11. INFECTION OF MAIZE BY DOWNY MILDFWS

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Introduction

The word 'infection' in this review is used for the process by which the downy mildew pathogen establishes contact and obtains nutrition from the susceptible maize tissues. A susceptible host, a virulent downy mildew pathogen and a favourable environment are necessary for successful infection. All the three factors are essential and no factor is less important than the others. Before the infection takes place, the inoculum has to reach the host and develop active growth on the surface and eventually penetrate and grow within the host. In the later stages the downy mildew pathogen may remain localized or it spreads and invades the plant organs partially or completely. The appearance of disease symptoms following infection depends upon the pathogen-host-interaction and environment.

The following species of downy mildews have been reported on maize so far: *Sclerophthora macrospora* (Sacc.) Thirum., *et al; Sclerophthora rayssiae* var. *zeae* Payak and Renfro; *Sclerospora graminicola* (Sacc.) Schroet.; *S. maydis* (Racib.) Butler; *S. sacchari* Miyake; *S. sorghi* (Kulk.) Weston and Uppal; *S. philippinensis* Weston; *S. spontanea* Weston; and *S. miscanthi* T. Miyake and Sacc. Of these nine species the last one is known to infect maize under laboratory conditions only. In this review, under each species, available information on spore germination, infection sites, mode of penetration, establishment of pathogen, incubation period, invasion of the pathogen into the tissues, role of environment factors on downy mildew infection and artificial methods of infecting maize will be discussed. Aspects which need greater attention by investigators will also be brought into focus.

Sclerophthora macrospora

Seed-borne mycelium can form a source of downy mildew infection in some cases. Ullstrup (32, 33) observed *S. macrospora* mycelium in the coleoptile, coleorhiza and scutellum of maize kernels harvested from infected plants and expressed the view that it does not constitute a hazard in the transmission of the pathogen under natural conditions. However, from mature seeds infected seedlings developed. Morphology of the mycelium and the infection process within the germling have not been observed.

Under natural conditions where downy mildew infection occurs regularly it has been observed that water-logging had taken place at some time during the period between sowing and until the plants were 4–6 weeks old (18, 33). The length of the period of soil saturation necessary for infection is not known. Some observations suggest that 24–28 hrs. of water-logging is sufficient for infection to become established (33). This may be the case with asexual sporangia but a longer period is essential if the source of inoculum is oospores, because oospore germination takes place over a period of 6–8 days (18). Saturation of the soil is a must for the zoospores to move to infection sites on the host. The optimum pH for spore germination is reported (18) to be between 5.5 and 6.5. There was a significant drop in oospore and sporangial germination below

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or above this range. Oospore germination occurs between 10–30°C the optimum being 22–25°C. At this temperature germination occurs rapidly. About 25 percent of the sporangia germinate after one hour and almost all the sporangia germinate within 3 hours.

The sources of inoculum for infection of maize are: oospores in the soil; asexual sporangia from early infected plants and from annual and perennial grass hosts. Whether the inoculum is oospores or sporangia the ultimate units which bring about infection are the zoospores released, into a water medium, from the asexual sporangia and the oosporic sporangia (24). Prof. Akai (1) has found that zoospores of *S. macrospora* showed a chemotactic reaction towards germinating rice seeds. Such observations have not been made during infection of maize by *S. macrospora.*

The information on the relationship between environmental factors and infection of maize is available mainly through observations in the field. The variation in the amount of infection and symptoms is related to the development of the host, pH of the water, temperature, duration of flooding and inoculum potential. Following infection, the pathogen may be completely or partially systemic with the fungal mycelium most abundant in the meristematic tissue. It is very likely that the morphology of the mycelium in maize tissues also varies characteristically as in finger millet (18) depending upon the inter-cellular spaces and the host organs it colonizes. In the embryonic tissues of finger millet the hyphae are thread-like, in the stems and leaf sheaths they become elongated and assume a plasmodial type in the leaf tissues.

