Acta Protozool. (2011) 50: 121–132
http://www.eko.uj.edu.pl/ap

ACTA
PROTOZOOLOGICA

Morphological Enumeration of Resting Spores in Sporosori of the Plant Pathogen *Spongospora subterranea*

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Summary. Plasmodiophorid sporosori (aggregations of resting spores) reach their most complex form in *Spongospora subterranea* f. sp. *subterranea*, the biotrophic plant pathogen which causes the economically important disease powdery scab of potato (*Solanum tuberosum*). Resting spores are the perennation life cycle stage of plasmodiophorids, allowing them to survive for long periods and infect subsequent host generations. Light microscopy was used to measure resting spores and sporosori of *Sp. subterranea*, and enumerate resting spores in individual sporosori. Mean resting spore diameters differed for two sporosorus collections, being 4.0 µm (from New Zealand) and 4.3 µm (from Switzerland). Counts of resting spores in 4 µm thick serial sections of sporosori from one collection gave a mean of 667 (range 155 to 1,526) resting spores per sporosorus. Number of resting spores per sporosorus was closely related to sporosorus volume, and could be accurately estimated using the formula; number of resting spores = 0.0081 × sporosorus volume (assuming sporosori to be spheroids). Using this formula, mean numbers of resting spores in sporosori from 37 *Sp. subterranea* collections from field-grown potato tubers from 13 countries were determined to range from 199 to 713. Differences in numbers of resting spores between the collections were statistically significant ($P < 0.05$), and independent of country or host cultivar of origin, indicating that enumeration should be carried out for individual sporosorus collections to accurately quantify inoculum. Morphology, using scanning electron microscopy, also showed that between 2 and 51% (average 20%) of resting spores released zoospores after exposure to roots of host plants. The formula for resting spore enumeration validated in this study can be used to standardise *Sp. subterranea* resting spore inoculum for plant pathology studies, and possibly to assist determination of soil inoculum potential for disease risk evaluations.

Key words: Microscopy, powdery scab, potato, *Solanum tuberosum*.

INTRODUCTION

Spongospora subterranea (Wallr.) Lagerheim f. sp. *subterranea* Tomlinson (Cercozoa, Phytomyxea, Plasmodiophorida, Plasmodiophoridae) (Cavalier-Smith and Chao 2003) causes powdery scab of potato (*Solanum tuberosum* L.). This disease is economically important because infection of potato tubers and roots by *Sp. subterranea* reduces tuber quality and yield (Falloon *et al.* 1996, 2004; Harrison *et al.* 1997; Lister *et al.* 2004). The pathogen is also capable of infecting several other host plants (Qu and Christ 2006), and is a vector of *Potato moptop virus* (Jones and Harrison 1969).

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In this paper we use the names *Spongospora subterranea* f. sp. *subterranea* (hereafter *Sss.*) for the pathogen that infects potato, and *Spongospora subterranea* f. sp. *nasturtii* Tomlinson (*Ssn.*) for the pathogen that infects *Nasturtium officinale* R. Br. (watercress).

Spongospora subterranea produces resting spores in sporosori. [We follow the terminology advocated by Karling (1981) and confirmed by Braselton (2001), and use **resting spore** (elsewhere "spore cyst," "cyst," "resistant cyst") and **sporosorus** (elsewhere "spore ball," "cystosorus")]. Resting spores are thick-walled cells that are the resistant propagation and perennation life cycle stages of members of the Plasmodiophoridae ("plasmodiophorids"), allowing long-term survival of these pathogens between host generations. Sporosori are aggregations of resting spores.

The plasmodiophorids are pathogens of plants, algae, fungi and stramenopiles (Karling 1968, Braselton 1995). Most members of this family produce resting spores in sporosori, which probably assist dissemination of these organisms. Sporosorus form in the plasmodiophorids has been described and illustrated by Karling (1968) and Dylewski (1990). Sporosorus complexity varies between plasmodiophorid genera, and sporosorus form is a key character for distinguishing the genera of the family (Karling 1968, Dylewski 1990, Braselton 2001). *Plasmodiophora* produces separate resting spores and does not produce sporosori. *Tetramyxa* produces sporosori that are generally tetrads of resting spores, while *Octomyxa* has octad sporosori. In *Sorodiscus* the sporosori are disc-shaped resting spore aggregations, and in *Sorosphaera* the resting spores are amassed in discrete hollow spheres. *Membranosorus*, *Polymyxa*, *Woronina* and *Ligniera* have sporosori of variable size and form, each containing many resting spores. *Spongospora* has the most complex sporosori of the known plasmodiophorids, with many resting spores aggregated in sponge-like structures of variable size. Resting spores or sporosori have not been observed in *Maullinia* (Maier *et al.* 2000).

Increasing sporosorus complexity in the plasmodiophorids has been postulated as an evolutionary progression (Karling 1968), and knowledge of sporosorus form in *Spongospora* will provide understanding of a point in this progression that is possibly the most highly evolved of the plasmodiophorids. Osborn (1911) presented an early description and illustration of sporosori of *Sp. subterranea*, and ultrastructural detail of sporosori and resting spores of the pathogen were described and illustrated by Lahert and Kavanagh (1985).

Resting spores in *Sss.* sporosori each produce a primary zoospore (Merz 1997), and zoospores initiate infections of host roots and, in potato, stolons and tubers (modified stolons). Sporosorus infestation of soil provides inoculum for subsequent infection of potato roots and tubers, while sporosori carried on seed tubers, in powdery scab lesions and/or as surface contaminants, transmit the pathogen to potato crops in previously uninfested land. Sporosori on seed tubers are the most likely means by which the pathogen has become widespread throughout most of the world's potato growing regions, and the disease is of economic importance in most areas where intensive potato production is carried out (Harrison *et al.* 1997, Merz 2008). Furthermore, resting spores and sporosori can survive in soil for many years (Kole 1954, Harrison *et al.* 1997), released into the soil after death of infected potato roots, stolons or tubers, or from infected or infested potato seed tubers.

