12. STORAGE, MAINTENANCE, AND VIABILITY OF MAIZE DOWNY MILDEW FUNGI

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The downy mildew fungi (Peronosporaceae, Oomycetes) are singularly notable in their obligate parasitism on both narrow- and broad-leaved vascular plants. On maize (*Zea mays* L.) at least six apparently distinct species as follows incite diseases, depredations due to which are of great magnitude particularly in the Oriental tropics: 1) *Sclerophthora macrospora* (Sacc.) Thirum., Shaw & Naras., 2) *Sclerophthora rayssiae* Kenneth var. *zeae* Payak & Renfro, 3) *Sclerospora sorghi* Weston & Uppal, 4) *Sclerospora sacchari* Miyake, 5) *Sclerospora maydis* (Racib.) Butler, and 6) *Sclerospora philippinensis* Weston. Of potential but at present of no grave concern on maize are *Sclerospora graminicola* (Sacc.) Schroet., *Sclerospora miscanthi* Miyake and *Sclerospora spontanea* Weston.

My task in this Symposium is to collate and review information relating to storage, maintenance and viability of the aforementioned peronosporaceous fungi attacking maize. Having been involved in this pursuit only in recent years, I must admit unabashedly that we have with us right now authorities, among others, Drs. R. Kenneth, M. M. Payak, R. A. Frederiksen and K. M. Safeeulla who are better versed on the subject than I and, to my mind, could make a more profitable presentation. Nonetheless I shall attempt to measure up to the task on hand if only to congratulate the sponsors and organizers of this Symposium, and also to express my appreciation to IACP which

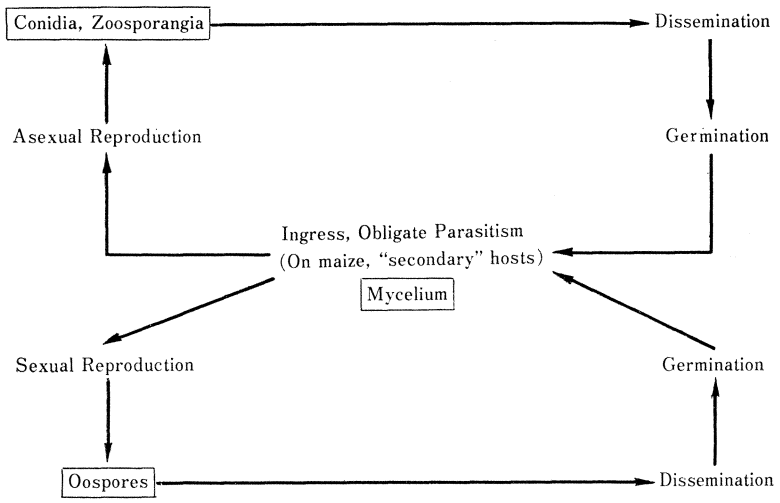

Fig. 1. Simplified life cycle of downy mildew fungi on maize. Propagules available for storage and inoculation are boxed

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made possible my participation.

Accelerated progress in the maintenance and storage of maize downy mildew fungi has largely come about only in the last 5–7 years following the initial success scored in disease control with chemicals and resistant varieties. Impetus has stemmed mainly from the pressing need for more definitive information on 1) processes involved in infection and disease development, 2) disease epidemiology, 3) mechanisms of host resistance to the disease, and 4) occurrence and role of biotypes or races of the pathogen.

One maybe apt to dismiss the subject as too basic, of no economic importance. But we can not afford to be complacent. The immediate need is not so much to know what is academic as it is to heed the call for practical and reliable inoculation techniques suited to the scale necessary in the search for resistant genotypes and in the regional testing of downy mildew resistant maize varieties (DMRs) derived therefrom.

Let us consider first the general life cycle of downy mildew fungi, centered as it is on maize, the "primary" host (Fig. 1). It is evident that only four types of fungal propagules as follows are available for either storage or maintenance, and thus for inoculation: 1) mycelium, 2) zoosporangia (*Sclerospora graminicola, Sclerophthora* spp.), 3) conidia (other *Sclerospora* spp.), and 4) oospores. Chlamydospores or gemmae are thus far not known.

Storage and Viability of Propagules

Mycelium. The thallus or mycelium is inconspicuous. It is generally distributed throughout the host tissues, its main axes often running parallel with the vascular bundles and, elsewhere, intercellular in the mesophyll and fundamental tissues of leaves, leaf sheaths, stem, ear shanks and cobs, tassels, glumes, and husks (Weston, 1920; Whitehead, 1958; Dalmacio & Exconde, 1969). Brace roots may also be invaded as in *S. macrospora* on maize (Ullstrup, 1970). In *S. rayssiae* var. *zeae* mycelia are found only in the leaves and leaf sheaths; none occurs in stalks, seeds and root system (J. P. Singh, *in* Payak, Renfro & Lal, 1970). Underground foots are probably rarely invaded, if at all, when initial infections are with air-borne propagules (conidia or sporangia). Germinated oospores in the soil, however, are expected to initiate direct peneration of roots as in *S. sorghi* (Anon., 1972).

It is doubtful if the mycelium *per se* plays a significant role in disease spread, except in host plants, "primary" or otherwise (e.g., *Saccharum* spp.), normally propagated vegetatively (Miyake, 1911; Hughes & Robinson, 1961; Chu, 1948). In the over-all epidemiology, however, mycelia sustained in so-called secondary or alternate hosts—mostly weeds—can not be overlooked.

