DESCRIPTION OF RANUNCULUS REPENS L. AND RANUNCULUS ARVENSIS L. POLLEN

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SUMMARY

A comparison of the morphology of the periporate, distinctly echinate pollen grains of *Ranunculus arvensis* L. and the tricolpate-pericolpate, microechinate-scabrate pollen grains of *R. repens* L. is made. The differences, studied with the aid of sections, are discussed in detail.

1. INTRODUCTION

Ranunculus arvensis L. pollen has been described by several authors, since its characters are significantly different from the other West European species. However, up to now the differential characteristics were never clearly compared; in the present paper *R. repens* L. is chosen as a representative of the more common *Ranunculus* type of grain. ERDTMAN, BERGLUND & PRAGLOWSKI (1961, p. 49–50), in their study of Scandinavian pollen, state that the pollen of *R. arvensis* differs distinctly from that of other species of *Ranunculus: R. arvensis* pollen is periporate, the pollen of the other *Ranunculus* species is usually colpate, the wall construction of *R. arvensis* is characteristic. In the more recent paper by TARNAVSCHI & MITROIU (1963), the pollen grains of *R. arvensis* and *R. muricatus* L. are not only incorrectly described as non-aperturate but also considered to be of the same type; *R. repens*, however, was correctly described as belonging to the more common colpate type of grain. Notwithstanding their early appearance, the papers of WODEHOUSE (1936) and KUMAZAWA (1937) are still very important contributions towards the study of the pollen morphology of Ranunculaceae. Wodehouse's account of *Ranunculus* pollen pertains to the more common type of grain, which is tricolpate or occasionally tetracolpate, hexacolpate, or irregular in various ways, the pollen of *R. arvensis* is not described. Kumazawa, who studied a variety of species, distinguishes three pollen types, *viz.*, a tricolpate-stephanocolpate type, a pericolpate type and a periporate type: *R. arvensis* belongs to the third type, while *R. repens* L. var. *major* Nakai and *R. muricatus* among others are reported to have the second, pericolpate type. The species belonging to the first type are of no importance for the present paper. As regards the pored character within the Ranunculacean family, both Wodehouse and Kumazawa are of the opinion that the pore of the porate grains may represent proliferated and shortened furrows. According to the former the occurrence of pores among the Ranunculaceae is probably polyphyletic; for that reason the taxonomical value of this character should not be overrated; other characters like the construction of the pollen wall may be of higher value. Kumazawa is of the same opinion considering the surface characters of

the pollen grains as being important for systematics. References to pollen sections of *Ranunculus* are scarce; only the diagrammatic representation of a section of a pollen grain of a not named *Ranunculus* species by UENO (1963) and the sections of the related *Ficaria ranunculoides* Moench by ROLAND-HEYDACKER (1964) have to be mentioned here. In the present paper the pollen morphology of *R. arvensis* and of *R. repens* is more thoroughly investigated and compared with the aid of sections.

2. MATERIAL AND METHODS

Material of *R. repens* was collected near Amsterdam and *R. arvensis* was obtained through the courtesy of the Plantenziektenkundige Dienst, Wageningen and supplemented by sampling the herbarium material from France and Central Europe, stored in the Hugo de Vries-Laboratorium. All flowers were boiled in KOH 10% followed by acetolysis; afterwards some preparations were chlorinated. One part of the pollen was embedded in a methacrylate mixture of 2 parts methyl-methacrylate and 8 parts butyl-methacrylate, and sectioned on a Sjöstrand Ultramicrotome type LKB 3314 with a glass knife, stained in safranin and mounted in Cedax; the other not sectioned part of the pollen was also stained with safranin and mounted in silicone oil. The observations were made with a Zeiss microscope (Standard GFL, obj. planapo 100 × immers., oc. Kpl 12.5) and a Leitz microscope (Laborlux, obj. 95 × immers., oc. periplan GF 10 ×). The terminology proposed by FAEGRI & IVERSEN (1964) is followed. The dimensions and structural relations in the figures were checked by means of microphotographs of the drawn specimen. As regards the measurements recorded, it must be emphasized that material of only a few localities was used.

