7. False smut (FSm) or green smut

Continued from Part II, Section 1, Chapter 4, p. 19

FSm was described at a very early date in the Chinese literature, probably during the Ming Dynasty (17th century), but then it was not given a scientific name (Ou 1985). Farmers believed that the presence of the disease, even in those early days, indicated a year of good harvest, as the conditions favoring disease development also favored good crop growth.

FSm of rice, also known as green smut or pseudo-smut (meaning, not a “true smut”, which refers to those caused by *Ustilago* or *Tilletia* spp.), was considered a minor disease in rice until recently. Intensive rice production, especially the introduction of hybrid rice, has increased the incidence and severity of false smut, which is now prevalent in major rice-growing countries in irrigated environments. False smut is more severe on japonica than on indica rice but is more important on the latter. The disease is characterized by production of “smut balls” on infected grains (**FSm Figure 1**).

It is difficult to assess the importance of false smut on rice production. Infected grains are transformed into smut ball, thus, assessing the weight of yield based solely on infected panicle weight may not reflect the actual yield of the grain. Such assessment needs to find a way to eliminate the weight of the grains with smut balls. A majority (around 52%) of the smut balls is observed in the middle portion of the panicle, while occurrence in lower and upper portions, is 35 and 13%, respectively.

FSm Fig. 1. Rice kernels infected with false smut.

Usually, a single panicle may have 1 to 50 grains infected, which, depending on the varieties, may even reach more than 100 (Singh and Pal 1992). Grains adjacent to infected ones may be sterile (Tsai et al 1990). In severe cases, the number of spore balls may increase and the number of plant hills infected may exceed 50%. The weight of diseased panicles decreases significantly and grains with a high abortive rate are noted (Hu 1985). The amount of milled rice decreases as the number of infected grains increases (Ding et al 1997).

The effect of FSm is more significant on the quality of rice grains. The infected grains contain alkaloids (Yamashita 1965) and ustiloxins (Koiso and Natori 1992, Koiso and Li 1994), which are toxic not only to rice plants but also to animals (Koiso and Natori 1992, Koiso and Li 1994). The smut balls of *U. virens* resemble, to some extent, the ergots formed by other "true" smut fungi (Yamashita 1965). Some evidence suggests that feeding FSm-infested bran to sows affects their reproductive capacity by decreasing litter size by 25%, litter weight by 42%, and weaning weight by 63% of the litter decrease (Shang et al 1985). In some cases, fetuses are born dead, mummified, and malformed. Feed infested with FSm bran also affects pigs' kidneys, liver, and spleen. If rice is severely infected with FSm, farmers are also repeatedly exposed to the orange dust during harvest, the effects of which can be allergenic and uncomfortable.

In recent years, extensive research on FSm has been conducted in China due to its wide occurrence on hybrid rice, especially hybrids derived from indica and japonica crosses. These include new methods of inoculation, new findings on infection process, functions of sclerotia and chlamydospores, and their potential as infection units and varietal resistance have been reported (Zhang et al 2003, Wang et al 1998).

7.1. Symptoms

The effect of the pathogen on the host is visible only after heading or before flowering, when the fungus invades the individual kernels. The invasion transforms individual grains of the panicle into greenish spore balls (pseudo-morphs) with a velvety appearance, and, sometimes, with more than twice the diameter of normal grains **(FSm Figure 1**). The spore balls are small at first and are visible between palea, lemma, and the glumes, growing gradually to reach 1 cm or more in diameter and enclosing the floral parts. Slightly flattened, the spores are smooth, yellow, and covered by a membrane, which may burst as a result of further growth. The ball becomes orange and later yellowish green or greenish black. The surface of the ball cracks as it grows to full size and matures. If infection is severe, a mist of orange dust spreads over the rice field during harvest.

A cross section of the smut ball shows that both the palea and lemma remain intact despite a layer of dense chlamydospores that fills the periphery of a mature smut ball, followed by a yellowish layer of hyphae. This appears to indicate that the invasion of the fungal pathogen into the floret is not due to penetration of the palea and lemma. The interface between the endosperm and the layer of hyphae is also distinct and the endosperm is filled with an abundance of starch granules. In some cases, the endosperm is not well developed (Chen et al 2007), suggesting that the fungal pathogen does not colonize the endosperm but may affect its development.

7.2. Causal organism

The fungus causing FSm was first described by Cooke in 1878 (cited in Ou 1985) based on a specimen received from India, and was named *Ustilago virens* Cooke. Patouillard (1887) named the fungus *Tilletia oryzae* Pat based on materials sent from Japan. Brefeld (1895) renamed it *Ustilaginoidea oryzae* (Pat.) Brefeld. The FSm pathogen produces both sexual ascospores and asexual chlamydospores in its life cycle.Takahashi (1896) in Japan revised the name of the anamorph form of the pathogen as *Ustilaginoidea virens* Cooke, which is now widely used and accepted. It is

an ascomycete fungus with the teleomorph Villosiclava virens (Nakata) E. Tanaka & C. Tanaka (Tanaka et al 2008).