The hyphal filaments are thin-walled, unpigmented, generally unisodiametric, easily plasmolyzed and unable to withstand long exposures to light and other desiccating agencies. Just how long the mycelium remain viable in the host tissues is not known. Since the fungi involved are obligately parasitic, the thallus most likely remains alive as long as nourishment is furnished by the host. Hot water treatment for 1 hr at 52°C is sufficient to kill the mycelium of *S. sacchari* in sugarcane seed pieces (Chu, 1948), while that of *S. sorghi* in Johnson grass (*Saccharum spontaneum* L.) is unable to withstand winter temperatures (Frederiksen, *in* Raychaudhuri, 1970b).

Chlamydospores have been reported in *S. maydis*, but these proved to be nothing more but protozoan cysts (Palm, *in* Semangoen, 1970). Indeed, it is fortunate that such hardy structures are not formed, for their presence will further complicate disease control considering, for example, that condemned diseased plants are now channeled to the livestock industry (Chang, 1972). These might end up in the manure, as do oospores, without loss of viability.

The presence of mycelium in seeds has been reported in nearly all downy mildew

fungi capable of infecting maize—in *S. maydis* (Purakusumah, 1965; Semangoen, 1970), *S. sacchari* on maize (Leece, 1941; Chang & Twu, 1965), *S. philippinensis* (Weston, 1920; B. A. Advincula, comm.), *S. sorghi* on sorghum (Bain & Alford, 1969) and maize (Jones, Leeper & Frederiksen, 1972), *S. graminicola* on *Eleucine coracana* ("ragi") (Anon., 1972), *S. rayssiae* var. *zeae* (Singh, Joshi & Chaube, 1968), and *S. macrospora* (Ullstrup, 1952; Ullstrup & Sun, 1969). Fortunately the disease is transmitted only when fresh seeds from diseased plants are used for planting. Drying to less than 20% moisture content (Leece, 1941; Chang & Twu, 1965) or prolonged storage at 1°C (Ullstrup & Sun, 1969) reduces infection from 50.6% (Semangoen, 1970) to nil (less than 1%) or, more commonly, zero infection. As Ullstrup (1970) points out, seed transmission becomes even less of a threat since diseased ears are malformed, hardly filled and thus, unlikely to be selected for commercial planting. I may add that ordinary growers in Southeast Asia also practice seed selection.

Zoosporangia. The morphological distinction between zoosporangia and conidia has already been underscored by Shaw (1970). The former are provided with a differentiated polar structure, the discharge papilla, dissolution of which effects zoospore discharge. I regard zoosporangia of downy mildew fungi to be inoperculate. As pointed out earlier (Dogma, 1972), operculation, in the sense it is used in the Ascomycetes and Chytridiomycetes can not be applied appropriately to zoosporangia of downy mildew fungi. No lid or cap, the operculum, is dehisced from the zoosporangium during or immediate y preceding zoospore liberation.

Sclerophthora rayssiae var. *zeae* appears to be the only zoosporangial downy mildew fungus of major economic importance on maize. Payak and Renfro (1967) described the zoosporangia as "large *Phytophthora*-like . . . sympodially produced on determinate (sic) sporangiophores . . ." Other pertinent information are given in the studies of Singh, Renfro and Payak (1970). Maximum initial crop of sporangia is produced within 3-3½ hr at temperatures optimum for sporulation (22°–25°C) on excised leaves in a moist chamber. The second peak of sporulation is reached in 9-9½ hr after removal of the first. Germination is highest in sporangia collected in the field from 12 noon to 4 pm, indicating that sporulation occurs during light periods of the day. However, when induction of sporulation on excised leaves is staggered at 2-hr intervals for 24 hr, maximum sporulation is always obtained within 3½ hr regardless of time and temperature. Moisture level or relative humidity, more than light, thus appears to influence sporulation. With rain water, host leaf extract, stream water, sterile tap water, well water, distilled tap water, and tap water, percentage germination decreases in that order from 78.5 to 58.5. This is to be expected since growth of zoosporic fungi is generally hindered in the presence of metal ions. R. S. Singh, *et al.* (1970) have shown that germination of sporangia of *S. rayssiae* var. *zeae* is reduced to 22% with Mn, Zn, and Fe at 100 ppm. Completely toxic at as low as 20 ppm are Cu, Bo and Mo.

J. P. Singh, et al (1970) also give interesting though as yet difficult to explain data of value for storage purposes. Percentage germination of sporangia tends to increase with time, the maximum (65–68%) obtained after 30 hr at 20°–22°C or a few degrees lower than for maximum sporulation. At 18° and 30°C germination occurs only after a lapse of about 24 and 20 hr, respectively. There is no appreciable difference in percentage germination after 30 hr at these temperature extremes. Pregermination chilling at 5° and 10°C for 30–120 hr also increases germination to about 21% after 120 hr at either 18° or 30°C. Chilling, however, has no statistically significant effect on germination at optimum temperature (20°–22°C).

Sporangia of *S. rayssiae* var. *zeae* on infected leaves wrapped in moistened paper are viable after 10 hr (J. P. Singh, *in* Raychaudhuri, 1970b). These release 4–8 zoospores at germination (Payak & Renfro, 1967). Sporangia scraped from naturally diseased

leaves may yield as much as 800,000 zoospores/ml after 24–30 hr at 20°–22°C. Temperature also influences zoospore motility. These biflagellate, bean-shaped swarm cells are most active at 22°–25°C.

