

テンサイそう(叢)根病の発生土壌から分離されたPolymyxa betae Keskin の宿主範囲

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Host Range of *Polymyxa betae* Keskin Strains in Rhizomania-infested Soils of Sugar Beet Fields in Japan

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Abstract

Host range of *Polymyxa* sp. isolated from various plants grown in rhizomania-infested soils of three different localities in Hokkaido was compared. Among 108 species of plants belonging to 23 families tested, 12 plants belonging to Chenopodiaceae, Amaranthaceae and Portulacaceae were infected with the fungus. The other 20 families including Gramineae were not infected. All isolates of *Polymyxa* sp. were identified as *Polymyxa betae* Keskin on the basis of their host range and morphological characteristics. The host range of the isolates was found to be limited to the family of the original host. Among the Chenopodiaceous isolates, that from sugar beet did not infect *Chenopodium album*, and those from *C. album* did not infect sugar beet. These *P. betae* isolates could be divided into several formae speciales; *Amaranthus retroflexus* isolates were identified as *P. betae* Keskin f. sp. *amaranthi* Barr, for the *Portulaca oleracea* isolates a new forma specialis *P. betae* Keskin f. sp. *portulacae* was proposed. Although sugar beet isolates and *C. album* isolates should be divided into different formae speciales, respectively, the classification of these isolates should await comparison with the Canadian isolate from *C. album*. From these results it is suggested that sugar beet isolates of *P. betae* which have a high infectivity to *Beta vulgaris* are associated with the incidence of rhizomania of sugar beet whereas the other two formae speciales and *C. album* isolates may not be involved.

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Key words : *Polymyxa betae*, host range, formae speciales.

Introduction

Rhizomania of sugar beet (*Beta vulgaris* L. var. *saccharifera*), which is characterized by the abnormal proliferation of rootlets as well as by the yellowing, wilting and stunting of leaves, was observed in Italy in the mid 1950's²⁾. It is now distributed in Europe, Asia and USA, causing serious damage to roots and loss of yields. In Japan the disease was reported first by Masuda *et al.*⁸⁾ in 1969 and since then it has spread throughout Hokkaido.

Rhizomania is caused by the beet necrotic yellow vein virus (BNYVV)¹⁰⁾ transmitted^{4,9,11)} by *Polymyxa betae* Keskin which belongs to the *Plasmodiophorales*. The host range of the fungus is limited to Chenopodiaceous crops and weeds. However, the occurrence of *P. betae* has been reported in plants belonging to *Amaranthaceae*^{1,5)}, and the name *P. betae* f. sp. *amaranthi* was proposed¹⁾.

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This paper reports the morphology and host range of *P. betae* isolated from plants grown in several rhizomania-infested soils in Hokkaido.

Materials and Methods

Soil samples and test plants. Rhizomania-infested soils were collected in fields located in Date, Tsubetsu and Shari in Hokkaido, where serious incidence of rhizomania had been recorded. Each one liter of soil samples was mixed with two grams of chemical fertilizer (N: 10%, P_2O_5 : 13%, K_2O : 4%, MgO : 2% and H_3BO_3 : 0.05%) and one gram of Hymexazol (Tachigaren, active ingredient 4%) to prevent damping-off caused by *Aphanomyces cochlioides*. The seeds of various plants (23 different families) were sown in soils placed in clay pots. The plants were grown in a greenhouse at about 20–30 C for three months or more. Infection with *Polymyxa* was determined by the presence of resting spore clusters in the rootlets under a light microscope.

Isolation and multiplication of *P. betae*. Several pieces of rootlets about 5 mm long containing only resting spores of *Polymyxa* were selected under a dissecting microscope. The suspension which were ground in a mortar with a small amount of sterile water, was taken on a glass slide, and resting spore clusters in the suspension were collected by sucking into a glass capillary under a light microscope. About 100 resting spore clusters were placed in the vicinity of the roots of healthy seedlings of the original host plant which were grown in sterile soil. After two months, infected rootlets with a large number of resting spores were thoroughly washed in running tap water and dried in air. They were served as inocula in the following experiments.

Inoculation. About 0.3 g of dried rootlets containing resting spores were cut into fragments about 1 mm long and mixed with steamed soil in a 12 cm clay pot. Usually one or two plants of each species were planted in a pot placed on a wire netting bed in a greenhouse 20 cm apart to prevent the contamination. In most experiments two to three pots were used for each plant.

