

# Recovery Plan for Philippine Downy Mildew and Brown Stripe Downy Mildew of Corn

Caused by *Peronosclerospora philippinensis*  
and *Sclerophthora rayssiae* var. *zeae*, respectively

Revised 2013

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This recovery plan is one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to insure that the tools, infrastructure, communication networks, and capacity required to mitigate the impact of high consequence plant disease outbreaks are such that a reasonable level of crop production is maintained.

Each disease-specific plan is intended to provide a brief primer on the disease, assess the status of critical recovery components, and identify disease management research, education and extension needs. These documents are not intended to be stand-alone documents that address all of the many and varied aspects of plant disease outbreak and all of the decisions that must be made and actions taken to achieve effective response and recovery. They are, however, documents that will help USDA guide further efforts directed toward plant disease recovery.

## Executive Summary

Philippine downy mildew of maize (PDM), caused by the oomycete *Peronosclerospora philippinensis* and Brown stripe downy mildew (BSDM) caused by *Sclerophthora rayssiae* var. *zeae* are destructive diseases of corn in tropical Asia. These are two of several downy mildew diseases that occur in China, India, Indonesia, Nepal, Pakistan, and Thailand. Neither disease has been reported within the United States. Because each was known to be very damaging to susceptible maize, and sources of resistance, if any, had not been established for U.S. maize varieties, both pathogens were designated select agents in 2002.

Corn is a common host for both species. PDM also infects cultivated sugarcane, some sorghum cultivars, and many weedy grass species, including wild species of sorghum and sugarcane, and common perennial grasses of the U.S., such as big bluestem (*Andropogon gerardii*) and little bluestem (*Schizachyrium scoparium*). BSDM has been reported to infect several species of crabgrass (*Digitaria*). The source of primary PDM infection in corn comes from spores produced by nearby infected hosts such as sugarcane or susceptible grass species at a time that maize plants are less than 4 weeks post emergence. PDM is most commonly spread by wind and rain. Production of spores requires night temperatures ranging from 70 to 79°F accompanied by free moisture. Wind dispersal of the downy mildew pathogens results in localized spread among fields in a given geographical region. Though the pathogen has been detected systemically in seed, it has been demonstrated that once the seed or grain is dried down below 14%, it will not produce an infected plant.

Annual yield losses in the Philippines from PDM on corn were often 40 to 60% on farms across the country before resistant varieties were widely available. On sweet corn, losses of 100% have been reported. It was estimated that the national yield loss in the 1974-1975 growing season was 8%, which was valued at U.S. \$23 million. Much less data are available on yield losses on sugarcane, but losses in the harvested and extracted sugar of 35% have been noted. Disease severity is highest in areas that receive 39-78 inches of rain annually and in tropical climates.

In the case of BSDM, the source of primary infection is from soil borne over-wintering spores which germinate in saturated wet soil producing both conidia and swimming zoospores which infect the young plants. Optimum temperature range for infection is 68 to 77°F. Wind and rain dispersed conidia produced on the surface of infected leaves are the source of secondary disease spread.

In the regions of India where the disease is prevalent, yield losses from BSDM reportedly range from 20-60%, occasionally reaching 70%. Disease incidence increases in tandem with annual rainfall, the most favorable being 40 to 80 inches.

Other tropical downy mildews that can reproduce on maize include *P. maydis*, *P. zeae*, *P. heteropogoni*, and *P. sacchari*, none of which are known to exist in the U.S. None of these is listed as a select agent, although *P. sacchari* may be the same species or very closely related to *P. philippinensis*. Although DNA-based diagnostic tools are being developed to distinguish among the species, introduction of any of these species could threaten maize production in the U.S. and would merit application of the same responses as described for PDM and BSDM. Similarly, should *P. sorghi* which is primarily a sorghum pathogen present in the U.S., gain

the ability to produce oospores rather than just asexual conidia on maize, many of the same response protocols and procedures would be applicable. Whole genome and mitochondrial sequencing projects are expected to clarify the relationships among maize-infecting downy mildews and to provide DNA-based diagnostic tools within the year.

Focused, national efforts are needed to determine the potential impact of these diseases if they were to infect the U.S. corn crop. In addition, a survey of available resistant germplasm is needed along with a categorization of efficacious fungicides that could be approved by EPA in case of a national need.

**Philippine Downy Mildew  
and Brown Stripe Downy Mildew of Corn**  
(caused by *Peronosclerospora philippinensis*  
and *Sclerophthora rayssiae* var. *zeae*, respectively)

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**Reviewer:** The American Phytopathological Society

## I. Introduction

Twenty-three species of downy mildew have been reported to be pathogenic to grass species (Shivas et al. 2011)). Of these, ten are reported as pathogens of corn. Three downy mildew diseases have been reported on corn in the United States: green ear downy mildew (*Sclerospora graminicola*), crazy top (*Sclerophthora macrospora*), and sorghum downy mildew *Peronosclerospora sorghi*).

Philippine downy mildew of maize (PDM), caused by the oomycete *Peronosclerospora philippinensis* and Brown stripe downy mildew (BSDM) caused by *Sclerophthora rayssiae* var. *zeae* are destructive diseases of corn in tropical Asia. These are two of several downy mildew diseases that occur in China, India, Indonesia, Nepal, Pakistan, and Thailand (Payak 1975). Both pathogens are obligate parasites so cannot be grown in pure culture for experimental purposes. Neither disease has been reported within the U.S.