Sclerophthora macrospora also attacks maize causing "crazy top", but this is considered of minor importance. The fungus, unlike *S. rayssiae* var. *zeae,* sporulates very sparingly in the field on maize, barley, or wheat (Ullstrup, 1952) and only fairly abundantly on other "weed" hosts of some 45 species (Semeniuk & Mankin, 1964, 1966). Not even exposures of young diseased maize plants in a fog chamber for 48 hr had induced sporulation. The sporangia of the fungus were in fact discovered 40 years after the description of the sexual stage by Saccardo in 1890. Ullstrup (1970) later found that sporulation can be induced rather simply. Pieces of diseased maize leaves floated or immersed partially in water form as many as 2,200 sporangia/cm^2 over a 6-day period at 28°C. Sporulation is fairly abundant between 12° and 28°C and sparse at 8°C. At 4° or 32°C, no sporulation is obtained. Each sporangium germinates to release as many as 117 zoospores. This is a staggering number—compared to 4–8 in *R. rayssiae* var. *zeae,* and 3–7 in *S. graminicola* (King & Webster, 1970)—compensating for the very infrequent sporulation obtaining in the field. Presence of infective zoospores in drainage ditches has been demonstrated by simply soaking wheat and barley seedlings (Semeniuk & Mankin, 1964). Direct germination of sporangia, i.e., by germ tube, does not occur over a temperature range of from 4° to 32°C.

Sclerospora graminicola, the type species, is sort of an anomaly, at least non-taxonomically. It is the only zoosporic member of the genus (Shaw, 1970). Its sporangia germinate within 1/2 hr at room temperature. At 5°C germination can be delayed for at least 24 hr (Safeeulla, *in* Raychaudhuri, 1970a). As stated above, only 3–7 zoospores are delimited in each sporangium.

Conidia. By far the most devastating downy mildews on maize are those caused by conidial Sclerosporas, viz., *S. sacchari* in Taiwan, *S. philippinensis* in the Philippines and North Celebes (Minahasa), Indonesia, *S. sorghi* in Thailand, India and Mexico, and *S. maydis* in Java, Indonesia. *Sclerospora sorghi* is equally virulent on sorghum in India, the USA, Mexico, East and West Africa, and Israel.

Observations by several competent investigators have firmly established that sporulation in conidial Sclerosporas is nocturnal, occurring generally from 12 midnight to shortly before dawn. Weston (1920, 1923) clearly recognized the intricate relationship between temperature and relative humidity necessary for the sporulation process in the evening. This has since been corroborated abundantly (Leece, 1941; Safeeulla and Thirumalachar, 1955; Tarr, 1962; Yang, Cheng & Matsumoto, 1962; Matsumoto & Yang, 1964; Exconde, Edralin & Advincula, 1967; Chang & Wu, 1969; Sun, 1970; Kenneth, 1970). Confirmation from field experiments has of late been provided in *S. philippinensis* (Dalmacio & Raymundo, 1972). At 90% relative humidity (RH) and above sporulation invariably takes place independent of temperature; however, temperatures lower than 80°F are necessary for sporulation at 80–89% RH. The presence of dew or rain water on the leaves has also been shown to be essential for sporulation (Exconde, et al., 1967; Sun, 1970; Semangoen, 1970). Dalmacio and Raymundo's study conducted over two planting seasons in 1970 and 1971 affirms the necessity of this microenvironment in the field over a temperature range of 71°–79°F and a RH range of 74–96.5%.

Since the requisite RH-temperature balance is in nature obtained during the night, darkness has likewise been intimated in relation to sporulation (Exconde, et al., 1967; Matsumoto & Yang, 1964). Yet plants kept in the dark after removal of the previous spore crop do not sporulate in total darkness even at optimum RH and temperature or in the presence of moisture on the leaves (Weston, 1923). The explanation, an unexpected one, came very recently from the independent investigations by Barredo (1972)

Table 1. Light requirement in sporulation, *Sclerospora philippinensis* (Modified from Barredo, 1972)

Time leaf excission	Pre-excission exposure to sunlight (hr)[1)]	Conidia/cm² (Ave., 5 reps)
6 AM	0	0
8	1.5	5,555
10	3.5	27,777
11	4.5	31,111
12 Noon	5.5	30,000

1) Supplied by author. Sunrise between 6 : 15 and 6 : 30; 6 : 30 taken as base

Summary Procedure:

Diseased leaves of PH 801 excised at times indicated, cut to 5–10 cm long pieces, washed, dispensed in culture dishes lined with moist paper at bottom, and exposed for 10 hr to dark conditions in growth chamber (23.3°C and 97% RH)

and Chang (1972). As it turned out, light is also important (Table 1). Whereas darkness is essential during *actual* sporulation, *presporulation* exposure to light is a prerequisite to it. A diseased plant kept in the dark during the day will not sporulate in the evening even if it sporulated vigorously the previous night. Table 1 indicates that exposure to light for at least 1 hr is necessary for sporulation in the evening. The amount of sporulation on excised maize and sugarcane leaves increases with increasing time of pre-sporulation exposure to light and also with increasing light intensity from 200 to 800 lux (Chang, 1972).

Just how this diurnal periodicity of sporulation is tied to the cyclic opening and closing of the stomatal pores during the day and night, or to the respiration of the plant is a most interesting facet worth exploring in the near future.