Morphological observation. Morphology of *Polymyxa* sp. in the rootlets of the inoculated plants was observed under a light microscope during the period from the 10 th to 60 th day after sowing. The rootlets were stained with lactophenol containing 0.1% cotton blue or 0.1% acid fuchsin.

Results

Infection of various plant species with Polymyxa sp.

One hundred and eight species of plants belonging to twenty three different families were planted in rhizomania-infested soils collected from three different localities. Twelve plants belonging to the Chenopodiaceae, Amaranthaceae and Portulacaceae families grown in Date and Tsubetsu soils were infected with *Polymyxa* sp. Amaranthaceous and Portulacaceous plants grown in Shari soils were not infected (Table 1). In the epidermis and cortex of the infected rootlets, resting spore clusters were produced abundantly in sugar beet (*Beta vulgaris* L. var. *saccharifera*), swiss chard (*B. vulgaris* L.

var. *cicla*) and *B. vulgaris* L. var. *rapa*, moderately in *Chenopodium album*, *C. murale*, *Amaranthus retroflexus* and *Portulaca oleracea*, and slightly in *B. procumbens*, spinach (*Spinacia oleracea*), *A. blitum* and *P. grandiflora*.

Table 1. Infection of various plant species with *Polymyxa* sp. grown in rhizomania-infested soils of three different localities

Plant tested	Severity of infection of <i>Polymyxa</i>		
	Date soil	Tsubetsu soil	Shari soil
Chenopodiaceae			
Sugar beet	+++ ^{a)}	+++	+++
Swiss chard	+++	+++	+++
<i>Beta vulgaris</i> L. var. <i>rapa</i>	+++	+++	+++
<i>B. procumbens</i>	•	+	•
Spinach	•	+	+
<i>Chenopodium murale</i>	+	++	+
<i>C. ficifolium</i>	++	++	+
<i>C. album</i>	++	++	+++
Amaranthaceae			
<i>Amaranthus retroflexus</i>	++	+	—
<i>A. blitum</i>	+	+	—
Portulacaceae			
<i>Portulaca oleracea</i>	++	++	—
<i>P. grandiflora</i>	+	+	—

a) Severity of infection expressed by the abundance of resting spore clusters in rootlets; +++ : Heavily infected, ++ : Moderate infection, + : Trace infection, — : No infection, • : Not tested.

The other eighty seven species of plants were not infected with *Polymyxa* sp. They were as follows : Chenopodiaceae [*Beta webbiana* Mog., *B. pattellaris* Mog.]. Amaranthaceae [*Celosia argentea* L.]. Compositae [*Lactuca sativa* L. (Head lettuce), *Zinnia elegans* Jacq. (Zinnia), *Tagetes erecta* L. (Marigold), *Chrysanthemum morifolium* Ramat. (Chrysanthemum), *Cosmos bipinnatus* Cav., *Dahlia pinnata* Cav. (Dahlia), *Petasites japonicus* Mir. (Japanese butterbur), *Chrysanthemum coronarium* L. (Garland chrysanthemum), *Ageratum houstonianum* Mill. (Mexican ageratum), *Bellis perennis* L. (English daisy), *Sonchus oleraceus* L. (Common sow thistle), *Taraxacum officinale* Weber (Dandelion), *Artemisia princeps* Pamp. (Common mugwort), *Brexa setosa* Kitam. (Canada thistle), *Sonchus brachyotis* DC. (Perennial sow thistle)]. Cruciferae [*Brassica campestris* L. (Chinese cabbage), *B. oleracea* L. (Cabbage), *Rorippa islandica* Borbas (Marsh yellow cress), *Erysimum japonicum* Makino]. Cucurbitaceae [*Cucumis melo* L. (Netted melon)]. Gramineae [*Oryza sativa* L. (Rice), *Triticum aestivum* L. (Wheat), *Hordeum vulgare* L. (Barley), *Zea mays* L. (Corn), *Panicum crus-galli* var. *frumentaceum* Hook. (Japanese millet), *Dactylis glomerata* L. (Orchardgrass), *Phleum pratense* L. (Timothy), *Bromus inermis* Leyss. (Smooth brome grass), *Lolium multiflorum* Lam. (Italian ryegrass), *Setaria viridis* Beauv. (Green panicum), *Poa annua* L. (Annual poa)]. Leguminosae [*Glycine max* (L.) Merr. (Soybean),