Corn is a common host for both species. PDM also infects cultivated sugarcane, some sorghum cultivars, and many weedy grass species, including wild species of sorghum and sugarcane, and common perennial grasses of the U.S., such as big bluestem (*Andropogon gerardii*) and little bluestem (*Schizachyrium scoparium*) (Bonde and Peterson 1983). BSDM has been reported to infect several species of crabgrass (*Digitaria*) (Bains et al. 1978) .

## II. Symptoms and Disease Cycle

Early field symptoms alone cannot be used for diagnosis of PDM or BSDM. Many plant pathogens, including some indigenous downy mildews and physiological conditions (fertility, weather, etc.) can cause similar symptoms. A non-indigenous closely related species, *Peronosclerospora sacchari*, also attacks corn, sorghum and sugarcane producing almost identical symptoms to that of PDM. With the exception of Australia where it has not been reported, *P. sorghi*, which causes downy mildews on sorghum can also spread to maize, and although it can become systemic and produce conidia, *P. sorghi* only rarely has been reported to produce oospores on maize (Bigirwa et al. 1998). However, absence of oospore production on maize is also the case for other *Peronosclerosporas*. Hence, caution is needed in concluding that plant symptoms shown below are caused by PDM.

The first symptoms of downy mildew on corn typically appear as chlorotic stripes at the first leaves as early as 9 days after planting (White 1999), (Figure 1). All leaves on a plant may show characteristic symptoms of long chlorotic (yellow) streaks (Figure 2). The surest indication of the disease is a downy covering on the underside of the leaves. This covering is the site of spore production and the source for secondary spread of the disease to other susceptible corn plants. (Figure 3). As the plant ages, leaves may narrow, become abnormally erect, and appear somewhat dried out (Figure 5). As the corn plant matures, tassels become malformed, ear formation is interrupted, and sterility of seeds results.

*S. rayssiae* produces very distinct BSDM symptoms without becoming systemic. It produces long-lived oospores which serve as the primary source of inoculum and the ability to overwinter in soil or plant debris. Seed transmission can also occur. For an excellent review of the limited information available for the physiology and transmission of BSDM, see (Putnam 2007a).

The National Plant Diagnostic Network has developed standard operating procedures for handling and diagnosis of brown stripe downy mildew.



Figure 1. Early symptoms of Philippine downy mildew on corn appear as chlorotic stripes on leaves. Photo courtesy of C. De Leon. Reprinted from Shurtleff, M.C. 1983, *Compendium of Corn Diseases*, Second Edition, American Phytopathological Society, St. Paul, MN.



a

b

Figure 2. a) Typical chlorotic streaks of Philippine downy mildew on corn. Courtesy of B.L. Renfro. b) Well defined lesions of brown stripe downy mildew on corn are initially limited by the leaf veins. Photo courtesy of A.J. Ullstrup and B.L. Renfro. Reprinted from Shurtleff, M.C. 1983, Compendium of Corn Diseases, Second Edition, American Phytopathological Society, St. Paul, MN.

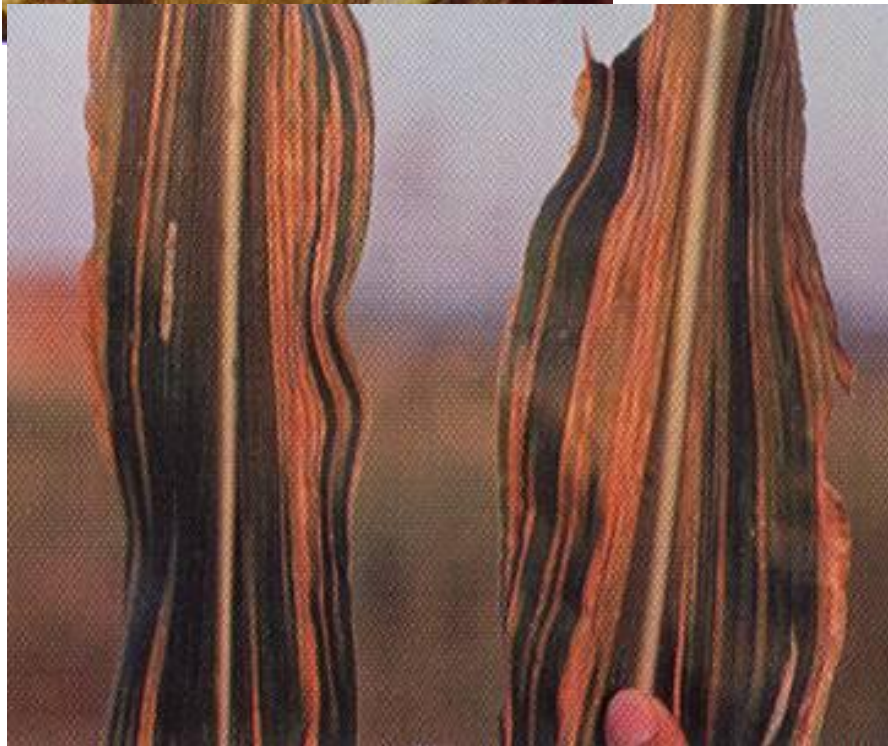


Figure 3. The greenish gray color of this leaf is a typical downy mildew symptom. Photo courtesy of C. De Leon. Reprinted from White, D.G. 1999, *Compendium of Corn Diseases*, Third Edition, American Phytopathological Society, St. Paul, MN.