Ustilaginoidea virens (Cooke) Takahashi is the name generally accepted for the fungus causing FSm in rice. The telemorph was discovered by Sakurai but Nakata (1934) wrongly recombined the anamorphic epithet *virens* with the teleomorphic genus *Claviceps* as *C. virens* (Cke.) Sakurai. Hashioka (1951) provided the name *Claviceps oryzae-sativae* for the teleomorph. More recent studies have shown that the FSm fungus does not belong to members of *Claviciptaceae* (Bischoff et al 2004) but is a member of the genera in a tribe Ustilaginoideae, which are distinct from teleomorphic genera of *Clavicipitaceae*. Ustilaginoideae should be recognized as a monophyletic group within *Hypocreales*. Thus, the FSm fungus is an ascomycete and does not belong to the order *Sphaeriales*, family *Clavicipiteae* as claimed by early taxonomists. The teleomorph is named as *Villosiclava virens* (Tanaka et al 2008).

The fruiting bodies of *U. virens* consist of sclerotia and chlamydospores, both are found in the smut ball or the pseudo-morph of the fungus. The smut balls are at first yellow to orange, later turning olive green to dark green. When young, they are fleshy inside. They become hard after some time and consist of a central hard mycelial tissue composed of thin, hyaline, septated hyphae. The central hard core is surrounded by three soporiferous layers. The innermost layer is pale yellow, the middle layer orange yellow, and the outermost layer olive green or dark green.

Later in the season, the mycelium mass in the smut or spore ball hardens and becomes pseudo-sclerotia. True sclerotia are formed on but not in the surface of the smut ball, one to several, which are irregular in shape and flat on the lower surface (Biswas 2001).

The sclerotium is the resting body of the fungus and was first discovered in Japan (Ou 1985). In the tropics, the true sclerotia were not reported until the 1980s, mostly because of a lack of active research and it may also be due to the fact that sclerotia are not common in tropical conditions compared to temperate environments. In India, for instance, sclerotia were discovered in the 1980s at an altitude of 1,200 m in and around the Kumaon and Garhwal hills of Uttar Pradesh with a climate similar to that of the temperate zone (Singh and Dubey 1984). In each of the pseudo-morphs or green spore balls, one to five, and most often two, sclerotia were found. Later, sclerotia of the FSm fungus were found in the plain of Assam in less than 1% of the FSm spore balls, at an altitude of 90 m in a rainfed lowland rice crop grown from July to December (Rathaiah and Bhattacharya 1993).

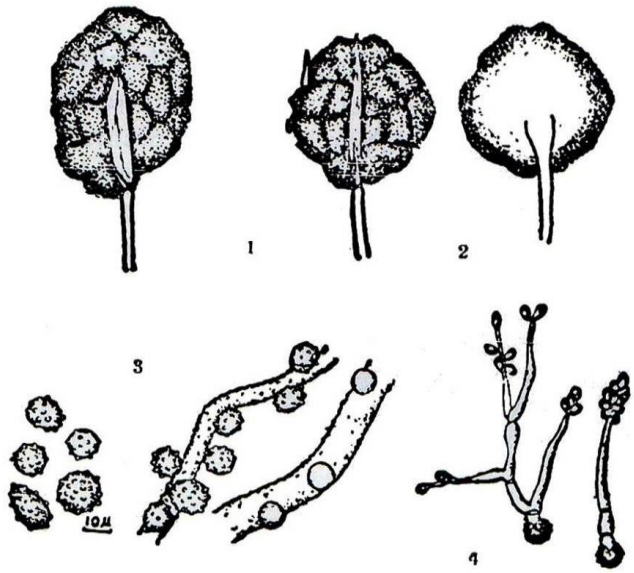
Sclerotia are more numerous and larger in the smaller greenish black smut balls. However, not all smut balls produce sclerotia. In China, sclerotia are found in most of the rice-growing provinces with varying degrees of elevation. Sclerotia have not been found in Guangdong Province, but 2-3% of the FSm has been observed in Yunnan and 5-11% in Fujian (Zhang et al 2003). These sclerotia remain attached individually or are sometimes joined to cover the surface of the pseudo-morphs. The outer tissue of the sclerotia is made of compact pseudo-parenchymatous cells, whereas the inner tissue is hyaline with parenchymatous cells.

