (1958).- Paper chromatography as a diagnostic aid in *Hymenomycetes*. *Ann. Acad. Regiae Sc. Upsaliensis* 2: 5-16.

GALUN, M. & A. SHOMER-ILAN (1988).- Secondary metabolic products. In M. Galun. *Handbook of Lichenology,* Vol. III, Chapter IX A: 3-8.

GROSS, G., RUNGE, A. & W. WINTERHOFF (1980).- Bauchpilze (*Gasteromycetes* s.l.) in der Bundesrepublik Deutschland und Westberlin, *Beih. Z. Mykol.* 2: 1-92.

GILL, M. & W. STEGLICH (1987).- Pigments of fungi. In Herz. W., Grisebach, H., Kirby, G. W. and Ch. Tamm. *Progress in the chemistry of organic natural products.* 51: 1-309.

HALE, M.S. (1983).- The Biology of Lichens, Edt. Arnold, 3rd edt., London, 190 pp.

LOCQUIN, M. (1984).- *Mycologie générale et structurale*. Edt. Masson, Paris, 551 pp.

MARTÍN, M.P. (1995).- *The genus Rhizopogon in Europe.* Universitat de Barcelona, 371 pp. (unpublished thesis).

PATERSON, R.R.M & M.A. RUTHERFORD (1991).- A simplified rapid technique for fusaric acid detection in Fusarium strains. *Mycopathologia* 113: 171-173.

PÉREZ-VALCÁRCEL, C. (1994).- *Flora liquénica del municipio de a Fonsagrada (Lugo)*. Univ. Santiago de Composela, 510 pp. (unpublised thesis).

SINGER, R. (1962).- *The Agaricales in modern taxonomy.* Edt. Cramer, New York, 915 pp. + 73 pl.

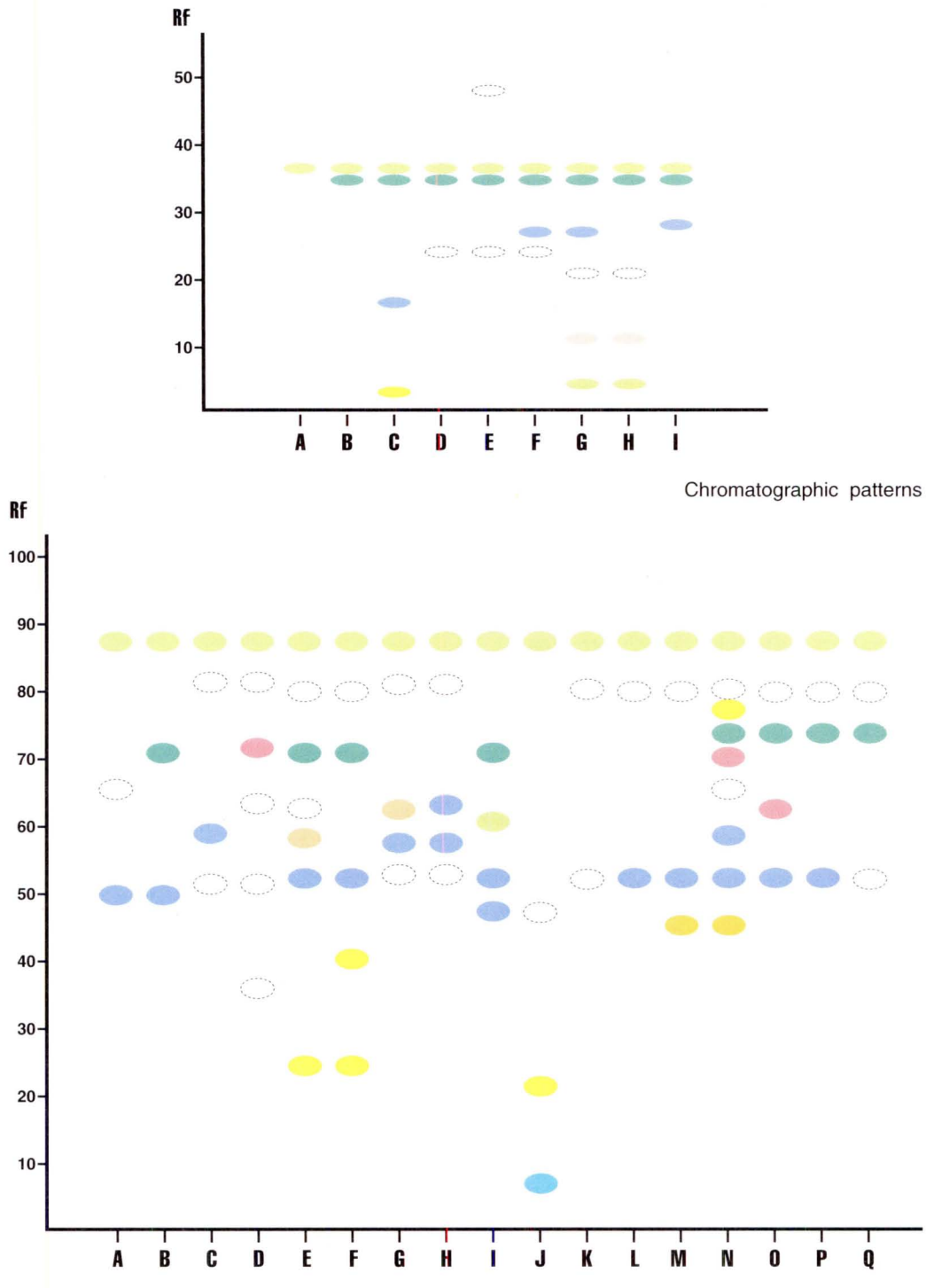

Figure 1. Diagrammatic representation of chromatographic patterns (capital letters) of ethanol extracts of 58 specimens of *Rhizopogon*. **Above**: Solvent C as developing system. **Below**: Solvent D as developing system. Details of specimens involved in each pattern are given in Table 1. (Filled oval: spots seen with UV-366 nm; dotted oval: additional spots seen under UV-245 nm).