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Infection Structures and their Surface Changes during Differentiation in *Uromyces fabae*

By

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With 12 figures

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Abstract

Germ tubes of *Uromyces fabae* differentiated into appressoria which were deliminated either by one or two septa. These appressoria developed rapidly on artificial membranes as well as on leaf surfaces.

Fluorescein-conjugated wheat germ agglutinin (WGA) bound strongly to the germ tube walls of *U. fabae* and its binding ability did not differ significantly in 3, 5 and 7 h old germ tubes. The binding of WGA was less on the appressorium. Measurement of fluorescence with a microscope photometer revealed two times more binding sites on germ tube walls than on appressoria. No binding of WGA was noticed on substomatal vesicles and infection hyphae. The binding of WGA was completely inhibited by preincubation with hydrolysate of chitin. Binding patterns of other lectins were quite different. *Canavalia ensiformis* lectin did not bind to any structure, the lectins of *Griffonia simplicifolia* and *Lotus tetragonolobus* bound weakly to all structures, the lectins of *Phaseolus vulgaris* and *Arachis hypogaea* bound only to substomatal vesicles and infection hyphae, and the *Ricinus communis* lectin bound only to the infection hyphae. Based on these observations it appears that infection structure surface of *U. fabae*, besides having chitin as a component, also contain some other carbohydrates such as galactose and fucose.

Zusammenfassung

Die Infektionsstrukturen und deren Oberflächenkohlenhydrate bei *Uromyces fabae*

Die Keimschläuche von *Uromyces fabae* differenzierten einmal die üblichen, endständigen Appressorien und solche mit 2 Septen aus. Diese Appressorien entwickelten sich auf künstlichen Membranen und auf der Blattoberfläche.

Fluorescein-markiertes Weizenkeimlektin (WGA) wurde von den Keimschläuchen ohne Rücksicht auf deren Alter gebunden. Mit dem Mikroskopphotometer wurde deutlich, daß die Appressorien jedoch nur halb so viele Bindungsstellen wie die Keimschläuche für dieses Lektin besitzen. Ein

Hydrolysat aus Chitin hemmte die Bindung. Keine Bindungsstellen für das Weizenkeimlektin konnten auf dem substomatären Vesikel und auf der Infektionshyphe nachgewiesen werden.

Das Lektin von *Canavalia ensiformis* wurde nicht, die Lektine von *Griffonia simplicifolia* und *Lotus tetragonolobus* wurden nur schwach von den Infektionsstrukturen gebunden. Die Lektine von *Phaseolus vulgaris* und *Arachis hypogaea* banden schwach an das substomatäre Vesikel und an die Infektionshyphe. Das Lektin von *Ricinus communis* haftete nur an die Infektionshyphe. Offensichtlich besitzt der Keimschlauch von *U. fabae* neben Chitin noch andere Oberflächenkohlenhydrate: z. B. Galaktose und Fucose.

Die Bedeutung der Oberflächenkohlenhydrate für die Wirtsspezifität wird diskutiert.

Rust fungi produce specialised infection structures which differ in their form and function (STAPLES and MACKO 1980). Production of such structures on artificial substrates and membranes has become a common practice (HARD-KARRER and RODENHISER 1947, SHARP and SMITH 1952, WYNN 1976, STAPLES *et al.* 1983) and has paved the way for the evaluation of environmental factors controlling their development and differentiation.

For the formation of a pathogenic relationship, plant and pathogen must come in contact at their cell surfaces where phenomena of specificity and recognition most probably occur. In recent years FITC labeled lectins have proved useful in mapping cell surface architecture and in studying distribution and mobility of lectin receptor sites on cell surfaces (SENGBUSCH *et al.* 1982, CHABOUD and LALONDE 1983, GALUN *et al.* 1976, MENDGEN *et al.* 1985). The present paper describes the results on carbohydrate binding patterns with lectins on the surface of infection structures of *Uromyces fabae,* the pathogen causing rust disease of *Vicia faba* L.

Material and Methods

Uromyces fabae (Pers.) de Bary, isolate KUF 1 was raised on ten day old plants of *Vicia faba* cultivar Frühe Weisskeimige. Uredospores were collected from 10—12 day old pustules and either used immediately or stored at 4 °C for 1—2 days.

Collodion membranes were prepared after MAHESWARI *et al.* (1967) and inoculated with uredospores in a settling tower to a density of 6000/cm^2. Seeded membranes were atomized with distilled water. The plates with the inoculated membranes were sealed with a strip of parafilm, wrapped in aluminium foil and incubated in a dark growth chamber at 20 °C (± 1) for varying lengths of time. At the end of each incubation period, 1 cm^2 pieces were cut out, separated from the agar block, and placed on microscope slides.