Phaseolus radiatus var. *aurea* Prain. (Adzuki bean), *P. vulgaris* L. (Kidney bean), *Pisum sativum* L. (Pea), *Trifolium pratense* L. (Red clover), *Trifolium repens* L. (White clover), *Medicago sativa* L. (Alfalfa)]. Plantaginaceae [*Plantago asiatica* L. (Broadleaved plantain)]. Polygonaceae [*Rumex obtusifolius* L. (Bitterdock), *R. acetosa* L. (Sour dock), *Polygonum lapathifolium* L. (Pale-persicaria), *P. nodosum* Pers., *P. nepalense* Meisn., *P. convolvulus* L., *P. aviculare* L. (Pink-weed), *Fagopyrum esculentum* Moench. (Buckwheat)]. Scrophulariaceae [*Antirrhinum majus* L. (Snapdragon)]. Solanaceae [*Solanum tuberosum* L. (Potato), *S. melongena* L. (Eggplant), *S. nigrum* L. (Black nightshade), *Lycopersicon esculentum* Mill. (Tomato), *Petunia x hybrida* Vilm. (Petunia), *Nicotiana tabacum* L. (Tobacco), *Physalis alkekengi* L. var. *franchetii* Mak. (Chinese lantern plant)]. Begoniaceae [*Begonia* sp. (Begonia)]. Umbelliferae [*Daucus carota* L. (Carrot), *Apium graveolens* L. (Celery), *Cryptotaenia japonica* Hassk. (Japanese hornwort), *Cnidium officinale* Makino]. Cactaceae [*Opuntia* sp. (Cactus)]. Caryophyllaceae [*Stellaria neglecta* Weihe, *Dianthus caryophyllus* L. (Carnation), *D. chinensis* L., *D. superbus* L.]. Commelinaceae *Commelina communis* L. (Asiatic dayflower)]. Ranunculaceae [*Aquilegia flabellata* Sieb. et Zucc. (Fan columbine)]. Equisetaceae [*Equisetum arvense* L. (Field horsetail)]. Labiatae [*Perilla ocymoides* L., *Salvia splendens* Sello ex Ness (Scarlet sage), *Elsholtzia ciliata* Hylander., *Mentha piperita* L. (Peppermint)]. Liliaceae [*Asparagus officinalis* L. (Asparagus), *Allium sativum* L. (Garlic), *A. tuberosum* Rottler (Chinese chive), *A. cepa* L. (Onion), *Lilium* sp. (Lily)]. Crasulaceae [*Sedum alboroseum* Baker]. Aizoaceae [*Tetragonia expansa* Murr.].

From the results of host range tests, it was suggested that the fungi detected in the three soil samples were *P. betae*.

Host range of *Polymyxa* sp.

The isolates of *Polymyxa* sp. isolated from seven different plants grown in Tsubetsu soil were selected as the inocula, and fourteen plants listed in Table 2 were inoculated.

Table 2. Host range of *Polymyxa* sp. infecting the plants grown in Tsubetsu soil

Test plants	Original host of <i>Polymyxa</i>						
	Sugar beet	Spinach	<i>C. murale</i>	<i>C. ficifolium</i>	<i>C. album</i>	<i>A. retroflexus</i>	<i>P. oleracea</i>
Sugar beet	+++ ^{a)}	+++	+++	+++	—	—	—
Swiss chard	+++	+++	+++	+++	—	—	—
Spinach	++	++	++	++	++	—	—
<i>C. murale</i>	+++	+	+++	+++	++	—	—
<i>C. capitatum</i>	++	++	+	++	++	—	—
<i>C. ficifolium</i>	++	+	++	++	+	—	—
<i>C. quinoa</i>	—	++	+	++	++	—	—
<i>C. album</i>	—	++	+	++	++	—	—
<i>A. retroflexus</i>	—	—	—	—	—	+++	—
<i>A. blitum</i>	—	—	—	—	—	+++	—
<i>P. oleracea</i>	—	—	—	—	—	—	+++
<i>P. grandiflora</i>	—	—	—	—	—	—	+++

a) Severity of infection expressed by the abundance of resting spore clusters in rootlets;
+++ : Heavily infected, ++ : Moderate infection, + : Trace infection, — : No infection.