Long-term survival of plasmodiophorid resting spores causes problems for management of the economically important diseases they cause. Many of the strategies for management of powdery scab of potato aim to avoid contamination of field soil by *Sss.* sporosori, or to prevent initiation of infection of host tissues by zoospores released from sporosori (Falloon 2008). Understanding the biology of sporosori, resting spores and zoospores is important, therefore, for development of effective management of powdery scab (Merz and Falloon 2009). Furthermore, enumeration of resting spores in sporosori is a necessary step in accurate determination of inoculum potential of the pathogen, a likely key component of disease risk assessment in potato cropping soils.

Several methods have been developed to detect inoculum of *Sss.* on seed potato tubers or in soil. These include bioassays (Merz 1989), antibody methods (Walsh *et al.* 1996, Merz *et al.* 2005a), techniques using polymerase chain reaction technology (Bulman and Marshall 1998, Bell *et al.* 1999, van der Graaf *et al.* 2003, Ward *et al.* 2004, Qu *et al.* 2006), and immunochromatography (Merz *et al.* 2005b). These methods are mostly qualitative. Where quantification has been attempted, this has been through correlative association between sporosori (rather than resting spores) and disease (Merz and Falloon 2009). Culturing techniques cannot be used for inoculum quantification because the pathogen is an obligate biotroph.

Sporosori are commonly used as inoculum in experiments with *Sss.* Accurate determination of the inoculum potential (numbers of potential zoospores in spo-

rosorus inoculum) is not possible, because the numbers of resting spores in each sporosorus cannot be easily counted. This problem is exacerbated by considerable variation in sporosorus size. Kole (1954) noted that he made "Unsuccessful attempts ... to count the numbers of resting spores in the sections of sporeballs," and Karling (1968) observed that enumeration of *Sss*. inoculum based on sporosori "would not give an accurate index of the numbers of spores present".

This paper reports a morphological study that aimed to determine the numbers of resting spores in sporosori of *Sss*. (and *Ssn.*) as a basis for accurate quantification of inoculum. Resting spores and sporosori have been measured, and the numbers of resting spores within sporosori have been determined using direct measurement with microscopy and appropriate calculations. The method for determining numbers of resting spores developed from these measurements has been applied to sporosorus collections from a number of different countries, to examine variation in numbers of resting spores they contain. A series of preliminary reports on this study have been presented previously (Falloon *et al.* 2005, 2006, 2007, 2010).

MATERIALS AND METHODS

Sources of sporosori

Most samples of sporosori used in this study were obtained from powdery scab lesions on field-grown potato tubers from different host cultivars. Sporosori of two collections were examined in detail. These were, respectively, from tubers of cv. Agria harvested at Lincoln, New Zealand, or cv. Estima harvested at Reckenholz, Switzerland. Both collections were made in 2004. Sporosori for scanning electron microscopy were from cv. Binje, grown in Switzerland. In addition, 37 collections of sporosori from tubers of different potato cultivars from 13 countries, and two collections of sporosori of *Ssn.* from root galls of laboratory-grown watercress plants (*Nasturtium officinale* R. Br.) (Table 2) were also used for measurement of sporosorus dimensions.

Sporosorus and resting spore dimensions (light microscopy)

Samples of sporosori from the potato cvs. Agria (New Zealand) and Estima (Switzerland) were placed in reverse osmosis purified water on glass microscope slides, and viewed with a compound microscope (× 1,000 magnification). Images of individual sporosori were captured with a digital camera, taking care to ensure that focus of each image was on the equatorial plane of the sporosorus. Dimensions (longest and shortest diameter) were determined from micrographs of 101 and 96 sporosori respectively from the two collections. A second sample of sporosori from each collection was mounted in trypan blue in lactophenol, and photographed (× 1,000 magnification). This preparation method, along with careful microscope focusing before image capture, gave good definition of individual resting spores within sporosori (see Fig. 4). Dimensions of resting spores were measured, taking a sample of five resting spores from each of six different sporosori from each of the two collections. All measurements were made using calibrations based on an image of a microscope stage micrometer captured at × 1,000 magnification.

Dimensions of sporosori from each of the other collections examined in this study (Table 2) were measured for a sample of 50 sporosori, using standardised micrometric methods (above).

Calculation of resting spore and sporosorus volumes and arithmetic enumeration of resting spores

Volumes of individual resting spores from the two collections from cvs Agria and Estima were calculated assuming them to be spheres (Weisstein 2009a), and volumes of sporosori were calculated assuming them to be ellipsoids (spheroids) (Weisstein 2009b). Estimations of numbers of resting spores in sporosori were made by calculation of the proportion of total sporosorus volume divided by resting spore volume, and different arbitrary proportions (Table 1) of sporosorus volume occupied by resting spores.

Embedding and sectioning of sporosori (light microscopy)

Sporosori from cv. Agria (above) were suspended in 2% molten water agar, which was cut into 1 mm^3 blocks when solidified. These were fixed in 2.5% glutaraldehyde, post-fixed in 2% osmium tetroxide, passed through an acetone dehydration series, and then embedded in resin (Spurr 1969). Sequential sections (4 µm thick) of the embedded blocks were cut with a microtome and glass knife, and mounted in order of cutting on glass microscope slides. The sections were examined at × 1,000 magnification with a light microscope, and images of all of the sections from each of 30 sporosori were captured with a digital camera.

Estimation of numbers of resting spores in sectioned sporosori

Images of sporosorus sections were printed, and numbers of resting spores in each section were counted from these micrographs. Total number of resting spores in each sporosorus was determined as the sum of resting spores in all sections. Two dimensions of the largest (equatorial) section of each sporosorus were determined micrometrically, and the third dimension was determined as the product of the number of sections cut for each sporosorus and 4 µm (section thickness). Sporosorus volume was calculated using two methods: ***Method a***, assuming sporosori to be ellipsoids (Weisstein 2009b) each with the three dimensions, and ***Method b***, by summing the cylindrical volume (height = 4 µm) of all of the sections making up each sporosorus (Fig. 1).