The germination of the true sclerotia of *U. virens*, which was first reported and demonstrated in Japan (Hashioka et al 1951), produces stalked stroma. Perithecia are embedded in the stroma with a partly protruding ostiole, and are ovate to pyriform,

measuring 150-430 x 86-193 μm. The perithecial wall is made of pseudoparenchymatous cells. There are numerous asci in the perithecia, each measures 160-220 x 4 μm, and contains eight cylindrical, single-celled ascospores (Zhang et al 2003). The ascus tip is hemispherical with a hyaline and a prominent cap. The upper portion of the ascus has a thick wall with a narrow pore, while the base is attenuated. The ascospores are filiform, hyaline, non-septate, measuring at 120-180 x 0.5-1.5 μm; 50-80 x 0.5-1.5μm, according to Hashioka et al (1951). The ascospores are released, apparently not through the narrow pore of the ascus tip but from the tip after it is dissolved and exposes the ascospores for release (Zhang et al 2003). The ascospores are reported to be of various sizes, which may depend on geographical locations or rice varieties (Wang et al 1998).

Chlamydospores, the thick-walled spores in the resting bodies of the fungus, are borne laterally on minute sterigmata on hyaline, septated hyphae on the outer layers of the smut ball. They are spherical, echinulate, and olivaceous, measuring at 4-6 x 3-5 μm. The younger spores are smaller, smooth, and lighter in color. Conidia are produced 12-24 hours after germination of the chlamydospores and are usually sub-globose, often globose to oblong, hyaline, and granulate. The conidia measure at 4-8 x 2-5 μm in size (Biswas 2001). In vitro, when chlamydospores germinate to form the "germ tube", two types of germ tube have been observed - long and slender, and short (Zhang et al 2003). Conidia are produced in cluster on the tip of the germ tube of either type (**FSm Figure 2)** and they continue to germinate to form secondary or tertially conidia (Zhang et al 2003). However, it is unknown whether or not this happens in nature.

At harvest in temperate environments, both sclerotia and chlamydospores fall with the smut balls on the ground of the field where they remain dormant until next year's rice crop season. Three color types of chlamydospores may be collected from smut balls in the field: yellow, yellowish green, and dark or black colored but all exhibit the shape and appearance of uredosopores produced by the "true" smut fungi. The yellowish green chlamydospores easily germinate on agar media and at a high rate. In contrast, the dark-colored chlamydospiores are quite matured and dormant, hence, more difficult to germinate. It is necessary to break their dormancy before germination (Wang and Lin 2008), which may be one of the reasons that causes difficulty in isolating the fungus, and producing consistent inoculum in artificial media in laboratory.



FSm Fig. 2. *U. virens:* 1. Smut balls; 2. Cross-section; 3. Chalmydospores and their attachment to the mycelium; 4. Germinating chlamydospores and conidia. After Brefeld (1895).

7.3. Host range

Ustilaginoidea virens has been recorded on other species of *Oryza* besides *O. sativa*, such as *O. officinalis* and others. It is found on maize (Haskell and Diehl 1929), *Chionachne koenigi* (Rao and Reddy 1956*), Digitaria marginata* (Shetty and Shetty 1985), and *Panicum trypheron* (Shetty and Shetty 1987). The importance of the alternative host in relation to false smut epidemics is not clear. In a systems approach to disease management, one needs to consider the role of weed species as contributors of primary inoculum in the *U. virens*-rice system.

7.4. Disease cycle

The disease cycle of FSm has not been completely understood, especially in the tropics. Some scientists categorize FSm as an “airborne” disease (Kulkarni and Moniz 1975) and the infection unit or units of the causal fungus, *Ustilaginoidea virens* (Cooke) Tak. are spores by nature, which can be carried by air current and gets deposited on host plant at an appropriate growth stage, to initiate an infection. However, the infection process is still a controversy as the linkage between the life cycle and the disease cycle is still not firmly connecting each other (Guo 2012).

Nevertheless, renewed interest in investigating this disease with histochemical analysis using serial semi- and ultra-thin sections and molecular tools have brought out some exciting information and have given a new twist to the disease progress, which is important in understanding the epidemiology of the disease. These are presented after dealing with the historical information gathered on the role of propagules such as sclerotium and chlamydospores produced by the pathogen in disease cycle. Some aspects of the disease cycle may be synthesized and proposed based on present available information (**FSm Figure 3, forthcoming**).

7.4.1. Sources of inoculum for primary infection. Ou (1985) considered the field observations made by scientists in Japan in early years and indicated that the false smut fungus survived the winter season by means of sclerotia and chlamydospores. In the absence of direct evidences, Ou (1985) postulated that the primary infection is initiated mainly by the ascospores produced from sclerotia, and play an important role in secondary infection which is the major part of the disease cycle. Later studies with additional information are discussed below.