Successful inoculation of maize has been accomplished by flooding potted seedlings to which infected leaves of grass had been added and by dropping a suspension of sporangia and oospores in the leaf whorls of young seedlings (33). Another method has been to place germinating kernels (2 to 3 cms. long), in a suspension of zoospores for 24 hours and then transferring germlings in soil. None of these methods, however, are suitable for large scale inoculation in the screenhouse or field. Therefore, an efficient artifical inoculation technique is important to study the mode of entry, sites of infection and aspects of host-pathogen interactions.

Sclerophthora rayssiae var. zeae

Seed-borne nature of *S. rayssiae* var. *zeae* has been demonstrated by Singh, *et al.* (26, 27). The primary source of infection is not only the oospores in soil but also the vegetative mycelium located in the seed particularly in the embryonic tissue. That the mycelium present in the seed is infective is demonstrated by cross inoculation experiments (27). After the establishment of primary infection several crops of sporangia are produced which bring about secondary infection (16). They found early infection appearing on the lower leaves in the form of minute round chlorotic flecks which subsequently developed into brown stripes, a characteristic symptom of the disease.

Singh, *et al.* (27) and Singh, *et al.* (25) studied the sporangial production, germination and infection process. According to them a film of moisture is essential in the prepenetration and peneration phases. A wetting period of 12 hrs. was sufficient to allow infection following inoculation with zoospores. On the contrary a wetting period of 6 hrs. was insufficient for infection. Germination took place at a very wide range of temperature (18–30°C) the optimum being 22–25°C.

Using oospores, sporangia and zoospores, different methods of artificial inoculation have been devised to infect maize plants for screening against downy mildew (8, 18, 24, 25, 27, 33). A detailed study of the oospores as the source of inoculum was made by Singh, *et al.* (28) by placing one-year-old infested leaf debris at varying depths in relation to the seed placement. The highest percentage of infection was seen when the oospores were placed in the soil not below 3.75 cm. Another method which gave high

infection was scattering oosporic material on surface. Bringing diseased leaves in contact with healthy plants also gave satifactory results. The most efficient method of inoculation, however, has been found to be spraying zoospore suspension all over the plants and holding them in moist chambers for 48 hours before transferring them to an open place. By resorting to this method the first symptoms of infection were noticed within 3 days. Good infection resulted in plants inoculated with zoospores during the months of July, August and September when the average temperature was between 28–30°C. Experimental results have indicated that at lower temperatures the infection was low (28). Consequently the germination and activity of the zoospores was influenced by temperature.

Age of the maize plant is an important factor in infection (25). Using 10 to 60-day-old maize plants in artificial inoculation tests it has been determined that infectivity was reduced with the increase in the age of the host plants. Zinc defficient soils are known to predispose the maize crop to the downy mildew infection (28).

Sclerospora maydis

One of the primary sources of maize infection by *S. maydis* is the mycelium located in the embryo of the kernels of infected plants (17). Rutgers (19) while reporting the mode of primary infection in his experiments observed 4 infected plants out of 5 kernels from severely diseased plants. According to Semangoen (23) if a seedling shows mildew symptoms on the very first leaf it is due to the seed-borne mycelium. However, he failed to see infection on the very first leaf in maize fields. Dried seeds developed only healthy seedlings although fresh ones gave a high percentage of diseased plants.

Infection of maize seedlings takes place through stomata (23). Upon germination conidia produce appressoria in the vicinity of the stomata and the entry, most probably, is by infection peg. The infectivity of the conidia is lost after ten hours of storage in saturated air of petri dishes. However, the conidia are reported to retain their infectivity even after 20 hours on young maize leaves (23).

The artificial inoculation method commonly used is to allow the conidial inoculum to fall directly on the plants. This method gave high degree of infection (23). Dropping of conidia in leaf funnels, on the other hand, did not give such good results. Infection is reported to take place within a few hours. It has been found (23) that in the infected leaves the fungus first appears on the leaf bases and eventually becomes systemic. If the fungus does not reach the whorl only local lesion symptoms are exhibited on the leaves.

Sclerospora sacchari

Seed transmission of this downy mildew has been reported by Weston (36) and Leece (13). That the mycelium observed with the kernels can be a source of primary infection has been suggested ever since the fungus was first described on maize. Chang (4) experimenting with kernels selected from diseased plants observed infected seedlings. He did not, however, get any diseased plant from infected seeds when the moisture content was brought below 20 percent.