Numbers of resting spores releasing zoospores

In a previous study (Merz 1997), sporosori of *Sss.* were exposed to roots of tomato plants (*Lycopersicon esculentum* Miller) for 5 or 8.5 h in a nutrient solution bioassay. Suspensions of sporosori were added to nutrient solution in which tomato plants were growing, and after the periods of exposure the sporosori were collected and

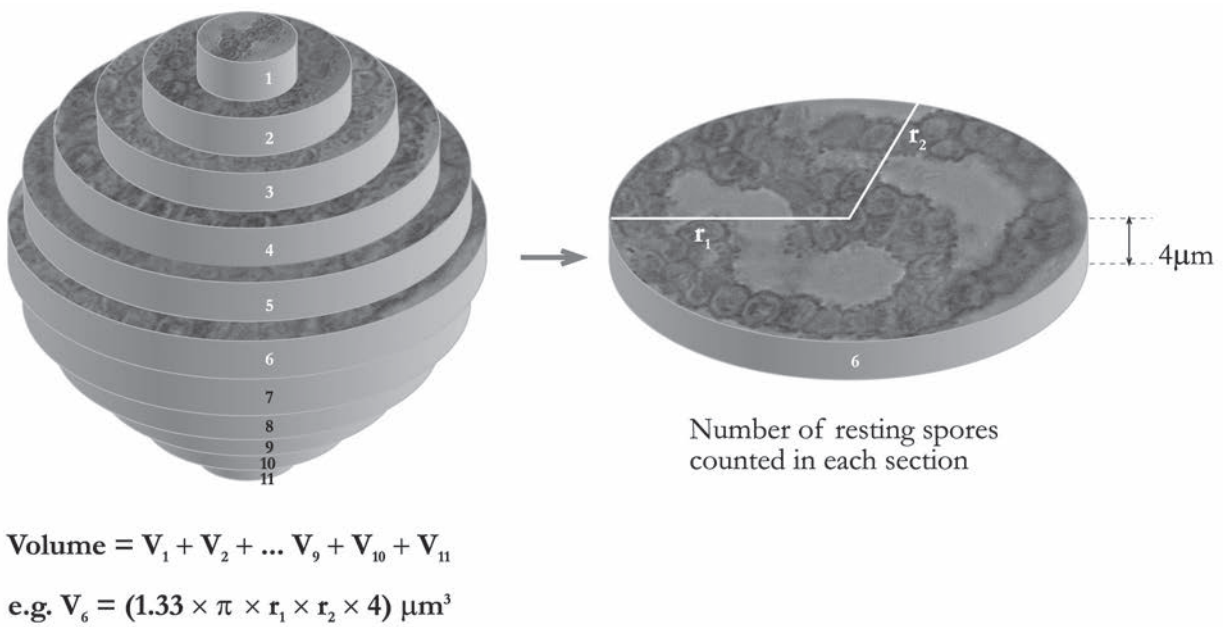

Fig. 1. Schematic indicating methods for calculation of *Spongospora subterranea* sporosorus volume, from summation of volumes of 4 µm thick sections of sporosori (left; see text, *Method b*), and counting resting spores in each section (right).

viewed with scanning electron microscopy. Electron micrographs from this study were reviewed to assess the numbers and proportions of resting spores in 25 sporosori that had released zoospores.

Numbers of resting spores in sporosori of different *Sp. subterranea* collections

The longest and shortest dimensions of 50 sporosori from each of 37 *Sss.* and two *Ssn.* collections were measured on micrographs (× 1,000 magnification) as described above, and the mean number of resting spores per sporosorus was calculated for each collection using the derived formula (***Equation 2***) outlined below. For comparison of collections, numbers of sporosori were analysed using a generalised linear model, with log link function and gamma error distribution. Least significant intervals (Snee 1981) were calculated for each collection on the log scale then back-transformed to the natural scale for presentation.

RESULTS

Sporosorus and resting spore form

Our detailed study of sporosori of *Sss.* has confirmed that these structures are complex aggregations of resting spores, which are "sponge-like" in overall form with holes on their outer surfaces leading to internal channels. Sporosori were usually spheroids, but were of variable form, from spheres to spheroids to more or less oblate or prolate spheroids (Weisstein 2009b). The complexity of internal channelling increased with increasing sporosorus size. In a detailed examination of one sectioned sporosorus, large internal spaces unoccupied by resting spores were obvious, and 47% of the sporosorus was occupied by resting spores. Variability in sporosorus form in one collection of sporosori is indicated by the examples in Fig. 2.

Fig. 3 illustrates general sporosorus form and surface ornamentation. Individual resting spores in sporosori ranged from circular to roughly hexagonal in cross section, and had punctate outer surfaces. They were arranged within sporosori in roughly double layers (Fig. 5). The punctate surface of each resting spore was always the exposed surface. Outer surfaces of resting spores observed with electron microscopy after exposure to tomato roots had open pores (see Fig. 7), where some of the resting spores had released zoospores. The resting spore arrangement within each sporosorus exposed each resting spore to open space, either on the external sporosorus surface or to an internal channel. All released zoospores would thus be given access to the environment external to the sporosorus.