7.4.1.1. Sclerotia: The fungus survives the winter by means of sclerotia as well as chlamydospores getting activated again in the succeeding season of summer or autumn (Ou 1985). In nature, in northern India, sclerotia germinate in June-July with the onset of monsoon rains and produce ascospores. The mature ascospores are released, coinciding with anthesis of the first rice crop (Biswas 2001). This facilitates the infection of wet-season (kharif) or first- or early-crop rice to show more incidence of FSm than the second-crop or boro (dry- or winter-season) rice. Ikegami (1960) suggested that sclerotia present in smut balls left in the field during harvest first germinate to produce stalked stroma, in which the flask-shaped perithecia are embedded and each of them contain nearly 300 cylindrical asci. At maturity, each ascus produces eight ascospores and these ascospores serve as a primary source of inoculum for initial infection in both the tropics and temperate environments, such as Japan. As the infection takes place during the flowering stage, the ascospores at ground level need to be airborne to land on the inflorescence to cause infection. However, there is no direct evidence until this

day that ascospores can cause infection directly after germination. However, vitro-germinated ascospores have been shown to produce conidia (Zhang et al 2003). Nevertheless, there is also no direct evidence to show whether conidia produced from germination of ascospores or both can initiate infection. On the other hand, inoculum prepared with ascospores or conidia separately caused infection, the infection efficiency of ascospores being less when compared to that caused by conidia, suggesting that both ascospores and conidia can serve as inoculum but conidia are more effective (Wang et al 1998, Zhang et al 2003). Thus, it is reasonable to assume that ascospores and conidia derived from ascospores can be primary inocula.

Although chlamydospores are airborne, they do not detach easily from the smut balls due to the presence of sticky material (Sreeramulu and Vittal 1966). There is no conclusive experimental evidence to show that chlamydospores are discharged into the air current and land on the panicles at booting or heading stage where they germinate to invade the grains through the opening of the glumes or cracks between the lemma and palea. Microscopic observation revealed that conidia produced by chlamydospores germinate and grow on the surface of the glume, and develop inside but do not directly penetrate through the glume wall (Dai et al 2005). The germinated conidia continue to grow in the young developing kernels of the spikelets.

The fungus overwinters in temperate environments or over rice-cropping seasons in subtropical conditions through the sclerotia. Dormancy helps in the survival of the fungus and is influenced by temperature. In laboratory conditions, sclerotia maintained at 15-25^{o}C over a six-month storage period would germinate in 19 days on sand culture with adequate moisture while those maintained at 4^{o}C germinated in more than 50 days. In temperate environments, sclerotia collected in the same year had to undergo a period of low temperature before germinating to produce stroma. Otherwise, they would germinate after storage in suitable conditions but fail to produce stroma (cited by Zhang et al 2003, of some studies in China). After breaking the dormancy, germination on sand culture with adequate moisture could produce mature stroma where perithecia are embedded.

In subtropical environments, it seems that sclerotia collected in the field after rice harvest would germinate only after certain treatments, which include radiation with ultraviolet light for 60 min, hot water treatment (at 40^{o}C) for 5 min, or storage at 5^{o}C for 8 weeks. The rate of germination was 50, 66, and 83%, respectively, while untreated sclerotia did not germinate at all (Singh and Dubey 1980). However, it should be noted that sclerotia are rare in tropical rice.

7.4.1.2. Chlamydospores: If the role of sclerotia is to provide inoculum then what is the role of chlamydospores? In reality, chlamydospres may play a more important role as a source of primary inoculum than sclerotia in both temperate and tropical environments. As mentioned earlier, germination of chlamydospores produce conidia. In vitro, germination of conidia may produce secondary or even tertiary conidia. Although it is unclear if this process also takes place in nature, it is safe to assume that conidia as an infection unit are produced through germination of chlamydospores. In artificial inoculation, conidia are more effective than chlamydospores in initiating false smut (Lu et al 1996, Zhang et al 2004). Thus, the role of chlamydospores as overwinter bodies is to produce conidia to initiate infection. Structurally, chlamydospores are produced in the smut ball. Functionally, chlamydospores allow many fungal species to survive in an

unfavorable environment. In the case of false smut, the green spore balls are full of chlamydospores. In addition, some reports indicate that the hardened smut balls (pseudo-morphs) may be structured for overwintering from one rice cropping season to another in temperate environments, and for over-season between crops in tropical environments (Hedge and Anahosur 2000, Biswas 2001).