The host range of the respective isolates was limited to the same family of the original host: i.e. the isolates from sugar beet, spinach, *C. ficifolium*, *C. murale* and *C. album* infected only Chenopodiaceous plants, those from *Amaranthus retroflexus* infected only Amaranthaceous plants, and those from *Portulaca oleracea* infected only Portulacaceous plants. Among the Chenopodiaceous isolates, there were some differences in host range. The isolates from sugar beet infected *Beta vulgaris* such as sugar beet and swiss chard and abundant resting spore clusters were produced in the rootlets, but *C. album* and *C. quinoa* were not infected. The isolates from *C. album* severely infected *C. album* and *C. quinoa*, but not sugar beet and swiss chard. And the isolates from spinach, *C. murale* and *C. ficifolium* infected all the Chenopodiaceous plants tested.

The infectivity of *Polymyxa* sp. parasitizing Chenopodiaceous plants in the above experiments was compared (Table 3, 4). The fungi in spinach, *C. murale*, *C. capitatum* and *C. ficifolium* infected sugar beet and *C. album*. The resting spore clusters produced in the infected rootlets were cross-inoculated to sugar beet and *C. album* (Table 3). The infectivity of the isolates from sugar beet and *C. album* was restricted to the respective host plants.

In the following experiments the infectivity of the isolates from sugar beet and *C. album* was tested. These isolates could attack *C. murale*, *C. capitatum*, *C. ficifolium* and spinach to produce resting spores. Moreover, if these resting spores were secondary inoculated to sugar beet and *C. album*, they could attack either sugar beet or *C. album* depending on the original host plant (Table 4).

Table 3. Infectivity of *P. betae* isolated from spinach, *Chenopodium murale*, *C. capitatum* and *C. ficifolium* to sugar beet and *C. album*

Inoculum		1 st		2 nd		
Resting spore clusters produced in		inoculation		inoculation ^{a)}		
Spinach (SO-T 19841)	→	→	sugar beet ^{b)}	→	sugar beet	<i>C. album</i>
		→	<i>C. album</i>	→	sugar beet	<i>C. album</i>
<i>Chenopodium murale</i> (CM-T 19842)	→	→	sugar beet	→	sugar beet	<i>C. album</i>
		→	<i>C. album</i>	→	sugar beet	<i>C. album</i>
<i>C. capitatum</i> (CC-T-19843)	→	→	sugar beet	→	sugar beet	<i>C. album</i>
		→	<i>C. album</i>	→	sugar beet	<i>C. album</i>
<i>C. ficifolium</i> (CF-T-19849)	→	→	sugar beet	→	sugar beet	<i>C. album</i>
		→	<i>C. album</i>	→	sugar beet	<i>C. album</i>

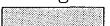
a) Resting spore clusters of *P. betae* propagated in 1st inoculation were reinoculated to sugar beet and *C. album*.

b) ; infected.
; not infected.

Table 4. Infectivity of *P. betae* isolated from sugar beet and *Chenopodium album* to spinach, *C. murale*, *C. capitatum* and *C. ficifolium*

Inoculum		1 st		2 nd	
Resting spore clusters produced in		inoculation		inoculation ^{a)}	
Sugar beet (TNS F 198554)	→	spinach ^{b)}	→	sugar beet	<i>C. album</i>
		<i>C. murale</i>	→	sugar beet	<i>C. album</i>
		<i>C. capitatum</i>	→	sugar beet	<i>C. album</i>
		<i>C. ficifolium</i>	→	sugar beet	<i>C. album</i>
<i>C. album</i> (TNS F 198555)	→	spinach	→	sugar beet	<i>C. album</i>
		<i>C. murale</i>	→	sugar beet	<i>C. album</i>
		<i>C. capitatum</i>	→	sugar beet	<i>C. album</i>
		<i>C. ficifolium</i>	→	sugar beet	<i>C. album</i>

a) Resting spore clusters of *P. betae* propagated in 1 st inoculation were reinoculated to sugar beet and *C. album*.

b) ; infected.