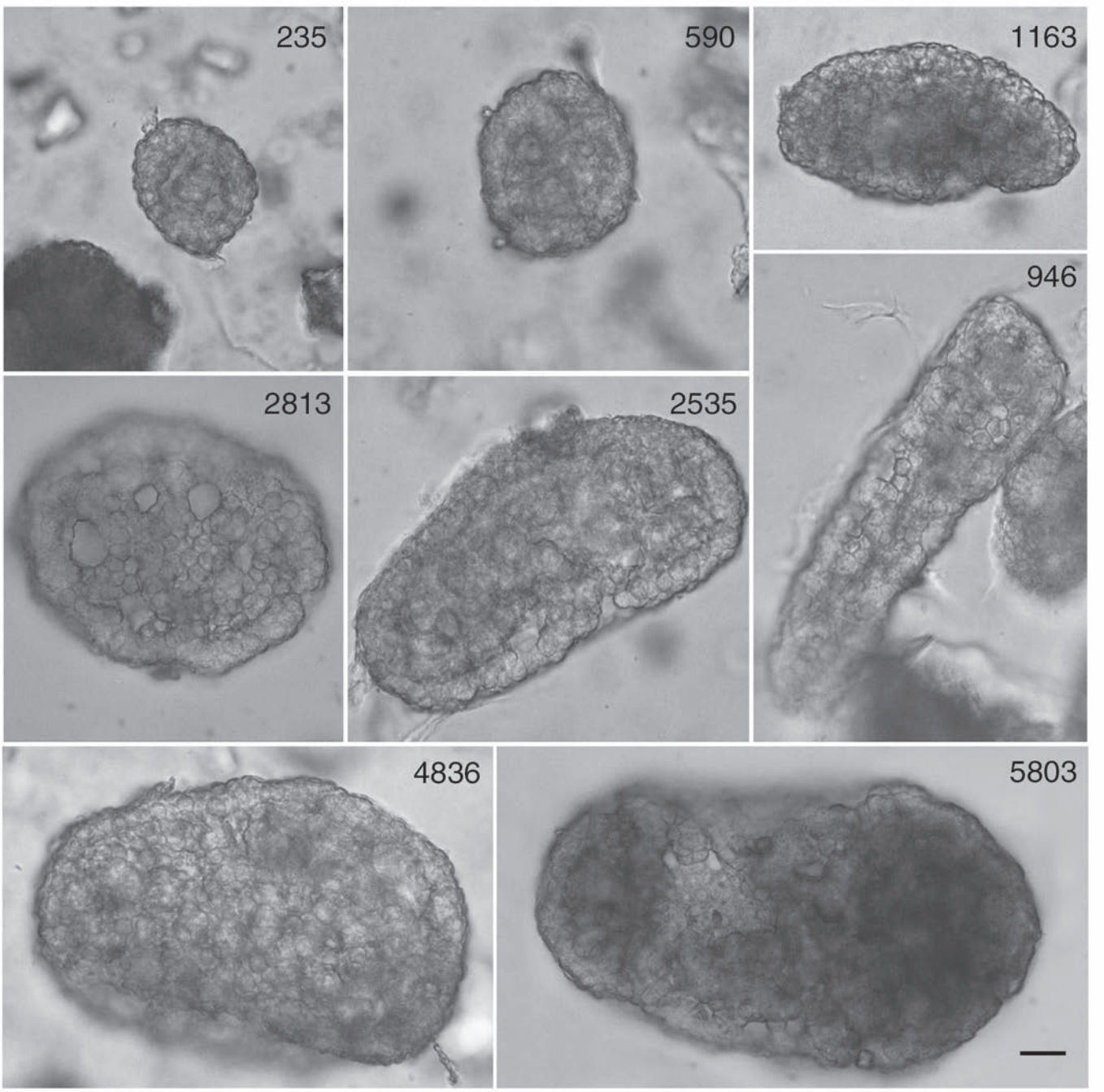

Fig. 2. Light micrographs of representative sporosori of *Spongospora subterranea* f. sp. *subterranea* from powdery scab lesions on potato tubers (cv. 'Agria', New Zealand 2004), indicating their range in size and overall form. Calculated numbers of resting spores (see text) for each sporosorus are indicated. Bar: 10 µm.

Sporosorus dimensions and volumes for two collections

Longest sporosorus diameters [(least) mean (greatest)] were: from cv. 'Agria', (20) 50 (100) µm, and from cv. 'Estima', (18) 51 (88). Statistical analysis of these data indicated that sporosori from the two collections had similar ($P = 0.73$) mean diameters. Calculated sporosorus volumes, assuming them to be oblate spheroids (Weisstein 2009b), were [(least) mean (greatest)]: from 'Agria', (2,667) 51,800 (235,300) µm^3; and from

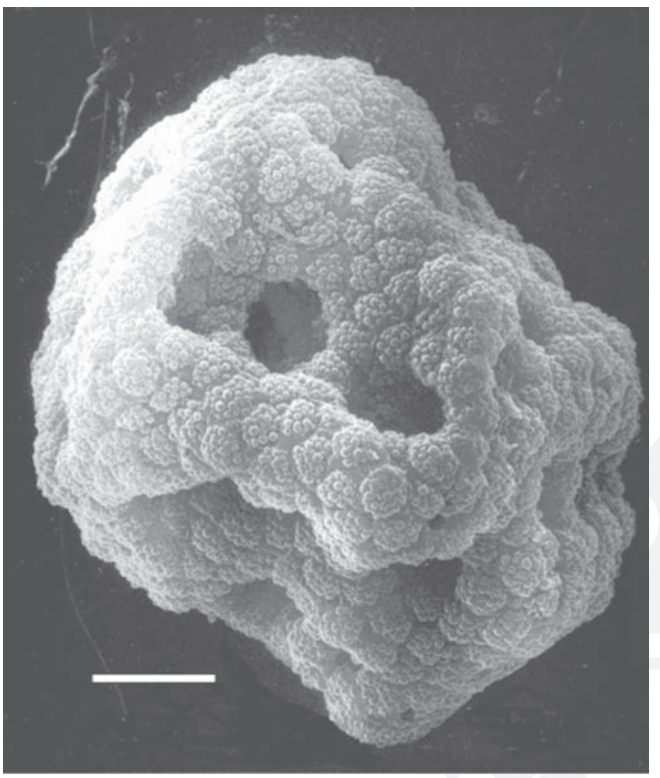

Fig. 3. Scanning electron micrograph of a *Spongospora subterranea* f. sp. *subterranea* sporosorus, showing individual resting spores each with punctate outer surface ornamentation. Bar: 10 µm.