From smut balls, immediate production of conidia from sclerotia is unlikely. First, sclerotia need to undergo a period of dormancy for several months prior to germination (Zhang et al 2003, Wang et al 1998).Second, after breaking dormancy, the sclerotia still need to go through the process of producing stroma then asci and from asci to ascospores, which germinate to produce conidia. In the same maner, the other pathway of conidia production is through germination of chlamydospores. A dormancy period is also reported for the chlamydospores (Zhang et al 2003). Germination of dormant chlamydospores is very low, usually less than 1% (Zhou et al 1999). To break the dormancy, chlamydospores are incubated at 26^{o}C under high humidity conditions for 20 days. The germination rate may increase to 30%. After breaking the dormancy, chlamydospores are favored to germinate at 25 to 28^{o}C with a pH of 5-7 to produce conidia (J.C. Zhang, pers. comm.. on work done by some Chinese scientists).

Chlamydospores are also considered as another resting structure of the FSm fungus. Chlamydospore germinates to produce conidia, causing infection of rice grains at booting or heading stage (Fujita et al 1989, Lu et al 1996). It is noteworthy that conidia can continue to germinate to produce secondary or tertiary conidia *in vitro*. However, despite failure of conidia to land on an infection court, they could still continue to produce secondary or tertiary conidia in nature. Together with conidia produced by ascospores, the conidia from chlamydospores are also discharged in the air and initiate infection after landing on the rice plant, thus, becoming the inoculum for primary infection.

Based on microscopic observations, some scientists believe that the infection was not initiated by ascospores but by the conidia produced from germinated ascospores (J.C. Zhang, pers. comm..), appearing to suggest that conidia are the fundamental infection units, regardless of their origin. Artificial inoculation using either conidia or ascospores together with chlamydospres at the booting stage has yielded the highest infection of FSm in the field (**FSm Table 1;** Lu et al 1996, Wang et al 1998), merely suggesting that the inoculum potential is enhanced by mixing the three types of spores. However, direct microscopic evidence is still lacking to convincingly establish that ascospores also serve as infecting propagules. The maximum number of conidia in the air was present during the time of heading (Sreeramulu & Vittal, 1966), thus, ready for primary infection. Although we may outline the disease cycle of FSm, additional information is still needed to support the disease cycle and life cycle of the pathogen proposed in **FSm Figure 3** (forthcoming).

7.4.2. Inoculum for secondary infection. According to Ou (1985), chlamydospores play an important role in “secondary infection”, thus, are important as “secondary inoculum”. In the early years, this was thought to be a major part of the disease cycle (Ikegami 1963). Although the term “secondary inoculum” seems to be used loosely here, as its original definition should mean “inoculum” produced from the

FSm Table 1. Germination of chlamydospores and sclerotia of *Ustilaginoidea virens* stored in field and indoor conditions. Source: Lu et al (1996).

	Chlamydospore germination (%) (day/month)						
Storage	12 Apr	15 May	16 Jun	19 Jul	17 Aug	20 Sep	17 Oct
Field conditions	14.2	14.9	12.7	17.6	27.9	26.8	17.1
Indoor conditions	0.7	3.9	5.2	0.3	2.1	2.7	6

	Time (days) required for sclerotia germination after storage					
Storage	12-Apr	25 May	19 Jul	17 Aug	21 Sep	17 Oct
Field conditions	26	20	6	5	8	no germination
Indoor conditions	180	40	no germination	29	70	no germination

Note: The sclerotia and chlamydospores collected from the smut balls after harvest in the previous year and maintained in the respective conditions for storage. Germination was done in the specified days of the month.

initial inoculum after primary infection. If the term is used in such a context, then chlamydospores are just another source of inoculum for primary infection. It is reported that chlamydospores “cannot easily move freely from the smut balls” (Biswas 2001) because a sticky substance is produced by the smut ball that “glues” them together, preventing them from moving freely from the smut ball. Spore sampling in the air at the rice heading stage is mostly in the conidial form (Kulkarni and Moniz 1975, Sreeramulu and Vittal 1966), suggesting that the chlamydospores cannot be discharged into the air and become airborne. The above may clarify the uncertainty about the “secondary inoculum” of the FSm pathogen causing secondary infection. Available information did not suggest a secondary infection of the fungus within the same rice cropping season, either through chlamydspores or ascospores.

It is, therefore, safe to suggest that *U. virens* is a fungus that causes a monocyclic type of disease and that the intensity of FSm in the field is closely related to the initial inoculum prevailing in the field at the booting stage. Both ascospores from germinated sclerotia and conidia from germinated chlamydospores are discharged into the air and become airborne before landing on the rice plant at the heading or flowering stages. In temperate and subtropical environments, the number of sclerotia is relatively higher in the second rice crop than in the first one. It is still unclear why germination of field-maintained sclerotia and chlamydospores is higher than those maintained in the laboratory.