; not infected.

These results suggest that the host ranges of sugar beet isolates and *C. album* isolates were different each other, though these isolates were able to infect spinach, *C. murale*, *C. capitatum* and *C. ficifolium*.

The results obtained with the isolates from Tsubetsu soil were the same as those with the isolates from Date and Shari soils.

Taxonomic comparison of *Polymyxa* sp.

The morphology of *Polymyxa* sp. isolated from Chenopodiaceous, Amaranthaceous and Portulacaceous plants grown in rhizomania-infested soils in Hokkaido is as follows; Sporangial plasmodia are lobed, elongated or irregular with small exit papillae, usually becoming markedly elongated and enveloped by membranes at maturity (Plate I). Zoosporangium is 5–20 μm in width, up to 100 μm in length forming many zoospores. Cystogenous plasmodia are globular or ovoid, 8–42 μm in diam., cleaving into cysts (resting spores) and forming cystosori (resting spore cluster) at maturity. One to several cystosori are formed in a cell and composed of 4 to more than 100 cysts, with a size and shape comparable to those of the host cell: globular, irregular, flattened, elongated, and sometimes composed of a linear series of cysts. Cysts which vary in size and shape, are usually globular, polyhedral, or slightly angular, 2.8–10.0 μm in diam. (avg. 4.5 ± 0.7 μm). In the sugar beet isolates a single primary zoospore is produced by the germination of a cyst, but in the other isolates zoospores have not yet been observed.

These morphological characteristics which do not enable to distinguish the fungi from one another were identical with those of *P. betae* described by Keskin⁷⁾. However, there

are differences in the host range. Therefore, it is possible to separate the fungi into several formae speciales. One of them was identical with *P. betae* Keskin f. sp. *amaranthi* Barr, and a new forma specialis was proposed for the *Portulaca oleracea* isolate, i. e. *P. betae* Keskin f. sp. *portulacae* (f. sp. nov.). Sugar beet isolates and *C. album* isolates should also be divided into different formae speciales. However, Barr¹⁾ reported that a Canadian isolate, *P. betae* f. sp. *betae* was highly infective to *C. album*, but not to sugar beet except in one inoculation experiment. Therefore, the authors prefer avoidance of assigning formae speciales to the sugar beet and *C. album* isolates, until they are directly compared with the Canadian isolate.

***P. betae* Keskin f. sp. *portulacae* (f. sp. nov.)**

The fungus which was isolated from Portulacaceous plants in Hokkaido, does not grow on Chenopodiaceous and Amaranthaceous plants, and its host range is clearly restricted to *P. oleracea* and *P. grandiflora*.

Herbarium material for holotype specimen has been deposited at the National Science Museum in Japan (TNS-F-198553).

Discussion

Fungi have generally been defined taxonomically on the basis of their morphological characteristics. However, in the Plasmodiophoraceae the use of the host range has generally been emphasized in their classification, especially in *Polymyxa* spp. *Polymyxa betae* cannot be readily distinguished from *P. graminis* on the basis of their morphological characteristics, but the host range is clearly different from that of *P. graminis* and restricted to the Chenopodiaceous plants^{6,7,12)}.

Based on the morphological characteristics and host range, *Polymyxa* sp. isolated from Chenopodiaceous plants grown in rhizomania-infested soils in Hokkaido belongs to *P. betae* Keskin. However, sugar beet isolates and *C. album* isolates were clearly different in their infectivity to *Beta vulgaris* and the genus *Chenopodium*. Sugar beet isolates did not infect *C. album* and *C. album* isolates did not infect sugar beet.

On the other hand, it has been reported that European isolates of *P. betae* Keskin infected sugar beet and *C. album*^{3,7)}. In Canada, Barr (1979)¹⁾ reported that the isolates of *Polymyxa* sp. isolated from *Chenopodium album* and *Amaranthus retroflexus* in Ontario were identical with *P. betae* in their morphological characteristics, and the names of *P. betae* f. sp. *betae* and *P. betae* f. sp. *amaranthi* were proposed due to the differences in the host range. The former did not infect sugar beet except in only one case and it was suggested that the European strain and the Canadian strain are different biotypes of *P. betae* f. sp. *betae*¹⁾.