Leaflets from 10—12 day old *Vicia faba* plants were detached and placed in the bottom of a petri dish (9 cm) containing about 5 ml distilled water. The leaflets were inoculated and incubated in the same way as were the membranes. After incubation, epidermal peels were stripped from the leaflets and mounted in lactophenol-trypan blue.

The fluoresceine (FITC) conjugated lectins (Table 1) were diluted with phosphate buffered saline (PBS, 0.01 M, pH 7.20) to give a protein concentration of 100 µg/ml. Of this dilution, 30 µl of each lectin solution were placed on the membrane with infection structures, and the slide incubated for 30 minutes under moist and dark conditions at room temperature according to MENDGEN *et al.* (1985). A control using lectin solution and inhibitory hapten (0.2 M, as used by MENDGEN *et al.* 1985) was also prepared, and 30 µl of this mixture placed on the membrane and incubated under the above described conditions. The lectin incubated membrane was washed about 10 times with PBS buffer, mounted in 80 % glycerol and observed immediately with a epifluorescence microscope using 460—490 nm excitation filter, FT 510 nm splitting filter and LP 520 barrier filter.

Table 1
Lectins used as probes, their sources, and hapten specificity

Lectin	Source	Hapten specificity
Con A	Concanavalin A agglutinin from *Canavalia ensiformis* (Sigma, Taufkirchen)	α-D-Mannose* α-D-glucose
GSA II	*Griffonia simplicifolia* agglutinin (Medac, Hamburg)	β-D-galactose
LTA	*Lotus tetragonolobus* agglutinin (Sigma)	L-fucose
PHA	Kidney bean agglutinin from *Phaseolus vulgaris* (Sigma)	N-acetyl-D-galactosamine
PNA	Peanut agglutinin from *Arachis hypogaea* (Sigma)	α-D-galactose
RCA I	*Rinicus communis* agglutinin (Medac, Hamburg)	β-D-galactose
WGA	Wheat-germ agglutinin (Sigma) from *Triticum vulgare*	N-acetyl-D-glucosamine

* All sugars from Sigma.

Fluorescence of germ tubes and appressoria of 10 uredospores was measured with a Leitz microscope photometer at 3, 5 and 7 hour intervals following the method described by MENDGEN *et al.* (1985) and using a standard fluorescein solution for calibration as described by JONGSMA *et al.* (1971). The values for ten germ tubes and appressoria were averaged. Variation is given as standard deviation.

To study nuclear behaviour, infection structured were fixed in 2 % glutaraldehyde in 0.05 M phosphate buffer (pH 7.0) for 1 hour, washed with phosphate buffer and stained with mithramycin (2.5 mg mithramycin, 25 ml phosphate buffer and 7.6 mg $MgCl_2$) modified after SLATER (1976) and examined with the fluorescence microscope as described above but using a 435 nm excitation filter. All photomicrographs were taken with Ilford HP 5 film.

Results

Uredospores of *Uromyces fabae* developed infection structures rapidly on collodion membranes generally on the schedule indicated in Table 2. By the eight hour, 90 % had developed appressoria, 63 % substomatal vesicles and 29 % infection hyphae. Appressoria — both on collodion membranes and on leaf surfaces — were either separated from the germ tube by a single septum (Fig. 1) and or it developed away from the germ tube tip and was deliminated by two septa (Figs. 2, 3, 8, 9). The substomatal vesicle of *Vicia* rust was a long and cylindrical structure that became narrowed into an infection hypha (Fig. 4).

After mithramycin staining we observed two nuclei in germ tubes (Fig. 5), four nuclei in appressoria (Fig. 6) and eight nuclei in the substomatal vesicles (Fig. 7).

After application of FITC-WGA, the fluorescence on germ tubes was brighter than on appressoria (Figs. 8, 9, 10). However, developing appressoria fluoresced brightly (Fig. 11), but the fluorescence intensity was reduced as soon as appressoria were deliminated by septa (Fig. 12). The substomatal vesicle