3. POLLEN MORPHOLOGY OF RANUNCULUS ARVENSIS L.

The pollen grains of *R. arvensis* are spheroidal, periporate and of various sizes (diam. 30–60 μ); consequently their structure looks likewise rather different. The group of smaller grains (mean diam. 35 μ approx.) has more obscure pores, particularly when the pore membrane is intact; their whole habit is more compact. The group of larger grains which give the impression of being somewhat swollen or blown up, (mean diam. 53 μ approx.) on the contrary shows their pores rather well. A grain of the smaller type is shown in plate II, fig. 1. In the larger type the pores together with the rather structureless part of the pollen wall around them form the meshes of a rather regular network-like pattern in which the large spines (echinae) seem to form the net. It is not clear whether these differences between the two types of grains are artificial and attributable to the treatment during preparation, or caused by a natural variation. In all slides examined, even in those that were not bleached or acetolysed, both types were present in varying percentages. The pores (about 25–30 in number) are suborbicular to faintly polygonal, the diameter is 3 to 6 μ in the smaller grains. The pore membrane bears up to six conspicuous spines apart from smaller

columella-like elements. The construction of the pollen wall is tectate, echinate. The echinae are supported by larger columellae, while smaller columellae are interspersed between the larger columellae; a concentration of smaller columellae is found around the pores.

Plate I, figs. 6–10, shows successive focussing levels of the pollen wall, for which a grain of the smaller type was chosen, as the authors are of the opinion that the smaller grains are more characteristic. The bright spots given in fig. 6, seen at the first level of focussing, correspond with the upper parts of prominent spines. The next level (*fig. 7*) shows the spines as larger bright spots with a dark lining, and directly surrounded by minute dark spots. The latter immediately turn bright when the level of focussing is lowered, and therefore represent tectal perforations, which are chiefly concentrated around the spines. The tectal perforations must occupy an oblique position towards the spine root as the perforations are characterized by darker minute spots more near the spine centre and bright spots more outwards when lowering focus. The spine with its ring of perforations is seen in a white surrounding band (*fig. 7*). At this same level the situation of the pores is seen rather vaguely, the pore membrane shows bright spots corresponding with the spines; one of which is visible somewhat below the centre and slightly to the right (see arrow). At a lower level (*fig. 8*) the white bands surrounding the spots representing the spine-supporting columellae, appear more greyish and contain white spots corresponding with smaller columellae. Usually each spine is individually surrounded by such an enclosing band, but also two or three spines can lie within a common enclosure (*e.g., fig. 8* bottom right). In the last case the enclosing band gives the impression of having originated from a coalescense of two or three "individual" enclosures. In the larger swollen type of grain such surrounding bands are imperceptible as a rule. As regards the structures responsible for the surrounding bands, more details will be given when dealing with the sections. When the pollen wall is examined at a level slightly lower than that of *fig. 8*, bright spots, similar to the bright columellar spots, previously mentioned in the greyish surrounding bands, and likewise indicating smaller columellae, occur scattered in between these surrounding bands with a concentration around the pores. At this same level the greyish surroundings change into darker bands. The pore membrane likewise shows small bright spots, which are slightly smaller than the spots on the wall representing real columellae, and besides larger darker spots are present that correspond with the bases of the spines on the membrane. The just mentioned small bright spots on the membrane are not drawn in this series, but are shown in *plate II, fig. 1*. The spine spots on the pore membrane have no surrounding bands and by this character the pore membrane can be distinguished from the normal wall surface. At a still lower level (*fig. 9*) dark spots representing the basements of the spine-supporting columellae, replace the bright spots of higher levels, corresponding with the spines. The surrounding bands, encircling the spines and their supporting columellae at higher levels of focussing, are here (*fig. 9*) dissolved into a number of small separate spots, which coincide approximately with the white columellar spots in these sur-