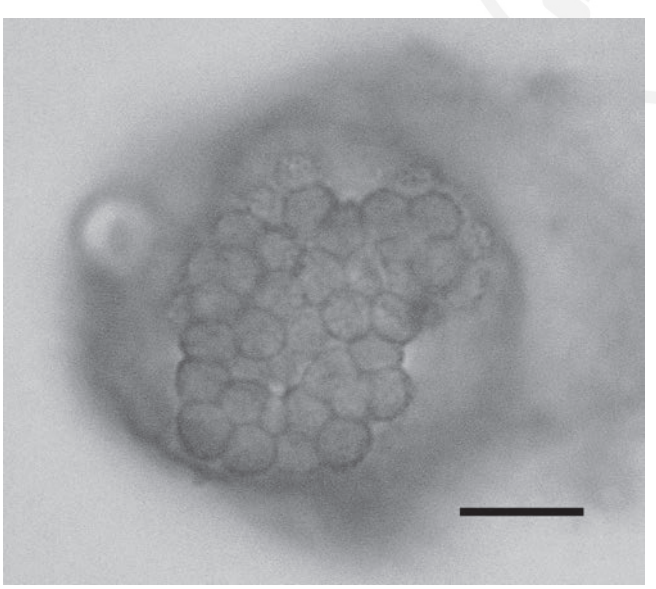

Fig. 4. Light micrograph of resting spores of *Spongospora subterranea* f. sp. *subterranea*. Bar: 10 µm.

'Estima', (3,311) 57,900 (224,500) µm^3. These represent an 88-fold volume range for 'Agria' and a 68-fold range for 'Estima'.

Resting spore dimensions and volumes for two collections

Fig. 4 illustrates resting spores of *Sss*. Mean diameters of resting spores from the two collections were: from cv. Agria, 4.0 µm, and from cv. Estima, 4.3 µm. Statistical analysis of data of resting spore diameters indicated that the means for each of the two collections were different (P = 0.03). Resting spores in each collection, however, were of very similar dimensions; the standard deviation of the resting spore diameters from different sporosori was 0.33 µm, and within sporosori, 0.25 µm. Mean calculated (spherical) resting spore volumes (Weisstein 2009a) for these two collections were 33.5 µm^3 from cv. 'Agria' and 42.8 µm^3 from cv. 'Estima', with the mean volumes for the two collections being different (P = 0.03).

Calculation of numbers of resting spores in sporosori

Table 1 presents the calculated numbers of resting spores in sporosori from the two collections, for different arbitrary proportions of sporosorus volume occupied by resting spores.

Numbers of resting spores and volumes for sectioned sporosori

Fig. 5 shows a section of a sporosorus which was included in the detailed determination of resting spore numbers. For the 30 sporosori which were examined using serial 4 µm thick sections, the mean number of resting spores per sporosorus was 667 (range 155 to

Table 1. Estimated numbers [(least) mean (greatest)] of resting spores in *Spongospora subterranea* f. sp. *subterranea* sporosori from two collections (from powdery scab lesions on potato tubers of cv. Agria from New Zealand and cv. Estima from Switzerland). Numbers are calculated from mean sporosorus and resting spore dimensions determined for the two collections, and for different arbitrary proportions of total sporosorus volume occupied by resting spores.

Proportion of sporosorus volume occupied	Collection	
	From cv. 'Agria'	From cv. 'Estima'
100%	(80) **1,548** (7,031)	(77) **1,354** (5,248)
75%	(60) **1,161** (5,573)	(58) **1,015** (3,936)
50%	(40) **774** (3,516)	(39) **677** (2,624)
25%	(20) **387** (1,756)	(19) **339** (1,312)

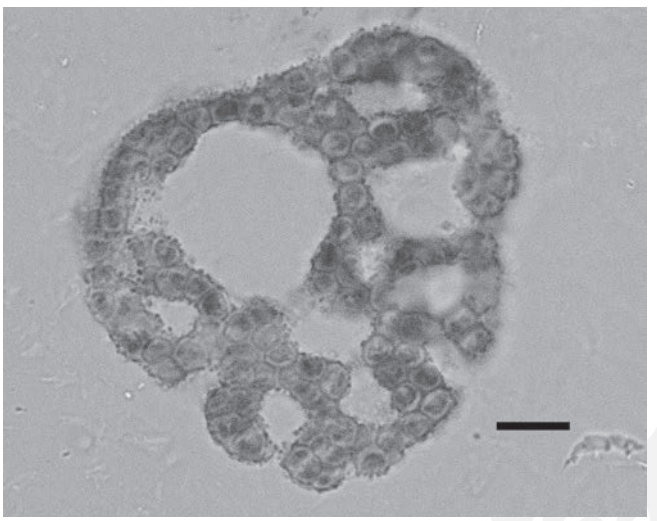

Fig. 5. Light micrograph of an equatorial section (4 µm thick) of a sporosorus of *Spongospora subterranea* f. sp. *subterranea*, in which 113 resting spores were counted. Bar: 10 µm.

1,526). Mean dimensions of these sporosori (with minima and maxima) were: length 64 µm (32; 101 µm); width 49 µm (29; 71 µm); depth 47 µm (24; 76 µm). Mean sporosorus volume (with minima and maxima), assuming they were spheroids (volume determination ***Method a***, above) was 82,050 µm^3 (11,660; 187,070 µm^3). The more precise method to determine sporosorus volume from serial sections (***Method b***, above) gave a mean volume of 77,060 µm^3 (13,960; 184,520 µm^3).

Fig. 6 illustrates the linear relationship between number of resting spores and sporosorus volume (determined using ***Method b***) for the 30 sporosori. From this we have calculated that the proportion of sporosorus volume occupied by resting spores (assuming resting spore volume = 33.5 µm^3) was 0.29 (s.e. = 0.022).