Optimum temperature for sporulation is 22°–25°C in *S. sacchari* (Chang, & Wu, 1969) and 21.6°–25°C in *S. philippinensis* (Barredo, 1972). In *S. sorghi* Kenneth (1970, p. 375) states that sporulation in the field on sorghum occurred when "temperatures never dropped below 23°C", but Safeeulla and Thirumalachar (1955) give the maximum at only 20°C. Conidia are developed and dehisced in 8–9 (–10) hr at 23.3°–24°C (Sun, 1970; Barredo, 1972; Visarathanonth, 1973). Presumably this includes the period for conidiophore formation since it is some 4–6 hr longer than the usually given "midnight to 4 AM" period. Weston (1920, p. 108) stated that "the process of condiophore emergence and conidia production begins about midnight and may continue a few hours after dawn..." In *S. sacchari* conidial formation in the field begins at 1–2 am and ends at 3–4:30 am (Chang, 1972). This agrees with our latest observations on this species on sugarcane and *S. philippinensis* on maize.

The relationships between RH-temperature combinations and sporulation, symptom expression, and disease incubation under controlled conditions in growth chambers have been worked out by Barredo (1972). These are summarized here in Table 2.

Conidial germination is always direct, i.e., by germ tube. This occurs in *S. philippinensis* at 6.5°–25°C (Weston, 1920), the optimum at 19°–24°C (Exconde, et al., 1967) or 20°C (Visarathanonth, 1973). From our recent and as yet unpublished studies, germination takes place over a wide range of pH, from 4–10. As low as 5–7% germination is obtained at pH 4, 76% at 5.5–6.0, and as much as 98% at 6.5 to 7.5. Conidia of *S. sacchari* germinate at 8°–32°C (Leu & Tan, 1970), the optimum at 19°–28°C (Chang & Wu, 1969). Maximum germination is obtained at 25°C (Yang et al., 1962) or 30°C (Visarathanonth, 1973).

Table 2. Maize downy mildew, *Sclerospora philippinensis*: Symptom expression and sporulation in growth chamber (Modified from Barredo, 1972).

°C-% RH		Conidia/cm²	Symptom Expressions			
Night	Day		Local		Systemic	
			% Infection	Incubation (days)	% Infection	Incubation (days)
20.0-91	26.6-69	16,666	98.5	2	98.5	9.5
21.6-94	28.3-72	32,222	98.5	2	92.5	8.0
23.3-97	30.0-75	33,333	98.5	2	89.5	7.5
25.0-98	31.6-78	30,000	88.5	4	88.5	8.5
26.6-98	32.0-81	4,000	97.5	3.5	93.5	8.5

Summary Procedure:

Inoculum—50,000 conidia/ml, prepared from leaf pieces of PH 801 induced to sporulate for 8 hrs. at 23.3°C-97% RH.

Plants—3-day old after emergence, 73-80 plants PH 801/night-day temperature-RH combination.

Inoculation—spray, 5 psi pressure; inoculum with 80 ppm Tween 80.

Incubation—12 days maximum, bagged with plastic first 4 days.

Sporulation—plants bagged for 8 hrs. after 10 days incubation to induce sporulation; spores counted with haemacytometer.

Conidia of Sclerosporas are difficult to store. These are colorless, the wall very thin and also colorless. Once mature, germination takes place in 1–2 hr under conditions obtaining during their formation. There is sufficient evidence to indicate that dispersal, inoculation, germination, ingress and initial infection all take place within the same period—midnight to shortly after dawn—associated with conidiophore emergence and actual spore formation (Weston, 1923; Semangoen, 1970; Sun, 1970; Dalmacio & Raymundo, 1972; Barredo, 1972). Extremely sensitive to desiccation, conidia, even germinated ones on the leaves in the field quickly shrivel and die within 1–2 (–4) hr of exposure to the morning sun (Weston, 1920, 1923; Leece, 1941). It is for this reason that aerial dissemination of infective conidia is limited to 1/4 or 1/2 of a mile (Hughes & Robinson, 1961) or to only 200 ft (Safeeulla, *in* Raychaudhuri, 1970a), not withstanding the drying effect of wind velocity and other meteorological factors (Exconde, Adversario & Advincula, 1968; Doggett, 1970).

In *S. sorghi* conidia germinate even while still perched at the sterigmatal apices (Miller et al., 1968; Kenneth, 1970). The same germination *in situ* has been observed in *S. miscanthi* on *Miscanthus japonicus* (Visarathanonth, 1973). The evidence on hand indicates that this peculiarity represents an attempt at repeated spore formation. This lessens the chances of natural infection of maize with *S. miscanthi,* a real threat revealed in cross inoculations (Exconde, 1970).

Conidia of *Sclerospora maydis* appear to be hardier than those of either *S. philippinensis, S. sorghi,* or *S. sacchari.* These remain viable and infective after 9 hr on glass slides in "saturated air" (Semangoen, 1970). Even more alarming is the finding that conidia held for 24 hr on maize leaves in saturated atmosphere are still infective when placed with water into the whorls of young seedlings. Moreover, germination is enhanced further by guttation water (Semangoen & Soemadi, 1971).

In *S. sacchari* spore viability at 25°C is lost completely in 1 hr at 95% RH, but not so in 3 hr at 100% RH (Yang, et al., 1962) although germination is reduced to 62% (Chang & Wu, 1969). Copper, Bo and Mo are completely toxic at as low as 20 ppm, but Mn, Zn and Fe reduce germination to only 27–60% at as high as 100 ppm (R. S. Singh et al., 1970). With *S. sorghi* viability is lost in 3–4 hr (Tarr, 1962), but can be

maintained for up to 6 hr at temperatures below 30°C. Spores of the previous night fail to germinate when kept at 20°C in an incubator the following day. On 5% carboxyl-methyl-cellulose, spores are still viable, but not those on glycerol (Kenneth, 1970).