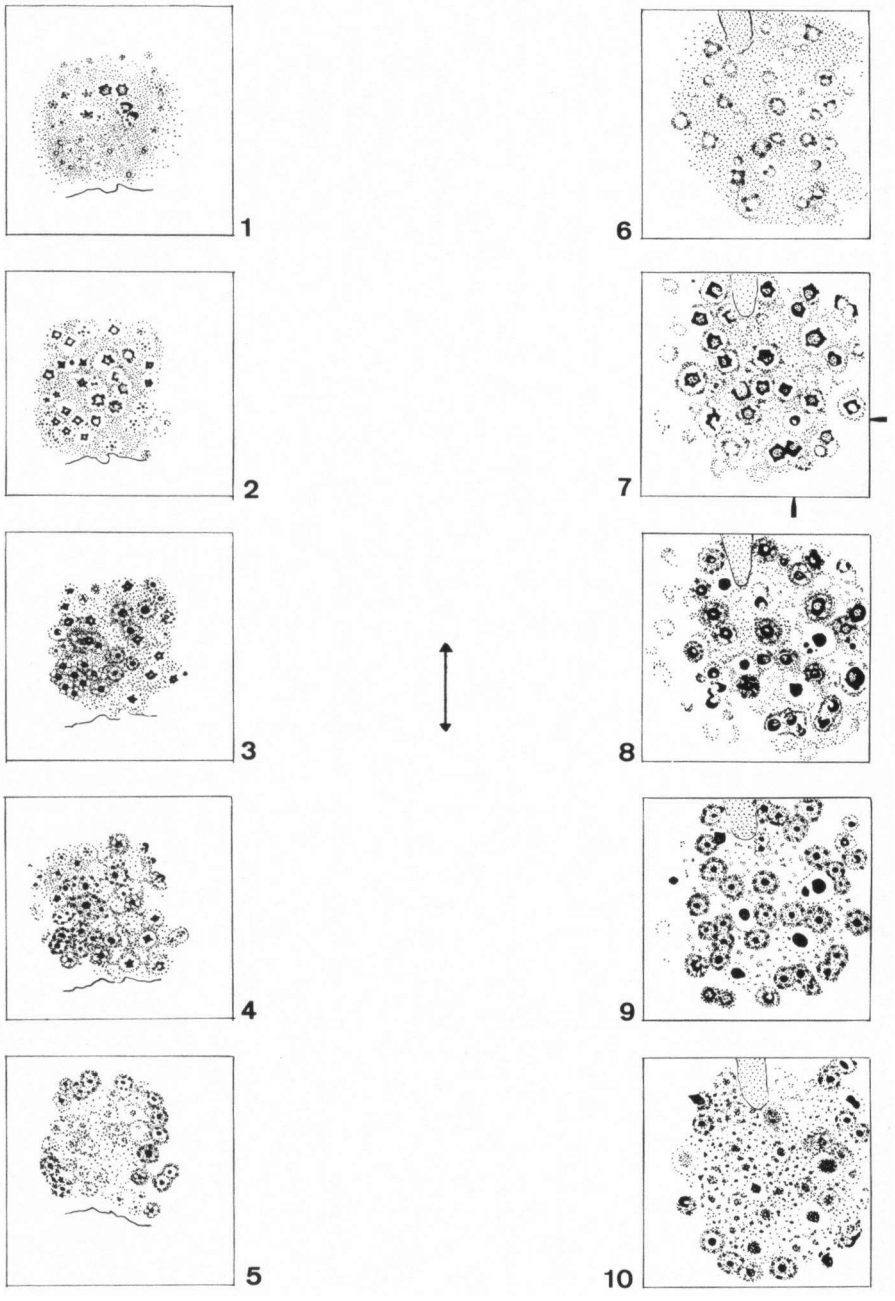

Plate I. Successive focussing levels of the wall of an intact grain (scale represents 10 μ)
Figs. 1–5, *Ranunculus repens* L.; Figs. 6–10, *R. arvensis* L.

rounding bands, depicted at *fig. 8*. The smaller columellar spots not covered by the surroundings now also appear dark. The clearest picture of the pores is obtained at this level of focussing. Within the pores the small bright columella-like spots (not drawn) turn dark, just like the large spine spots on this membrane. At the lowest focus, shown for the sake of completeness (*fig. 10*) the basements of the smaller as well as the larger columellae and the pores with their spines are but vaguely seen.