7.4.2. Mode of infection. How does the FSm fungus invade the grain and turn it into a smut ball? Knowledge on the infection process, particularly in complex diseases such as FSm of rice would not only aid to understand the epidemiology of such

diseases but also in devising effective methods for managing them to avoid yield losses. Based on the expression of FSm in the florets and grains, it was thought that rice plants are more vulnerable to infection during booting to heading stage. Bagging the plants at the heading stage up to the boot leaf stage before the emergence of the inflorescence prevented the infection and smut formation, in contrast to plants bagged after the emergence of inflorescence, which were infected naturally by the pathogen and formed high proportion of smit balls (J.C. Zhang, pers. comm. on the work by Chinese scientists).

Based on histo-pathogical studies, Raychaudhuri (1946) described two types of infection processes that transform the grain into a smut ball. In the first type, infection took place at a very early stage of flowering. In this process, infection was through the ovary, which was destroyed but other parts of the flower structure, such as the style, stigmas, and anther lobes, remained intact, which, in the end, were all buried by the spore mass. The second type took place when the grain was mature. As Raychaudhuri described it, “spores accumulated on the glumes, absorbed moisture, swelled, and forced the lemma and palea apart.” Finally, the invading hyphae colonized the endosperm and ultimately the entire grain was occupied and filled by the fungus.

Scanning electron microscopy showed that both lemma and palea remained intact with no sign of infection or penetration by the fungus. It is, therefore, unlikely that the fungus could penetrate the wall of rice husk (Chen et al 2007). In the second type of infection, the fungus enters the grain through opening of the glumes or the cracks between the lemma and palea (Chen et al 2007).

With a more advanced technique, Azhizawa et al (2012) traced the infection in the florets inoculated with a conidial suspension of a laboratory strain of *U. virens* expressing a green fluorescent protein gene by the injection method. Conidia were initially present on the surface of the spikelets by 48 hr post-inoculation (PI) after germination. The hyphae gradually extend themselves by 120 hr PI and invade the spikelets through their apices via the small gap between the lemma and palea engulfing all floral parts by 144 hr PI. When the infecting hyphae develop inside, a distinctive interface between a layer of hypha and endosperm was seen. The absence of hyphae in the endosperm suggested that the pathogen colonized the grain more like a neotrophic rather than as a biotrophic parasite (Chen et al 2007) and that infection took place before gain maturity. Chlamydospores overwintering in paddy soil germinate and infect the coleoptiles’ epidermal cells of the rice seedlings. Ikegami (1963) postulated that the infection hyphae reaching the meristematic tissue of the tillers during the vegetative stage may move to the young panicles still inside the leaf sheaths to infect the florets. Considering this, it cannot be ruled out that the chlamydospores serve as the primary source of infection.

In the field, artificial inoculation using conidia has shown that infection occurs at the late booting stage (Li et al 1986, Zhang et al 2003, Dai et al 2005). The booting stage of rice has been the most sensitive stage of *U. virens* infection and conidia have been the most effective inoculum (Lu et al 1996). Early observations also indicated that most (99.6%) smut balls contain intact anthers, indicating that most infection takes place just before flowering (Hashioka et al 1951). It appears, therefore, that successful infection takes place through a very narrow window of time at heading or before flowering (Yoshino and Yamamoto 1952).

It had been studied earlier whether or not the pathogen can infect the plant before the heading stage including right from the seeds by treating them with the fungal inoculum. Plants were inoculated at different stages to explore the possibility of systemic movement of the pathogen within the plant and the expression of the disease in the panicles. Ultimately, infection did not occur. However, the ability of *U. virens* to infect the roots at the seedling stage and lead a asymtomatic colonization of the entire plant has been shown more convincingly with the aid of molecular detection of the pathogen and histological observation (TeBeest 2010, Schroud and TeBeest 2005).

This pathogen also infects rice coleoptiles intercellularly at the early growth stage (Tang et al. 2012). This led to the conclusion that the pathogen infects the plant at either the booting stage or early flowering stage. Interestingly, Tang et al. (2012) histochemically examined the infection process using serial semi and ultra thin sections of the plant inoculated with the pathogen. This elegant study has shown that the primary infection sites for the pathogen are the upper parts of three stamen filaments located between the ovary and the lodicules. The stigma and the lodicules are also occasionally infected to a lesser extent. The pathogen in this process infected the filaments intercellular lay and extended intercellularly along the filament base. Though the host cells are degraded gradually, the pathogen does not penetrate host cell walls directly and form haustoria. In the smut balls, the ovary remain alive and is never infected suggesting that the pathogen is a biotrophic parasite that grows intercellularly in vivo contradictory to the view held earlier that it is a necrotrophic pathogen.

7.5. Control measures

Although FSm has become an important constraint in intensive rice systems with high inputs and especially with hybrid rice, no systematic research effort has been devoted to developing appropriate measures for its control. Both sclerotia and chlamydospores are resting bodies that can produce primary inoculum, conidia, causing initial infection in the following crop season. Thus, there is a need to pay more attention to the density of smut balls falling in the fields during harvest. Field sanitation and good crop husbandry should be components of an integrated approach to manage false smut and to reduce smut ball density in the field.