Estimation of numbers of resting spores in sectioned sporosori

Comparison of the two methods for determining resting spore volumes showed that numbers of resting spores in a sample of sporosori can be accurately esti-

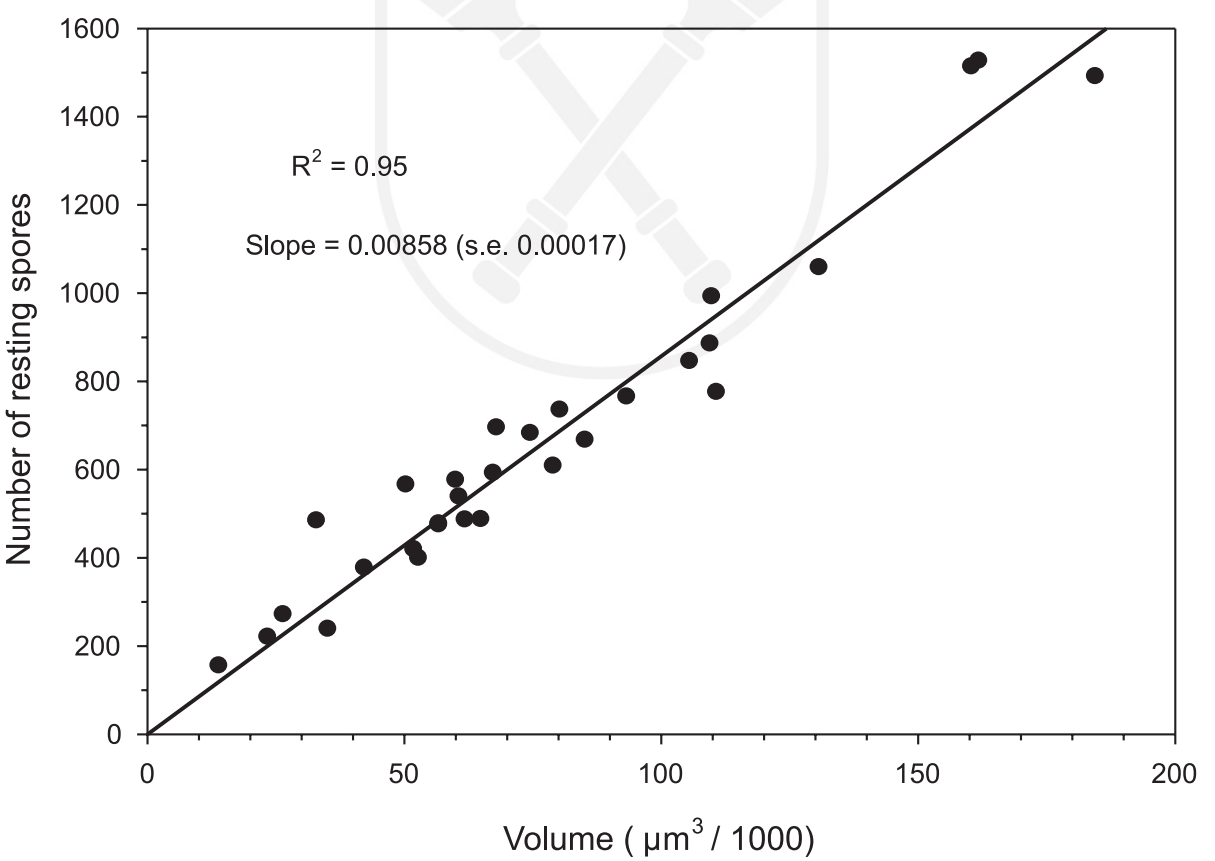

Fig. 6. Relationship between numbers of resting spores and sporosorus volumes (estimated from serial sections) for 30 *Spongospora subterranea* f. sp. *subterranea* sporosori.

mated assuming sporosori to be oblate spheroids (***Method (a)*** above) and applying a correction factor of 0.945 to the relationship illustrated in Fig. 6. Thus, numbers of resting spores for a particular collection of sporosori can be estimated by calculating mean sporosorus volume (***Equation 1*** below) and applying a multiplication factor from this linear relationship (***Equation 2***).

Equation 1: **sporosorus volume**
= 1.33 × π × r_1 × $(r_2)^2$
[where r_1 is the longest mean sporosorus radius and r_2 is the shortest mean sporosorus radius].

Equation 2: **numbers of resting spores per sporosorus**
= 0.0081 × sporosorus volume.

Numbers of zoospores released from sporosori

Fig. 7 shows a sporosorus recovered after exposure to tomato roots in a bioassay. Open pores in some of the resting spores indicate those from which zoospores have been released. From similar images, each of one sporosorus, it was determined that between 2 and 51% of resting spores in 25 sporosori had released zoospores. Mean proportions of resting spores with release pores were similar for two groups of sporosori which had been exposed to tomato roots for different periods; 18% for sporosori exposed for 5 h and 21% for those exposed for 8.5 h. The overall mean proportion of resting spores that had released zoospores was 20%.

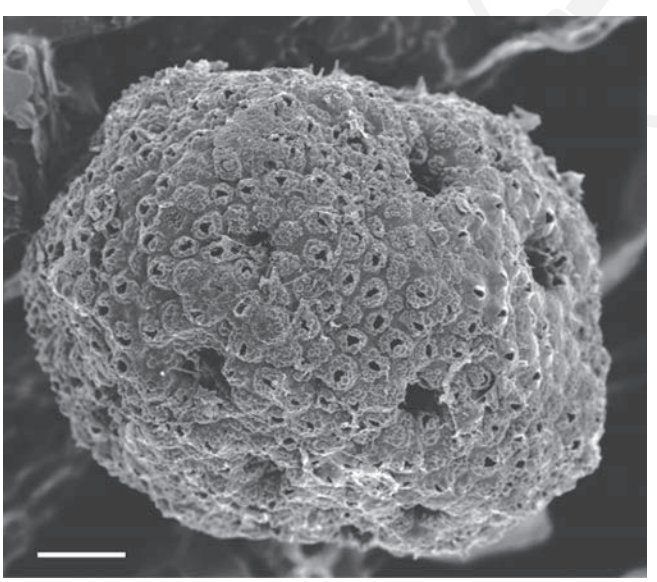

Fig. 7. Scanning electron micrograph of a *Spongospora subterranea* f. sp. *subterranea* sporosorus from which zoospores have been released from individual resting spores. Bar: 10 μm.