The sections (*Plate II, figs. 2–7*) enable a better interpretation of the construction of the wall. The larger columellae supporting the rather large spines (length 1.5–2 μ approx.) taper from the basement towards the middle where the smallest diameter is found (about 1 μ). The length of spine and supporting columella is 3–4 μ. The spines are surrounded at their base by oblique tectal perforations, as was already concluded from the intact grain wall. The spine-supporting columellae are much longer than the others. The tectum, about 0.2 μ thick, is distinctly undulate: from the spine top it bends downwards and as a result surrounds the long columella like a dome; the dome consequently is formed by the spine top together with the heightened tectum. There is one dome around each columella, but often two or more columellae are capped by a single dome. The base of the dome corresponds with the surrounding band seen around the spine in not sectioned grains. The diameter of the surrounding band in surface view and the width of the dome measured from sections agree quite satisfactorily (the mean value of 10 measurements was 2.68 μ in both cases). The dome-shaped structures are inconspicuous in the larger swollen grains. The swelling of these grains effects a tension in their wall so that the domes are more flattened and the optical effect responsible for the surrounding band fades. The optical effect of the surrounding band has not completely disappeared in moderately swollen grains, where parts of the surrounding bands are visible near the optical outline of the grain and only here. This sustains the opinion that surrounding bands are effected by dome bases as only these structures, oblique by tension but near the outline only parallel to the optical axis, can give the mentioned effect. The base of the domes is supported by small columellae, which appear in a surface view of the whole grain as the small bright spots in the surrounding bands (*Plate I, fig. 8*). ERDTMAN (1961, p. 50) describes the domes as "densely and irregularly spaced spinules" of which "the base forms a platform for the small upper, pointed part of the spinule. The platforms often amalgamate". The domes are not recognized by him, as the spine base, "the platform", is in reality supported by a ring of elevated tectum, here reflected to as dome base. In some cases it could be observed that the supporting columella is connected with the inside of the dome by some minute bars (*Plate II, figs. 6, 4*). Apart from the smaller dome-supporting columellae, columellae of about the same size (length about 1 μ, diameter under 0.5 μ) are found scattered, with concentrations around the pores. In the sections the tectum shows minute spine- or granula-like sculptural elements on top of these smaller columellae. The endexine, usually thinner than 0.5 μ, seems to be discontinuous. In the sections the pores do not become very distinct either. The thin closing membrane is tectate (*Plate II, figs. 3, 5*) and does