The inoculum for primary infection of maize in some countries is reported (29) to come from the diseased sugarcane which is a host for *S. sacchari*. Infected maize plants produce several crops of conidia starting a couple of weeks after infection. These conidia are capable of spreading the disease under favourable environmental conditions. Temperature, moisture, light, and age of the host plants are some of the important factors in infection, establishment and development of the sugarcane downy mildew of maize. The optimum temperature for the production and germination of conidia is about 25°C although they are germinable at temperatures ranging between 10°C and

34°C. Chang (5) has related the effect of temperature on the time necessary for infection. His experiments have indicated that 2 hours are sufficient for infection to take place at 25°C and 28°C while 3 hours were necessary at 19°C and 22°C. Infection by conidia has been observed on leaves (14). Sun (29) studied the penetration of germ tubes through stomata and observed the spread of the hyphae intercellularly by the development of knob-shaped haustoria into the host cells. Penetration sites on maize leaves were identified as tiny chlorotic round spots. Such spots were rarely observed on naturally infected crop. Microscopic examination of the chlorotic spots revealed loosely woven hyphae. Depending upon the host, parasite and environment interactions symptoms of systemic infection may appear in the form of chlorotic streaks in the second leaf onwards. Normally the older leaves are partially invaded by the fungus. After the mycelium reaches the growing point the newly formed leaves are completely invaded by the pathogen. Age of maize plants is found to be important for infection. One-month-old plants are highly resistant to downy mildew infection (30). Chang's observations (5) have indicated a significantly higher incidence of downy mildew in the zinc deficient maize fields.

Sclerospora sorghi

Primary infection through seed has been demonstrated both in sorghum (11) and maize (10, 20). Jones, *et al.* (10) observed hyphae in the style, ovary wall, and carpellate flowers of maize flowers inoculated with *S. sorghi*. In the mature seeds they located the mycelium in the pericarp and pedicel. Safeeulla and Shetty (20) have determined the presence of *S. sorghi* mycelium in the endosperm and embryonic tissues of the infected maize plants. Infested and mature kernels harvested and stored under laboratory conditions developed downy mildew symptoms after they were grown in sterilized garden-soil.

Infection by oospores in the soil is through maize roots (21). Oospores germinate in response to the presence of susceptible host seedlings. The mode of peneration of oosporic germ tubes in the root is not yet determined nor the actual sites of infection.

Systemic infection in maize seedlings is characterized by chlorotic symptoms which normally appear two weeks after sowing. The first leaf is invariably free from infection. Two reasons may be attributed to this situation, either the first leaf overgrows the pathogen which takes time to penerate the root and invade the stem tissues or a passive defence mechanism in the first leaf prevents the entry of the fungus. There seems to be close correlation between the age of the host leaves and the appearance of the disease on them (21). If no infection is seen within two weeks the second leaf escapes infection while the younger leaves may develop symptoms subsequently. Likewise, if the 4th, 9th and 11th leaves do not show infection by the 4th, 6th and 8th week respectively, the expression of the disease symptoms does not take place and the leaves below this region escape infection (21). The variation in the time of disease expression is due to the factors like: inoculum load, temperature, soil moisture, age of the host plant and the cultivar. Late expression of disease symptoms in the younger leaves while the lower leaves remain free from infection is likely to be mistaken for secondary infection. The mycelium of the fungus soon after penetration invaded the parenchymatous tissue and subsequently reached the shoot. More hyphae were present in the nodal than in the internodal regions. In some of the sections examined the mycelium from the nodal region extended to the leaf primordia. Extensive invasion of the fungus took place only after its entry into the leaf tissues. In a completely chlorotic leaf only the vascular bundles were found uninvaded. Artificial inoculation techniques have been very successful with conidia as well as oospores (6, 9, 12, 22, 34). Upto 100 percent of the inoculated susceptible maize cultivars have been infected. Appressorial

formation and infection through stomata by infection pegs have been noticed on sorghum (11). Several attempts to observe entry through stomata when conidia were germinated on maize leaves have been unsuccessful. Germinating conidia rarely produced appressoria and the germ tubes did not show any tendency to grow towards the stomata. This perhaps explains the absence of secondary infection on maize leaves by *S. sorghi.*