Figure 4. a) Older leaves of plants infected with Philippine downy mildew are often abnormally erect and appear somewhat dried out. Photo courtesy A.J. Ulstrup and B.L. Renfro. Reprinted from 1973, A Compendium of Corn Diseases, American Phytopathological Society, St. Paul, MN.



b) Advanced symptoms of brown stripe downy mildew on corn. Photo courtesy C. De Leon. Reprinted from White, D.G. 1999, Compendium of Corn Diseases, Third Edition, American Phytopathological Society, St. Paul, MN.

### III. Biology, Inoculum Source and Spread

The source of primary PDM infection in corn comes from the fragile spores (conidia) produced by nearby infected hosts such as sugarcane or susceptible grass species. Although PDM was reported in 1967 to produce an over-wintering spore form (oospores) on corn leaf (Acedo and O.R. 1967), no subsequent reports have appeared. Consequently, no role for oospores in the lifecycle or disease has been established. Shaw (Shaw 1978) mentions a conflict in descriptions of how *P. philippinensis* oospores germinate, raising the possibility that another *Peronosclerospora* species may have confounded the sample. *P. sorghi* has also been reported to produce oospores on maize, but that report was from Africa, not Asia (Bigirwa et al. 1998). The PDM is most commonly spread by wind and rain. Production of conidia requires night temperatures ranging from 70 to 79°F accompanied by free moisture. Disease severity is highest in areas that receive 39-78 inches of rain annually and in tropical climates. Wind dispersal of the downy mildew pathogens results in localized spread among fields in a given geographical region. Though the pathogen has been detected systemically in seed, it has been clearly demonstrated that once the seed or grain is dried down below 14% it will not produce an infected plant (Adenle and Cardwell 2000).

In the case of BSDM, the source of primary infection is from soil borne oospores (over wintering stage) that germinate in saturated wet soil producing both conidia and swimming zoospores capable of infecting young plants. Optimum temperature range for infection is 68 to 77°F. Wind and rain dispersed conidia produced on the surface of infected leaves are the source of secondary disease spread (Putnam 2007a). Disease incidence increases in tandem with annual rainfall, the most favorable being 40 to 80 inches.

### IV. Diagnosis

While diagnosis of the disease downy mildew is simple, classification as to the causal species is problematic, but improving. The obligate nature of the pathogen and general failure to produce oospores on maize makes classification via classical measures especially difficult.

Differentiating PDM from the endemic maize and sorghum pathogen (*P. sorghi*) can be accomplished microscopically based on differences in the conidiophore morphology. Likewise, BSDM can be differentiated from all of the corn infecting *Peronosclerospora* spp. based on spore morphology. Due to the overlap in morphology, intraspecific variability in symptoms, and pathogenicity, separation of PDM and *P. sacchari* may prove to be the most difficult. Some progress has been made in the use of molecular diagnostics to differentiate the downy mildew *Peronosclerospora* spp (Micales et al. 1988). In the past few years, the use of DNA sequence information has enabled researchers in Australia to identify two new *Peronosclerospora* species that can infect *Zea mays* bringing the total to ten (the others are *P. heteropogoni*, *P. maydis*, *P. miscanthi*, *P. philippinensis*, *P. sacchari*, *P. sorghi*, *P. spontanea* and *P. zae*) Through the use of *cox2* (cytochrome oxidase subunit II) and nrLSU (nuclear ribosomal large subunit) sequences, Telle et al (Telle et al. 2011) were able to demonstrate that *P. eriochoae* (primary host is *Eriochloa pseudoachrotricha*) can also be isolated from maize. A year later Shivas et al (Shivas et al. 2011) used the same technique to show that *P. australiensis* can also be recovered from maize. The primary hosts for *P. australiensis* are *Sorghum plumosa* and *Sorghum timorense*, both sorghums native to Australia. In addition, their studies strongly suggested that what had been assumed to be *P. maydis* was not the

same as the *P. maydis* that causes Java downy mildew on maize in Indonesia.

Earlier studies also noted DNA-based differences in the *Peronosclerosporas*. Yao found size differences in ITS-2 (internal transcribed spacer-2, a segment of the ribosomal RNA transcript that is cleaved to create the small, large and 5.8s ribosomal RNA components) when comparing *P. sorghi*, *P. sacchari*, *P. maydis* and *P. sorghi* Thailand isolate (now *P. zaeae*) (Yao et al. 1992). He was also able to differentiate among isolates on the basis of restriction fragment length polymorphisms since probes from one species hybridized to DNA from each of the others (Yao et al. 1991) and was able to develop a pair of PCR primers that amplify a segment of mitochondrial DNA only from *P. sorghi* (Yao et al. 1991). He also defined another primer pair that would amplify from all *Peronosclerospora spp.* available at the time but not from other pathogens of sorghum. For his work, Yao was able to extract DNA of *P. maydis*, *P. philippinensis* and *P. sacchari* from samples stored in the Foreign Disease-Weed Science Research Unit at Ft. Detrick, MD which is equipped to handle potentially damaging pathogens. A problem at the time that has become even more limiting since 9/11, is that very few samples of the Asian species are available in the US, meaning that the ability to examine DNA variation within those species is also limited. In 2013, the Ft. Detrick laboratory was able obtain samples of downy mildew from the Philippines for examination (Gary Peterson, personal communications). If confirmed as *P. philippinensis*, the ability to define rapid diagnosis via species-specific DNA sequences or other techniques should soon be available.