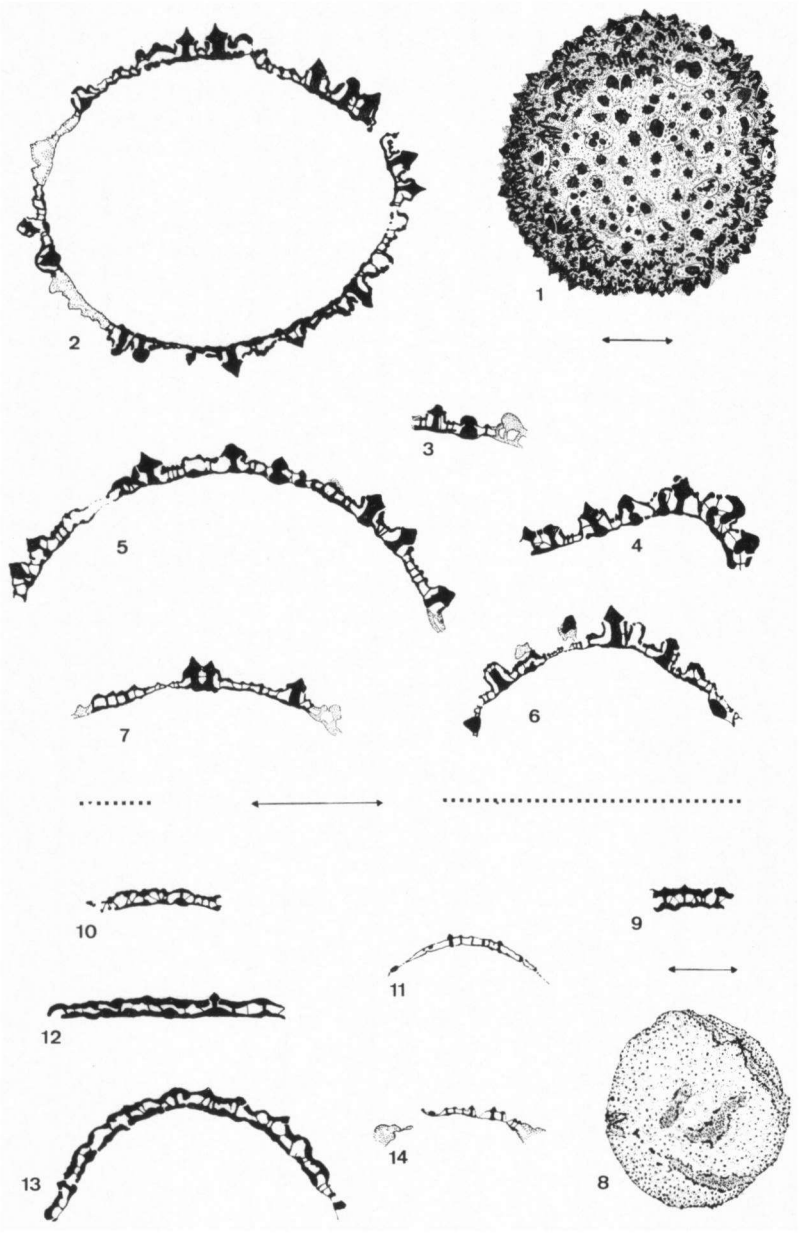

Plate II. Intact grain and wall sections (scale represents 10 μ, all sections are enlarged to the same degree, the enlargement of the intact grains is less). Figs. 1–7, *Ranunculus arvensis* L.: fig. 1 intact grain; fig. 2 wall section; fig. 3 section chiefly through pore membrane; figs. 4–7 wall sections. Figs. 8–14, *R. repens* L.: fig. 8 intact grain; figs. 9, 10, 12, 13 wall sections; figs. 11, and 14 sections through colp membrane.

not differ appreciably from the surrounding pollen wall; this is effected by the smaller columellae of the wall, and smaller columella-like elements of the membrane which resemble each other (*Plate II, fig. 2*). Spine-like elements, originating from the lower skin but not supported by domes, are in addition present on the pore membrane *(Plate II, fig. 3)*.

4. POLLEN MORPHOLOGY OF RANUNCULUS REPENS L.

The pollen grains of *R. repens* L. are subspheroidal (Lg/Lt varies between 0.83 and 1.10, the length varies between 20 and 35 μ); smaller grains probably have a somewhat thicker exine. The shape of the grains depends very much upon the expansion of the colp membrane (WODEHOUSE 1936; KUMAZAWA 1937). As in most *Ranunculus* species the pollen is 3-colpate, 4-colpate or pericolpate; the colpi are long drawn, their ends are mostly rounded. The polar area is small (the index is usually less than 0.25). The harmomegathic mechanism and its function have been amply described by WODEHOUSE (1936). The irregularly running margins and the ektexine elements on the aperture membrane render the colpi less clear (*Plate II, fig. 8*). In 6-pericolpate grains four colpi occupy a stephanocolpate position and the remaining two colpi are situated at the proximal and distal poles of the grain (*Plate II, fig. 8*). The 3- and 4-colpate grains are mostly stephanocolpate. The pollen wall is tectate, microechinate-scabrate.

Sculpture and structure patterns of the pollen wall of an intact grain in successive focus are given in *Plate I, figs. 1–5*. At the highest level minute bright spots representing the microspines (scabrae, microechinae) are seen (fig. 1, at the bottom), which at a somewhat lower focus get a dark encircling, while the bright centre becomes larger (*fig. 1*, centre). Almost at the same moment minute dark spots are added to these encirclings (*fig. 2*, centre), which turn bright by lowering of focus (not drawn); these spots correspond with tectal perforations. Around the encircling with added dark (or at a slightly lower focus bright) spots there is a bright band (*figs. 2, 3*). Just below this level the bright band is replaced by a circle of bright spots of the smaller columellae (*fig. 3*, bottom left). Focus is now lowered so far that in the area where first the bright spots of the microspines were visible the dark optical section of the supporting columella is met. The picture of the bright spots of the smaller columellae, and of the band in which they are grouped, turns darker to vanish at the lowest level of focussing, (*fig. 3*, centre; *fig. 4*, at the left), so that all columellae are represented by dark spots at approximately the same places where first the bright spots were seen.