With *S. philippinensis* we have attempted in various ways to maintain the viability of conidia (Exconde, Dogma & Elazegui, 1972). Germination of newly dehisced conidia occurs within 20 min to 2 hr in distilled water or on water agar, percentage germination ranging from 95 to 100. When newly deposited spores on agar are flooded with extract from leaves of a resistant corn variety (MIT S-2, popcorn), germination is reduced to as low as 10% compared to 85–95% with similar extract from a susceptible variety (PH 801, sweet corn). The extracts (1 gm fresh leaves: 5 ml distilled water) are prepared by maceration in a Waring Blendor and subsequent filtration, first with adsorbent cotton and later with Seitz filter. A few spores get plasmolyzed, but the majority show no apparent morphological distortion due to the extracts. There is no appreciable difference in the pH of the extracts, 5.0 for PH 801 and 5.3 for MIT S-2. Conidia suspended for 1 hr in full strength MIT S-2 extract could not be induced to germinate when centrifuged, washed and resuspended in water. Diluted with water from 1:1 to 1:5, the extract fails to appreciably inhibit spore germination.

Similar suppression of conidial germination in *S. philippinensis* has been found with leaf extracts from Phil. DMR 2 and Aroman 206 (Raymundo & Exconde, 1972). Kusdiarti and Semangoen (1972) extended the study with *S. maydis*, but they found little significant difference in germination with distilled water and leaf extracts from susceptible and resistant local varieties. Nevertheless, correlation is obtained between degree of susceptibility to infection and percentage spore germination in leaf extracts. Just what chemical(s) in the extract brings about suppression of spore germination is yet to be identified. All that can be said at this point is that there appears to be quantitative differences in the amount of this germination-repressor substance in different resistant corn varieties.

We have also tried freezing (−8°C) newly formed conidia on leaf pieces in small amounts of water or peanut oil (commercial grade) in small vials. Less than 10% germination is obtained after 24 hr. Conidia in peanut oil kept at 20° and 30°C likewise loss viability after 1 day.

What has thus far worked to assure us of fresh viable spores is controlled induction of sporulation worked out recently by Barredo (1972). This maybe outlined as follows: Naturally diseased leaves are gathered and cut to convenient lengths around 11 am to 12 noon after sufficient exposure to sunlight. These are bagged in plastic or placed upside down in culture dishes lined with moistened cotton or paper at the bottom. Sporulation is induced in the dark inside growth chambers set at 23.3°C and 97% RH. As many as 31,000 conidia/cm^2 are produced in 8–10 hr. Since maximum infection on 2-3-day old seedlings is obtained in spray inoculation with 50,000–60,000 conidia/ml, supply of fresh spores is assured. Moreover, now that the significance of pre-sporulation exposure to light is known it is possible to induce sporulation at will and inoculate at a time more convenient than the usual cumbersome early morning hour (4–5 am) schedule.

Oospores. These sexual spores have at one time or another been associated with every species of downy mildew fungi attacking maize. Claims of their occurrence in *S. maydis* have proved erroneous (Palm, *in* Semangoen, 1970), and there are reasons to believe (Dogma, 1972; Exconde, Dogma & Elazegui, 1972) that those made for *S. philippinensis* (Napi-Acedo & Exconde, 1967; Elazegui & Exconde, 1968) fall in this category.

Oospores have been given much attention in recent years in view of their growing importance in primary infection. These are found internal in host tissues, in the leaves

generally among cells between vascular bundles in *Sclerospora* (Pedrosa, 1970a; Frederiksen, et al., 1970) and mainly in the bundles and their parenchymatous sheath cells in *Sclerophthora* (Thirumalachar, et al., 1953; Kenneth, et al., 1964). In *S. rayssiae* var. *zeae* oospores are scattered in the mesophyll and at times present also in the stomatal chambers (Payak & Renfro, 1967). As yet out knowledge of oospore formation is still very meager (McDonough, 1946; Whitehead, 1958; Pedrosa, 1970b), no doubt partly because of the opacity of the host tissues.

Just as conidia and sporangia are difficult to maintain, oospores, by virtue of their thick enveloping walls and assumed physiological dormancy, are seemingly easy to store with little fear of diminished viability. Infected leaves in which they abound are allowed to dry and stored as they are in the laboratory (Kenneth, 1970), or these may be powdered (J. P. Singh, et al., 1970; Sangam, *in* Raychaudhuri, 1970a), or simply aged in the soil (Safeeulla, *in* Raychaudhuri, 1970; Anon., 1972). Viability is prolonged by storage at temperatures below or slightly above freezing (−10° to 5°C). Oospores of *S. graminicola, S. macrospora* and *S. sorghi* are said to remain viable longer when mixed with garden soil than when simply kept dry in the laboratory (Anon., 1972).

Stored oospores have lately been utilized successfully for inoculation purposes. Incorporated in or on soil at seeding time or shortly thereafter, 12.5–93.8% infection at 28°–32.5°C is obtained with *S. rayssiae* var. *zeae* (J. P. Singh, et al., 1970), 80% or more with *S. graminicola* (Sangam, *in* Raychaudhuri, 1970a) and 6.0–51% in *S. sorghi* at 24°–29°C (Kenneth, 1970; Frederiksen, et al., 1970). When sprayed over the plants or placed into the whorls, infection is 15–20% in *S. rayssiae* var. *zeae* (J. P. Singh, et al., 1970) and 80% in *S. sorghi* (Sundaram, *in* Raychaudhuri, 1970b).