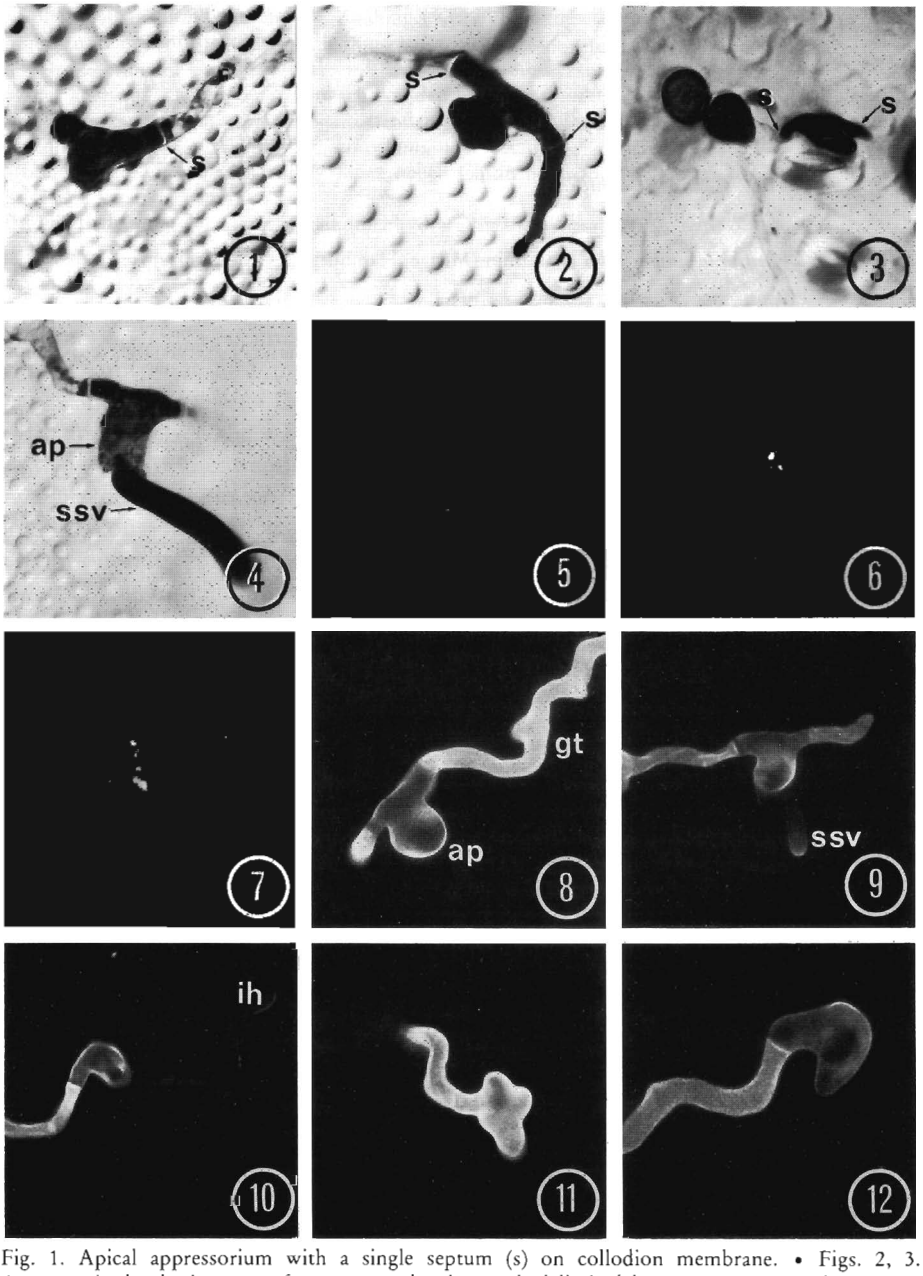

Fig. 1. Apical appressorium with a single septum (s) on collodion membrane. • Figs. 2, 3. Appressoria developing away from germ tube tips, each delimited by two septae (s). Fig. 2, on a collodion membrane; Fig. 3, on a leaf surface. • Fig. 4. Fully differentiated appressorium (ap) and substomatal vesicle (ssv). • Figs. 5, 6, 7. Nuclei in infection structures, fluorescing after staining with mithramycin. Fig. 5, two nuclei in germ tube; Fig. 6, four nuclei in appressorium; Fig. 7, eight nuclei in substomatal vesicle. • Fig. 8. Germ tube (gt) and appressorium (ap) showing differential fluorescence with wheat germ agglutinin (WGA). • Figs. 9—10. Substomatal vesicle (ssv) and infection hypha (ih) showing no fluorescence with WGA. • Fig. 11. Germ tube (gt) and the young appressorium (ap) showing bright fluorescence with WGA. • Fig. 12. Reduced fluorescence in appressorium relative to germ tube after septum formation

Table 2
Time course of differentiation of infection structures of *Uromyces fabae* on collodion membrane at 20 °C

Infection structure	Time taken to develop
Germ tube	2—3 h
Appressorium	3—4 h
Septum/infection peg	5—6 h
Substomatal vesicle	6—7 h
Infection hyphae	7—8 h

(Fig. 9) and the infection hypha (Fig. 10) had only very faint fluorescence. Binding of FITC-WGA was prevented by chitin hydrolysate.

GSA II and LTA binding on germ tubes, appressoria, substomatal vesicles and infection hyphae was weak as indicated by generally faint fluorescence, PHA and PNA also bound weakly and the binding was restricted to substomatal vesicles and infection hyphae. RCA I bound only to the infection hyphae and here too very faint fluorescence was detected. Con A did not bind to any structure.