Studies among isolates of *P. sorghi* from sorghum (these are readily available since the pathogen is present in the U.S.) show that a new pathotype (P6) that recently arose in a metalaxyl resistant strain (Isakeit et al. 2003; Isakiet and Jaster 2005) most likely originated from P3, the prevalent pathotype in the area (Perumal et al. 2006). The new race was found on sorghum in the Gulf Coast region of Texas and although *P. sorghi* can also cause disease in maize, no problems with downy mildew in maize have since been reported from the region. Another advance in prospective PCR-based determination of species from undefined samples comes from a series of 55 primer pairs that flank simple sequence repeats (SSRs) in *P. sorghi* DNA (Perumal et al. 2008). These di- and tri-nucleotide repeats, also referred to as STRs (short tandem repeats) or as microsatellites, have been shown to expand or contract during DNA replication at a frequency that makes them useful for comparisons within or between species. In this study, a number of the primer pairs could also be used to amplify DNA samples from *P. maydis*, *P. sacchari* and the only available sample of *P. philippinensis*. Subsequent analysis revealed the species sorted into separate clusters (Perumal et al. 2008). These SSR primers have also been used along with amplified ribosomal DNA sequencing to show that there are at least two distinct populations of maize downy mildews in Indonesia, possibly a second species besides *P. maydis* (Lukman et al. 2013). Sequences for one or two isolates each of *P. maydis*, *P. sacchari*, *P. philippinensis* and *P. sorghi* for segments of the *cox* spacer, eukaryotic translation elongation factor (*eef1-a*) and *b*-tubulin gene also suggest potentially useful species-specific targets for use in PCR-based identification (Luster, personal communications), but again more samples are required for verification.

Two projects now underway should soon provide a direct framework for rapid species identification in the event that DNA from other *Peronosclerosporas* becomes available. Richard Michelmore, Director of the UC Davis Genome Center is using his high capacity sequencing equipment to sequence the genome of *P. sorghi* (possibly both pathotypes 3 and

6), and Frank Martin, a USDA Scientist at Salinas CA, is sequencing the mitochondrial DNA of as many of the downy mildew-causing oomycetes as he can obtain, including *P. sorghi*. He believes the mt-DNA is an ideal target for developing PCR-based diagnostic tools.

The ultimate authority for confirming a diagnosis of the disease rests with the Plant Protection and Quarantine (PPQ) division of APHIS: <http://www.aphis.usda.gov/ppq>

## V. Survey and Detection

The most likely scenario for discovery of a downy mildew select agent in maize in the U.S., whether introduced deliberately or accidentally, is notice of symptoms by a grower that leads to contact with a county agent or farm management professional for diagnosis. Ideally, the professionals will immediately alert the National Disease Diagnostic Network of the potential threat. The Cooperative Agricultural Pest Survey (CAPS) program (<http://caps.ceris.purdue.edu/>) does include survey site information for both pathogens as part of the Pest Tracker National Agricultural Pest Information System. The PDM site is at <http://pest.ceris.purdue.edu/pest.php?code=FFABPMF> and that for BSDM is available at <http://pest.ceris.purdue.edu/map.php?code=FFABSET>. Neither pathogen currently ranked as high priority on the Analytic Hierarchy Process (AHP) Prioritized Pest List. [http://caps.ceris.purdue.edu/guidelines/2014/apdx\\_g1](http://caps.ceris.purdue.edu/guidelines/2014/apdx_g1).

If any of the non-resident downy mildew pathogens reaches the US or neighboring countries it would be appropriate to consider a program such as that constructed for soybean rust (APHIS, 2005). The framework for corn downy mildews would be delivered with consensus-building and commitment of cooperating parties such as USDA, Land Grant Universities, the Seed Corn Industry and State Government. Three basic deliverables of the framework include i) Surveillance and monitoring program; ii) Web-based system for information management; and iii) Prediction modeling. Development of decision criteria for management and training for cooperators would also be important components of the framework.

1) Deliver a surveillance and monitoring network to provide timely information of the incidence and severity of corn downy mildew in the United States and off-shore source areas such as the Caribbean Basin.

A surveillance system may be composed of a number of separate components including sentinel plots, mobile teams, passive surveillance, industry data and spore sampling. Sentinel plots are susceptible plantings kept unsprayed and surveyed periodically by scouts. Mobile teams are groups who target specific locations or areas based on weather or pest models. Scouting data is also contributed by industry. Diagnostic clinics including Federal (APHIS and ARS), State (including the National Plant Diagnostic Network (NPDN) provide diagnostic data. Diagnostic samples may originate from walk-ins, from sentinel plots, mobile teams or from first responders. Industry also provides another source of surveillance data. The surveillance system is organized by the lead USDA agency, who liaises with state and federal agencies to coordinate a leadership structure on a state by state basis. Off-shore observations may also be important for establishing source areas for predictive modeling efforts.