A slight shift in the position of the bright and dark spots is due to a slightly oblique orientation of the columellae. Only a small number of columellae not associated with spines or spine-encircling structures is present; they are seen like the other columellae, bright at a higher focus and dark at a lower level. The band in which the bright spots of the smaller columellae became visible, may be effected by a fusion of the tops of the smaller columellae encircling the larger central one, as well as by the slightly elevated sculpture around the base of the microspine.

The sections of the wall (*Plate II, figs. 9–14*) reveal its construction more clearly. The tectum (0.3–0.5 μ thick) is only slightly undulate, its highest points being supported by larger columellae; here microspines are found, total length of supporting columella and microspine 1.5–2 μ. Around the larger columellae the tectum slopes downwards and continues over the smaller columellae. On top of all smaller columellae "microscrabrae" are found. These smaller columellae are based either on the endexine or on the enlarged base of one of the larger columellae. Tectal perforations are only occasionally observed around the microspines. The endexine, (0.5 μ thick or less) appears as if interrupted. The colp membrane (*Plate II, figs. 11, 14*) is tectate and provided with smaller and larger columella-like structures, the larger ones bear spine-like elements while the smaller ones are not correlated with such elements. *Figs. 1–5* of *Plate I* do not show an intact colp membrane, but its characters can be observed in *Plate II, fig. 8.*

5. DISCUSSION

The pollen grains of both species discussed are tectate; the tectum being supported by two types of simple columellae, *viz.*, by larger and by smaller ones. In *R. arvensis* the smaller and larger columellae differ more in length than they do in *R. repens;* in the latter species the two types of columellae chiefly vary in slenderness. In *R. arvensis* pollen the larger columellae each support a rather large spine; in *R. repens* they each bear a microspine. In *R. repens* the majority of the smaller columellae are on the one hand normally fused at their tops but on the other hand it is noteworthy that these fused columellae are fused concentrically around the larger columellae, these ring-shaped concentrations inside the tectum form a reticulum-like pattern. In *R. arvensis* a great number of the smaller columellae support the "domes" encasing the larger columellae. The dome-bases may perhaps be considered to be homologous with the mentioned ring in *R. repens.* In both species there is a circle of perforations around each spine or microspine. The footlayer could not be distinguished after applied staining. In both species the aperture membrane is tectate and contains columella-like elements. The difference between both membranes is formed by the development of the pointed elements originating from the lower membrane; in *R. arvensis* conspicuous spines are replacing the smaller warts of *R. repens.* The two species differ in size and aperture type as discussed. Moreover, conspicuous characters are not found in the pollen wall of *R. repens,* whereas *R. arvensis* shows the typical undulating tectum, the longer and stouter columellae with their typical domes and distinct spines.

The present authors could not detect the presence of a slight thickening of the ektexine along the colpi in *R. repens,* which was described by ERDTMAN c.s. (1961). The simplified diagram of a section of *Ranunculus* spec. given by UENO (1963) did not lend itself to a comparison. The section of *Ficaria ranunculoides* Moench pollen described by ROLAND-HEYDACKER (1964) is the only section known that can be compared well with the sections of *R. repens;* the pollen

grains of the two species resemble each other very much, which resemblance is reflected in the sections. The sections of *F. ranunculoides*, studied with the aid of an electron microscope, have the advantage that they clearly show the presence of a footlayer. This footlayer proved to be absent in the aperture membrane. The tectum and the columellae, though very thin here, are not lacking in the colp. The colp membrane can, therefore, be considered to be tectate; pointed elements are found on this membrane and thus the membrane in *R. repens* is similar. The endexine increases considerably below the colp in *F. ranunculoides*, a feature which could not be demonstrated in *R. repens*. In both species the perforate tectum is microechinate-scabrate, in *F. ranunculoides* the microechinae are probably also supported by larger columellae. A differentiation of the columellae into larger and smaller ones (the latter surrounding the larger) is not visible in the sections of *F. ranunculoides*.