Quantitative data on the WGA-FITC fluorescence of germ tubes and appressoria show that the former had twice as much fluorescence as the latter (Fig. 13). The intensity of fluorescence did not change significantly in germ tubes or fully developed appressoria from 3 to 7 hours after the start of germination.

Discussion

By suitable manipulation of environmental conditions, germinated uredospores of many rust fungi develop infection structures on artificial substrates and

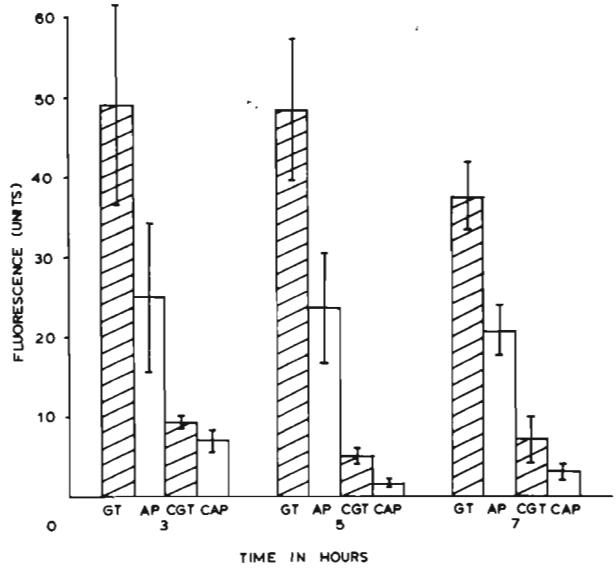

Fig. 13. Fluorescence intensity of WGA on germ tube (gt) and appressorium (ap) at 3, 5 and 7 hours after incubation of uredospores of *Uromyces fabae* on collodion membranes at 20 °C and their controls (CGT and CAP) including WGA and the inhibitory hapten

membranes (HURD-KARRER and RODENHISER 1947, EMGE 1958, PAVGI and DICKINSON 1961, MAHESWARI *et al.* 1967). According to these reports, an appressorium develops at the tip of the germ tube from which it is separated by a single septum. Present studies on *Uromyces fabae* show that appressoria also develop somewhat removed from the germ tube tip. These appressoria are delimited by two septa, instead of one. The part of the germ tube, which is confined by the septa, enlarges into a wedgeshaped structure and is eventually transformed into an appressorium. Such appressoria not only developed on artificial membranes but also on leaf surfaces. The frequency with which appressoria were formed, in different ways, indicate that this is a regular feature of *Vicia* rust, at least under the incubation conditions used here. Nuclear events during the process of infection structure differentiation further showed that there were no cytological anomalies in this rust.

Uredospore germ tubes of *Puccinia graminis* f. sp. *tritici* and *Uromyces appendiculatus* contain lipid, proteins and sugars (JOPPIEN *et al.* 1972, TROCHA *et al.* 1974, TROCHA and DALY 1974). The fate of these substances during the process of infection is not well established. Some clues to events during infection structure development can be obtained by the application of lectins as bioprobes (LECHEVALIER and LECHEVALIER 1979, NEWCOMB *et al.* 1979) which can identify surface carbohydrates. WGA has a specific binding affinity for chitin oligomers (ALLEN *et al.* 1973, LOTAN and SHARON 1973). Our studies revealed that, per unit area, the germ tube walls of *Vicia* rust have two times more receptor sites for WGA than do the walls of fully developed appressoria. This is in contrast to the observations with *U. appendiculatus* and *P. coronata* (MENDGEN *et al.* 1985) where germ tube and appressorium were similar in number of receptor sites for WGA. The presence of reduced amounts of this carbohydrate in the walls of appressoria of *Vicia* rust would suggest that only a part of it is mobilised from the germ tube walls as appressoria and substomatal vesicles are produced. Chitin oligomers have also been found to occur in the walls of other fungi (GALUN *et al.* 1976, CHABOUD and LALONDE 1982, BARRAQUETA and SCHAUZ 1983).

The results with the lectins indicate that — besides chitin — there were no residues of glucose or mannose (Con A), some terminal N-acetyl-glucosamine (GSA II), very little galactose (PNA, PHA, RCA I) and some fucose (LTA) on the surface of infection structures of *U. fabae*. This also contrasts with our earlier observations (MENDGEN *et al.* 1985), where Con A did bind to the infection structures, but RCA I and PNA did not bind to the infection structures of *U. appendiculatus* and *P. coronata*.

It is apparent from the present and earlier studies (MENDGEN *et al.* 1985) that infection structure surfaces of rust fungi are not identical in composition and that they undergo modification during the process of their differentiation. Perhaps the surface changes are pathogen specific and relate to their ability to recognize host surfaces (MENDGEN 1982).

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