2) Provide a web-based system (USDA Corn Downy Mildew Monitoring and Prediction System) for information management of monitoring observations, forecasts, and decision criteria to stakeholders.

Field and diagnostic observations need to flow into a web-based information system based on sites constructed for other exotic pests such as soybean rust and citrus greening. The Corn Downy Mildew Monitoring and Prediction System (CDM-MPS) web site would provide a powerful on-line database with tools for viewing geographic data sets, including both a password protected web site for exchange of site-specific and sensitive information and a public web-site to communicate with stakeholders. The maps on the public site show counties that are positive as posted by the State Plant Health Director and includes national commentary and links to other sites. The password restricted web site contains detailed geocoded data and consists of separate user accounts for observers, state specialist, researchers and associates. The observer sites consist of a series of on-line tools for uploading data. These tools include on-line forms, uploadable spreadsheets and PDA software. The specialist sites contain a set of tools for updating the public map and documents for stakeholders that appear on the system when a user zooms into the state. The researcher sites allow experts to provide national guidance to state specialists. This might include interpretation of prediction model output or commentary. Associate access allows users such as administrators to view data and maps but not to make changes or to upload data.

### 3) Prediction modeling

An important part of the framework is the delivery of a predictive model for corn downy mildew. These maps are delivered within the password protected web-site. Models vary from simple to complex. At the simple end of the scale the model may represent the climatological likelihood that the pathogen could establish in the United States. More complex models could also be deployed through the site and might include sophisticated aerobiological tools. For soybean rust, APHIS cooperators at PSU, NCSU and ISU have developed aerobiological models that utilize hourly weather data to predict source production, transport, deposition and infection. The models include host phenology and distribution allowing the model to provide real time and forecast output at a 10km<sup>2</sup> resolution. The creation of a suitable prediction model for corn downy mildew may require the collaboration of scientist from ARS, APHIS and land grant universities. Importantly, experience has shown the successful model operation requires feedback from field observations.

## **VI. Economic Impact and Compensation**

U.S. corn production now averages about 300,000,000 metric tons (over 12 billion bushels) per year (<http://www.indexmundi.com/agriculture/?country=us&commodity=corn&graph=production>) and increased demand has raised the price to near \$7 per bushel. Thus while it is impossible to predict an epidemic or the economic consequences of lost production, even a small yield loss would have significant impact. A worse case scenario, where the pathogen was introduced in Florida, the U.S. hybrids were highly susceptible and ideal weather conditions allowed the disease to progress from the south through the midwestern cornbelt

as was the case for Southern leaf blight caused by *Bipolaris* (then *Helminthosporium*) *maydis* race T in 1970 (Tatum 1971), losses would be extraordinary. Interestingly, the realization that maize with Texas male sterile cytoplasm was extremely susceptible to *B. maydis* was originally discovered and reported in the Philippines. (Mercado and Lantican 1961). A more likely situation, especially if spores cannot survive over winter would be outbreaks limited to areas where conditions are favorable for dew at the seedling stage and a new cycle of infection can be initiated from an alternate host. Before resistant cultivars were available, annual yield losses in the Philippines from PDM on corn were 40 - 60% on farms across the country. On sweet corn, losses of 100% have been reported. It was estimated that the national yield loss in the 1974-1975 growing season was 8%, which was valued at U.S. \$23 million. Much less data are available on yield losses on sugarcane, but losses in the harvested and extracted sugar of 35% have been noted.

In India, yield losses from BSDM reportedly range from 20-60%, occasionally reaching 70% in certain areas. Economic losses in 1976 were estimated at \$1.1 million.

Compensation to US producers for crop losses is generally available as a form of 'multiperil' insurance, with costs dependent on the basis of the percent crop protection or adjusted gross revenue insured. In either case, producers must have records to substantiate production and revenue history. The programs are backed by USDA's Risk Management Agency through the Federal Crop Insurance Corporation through contracts with Approved Insurance Providers. Options for 2013 for corn include:

- Group Risk Income Protection - Corn Crop Provisions (06-grip-corn)
- Group Risk Income Protection - Harvest Revenue Option Endorsement (04-grp-hro).
- Group Risk Plan - Corn Crop Provisions (00-141).
- Yield Protection, Revenue Protection and Revenue Protection with Harvest Price Exclusion  
- Coarse Grains Crop Provisions (11-0041).

(<http://www.rma.usda.gov/policies/2013policy.html>)

## VII. Mitigation and Disease Management

Any disease mitigation strategy that is utilized should be coordinated with Federal, State and local regulatory officials.

**Chemical control:** Several products are available, including Deuter and Dithane M-45, Dexon, Cela, azoxystrobin, chlorothalonil, metalaxyl, and mefenoxam. Mefenoxam and metalaxyl are curative and protectant systemics. Control of these downy mildews could potentially be accomplished with a seed treatment with mefenoxam or metalaxyl (Bock et al. 2000) . Because maize downy mildews have not been a significant problem in the U.S., these products may not be directly labeled for use on corn for downy mildew control. However, most hybrid seed corn sold in the U.S. is treated with a combination of fungicides including metalaxyl, fludioxonil, or related compounds targeted to *Pythium*, another oomycete. Metalaxyl has also shown to be effective in controlling BSDM when used in foliar applications post-emergence (Sangam et al. 1980).