In the field oospores are released by splitting or shredding of infected plant parts, generally the leaves in *Sclerospora*. This takes place at 120°–140°F in *S. sorghi* on sorghum (Frederiksen, et al., 1970). Shredding apparently ensures not only liberation and dispersal but also infectivity. Kenneth (1970), for example, found that oospores within the leaf tissues caused little or no infection, but when liberated by maceration heavy infection was obtained. Release from host tissues is also effected by grazing animals or even insects (e.g., grasshoppers) in whose excreta oospores end up without apparent loss of viability and infectivity (Tasugi, 1953; Kenneth, *in* King and Webster, 1970; Anon., 1972). This poses danger in newly opened or reclaimed areas where soil manuring is practiced. Oospores in soils are known to remain viable for several seasons, but no definitive data on the matter are available.

Despite the aforementioned successes with oosporic inoculum, we are still very much in the dark regarding oospore germination. These sexual spores are notoriously tardy in germination. It is this problem that makes oosporic inoculation unreliable especially when standardized inoculum potential is desired. Laboratory trials with various reducing and oxidizing agents, hormones, leaf extracts, swelling agents, snail extracts, dung filtrates, bubbling with oxygen, temperature treatments, others have all been tried without success with oospores of *S. sorghi, S. spontanea, S. sacchari, S. philippinensis, S. miscanthi, S. graminicola,* and *S. macrospora* (Cosper, *in* Frederiksen, et al., 1970; Safeeulla, *in* Raychaudhuri, 1970a; Pedrosa, 1970c). Only a few successful attempts are on record. Akai (1959) germinated oospores of *S. macrospora* with 10 r/ml streptomycin or 1.0 r/ml gibberellin. Recently, the group of Dr. Safeeulla (Anon., 1972) has determined the factors essential for oospore germination in this species on finger millet. Oospores of *S. sorghi* have also been germinated using root exudates from susceptible sorghum varieties, but not from resistant varieties. Soil extracts similarly induced germination.

Culture and Maintenance

Culture on Excised Leaf Pieces. This method is similar to the one being used with the equally obligately parasitic powdery mildew fungi. It relies upon the potential of the fungus to sporulate on pieces of naturally diseased leaves maintained in nutrient solutions. In other words, it is natural sporulation in the micro-environment possible in a culture plate. At best, light, temperature and humidity can be controlled; however, purity of the harvest can not be guaranteed and sporulation itself lasts for only a few days.

Diseased leaf pieces (*Sclerophthora rayssiae* var. *zeae*) floated in 5% sucrose plus 20 ppm kinetin yield good harvest of sporangia after 24–30 hr at 20°–22°C (J. P. Singh, et al., 1970). The initial crop of sporangia is produced in 3 hr at 25°C, the second crop in 9 hr and 25 min after removal of the first. Since the fungus is zoosporic, inoculum potential is incredibly high (600,000–800,000 zoospores/ml). Sixty to seventy percent infection is obtained in 3 days, or 4–7 and 1–3 days earlier than with oosporic and sporangial inoculum, respectively. On disease-free leaf pieces in the same sucrose solution, J. P. Singh (*In* Raychaudhuri, 1970b) claims that symptoms appear also in 3 days at 25°C.

For the conidial *Sclerospora sorghi,* Kenneth (1970) used 60–200 ppm benzimidazole. Diseased leaf pieces are supported with cotton wool and the cover disc lined with moist paper. Sporulation at 20°C occurs nightly for 7 days, or 2 days longer than in plain water provided each previous spore crop is removed. Healthy leaf pieces placed over sporulating ones turn purplish in 2 days, but infection has not been established. Likewise, with *S. philippinensis* we failed to get infection with single or multiple conidia on either surface of the first true leaf of maize suspended in 5% sucrose solution with or without kinetin at 20 ppm. The leaf remained green and vigorous for 7 days at 20°–25°C.

Culture on Callus Tissues. One object clue on the nature of obligate parasitism may be drawn from Cutter's (1961a, 1961b) experience in taming *Uromyces* and *Gymnosporangium.* He was able to grow these rust fungi on synthetic media only after a period of conditioning on cultured host tissues. In other words, what seems essential is a gradual but persistent shifting from a strictly parasitic mode of nutrition to one of saprophytism. Tiwari and Arya (1969) evidently recognized such a transition in their effort to explain the "sudden appearance" of saprophytic growth of *S. graminicola* on modified White's basal medium reinforced with casein hydrolyzate and several growth-promoting substances (e.g., kinetin, 2, 4-D, NAA, vitamins). This downy mildew fungus had earlier been maintained for 4 years on callus tissues of pearl millet (*Pennisetum typhoides*) cultured on the same medium.

Dr. Safeeulla's group at the University of Mysore (Anon., 1972) appears to have succeeded also in growing *S. graminicola* on callus tissues of "bajra" (pearl millet) on modified White's medium. Both sexual and asexual structures of the fungus are produced, just as they were in Tiwari and Arya's cultures from proliferated floral pieces. Mycelium from the culture infects seedlings and healthy callus of bajra. Moreover, it grows on healthy callus of *Eleucine coracana* ("ragi" or finger millet), a "non-host species and genus".

It seems obvious that, in the present absence of any available axenic cultures of fungi causing downy mildew on maize, the same approach as above should be tried with Sclerophthoras and other Sclerosporas. Already several excellent techniques have been worked out for *in vitro* culture of corn tissues, utilizing either the embryo, meristem, or endosperm (Nitsch, 1951; Strauss & la Rue, 1954; Tamoaki & Ullstrup, 1958; Strauss, 1960; Sun and Ullstrup, 1971).