Chemical control has been recommended (Biswas 2001, Wang and Lin 2008). So far, a copper compound has been found to be very effective, which is best applied at the booting stage (Zhang et al 2003, Wang et al 1998).

Biological control using a *Bacillus subtilis* strain 916 formulation has been found effective against both *Rhizoctonia solani* and *U. virens*. This is widely used by farmers in Jiangsu Province, China, and provides effective control against false smut (Z. Chen, pers. comm.).

Planting resistant varieties is another feasible measure but little is known or done on the resistance of rice varieties to the fungal pathogen.

7.5.1. Host-plant resistance (HPR). Varying degrees of the disease were observed in field observation of a large number of rice varieties infected with FSm. Some appeared to have fewer smut balls than others. Because of the increasing prevalence of FSm in rice, research on HPR had drawn a lot of interest but with limited progress. To evaluate rice varieties for resistance to FSm, a few considerations are

needed. Foremost are the method of inoculation, inoculum production, and the appropriate growth stage of the rice plant for inoculation to obtain a good level of infection to select true varietal differences. Adequate information on pathogen variation and understanding host-pathogen interactions can be the bases for host resistance.

7.5.1.1. Methods of inoculation: Different methods of inoculation have been devised to inoculate rice plants at the heading stage with fungal spore suspensions. Although these methods are all successful to some degree in producing FSm on plants, the efficiency depends on the purpose of the study. In evaluating rice varieties for resistance, natural infection in the field is often practiced. However, variable results had been obtained because of shortcoming of the method. The advantage of the method is its conduct in "hot-spot" areas (i.e., sites or localities of rice fields) where satisfactory levels of natural infection can provide test for a large number of rice germplasm. Field sites can provide more useful information than anticipated when used in the study of pathogen variation. For varietal evaluation, field evaluation based on natural infection ideally must be combined with artificial inoculation on selected entries from a field test in the greenhouse.

Varietal evaluation based on artificial inoculation is also done. All of the following methods have been used by different scientists for various purposes.

- Artificial injection inoculation: This is the most commonly used method for inoculation. Inject a spore suspension with a hypodermic needle into the leaf sheath at the booting stage of the rice plant.
- Spray inoculation: Use an atomizer with a spore suspension and spray the inoculum directly onto the plants at flowering or heading stage.
- Smear inoculation: Use a camel-hair brush and dip it into a spore suspension and smear this directly on the panicles at heading stage.
- Natural infection in field "hot-spot" areas.

For resistance evaluation or breeding, every method above has its advantages and disadvantages. Artificial inoculation is most effective in producing disease but it is tedious and may not be efficient in testing a large number of plants, especially segregating populations. Spray and smear inoculations are not always effective in producing disease but operationally are more efficient than artificial inoculation. To be very effective, field testing on natural infection must be based on a good understanding of the disease epidemic process and conditions. The methods used by Ashizawa et al (2011) and by Lu et al (2009) appeared adequate for this purpose.

7.5.1.2. Inoculum preparation: Different types of inoculum consisting of crushed smut balls, mycelium from the culture, mycelium plus chlamydospores and conidia, and mycelium plus ascospores have been used for artificially infecting rice plants. All have been reported to cause the disease but in varying degrees. As discussed in the section on the Disease cycle, there are missing links in the disease cycle. Lu et al (1996) and Zhang et al (2003) tested the efficiency of FSm development using various infection methods in inoculum production. These methods included:

(1) Chlamydospore suspension. Collect fresh smut balls and wash them vigorously with an adequate amount of water in a beaker to obtain chlamydospores at 100 per micro-sphere.

(2) Conidial suspension. Conidia may be produced on culture medium (for instance, potato sucrose [PS] liquid medium) in 5 to 10 days. Filter the culture using a double-layer cheese-cloth to obtain the concentration needed.

(3) A mixture of mycelium-conidia. The fungus culture is prepared as in method 2. Instead of using filtration, the entire medium culture is placed in a blender to homogenize the culture into a mycelium-conidium mixture. In this mixture, the conidial concentration is maintained at 120 per micro-sphere. Based on conidia production, the rice grain medium is the best one, followed by Wakimoto's medium and PDA, among the solid media (Zhou et al 1999).

Among these methods, the third one is most effective. This method also seems to suggest that the primary inoculum of FSm is the conidia.