Numbers of resting spores in sporosori of different *Sp. subterranea* collections

Table 2 contains data of sporosorus dimensions and calculated numbers of resting spores in sporosori (***Equation 2***) from 37 *Sss.* collections from potato and two *Ssn.* collections from *N. officinale*. Sporosorus dimensions differed among the collections ($P < 0.05$). The calculated mean number of resting spores per sporosorus for *Sss*. also differed among the collections (deviance ratio = 17.5; d.f. = 36, 1,813; $P < 0.001$). The mean calculated number of resting spores in these collections ranged from 199 to 713. Sporosori from the two collections of *Ssn.* were smaller than those of *Sss.*, and the calculated mean number of resting spores per sporosorus for these two collections (97 and 166, respectively) were less ($P < 0.05$) than for the *Sss*. collections.

No patterns of numbers of resting spores per sporosorus relating to country of origin or potato cultivar were apparent from these data. The 16 collections from New Zealand and the five collections from Switzerland all spanned the full range of sporosorus size and numbers of resting spores. Similarly, the six collections from the cultivar 'Agria' spanned a broad range of numbers of resting spores per sporosorus.

Within individual *Sss.* sporosorus collections, calculated numbers of resting spores per sporosorus varied considerably (Table 2). The greatest variation within a collection was 63-fold (18 to 1,137) in a New Zealand collection from the potato cultivar 'Riverina Russet', and was commonly 10- to 20-fold in other collections. The smallest variation was 7-fold (74 to 531) in a New Zealand collection from cv. 'Rua'. The overall mean for the 37 *Sss*. collections was 412 resting spores per sporosorus.

DISCUSSION

This study followed a sequence of first calculating numbers of *Sss.* resting spores in sporosori from simple measurements of resting spore and sporosorus volumes, then determining numbers from detailed examination of sectioned sporosori. From this, a formula was derived which accurately estimated numbers of resting spores in sporosori of this organism.

The general equation we have derived for calculating numbers of resting spores in sporosori will allow inoculum enumeration to the level of potential zoospores, which are the infective units of this plant pathogen.

Table 2. Mean longest (r_1) and shortest (r_2) radii of sporosori of *Spongospora subterranea* f. sp. *subterranea* and *Sp. subterranea* f. sp. *nasturtii* collected from different countries. Dimensions were measured for 50 sporosori from each collection. Calculated mean sporosorus volumes (see text, ***Equation 1***) and numbers of resting spores per sporosorus (***Equation 2***) are also presented.

Country	Host cultivar	r_1 (μm)	r_2 (μm)	Volume (μm^3)	Calculated numbers of resting spores				
					smallest	largest	mean	LSI† (lower)	LSI† (upper)
***Spongospora subterranea* f. sp. *subterranea* (from *Solanum tuberosum*)**									
New Zealand	'Agria'	22.6	15.5	24 563	38	521	**199**	176	225
Sri Lanka	Unknown	20.4	16.7	25 194	57	480	**204**	180	231
Netherlands	Unknown	20.1	17.0	26 910	64	721	**218**	193	247
Switzerland	'Agria'	22.5	16.9	29 775	46	738	**241**	213	273
South Africa	'Mondial'	23.2	17.0	31 599	41	786	**256**	226	290
Korea	'Superior'	22.3	17.6	31 721	47	647	**257**	227	291
Japan	'Irish Cobbler'	22.7	17.6	32 240	56	772	**261**	231	296
New Zealand	'Rua'	25.8	17.3	33 847	74	531	**274**	242	310
Netherlands	Unknown	23.1	17.8	34 224	77	778	**277**	245	314
Korea	'Superior'	23.9	17.7	34 388	76	731	**279**	246	315
Iceland	'Red Icelandic'	23.5	18.0	34 759	41	652	**282**	249	319
New Zealand	Unknown	21.6	18.6	35 283	55	1 453	**286**	253	323
United States	'Beacon Chipper'	23.8	18.2	36 473	43	840	**295**	261	334
New Zealand	'Agria'	24.0	18.1	37 118	73	914	**301**	266	340
New Zealand	'Rua'	22.9	18.9	38 100	73	947	**309**	273	349
Colombia	'Criolla'	25.1	19.0	43 849	71	1 396	**355**	314	402
Switzerland	'Agria'	25.5	18.6	43 888	34	1 935	**355**	314	402
New Zealand	'Riverina Russet'	26.9	18.5	45 083	18	1 137	**365**	323	413
New Zealand	'Markies'	24.8	19.4	46 077	20	1 226	**373**	330	422
United States	"ND 1496-1"	26.0	22.0	56 966	134	1 060	**461**	408	522
Colombia	Unknown	26.3	21.2	58 010	91	1 933	**470**	415	532
New Zealand	'Nadine'	26.2	20.9	58 178	41	1 778	**471**	417	533
New Zealand	'Agria'	28.0	21.3	58 243	106	1 542	**472**	417	534
Scotland	'Nadine'	29.0	21.1	58 957	67	1 298	**478**	422	540
New Zealand	'Sinora'	26.9	21.6	59 454	54	1 256	**482**	426	545
New Zealand	'Innovator'	27.7	20.5	60 947	33	1 844	**494**	436	559
New Zealand	'Ilam Hardy'	27.1	21.7	61 666	45	1 389	**500**	442	565
New Zealand	'Russet Burbank'	29.3	20.8	61 905	57	2 757	**501**	443	576
Switzerland	'Bintje'	27.9	22.3	63 055	141	1 455	**511**	451	578
New Zealand	'Innovator'	28.4	22.1	64 153	97	1 180	**520**	459	588
Switzerland	'Agria'	28.6	23.0	66 286	166	1 464	**537**	474	608
New Zealand	'Rua'	28.4	22.6	72 160	87	2 709	**585**	517	661
New Zealand	'Nadine'	30.8	23.5	78 929	91	1 642	**639**	565	723
Japan	"KNP"	30.5	24.2	80 449	231	1 853	**652**	576	737
Switzerland	'Bintje'	29.7	24.9	85 195	162	2 714	**690**	610	781
Peru	Unknown	31.1	24.7	85 534	230	2 348	**693**	612	784
Sweden	"Cultivar 162"	30.6	24.5	87 991	99	2 116	**713**	630	806
***Spongospora subterranea* f. sp. *nasturtii* (from *Nasturtium officinale*)**									
Switzerland		15.4	13.0	11 991	13	195	**97**	86	110
Switzerland		18.6	15.6	20503	46	326	**166**	147	188

† Least significant intervals ($P = 0.05$).