Events since the original plan suggest the need for alternative chemical controls. Seed treatment with metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alaninemethyl ester]

sold under various trade names including Ridomil (Novartis & Alibaba), Subdue or Apron (Syngenta), and Acrobat (BASF) and often mixed with other fungicides, has been used as a control SDM for years. However, a metalaxyl resistant strain of *P. sorghi* has evolved in the coastal bend area of Texas (Isakeit et al. 2003), leading in turn to a new pathotype able to cause disease on a previously resistant sorghum cultivar (Isakiet and Jaster 2005). While not as precisely documented, metalaxyl resistant strains of *P. maydis* and/or *P. philippinensis* also appear to exist. In Indonesia, where spore shape suggests that *P. maydis* is present in Java and Sumatra but that *P. philippinensis* is more common in Sulawesi (Dewa et al. 2004), DM is the greatest biotic problem. Farmers there typically use both Ridomil and resistant varieties to protect yields (Dewa et al. 2004). In 2009, BASF released Acrobat 50 WP (a mixture of dimethomorph and mancozeb) for use as seed treatment in Indonesia to control DM. According to the press release made at the time, “the disease has shown indications of resistance in the field to some existing corn seed treatments”. The article also claims that Acrobat 50 WP is also curative when used as a foliar application. Similarly, an unpublished report available at the University of the Philippines at Los Banos suggests a resistant strain of *P. philippinensis* is present in one location based on infection in some cultivars at that site despite seed treatment with Apron. The same cultivars did not develop symptoms at other locations (Manceras et al. 1991).

In 2012, Syngenta received European Patent EP2231614 for ‘Quinoline derivatives and their use as fungicides’ that specifically mentions *P. philippinensis* and *P. sorghi* on maize, sorghum and other hosts. <http://www.freepatentsonline.com/EP2231614.html>

Another company has submitted a new chemical for registration for use against DM in the U.S. Valent U.S.A. expects EPA registration for AP3 a ‘fungicide’ that includes ethaboxam as an active ingredient, which has a different mode of action than metalaxyl. Their press release claims ‘Studies are showing AP3 is providing systemic and contact protection against oomycete pathogens such as *Pythium*, *Phytophthora*, downy mildew, and *Aphanomyces* spp., including resistant and uncontrolled types’. Registration is being sought for use on corn and sorghum along with other crops.

<http://www.valent.com/newsroom/newsreleasesbyyear/2012/valent-reports-positive-results-from-ap3-fungicide-system.cfm>

Ethaboxam was developed and has been used for some time in Korea (Kim et al. 2004) and has been registered for use on grapes in the U.S. since 2006. Several other companies have recently released new compounds for use on downy mildews of vegetables, suggesting they may be effective against other oomycetes.

***Host resistance: Sources, incorporation and stability of genetic resistance:*** Little to no public information concerning response of U.S. maize breeding lines is available for either of the select agent downy mildew pathogens. However, host resistance has been a mainstay for production in areas where each disease is prevalent.

*P. philippinensis*: Early success in developing maize with resistance to downy mildew in Thailand and the Philippines was attained through selection from deliberately created open pollinated composites. Suwan-1, developed in Thailand (Sriwatanapongse et al. 1993), included Philippines DMR composites 1 and 5 as a source of resistance (De León et al. 1993): these composites have served as sources for DM resistance in many subsequent

studies. However, it is not always clear that the resistance is actually to *P. philippinensis*, especially when other species of Peronosclerospora may also be present as in the case of Thailand. Several studies have shown that lines resistant to one downy mildew-causing species may or may not be resistant to another. As an example Rashid et al. (Rashid et al. 2013) tested lines developed by CIMMYT (CMLs) that were released as having resistance to *P. zea* (formerly identified as a Thailand isolate of *P. sorghi*) for resistance to *P. sorghi*, and found that only 4 of 19 were resistant. However, they were able to select progeny lines from bi-parental crosses that were resistant to both *P. sorghi* and *P. heteropogoni* (Rajasthan downy mildew) as determined in tests conducted in locations that provide environmental conditions favorable to each of the two species. Attempts to analyze mode of inheritance of DM resistance also give varied results. Kaneko and Aday (1980) reported that mode of inheritance of resistance to *P. philippinensis* varied with the age of the plant and inoculation density. Sabry et al. (2006), although expecting to identify markers linked to QTLs (quantitative trait loci) associated with resistance in a line developed from Suwan background, instead identified a single gene on maize chromosome 2 that was effective against DM in Texas, Mexico, Egypt (*P. sorghi*) and Thailand (*P. zea*?). In a report from work done in the Philippines made at a 2011 conference, Galvez et al. (2011) used SSR markers from all 10 chromosomes of maize to identify co-segregating QTLs for DM resistance in a BC<sub>1</sub>F<sub>7</sub> population derived from a cross between the parental lines Pi23 and P345. A major QTL was identified on chromosome 8, and a lesser QTL on chromosome 1, both of which functioned at 2 'hotspot' test locations. Other QTLs on chromosomes 9 and 4 were location-specific, suggesting different races or species of the pathogen may be present. The researchers were then able to use marker assisted selection (MAS) to introduce the QTLs into progeny of crosses to other lines to create 4 hybrids that had 0% DM, with 16 more hybrids still to test. In another QTL study with disease response analysis conducted at DM nurseries in India (*P. sorghi* and *P. heteropogonia*), Indonesia (*P. maydis*, Java DM), Thailand (*P. zea*), and the Philippines (*P. philippinensis*) to identify QTLs for resistance in a recombinant inbred population derived from cross between Ki3 (a downy mildew resistant line developed from Suwan) and CML139 (susceptible), George et al. (2003) identified a gene on chromosome 6 that significantly lowered disease at all locations, along with genes on chromosomes 1, 2, 7 and 10 that are effective in some environments.