Sclerospora philippinensis

Although, Weston (35) observed *S. philippinensis mycelium* in the seed coat and endosperm, the role of the vegetative mycelium in the primary infection is unknown. However, he studied in detail the development of the disease in infected plants. He observed that the first three or four leaves were either free from infection or only partially infected. Above this level all the leaves were invaded with the pathogen and appeared yellowish or whitish. Following invasion the growth of the stem was retarded. Sometimes secondary shoots or suckers which developed from the infected plants became rapidly infected. Abundant mycelium was found mostly in the leaf tissues occupying intercellular spaces. Two types of hyphae were seen. In one type the hyphae were long, slender and sparsely branched and the other type was characterized by irregularly branched crooked hyphae of varying dimensions. Haustoria were observed to be simple, papillate to tubular or even lobed. Weston (35) reported the collapse of badly infected cells and the destruction of chloroplasts of the parasitized cells resulting in the characteristic pale yellow colour on the diseased leaves. Secondary infection took place by the formation of conidia which germinated readily on moist leaves. The susceptibility to infection was the greatest in the young seedlings and it decreased as the plant aged. Suryanarayana (31) inoculated the seedlings in the two-leaf-stage with fresh conidia and observed the penetration of conidial germ tubes through stomata. Subsequently, appressoria developed and infection pegs facilitated the entry into the sub-stomatal spaces. After reaching the mesophyll tissue the hyphae became branched and invaded the intercellular spaces rapidly and the chlorotic symptoms became evident on the 4th day of inoculation.

Carangal, *et al.* (3) accomplished artificial inoculation from the 3-leaf to 3-week-old plants by dropping infected leaf bits to leaf funnels and described it as the most efficient method. Dalmacio, *et al.* (7) studied the mode of peneration and infection by *S. philippinensis* on artificially inoculated seedlings and naturally infected plants. As described by Suryanarayana penetration was strictly through stomata. They traced the spread of the mycelium down through the leaf sheath to the stem. Once the mycelium invaded the shoot apex it remained active throughout the life of the plant. All the leaves which grew out of the shoot apex became infected completely.

Sclerospora spontanea, Sclerospora miscanthi and *Sclerospora graminicola* are not of any economic significance at least so far. There is very little information on these species with regard to the mode of infection and colonization within the maize tissue which warrants a discussion in this paper. Melhus, *et al.* (15) succeeded in inoculating maize with *Sclerospora graminicola* oospores.

Discussion and Conclusions

Infection of maize by downy mildews takes place by the vegetative mycelium located in the seed and by propagules in the rhizosphere and on the host surface. It is well known now that undehydrated seeds of corn are capable of transmitting downy mildew as the mycelium remains viable in the seed but the fungus loses its viability after drying. On account of the delicate nature of the mycelium it has escaped the attention of many seed pathologists. Details of development, mode of penetration into the seedling tissues and subsequent establishment are still obscure.