P. betae isolated from Chenopodiaceous plants in Hokkaido, as mentioned above, are different from the European and Canadian strains in their infectivity to sugar beet and *C. album*. Therefore, the sugar beet and *C. album* isolates of Hokkaido should be classified as different formae speciales. However, in this report the authors withhold the assignment of formae speciales to these isolates, because they have not yet been directly compared with the Canadian strain.

Spinach, *C. murale*, *C. capitatum* and *C. ficifolium* are common host plants for the

sugar beet and *C. album* isolates.

The morphology and host range of *P. betae* f. sp. *amaranthi* from Hokkaido are similar to those of the strains from Canada¹⁾.

The morphology of the *Portulaca oleracea* isolates was similar to that of *P. betae* Keskin and their host range was restricted to Portulacaceae plants. Portulacaceae and Chenopodiaceae are taxonomically close, as is the case for the Chenopodiaceae and Amaranthaceae. Therefore, a new forma specialis of *P. betae*, i. e.; *P. betae* Keskin f. sp. *portulacae* was proposed for these isolates.

On the basis of the results obtained it is suggested that sugar beet isolates of *P. betae* which show a high infectivity to *Beta vulgaris* are associated with the occurrence of rhizomania of sugar beet whereas the other formae speciales and *C. album* isolates may not be involved.

Further data on the association between these isolates of *P. betae* and beet necrotic yellow vein virus will be reported elsewhere.

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和 文 摘 要

阿部秀夫・宇井格生：テンサイそう（叢）根病の発生土壌から分離された *Polymyxa betae* Keskin の宿主範囲

北海道のテンサイ畑に生息する *Polymyxa* 属菌の同定および分類を行なうため、そう根病の発生土壌に植えた各種植物に寄生した *Polymyxa* の形態および宿主範囲を比較した。用いた植物は23科108種で、そのうちアカザ科、ヒユ科およびスベリヒユ科植物の細根表面の細胞および皮層に *Polymyxa* sp. の寄生が認められた。これら菌株の形態には互に差がなく、*P. betae* Keskin の原記載と同様であった。テンサイ、ホウレンソウ、*Chenopodium murale*, *C. ficifolium*, シロザ、アオビユおよびスベリヒユから分離された菌株を用いてそれぞれの分離源植物に交互接種を行い、寄生性を比較した結果、各菌株の寄生は分離源と同じ科の植物に限られた。ただし、アカザ科植物から分離された菌株はアカザ科の多くの植物を共通宿主とするものの、テンサイ菌株はシロザに、シロザ菌株はテンサイにそれぞれ寄生しなかった。これらの結果から北海道の *P. betae* はいくつかの分化型に分かれるものと考えられた。即ちアオビユから分離された菌株は *P. betae* f. sp. *amaranthi* と同定され、スベリヒユから分離された菌株は新しい分化型、*P. betae* f. sp. *portulacae* として記載した。テンサイとシロザから分離された菌株はそれぞれ別の分化型にすべきであるが、両菌株の分類はカナダのシロザ菌株との直接的な比較を行ってから決めたい。以上の結果、そう根病の発生にはテンサイ、フダンソウなど *Beta vulgaris* に高い寄生性を持っているテンサイ菌株が関係し、他の2つの分化型およびシロザ菌株は関係

していないと考えられる。

Explanation of plate

Plate I

- Fig. 1. Plasmodia of *Polymyxa betae* in sugar beet root cells.
 - Fig. 2. Zoosporangia of *P. betae* in sugar beet root cells.
 - Fig. 3. Resting spore clusters of *P. betae* in sugar beet root cells.
 - Fig. 4. Zoospores released from zoosporangia of *P. betae* in sugar beet root cells.
 - Fig. 5. Zoosporangia of *P. betae* in *Chenopodium album* root cells.
 - Fig. 6. Zoosporangia of *P. betae* in *Portulaca oleracea* root cells.
 - Fig. 7. Resting spore clusters of *P. betae* in *C. album* root cells.
 - Fig. 8. Resting spore clusters of *P. betae* in *P. oleracea* root cells.
- Bar in each Figure represents 50 μ m.

Abbreviations

HW: host cell wall, p: plasmodium, zg: zoosporangium, ew: enveloping wall, z: zoospore, f: flagellum, r: resting spore cluster.

Plate I