Lately we tried the modification of Nitsch's medium recommended by Sun and Ullstrup (1971) with tissues of both the endosperm and meristem. Callus formed from either healthy or mildewed plants (*S. philippinensis*) was very small and did not appear capable of increasing beyond twice the size of the original explant after 3 weeks of incubation at 24°C under light or dark conditions. We hope to bring the callus to an acceptable size for inoculation with single conidia.

Agar Culture. Advances in culture techniques have in the last three decades narrowed the gap between obligate and facultative parasitism among fungal plant pathogens. A number of smut and rust fungi previously regarded to be obligate parasites have already been divorced of their host and brought into cultivation on complex agar media. Outstanding examples are *Ustilago zeae, Puccinia malvacearum, Gymnosporangium juniperivirginianae, Uromyces aritriphylli* and lately, *Puccinia graminis* f. sp. *tritici* (De Vay, 1954; Cutter, 1960, 1961 a & b; Hotson and Cutter, 1951; Williams, et al., 1966).

There can be no doubting the need for similar cultures of downy mildew fungi attacking maize and related economic crops. Their importance in phytopathological or mycological studies, pure or applied, is paramount and need not be re-emphasized. Suffice it to state here that axenic cultures of these pathogens would bare open heretofore unexplored research venues, notably physiological and nutritional, genetical, development, etc. I strongly hope that investigators working with the downy mildew in their respective countries will not tire exerting efforts along this line. The task is certainly formidable, tedious and time consuming, but I believe it is also most rewarding and, as Dr. Safeeulla remarked elsewhere in India in 1969, a thrilling experience.

Success with maize downy mildew fungi appears to have been scored first by Katsura (1952) with an isolate of *Sclerophthora macrospora* from rice in Japan. Akai (1959) duplicated the feat with the same fungus on the same sucrose agar medium with extract of either watermelon, oatmeal or potato. Later subcultures of Akai's material however proved to be non-infective on rice or maize (Ullstrup, 1970). It could be that the fungus had mutated as to have lost its pathogenic ability over the years that it has been in cultivation on agar. Indeed this is a well-known phenomenon among fungal plant pathogens, especially *Fusarium* species. Katsura clearly indicated infection of rice with his material; however, symptom expression was not given. Tiwari and Arya's (1967, 1969) success with *Sclerospora graminicola* appears to be the best documented. They used an agar medium consisting of White's basal mineral salts, growth promoting substances (NAA kinetin, 2-4-D), glucose, several vitamins (thiamine, cyanocobalamin, folic acid, etc.), glycine and casein hydrolysate. They cautioned that growth was"... limited in character, and proper nutritional requirements of the fungus need to be worked out to obtain continuous copious growth". Lately, Shaw and Safeeulla (1969) reported on the cultivation of both *S. graminicola* and *S. sorghi* on a semi-synthetic medium; however, details seem not to have appeared as yet.

J. P. Singh, et al., (1970) tried to grow *S. rayssiae* var. *zeae* on the vegetable agar media used by Katsura, but they failed as did Ullstrup (1970) with *S. macrospora* from maize, and Srinivasan and Thirumalachar (1962) with *Sclerophthora cryophila* on oatmeal French bean, potato dextrose, yeast-glucose and corn meal agar.

Our efforts with *S. philippinensis* have likewise met with frustrations. We tried Czapek's salts with sodium citrate, modified White's and Nitsch's medium, and malt-yeast extract plus thiamine. All but the first afforded excellent spore germination (90–100%) under light or dark conditions at room temperature (26°–28°C). With modified White's medium under dark the culture plates were half-filled with pearly-white mycelial growth, but this did not progress further after 5 days. In another set of trials, the medium was dispensed in 250-ml flasks, inoculated with blocks of water agar with several germinating conidia, and incubated for 2 weeks at 22°C. Illumination

was not controlled although the set-ups were in total darkness except during occassional opening of the incubator. The growth obtained was very sparse and limited to no more than 1.5 cm from the edges of the agar block used for seeding. Again, this failed to progress after another 7 days of incubation at room temperature or after "rescue" attempts by flooding the agar surface with full-strength or half-strength White's solution. The hyphal filaments turned out to be either dead or moribound when examined. It is possible that the fungus was never able to utilize the medium, the observed growth then representing the maximum afforded by the spores own food supply.

The littany of failures above with different downy mildew fungi of maize points to the need for information concerning the chemical composition of the fungus cell wall. In view of present limitations in obtaining sufficient quantity of mycelium, conidial or sporangial wall can well serve this purpose. From such an analysis, perhaps some growth factors, possibly not supplied or supplied in toxic levels in the variety of media used, can be identified.

Monospore Culture on Intact Seedlings. Just as we are frustrated thus far in growing *S. philippinensis* on excised leaf pieces and maize callus tissues, we have had good fortunes in parallel attempts to establish on intact seedlings homogenous populations of the pathogen (Dogma, 1972; Dogma, et al., 1973). Analysis of earlier cross inoculations with *S. philippinensis* and three (3) other Sclerosporas (*S. sacchari, S. spontanea* and *S. miscanthi*) reported to attack maize in the Philippines clearly indicates the very likely though as yet unproven possibility in the field of simultaneous infection with at least two species. To make certain then of what fungus we are dealing with in the laboratory, it is imperative that pure populations are used. In January 1972, such a pure population of *S. philippinensis* was established on seedlings of a highly susceptible sweet corn variety (PH 801). It may be profitable here to elucidate on the procedure: Newly formed conidia on naturally diseased leaves are allowed to lodge onto 2% water agar in culture plates. Small blocks of agar, each with a conidium in early germination, are cut out under a stereoscopic dissecting microscope and, with a drop of sterile water, introduced into the whorls of 3-4-leaf stage seedlings. The inoculum may also be placed on the remnant of the coleoptile. All treated plants are bagged with plastic and dispensed for 6 days in a growth chamber adjusted to night conditions of 23.5°C and 97% relative humidity, thereafter to day conditions of 30°C and 75% RH. Systemic infections are noticeable 9–15 days after inoculation, or 2–8 days later than would obtain with mass spore inoculation. Success is fairly low, 7–22% (13% ave.); however, a single seedling with systemic infection is all that is necessary. The fungal population can then be maintained easily by inoculating other seedlings by the mass spore technique, the inoculum coming from the plant previously infected with a single spore (See p. 13 for induction of sporulation.).