Oospores constitute the main source of primary inoculum to maize infection by *S. macrospora, S. rayssiae* var. *zeae* and *Sclerospora sorghi*. The amount of infection caused by these root infecting fungi is determined by the total mass and distribution of viable inoculum and also by the environmental conditions. *S. macrospora* is less specialized than the other two and its resting spores germinate in water without any host stimulus. However, saturation of soil for 5–7 days, a pH of about 5.5 and young host seedlings are necessary for infection. If no synchronization of these factors takes place infection fails to appear. Instances are very common when susceptible host cultivars remain completely free from infection in heavily infested soils. Evidently, the root system and other host surface escape infection during the short but critical period of immaturity. Spontaneous germination without a host stimulum may result in loss of inoculum in the absence of a suitable host. Materials of host origin apparently effect oospore germination in *S. sorghi* (11). Until we know the chemicals involved, it would be premature even to speculate the physiologiocal changes in the resting spores leading to germination. There are indications that amino acids and other growth factors released from the roots effect spore germination and the growth of germ tubes. Such a mechanism undoubtedly provides a greater survival value to the fungus which requires a specific host. Diffusible host materials secreted from the host roots may extend their influence beyond their surface, induce germination and the resulting germ tubes reach the host by active growth through the soil. When the germ tube comes in contact with the host roots infection can take palce. *S. sorghi* oospores have a dormancy to overcome the normal fiuctuations of environment in the absence of a susceptible cultivar and yet are sensitive to host factors which ensure rapid germination. It is likely that only a small fraction of the soil inoculum is sufficient for the infection process to establish on the host. Very little attention has been given to the problems of how downy mildews find their hosts in the soil and what determines the choice of a particular site for entry. In any case spore germination is followed by hyphal elongation and attachment to the host surface. The pathogens may eventually enter the roots directly through intact surface, root hairs or wounds. The actual penetration may be accomplished by an infection peg or by means of enzyme action. But there is no evidence to prove that downy mildews of maize enter roots by the action of cell wall dissolving enzymes. There is no information on this and very little on the infection mechanism by way of roots. It is certain that disease escape due to the local absence of oospores is a common phenomenon. This happens more often than not and is very essential to the survival of the host seedlings. As in many soil-born fungi the possibility of disease is very real in downy mildews.

After successful infection and penetration, the susceptible host tissue provides a congenial environment for the establishment of the pathogen. The host factors involved in such a relationship are decidely numerous and complex. During and subsequent to infection the downy mildews normally do not kill the host cells but affect the structure and physiology of the invaded tissue. The hyphae in the root and stem are scanty. Infected roots and stem appear healthy. The mycelium in these parts is elongated and morphologically different from the plasmodial type in the leaf tissues. The existence of the pathogen in the nodal regions provides inoculum for the differentiating buds of that region. Extensive invasion of the tissue takes place when the mycelium reaches the leaf. The systemic distribution may be thorough or may involve only a part of the infected plant. In some plants gaps of tissues and fungus free organs may be found. In some others newly produced meristematic tissues may be colonized very early. Cases are known where the growing points of stems and roots of affected plants remain free from downy mildew mycelium. If the growth rate of the mycelium is faster, then the meristematic tissue is soon invaded. On the other hand, if the apical meristems

overgrow the fungus, many older leaves reach a stage of maturity at which infection is checked. Such expressions of symptoms are common in downy mildew maize plants.

In species of downy mildews where oospores are not found or are unimportant in disease cycle, the primary source of inoculum (conidia) comes from early planted maize plants or other collateral hosts. By and large the asexual spores of downy mildews germinate readily in water without any stimulation from the host. In most of the cases the germ tube development and appressorial formation are free from host influence. The appressoria form the spearhead of attack into which the inoculum potential from the conidia passes through the germ tube and penetration of the host may take place through stomata. Sites other than stomata are not known for the entry of the conidial germ tubes. However, the presence of enzymes secreted by the pathogen at the peneration site and the softening or dissolution of the host surface by enzymatic action cannot be ruled out.

Some poorly known but important aspects of infection by downy mildews should be studied. Areas which require immediate attention are: (1) morphological and physiological changes in infected plants; (2) behaviour of downy mildew on maize cultivars which show different degrees of resistance; (3) the effect of soil environment on infection; (4) the microbiological interactions of root infecting downy mildews with soil-microorganisms; and (5) the metabolic pattern of the diseased and healthy plants. A comparison of host varieties with resistant and susceptible reactions would be of immediate interest. One of the most rewarding and difficult areas would be to find out the complex phenomenon of host specificity by investigations on the initial establishment of the parasite. The nutritional approach to the study parasitism in regard to maize downy mildews would be useful. New avenues of research in this field have opened up by the recent success in obtaining pure cultures of some downy mildew fungi which have retained infectivity even after several years of sub-culturing (2). Furthermore, the efficient artificial inoculation techniques devised by many downy mildew pathologists would be most beneficial. I hope a few of these problems will be solved in the near future and this review will stimulate further efforts towards understanding of the very complex downy mildew-maize relationships, particularly the infection mechanism.