One can thus conclude that, although the pollen of *R. arvensis* L. differs distinctly from *R. repens* L. pollen, the wall construction may be in principle the same in both species, but it is in any case much more developed in *R. arvensis*. The pollen of *Ficaria ranunculoides* Moench (see ROLAND-HEYDACKER 1964) closely resembles the pollen of *R. repens*. The pollen of *R. muricatus* L. is not of the same type as that of *R. arvensis* and should be re-investigated.

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SOMMAIRE

Les grains du pollen du *Ranunculus arvensis* L. et *R. repens* L. ont été comparés. Quoique le pollen de ces deux espèces diffère considérablement, une analogie du schéma de la construction est possible.

Le pollen du *R. arvensis* est subsphérique (le diamètre varie entre 30 et 60 μ), periporate, tectate-perforate, échinate. Les épines sont fixées aux columellae robustes (hauteur de la columella et de l'épine ensemble 3–4 μ), tandis que des columellae plus courtes groupées autour des columellae robustes, supportent des "dômes". Le tectum est assez ondulé, au-dessus des columellae courtes le tectum est bas, et autour d'une columella robuste il s'élève formant la base d'un dôme. Celui-ci se compose donc de l'épine et du tectum élevé; chaque dôme contient une columella robuste, mais aussi deux ou trois columellae robustes peuvent se trouver dans un seul dôme donnant l'impression que celui-ci est né d'un fusionnement de deux ou de trois. Des perforations menues se trouvent autour de l'épine. Le membrane de l'aperture est tectate, et contient des columellae, il est orné de vraies épines.

Le pollen du *R. repens* est subsphérique (Lg/Lt se trouve entre 0,83 et 1,10, la longueur entre 20 et 35 μ), 3-, 4-colpate ou pericolpate, tectate-perforate, microéchinate-scabrate. La région polaire est assez petite (indice de moins de 0,25). Les microscabrae se sont implantées aux columellae assez robustes (hauteur 1,5–2 μ, micro-épine comprise), quoiqu'il n'existe pas une

grande différence entre les deux types de columellae. Il n'y a pas de dômes, les columellae du type petit se sont aussi groupées en cercle autour des columellae du type robuste, mais le tectum s'élève à peine tandis qu'il est seulement un peu ondulé. Il y a aussi des perforations menues autour de la micro-épine. Le membrane du sillon est tectate contenant des columellae et il est orné de minces pointes. En général la construction de l'exine n'est pas très distincte chez le *R. repens.*

Le pollen de la *Ficaria ranunculoides* Moench (cf. ROLAND-HEYDACKER 1964) se rapproche du pollen du *R. repens.*

Le pollen du *R. muricatus* L. est d'un autre type que le pollen du *R. arvensis.* Il faut que le *R. muricatus* soit réexaminé.

REFERENCES

ERDTMAN, G., B. BERGLUND & J. PRAGLOWSKI (1961): An Introduction to a Scandinavian Pollen Flora. *Grana Palynologica* **2** (3): 3–92.

FAEGRI, K. & J. IVERSEN (1964): *Textbook of Pollen Analysis*, Second Revised Edition, Copenhagen.

KUMAZAWA, M. (1937): Pollen Grain Morphology in Ranunculaceae, Lardizabalaceae and Berberidaceae. *Jap. J. Bot.* **8** (1): 19–46.

ROLAND-HEYDACKER, F. (1964): Morphologie du Pollen de *Ficaria ranunculoides* Moench. Ranunculaceae. *Pollen et Spores* **6** (2): 373–378.

TARNAVSCHI, I. T. & NATALIA MITROIU (1963): Cercetări Palinologice asupra Ranunculaceelor din R.P.R. (with french and russian Summary). *Probl. Biol.* Ed. Acad. Rep. Popul. Romîne (1962): 79–122.

UENO, J. (1963): The Stratigraphical Structure of the Pollen Walls of Dicotyledoneae I: Ranales and Amentiflorae (in japanese, with english Summary). *Acta Phytotax. Geobot.* **9** (4–6): 137–141.

WODEHOUSE, R. P. (1936): Pollen Grains in the Identification and Classification of Plants. VII The Ranunculaceae. *Bull. Torrey Bot. Cl.* **63** (9): 495–514.