This precision has not been attempted previously, as inoculum standardisation has been achieved at the level of sporosori.

Sporosorus size in *Sss.* is highly variable, and numbers of resting spores are closely related to sporosorus volume. Estimation of numbers of resting spores in sporosorus inoculum can be achieved by measuring the dimensions of an appropriate sample of sporosori. Using the conversion factor determined in the present study, an accurate estimation of the numbers of potential zoospores in the inoculum can be obtained. Our study has shown that different collections of the pathogen are likely to have different sporosorus dimensions, so we recommend that measurements are made of sporosori, and resting spore numbers are calculated, for each collection. If less precise estimates of resting spore numbers are appropriate, the overall mean number of resting spores per sporosorus we obtained (412) could be used to estimate resting spore numbers. In any case, these approaches to inoculum quantification will give a more precise estimation of inoculum potential than quantification of sporosori.

We have confirmed previous reports that have outlined the general form of *Sss.* sporosori (*e.g.*, Osborn 1911, Jones 1978, Lahert and Kavanagh 1985). Sporosori of this organism are the most complex of the plasmodiophorids, and are probably the most advanced along an evolutionary path towards complexity. Each *Sss.* sporosorus contains many resting spores, and large numbers of sporosori are produced in each *Sss.* lesion (root gall or tuber scab) on host plants.

The evolutionary advantages of complex sporosori in the plasmodiophorids are difficult to identify. Sporosori, particularly the relatively large ones of *Spongospora*, *Woronina* and *Ligniera*, probably assist dispersal in wind, soil or water, and on host propagation material such as seed potato tubers. They may also resist degradation in soil or water environments, or help prevent desiccation in dry conditions. This could be especially the case for the internal resting spores within complex sporosori, which are protected by the outer sporosorus layers. However, all of the plasmodiophorids are successful pathogens, of plants, algae, straminipiles or fungi. The most economically important are *Plasmodiophora* which causes clubroot of *Brassica* spp., *Spongospora* which causes powdery scab of potato and crook root of watercress and vectors viruses of these hosts, and *Polymyxa*, which vectors viruses of sugar beet (causing rhizomania) and gramineaceous crops. These economically important pathogens of food and forage crop plants have sporosori that span the full spectrum of complexity of resting spore aggregation. Thus, sporosorus complexity may not be the only factor responsible for pathogenic success. The ability to produce resting spores in numbers great enough to survive between host generations, recognise susceptible hosts and release zoospores that initiate infection are characteristics more likely to determine pathogenic success, and these are features intrinsic in resting spores, rather than sporosori.

The question remains as to whether evolution in plasmodiophorids could be towards sporosorus complexity. Sporosori develop within single host cells, from sporogenic plasmodia. These plasmodia are multinucleate, which develop into uninucleate resting spores. The considerable variation in sporosorus size within individual plasmodiophorid species which produce large, complex sporosori is probably a reflection of the variable size (volume) of the respective host cells in which they develop. Sporosorus differences between the different plasmodiophorid species are likely to be affected by the differences between hosts, for which cell size (at least) is likely to be different. For example species of *Worononia* are pathogens of straminipilous fungi, while *Spongospora* and *Polymyxa* parasitise higher plants. Plasmodiophorids also generally cause host hypertrophy to greater or lesser extents, which affects host cell size and also (presumably) sporosorus complexity of the pathogens. Thus, host factors are likely to considerably influence sporosorus complexity within the plasmodiophorids.

Our examination of different collections of *Sss.* from potato showed that resting spore size differed only slightly between two collections of the pathogen, but sporosorus size differed greatly between different collections of the pathogen from particular locations (countries) and potato cultivars. These results strongly suggest that environmental (rather than host) factors affect sporosorus size within *Sss*.

Determination of resting spore viability cannot be achieved by currently available methods. We have determined in a limited study (again using morphology) that low proportions (an average of 20%, but ranging from 2 to 51%) of resting spores released zoospores over a period of a few hours. This may indicate either a mechanism by which the pathogen maintains inoculum potential over long periods by not releasing all potential infection units at any one time, or that not all of the resting spores in each sporosorus were viable. Detailed study of zoospore release is required to accurately determine resting

spore viability or if some mechanism delays zoospore release to maintain inoculum. In either case, zoospore release is likely to be affected by environmental, host and/or pathogen factors. Bioassays and electron microscopy, as described by Merz (1989, 1997), could be further used to determine proportions of released zoospores. This approach could also indicate factors affecting the ability of the pathogen to release zoospores and infect host tissue, and may reveal important information on the epidemiology of powdery scab of potato.

Acknowledgements. This research was funded by the New Zealand Foundation for Research, Science and Technology and a New Zealand Institute for Crop & Food Research "Eureka" award. Mr Robert Lamberts gave helpful advice on image analysis. Sporosorus samples examined in this study were provided by Georg Babu, Celsa Garcia, Hans-Reinhard Hofferbert, Kerry Hughes, Joem Soon Kim, Alison Lees, Krishna Mohan, Takato Nakayama, Xinshu Qu, Maria Sandgren, Halldór Sverisson, John Stanley, Herbert Torres and Jessica Wright.

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Received on11th November, 2010; revised on 11th February, 2011; accepted on 13th February, 2011