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Question and Answer

M. Fukutomi, Japan: We reported about the host range of the downy mildew fungus of rice plants, *Sclerophthora macrospora* (Syn.=*Phytophthora macrospora*) in 1964.

In the conclusion of the report, we indicated thought that this fungus caused downy mildew on 43 genera and 72 species of Graminae plants.

Furthermore, the asexual and sexual organs were very variable in size depending on hosts and environmental conditions. I would like to ask that the classification of such fungus be based on morphological characteristics.

For example, range of the zoosporangial size of *S. macrospora* in various host plants was 44.0—11.40×19.1—68.4 μ according to our observations.

Answer: *S. macrospora* has more than 140 hosts. Last year we added 8 more.

Environment plays a part in sporulation and also in spore morphology. Dimensions may vary with host matrix. So far we have identified only one species of *S. macrospora*. It is likely that there are more than one, on the basis of biometric studies. Cross inoculation will determine this.

Sujin Jinahyon, Thailand: What is the average number of germ tubes produced by each germinating conidium? In our preliminary study we observed more than one germ tube in *S. sorghi*.

Answer: It is variable. One or more.

Sutat Sriwatanapongse, Thailand: 1) In Thailand, oospores have not been found. The primary source of inoculum seems to have come from the early planted corn plants and the collateral hosts. However, in the newly developed and isolated land, downy mildew is also found. So I still believe that seed transmission is an important means of spreading the disease. I would like to have your comment on this.

2) The moisture level in the seed, which you quoted from Chang's paper and which in necessary in order to control this disease is too high (less than 20%). Do you not think that it should be lower than this? In Thailand it was found that it should be below 12%.

Answer: 1) I also feel that in a few cases seed transmission of downy mildew (in the form of mycelium) is possible.

2) Chang failed to get infection when the kernels had 20% moisture level. A lower moisture level may be helpful in inactivating the fungal mycelium.

R. A. Frederiksen, U.S.A.: Have you inoculated 1st and 2nd maize leaves with conidia? If you have you will find an abundance of local lesions.

Answer:: Yes by artificial means.

S. Y. Mah, Malaysia: Ref. p. 4, 1st paragraph. You have stated that in *S. sorghi* observation of entry through stomata on maize leaves have been unsuccessful. Also, appressoria are rarely produced. Could you then elaborate on the mode of entry of

the fungus into leaf tissue in such a case?

Answer: One month-old maize plants did not show local lesion symptoms. When the seedlings are young, infection takes place, but the mode of infection has not been studied.

N.Mochizuki, Japan: 1) May I know the possibility of producing oospore from mycelium during sun-drying of seed in relation to seed transmission?

Answer: Not in maize. It is possible in sorghum.

I. J. Dogma, Jr. Philippines: Could you please elaborate further on the "plasmodium-like" state of the mycelium of *S. rayssiae var.* zeae in the mesophyll tissues? Have you done plasmolysis work to show that those "plasmodium-like" mycelia lack cell walls as would be expected in a true plasmodium?

Answer: We have not seen this in *S. rayssiae.* The cell wall is very thin and delicate that when the leaves are sectioned the protoplasm oozes out.

Sangam Lal, India: What would be the probable infection site(s) in the case of the primary infection of *Sclerospora sorghi* or related pathogens?

Answer: Through roots by oospores. By conidia through stomata in the leaves.

R. Kenneth, Israel: 1) In 1968, some speakers at NAINITAL cautiously mentioned "PLASMODIAL-TYPE BODIES" in stricken tissues. Is anything new known about this phenomenon?

2) We found that in maize in which chlorosis began as late as the 7th or 8th leaf-stage, the 1st, 2nd and 3rd first-elongating internodes were shorter than in healthy plants. This shows that infection occurred in very young plants, long before chlorotic symptoms appeared. I would call it "latency". Have you found anything like this?

Answer: 1) 'Plasmodium-like' mycelium is an important stage in the development of the fungus. It is a prerequisite to the development of the asexual and sexual phases. This is more definite now.

2) Similar symptoms have been noticed on maize infected with *S. sorghi* at Mysore, India.