*Sclerophthora rayssiae* (Brown Stripe Downy Mildew) has not received near the attention of the Peronosclerosporas. After the initial outbreak, several publications in 1970 identified resistant varieties but there was then a gap until 1981 when Khera et al. (1981) used mass selection and sib selection to significantly improve resistance with no associated loss of production in the absence of disease. After another long gap, Singh and Singh (2011) identified 10 highly resistant and 22 resistant stocks in a screen of 221 hybrids and composites in tests conducted over 2 years under epiphytotic conditions. Surprisingly, several patent applications filed since 2009 identify *Sclerophthora rayssiae* of maize (among many other pathogen/host combinations) as a potential target for the use of transgenes to prevent disease, either by adding a gene that encodes an inhibiting protein or by creating a hairpin RNA designed to induce RNAi destruction of mRNA made by a gene essential for pathogenicity.

Alternate sources of resistance to other downy mildews have also been identified beyond those previously mentioned. Studies in India have identified alternate sources of RDM and SDM resistance as well as lines resistant to both (Nair et al. 2005; Sudha et al. 2004). A



recent study in Thailand used association mapping with a set of 60 inbred lines with varying degrees of DM resistance to identify 3 SSR markers that are at different map locations from any previously identified resistance loci (Phumichai et al. 2012), suggesting that untapped sources of resistance are still available. An earlier study by George et al. (2004) had shown no loss of genetic diversity among 60 SSR loci in the resistant lines, indicating that selection for DM resistance had not significantly narrowed the overall diversity as would be predicted if the same genes were being selected.

In summary, it appears that multiple sources of resistance are available, including some genes that appear to confer resistance across species. Even though resistance is often multigenic, a number of contributing QTLs have been tagged with DNA-based markers. These markers, along with the availability of the entire maize genome sequence and advanced technology that allows simultaneous detection of multiple SNPs or SSRs and even ILLUMINA sequencing should greatly speed transfer of resistance into high yielding hybrids. Large seed companies are in the forefront in the use of such technology. In 2011, both Pioneer and Syngenta (NK) seed companies greatly expanded hybrid seed production facilities in the Philippines. PT BISI International TBK seeds has about 50% of the market share for hybrid maize seed in Indonesia. The company reports that sales are up 38% in 2013, in part due to the release of a new *P. maydis* resistant hybrid, BISI 222. Unfortunately, any information that these companies may have on the status of resistance of inbreds or high yielding hybrids popular in the U.S. is considered confidential. The official response to a query about such information from Pioneer was "*Pioneer has plant breeding activities in those countries where these diseases occur and have sources identified. The risk of there being a serious outbreak in the continental USA is extremely low to nil.*" So far no response has been received from Syngenta.

In areas where sorghum and maize are grown in adjacent fields, it will be critical to also be sure that resistant sorghum cultivars are used. This may become more of a problem with SDM if fodder type sorghum species are being grown in dense populations for use as biofuels. A classic example occurred some years ago in Egypt, where the borders of small corn plots were planted with borders of SDM susceptible sorghums that effectively served as spreader rows, creating alarm that a new maize DM had arisen. Sabry (2003) was able to use *P. sorghi* specific primers to show that the pathogen was *P. sorghi*. Also, global warming could allow overwintering farther north than is likely the present case.

**Biological control:** At this time there are no known biological controls available for the prevention or treatment of the corn downy mildews.

**Cultural control:** Early research showed U.S. corn cultivars, especially sweet corns, had little or no resistance to PDM. As discussed previously, resistant cultivars are available from foreign sources. Drying seed to < 14% moisture will prevent seed transmission of the disease.

There is still little or no public information available regarding the resistance of US cultivars to BSDM. However resistant germplasm is available from foreign sources. Since infection requires water-saturated soil, planting outside the rainy season will reduce disease incidence.

## VIII. Research, Education and Extension Priorities

### *Research priorities – short term*

1. Obtain geographically distinct isolates of *P. philippinensis* and *Sclerophthora rayssiae* var *zeae* and near neighbor species with density and distribution represented in collections as needed for population studies and molecular finger printing.
  - a) To accomplish this, establish cooperative agreements with scientists in originating countries (India, Philippines, China, and Taiwan, Indonesia et al) to obtain and establish viable isolates at ARS FDWSR, Fort Detrick, MD.
2. Use molecular sequence information from isolate and species collections to develop species-specific molecular detection tools, focusing on real-time PCR diagnostic assays for deployment at NPDN clinics.
3. Based on known downy mildew biology, develop beta versions of predictive models for spread and establishment.