Essentially the same results are obtained if single conidiophores bearing mature conidia are used as inoculum in place of single conidia. Of course, chances of success are increased by as many times as there are conidia per conidiophore (15–20 on the average).

We have to date propagated as above on maize countless asexual generations of two isolates of *S. philippinensis* and one of *S. sacchari.* Homogenous populations of these two species have been utilized in cross-inoculations and in developmental and cytological studies (Visarathanonth, 1973). We hope to accomplish the same with *S. miscanthi* and *S. spontanea* to complete the list of known Sclerospora in the Philippines. Single or multiple spores of these species have thus far failed to infect maize systemically.

Dr. M. H. Sun (*in* Raychaudhuri, 1970b) mentioned in passing his work of a similar nature with *S. sacchari* in Taiwan. Success, according to him, is about 50% on the average after incubation in a moist chamber.

From field and laboratory observations, there is general consensus among workers on the occurrence of physiologic races of the downy mildew fungi attacking maize. The literature is certainly replete with this sentiment. Preliminary experimental evidence indicates that this is in fact the case in *S. philippinensis* (Titatarn, 1973). It is therefore conceivable that work along this line will commence within the next 5 years. Already, several inbred lines of maize are available for the purpose. I would like to believe that monospore culture on intact seedlings will prove invaluable in this undertaking, at least until such time as we can rely on single spore techniques with the excised leaf, tissue culture, and axenic agar culture methods.

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Question and Answer

Ampol Senanarong, Thailand: In which country was it reported that oospores excreted from grasshoppers could transmit *Sclerospora?*

Answer: Tasugi reported in Japan, in 1953, the presence of viable and infective oospores of *Sclerophthora macrospora* in excreta of grasshoppers.

E. C. Johnson, CIMMYT: With reference to the relationships of fungal sporulations to light, might not the real relationship rather be the essential nutritional requirements of the fungus to obtain the photosynthates produced by the

host plant? In the absence of light, the host plant cannot elaborate photosynthates in the cells from which the fungus derives its nourishment—thus, no food supply, no spores?

Answer: Certainly, the observed correlation between nocturnal sporulation of the fungus and pre-sporulation exposure to sunlight of the infected plant could well be due to the replenishment of the host's photosynthates during the day and subsequent utilization of these substances by the fungus in the evening. The issue is an open one, and we cannot exclude the other possibilities I mentioned earlier. A lot of work needs to be done. Obligate parasitism is one complex organic and nutritional relationship between the pathogen and host, the mechanism(s) of which is stiill very much obscure.

C. G. Shaw, U.S.A.: I have observed detachment of the "operculum". Have you observed dissolution of the "papilla"?

Answer: We don't have zoosporic downy mildew fungi on maize or any grass in the Philippines and consequently, I have not personally witnessed dissolution of the discharge papilla. But neither have I encountered any authentic report of operculation in *Sclerospora graminicola* or species of *Sclerophthora.* By operculation I mean the presence of a lid, the operculum, which flips up along a circumsissile line of dehiscence at the polar discharge papilla. Judging from published reports, discharge in these fungi is inoperculate as in *Phytophthoras,* i.e., by dissolution or *possibly* rupture of the wall of the discharge papilla. The lens-shaped non-sporogenous substance immediately below then exudes out followed closely by the mass of zoospores. I imagine that Dr. Shaw is the first one to have observed detachment of an "operculum" in *Sclerospora graminicola* or *Sclerophthora* spp. This is certainly something new.

R. G. Kenneth, Israel: It is true, as you mentioned, that I had written that sporulation in *S. sorghi* in the field on sorghum occurred when "temperature never dropped below 23°C". I did not mean that it does not occur at lower temperatures—it does in Israel, often with more profuse sporulation. I meant to show that it was shown capable of sporulating at a higher temperature than was then known. Today, in Thailand, it can sporulate even at 28°C, which does not happen in Israel. Are they different strains, possibly?

Answer: Yes, you did write that natual sporulation of *S. sorghi* on sorghum in Israel occurred when temperatures never dropped below 23°C. I have quoted this. Of course, sporulation of the fungus at lower temperatures elsewhere is not excluded. For example, Drs. Safeeulla and Thirumalachar got sporulation at up to 20°C only, while our Thai colleagues now reveal that sporulation can occur at as high as 28°C. It is evident that *S. sorghi* is the most widespread of conidial *Sclerosporas* on Gramineae, it spreads seemingly towards warmer regions (e.g., Africa, South America, Southeast Asia). You may be right that strains are involved here, and downy mildew workers are generally agreed on this. But sentiment is different from experimental results, the latter exceedingly few in past years. The matter of biotypes or races of downy mildew fungi on graminicolous hosts is one whole area hardly explored up to now.