More recently, detailed procedures for inoculum preparation using single conidial isolates have been developed by Lu et al (2009) and Ashizawa et al (2011). They describe not only culture preparation from single conidial isolates but also methods for inoculum preparation and inoculation. The inoculum production method of Ashizawa et al (2011) uses conidial suspension only while that of Lu et al (2009) consists of a mixture of blended conidia and mycelia. Either method seems to produce good results for successful inoculation of plants for evaluating resistance.

For varietal evaluation, both methods employ injection of conidial suspension into the boots of the rice plant. To estimate resistance among the tested rice varieties, the method of Lu et al (2009) measures disease intensity or incidence by obtaining the percentage of diseased panicles over the number of plants inoculated per variety, and grains per panicle per plant infected with the FSm fungus. In comparison of tests in the greenhouse and the field, Ashizawa et al (2011) showed that results obtained from the greenhouse were more reproducible than those from the field.

7.5.2. Pathogen diversity. The results from studies on genetic diversity of *U. virens* seem to be relatively simple. No specific DNA pattern was found among strains collected from the same rice varieties from the same field or even from different geographic sites. The population structure and genetic diversity of 56 strains of *U. virens* in China from the northern, southern, and central rice-growing regions collected in 1992, 1996, and 2001 were analyzed using random amplified polymorphic DNA (RAPD) (Zhou et al 2004**).** Analysis of 223 DNA bands amplified with 32 primers showed tight clustering based on a geographical of strain origins and sampling time, suggesting that *U. virens* in China was not highly diversified. Analysis using AFLP (amplified fragment length polymorphism) of isolates collected from different rice varieties and breeding lines from the same site obtained a similarity coefficient of over 0.72 (Pan et al 2006). It is possible that there was no specific interaction between the rice genotypes (or that the rice plants used had similar genetic background) and *U. virens*.

There were no data to indicate pathogenic specialization of the fungus on specific rice varieties (Pan et al 2006). The *U. virens* isolates were virulent to some of the test varieties while others were avirulent (Zhang et al 2004). Among varieties resistant to the virulent strains, a range of disease index from high to low was revealed, which appears to confirm that the interaction is not specific. Results obtained by Lu et al (2009) indicated more interesting interactions. Their study has shown the possibility of strong interaction between isolates of *U. virens* and hybrid rice carrying different R

genes (**FSm Table 2**). Pathogen isolates from hybrid rice grown across Sichuan Province, China, indicated that disease severity was significant between isolates and hybrids, and a strong interaction between the two. Resistance of both the female and male parents influenced the isolate groupings. Six groups of the isolates were identified based on their virulence on three rice hybrids that differed in resistance.

FSm Table 2. Disease index of three rice hybrids and their response to isolates of *Ustilaginoidea virens* in Sichuan, China. Source: Lu et al (2006), unpubl. data.

Isolates	Disease index (%)* Gangyou 182	Gangyou 94-11	Yixiang 2292	Isolate group
UV56	98.2 a	91.1 a	19.6 bcde	I A
UV25	76.7 b	65.2 b	0.01 e	I B
UV24	62.6 bc	53.1 bcd	11.1 bcde	I A
UV29	57.8 bcd	62.6 bc	29.6 abc	I A
UV28	52.2 cd	55.6 bcd	0.01 e	I B
UV17	51.8 cd	44.8 bcd	16.3 bcde	
UV55	41.8 cd	35.6 def	13.1 bcde	
UV18	40.4 cde	21.1 fgh	15.9 bcde	
UV46	38.1 cbe	19.6 fgh	0.01 e	
UV36	27.4 def	51.5 bcd	35.2 ab	II
UV42	5.9 fghi	41.8 cde	3.3 cde	III
UV16	0.01 i	0.01 j	60.4 a	IV
UV01	1.5 ghi	0.7 j	36.3 ab	IV
UV21	0.01 i	0.01 j	38.1 ab	IV
UV27	4.1 ghi	5.2 ij	1.5 de	V
UV31	1.8 ghi	0.01 j	1.5 de	V
UV51	0.01 i	0.01 j	1.1 de	V
UV32	0.4 hi	0.01 j	0.01 e	V

*The values are the mean of three replications and, in each column, figures followed by the same letter are not significant (p=0.01) by Duncan's multiple range test.

In analyzing the genetic diversity of *U. virens* isolates, it seems necessary to keep in mind a few issues. The infection units of *U. virens* in rice may come from two sources, conidia derived from the ascospores (sexual) of sclerotia origin and conidia (asexual) derived from chlamydospores. In principle, one would expect a more diverse pattern than detected, suggesting that the origin of isolate collection is important. The genetic diversity of isolates from Sichuan Province (Lu et al 2009) may be an interesting sample set to be included in genetic analysis.

Thus, research on host plant resistance to *U. virens* seems to be an interesting area for further studies in the near future. The use of resistant varieties is the priority in controlling FSm while resistance to *U. virens* should be on the top of the agenda in research.

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