### *Education Priorities – short term*

4. Compile and develop digital images of disease symptoms and pathogen morphology on relevant hosts and distribute via the NPDN.
5. Develop a short course for first detectors indicating what to look for and how to recognize downy mildews.
6. Compile bibliography of downy mildew related scientific and technical literature, including collections from originating countries. Develop database of citations and distribute via the NPDN and researchers.

### *Extension Priorities – short term*

7. Gather current information on industry activities related to the testing of more recently developed fungicides (e.g. strobilurins, azoles, etc,) for maize downy mildew control in Asia.
8. Determine fungicide product label registration status for maize, sorghum and sugarcane based on chemistries in use in Asia.
9. Deploy first detector training course.
10. Prepare picture cue field guides and educational materials for the extension and crop advisor community, advising them to be on the lookout for mildews in corn and how and where to take suspicious samples.

### *Research priorities - long term*

1. Confirm or refute published assertions that *Peronosclerospora. philippinensis* does not produce the oospore over-wintering stage in sugarcane or maize.
  - a. Determine if *P. philippinensis* produces oospores in the known wild grass alternative

- hosts common in the US maize growing regions.
- b. Determine the potential role of oospores produced by *P. sacchari*, the closely related sugarcane and maize pathogen, in the lifecycle of the disease.
2. Arrange for Asian testing of U.S. maize lines for *P. philippinensis* and *S. rayssiae* var *zeae* resistance (Philippines, Papua New Guinea, India?).
  3. Investigate recent reports of *P. philippinensis* metalaxyl resistance in the Philippines and implications for US maize production.
    - a. If warranted, conduct fungicide efficacy experiments for *P. philippinensis* and *S. rayssiae* var *zeae* in the Philippines and India.
  4. Develop geophytopathological models to predict the establishment and spread potential of *P. philippinensis* and *S. rayssiae* var *zeae* in the U.S.
    - a. Identify available sources of maize resistance suitable for growing in highest risk areas of U.S.
    - b. Develop an action plan (Section 18 emergency registrations) for fungicides in states within highest risk areas of U.S.
  5. Characterize the survival potential of *Sclerophthora rayssiae* var *zeae* oospores in soil
  6. Determine the effects of soil temperature and moisture on germination of oospores of *S. rayssiae* var *zeae* and disease potential.
  7. Determine the relationship between maize susceptibility to sporidial and zoospore infection and plant growth stage.
  8. Determine the internal and external maize seed transmission potential of *S. rayssiae* var *zeae*.
  9. Determine effects of temperature and dew period on spore viability, germination, and infection of maize by sporidia and zoospores of *S. rayssiae* var *zeae*.

#### *Education Priorities – long term*

1. Educate a new cadre of plant pathologists in the epidemiology of phycomycetes, and the population dynamics that cause loss of pathogen sensitivity to fungicides.
2. Develop training courses on management of downy mildew diseases and management of fungicide resistance

#### *Extension Priorities – long term*

Educate county extension, growers and crop advisors in the utility of map-based tracking and information systems such as the Pest Information Platform for Education and Extension (PIPE).

## **Additional Recommendations**

Characterize the risk potential of other non-endemic, closely related maize downy mildews to U.S. Agriculture. The workshop group targeted a potentially new and little studied, emerging species of maize downy mildew in southern Nigeria.

## **IX. Infrastructure and Experts Listing**

Infrastructure to handle research on the pathogens of the maize downy mildews is limited due to their select agent status. To conduct experiments with the pathogens, registration is required with USDA/APHIS. Registration is approved on a site-specific basis, taking into account geographical location, research objectives (i.e. if plant inoculation will be conducted), security measures, and a variety of other factors. In addition, the possession, use, or transfer of maize downy mildew or its pathogens requires entity registration. Permits are only required if movement of cultures or infected plant materials will occur across state or international lines.

Research projects concerning maize downy mildew are active at the USDA-ARS facilities in Ft. Detrick Maryland.

The following experts on the maize downy mildews have been identified:

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## **XI. Web Resources**

NPDR Posters online:

PDM (Byrne and Hammerschmidt 2007)

<http://www.plantmanagementnetwork.org/proceedings/npdn/2007/posters/05DownyMildew.pdf>

BSDM: (Putnam 2007b)

<http://www.plantmanagementnetwork.org/proceedings/npdn/2007/posters/02BrownStripeDowneyMildew.pdf>

Host (maize)

[http://www.nappfast.org/caps\\_pests/maps/2010%20Matrix%20Host%20Map%20PDFs/Peronosclerospora%20philippinensis%20Host%20Map%20Final.pdf](http://www.nappfast.org/caps_pests/maps/2010%20Matrix%20Host%20Map%20PDFs/Peronosclerospora%20philippinensis%20Host%20Map%20Final.pdf)

**Pest Tracker maps:** <http://pest.ceris.purdue.edu/pest.php?code=FFABPMF>

**Surveyed counties since 2010:**

PDM:

<http://pest.ceris.purdue.edu/pest.php?code=FFABPMF>

BSDM:

<http://pest.ceris.purdue.edu/map.php?code=FFABSET>