

Formation and Histopathology of Galls Induced by *Ustilago esculenta* in *Zizania latifolia*

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ABSTRACT

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"Kah-peh-sung," or "water shoot," widely used as a vegetable in the Orient, is a gall induced by *Ustilago esculenta* in the stem tissues of *Zizania latifolia*. Histological studies and tissue isolations revealed hyphae of the fungus distributed systemically throughout the stem tissues, particularly near the apical meristem. No hyphae were found in leaf and root tissues. The hyphae grew intracellularly and intercellularly. They were present mostly in the parenchyma,

but invasion of vascular bundles in young tissues was common. In the gall, the hyphae disintegrated the cells by digesting the parenchyma cell wall. Subsequently, the hyphae grew into the increased space to form a hyphal cluster. Then the hyphae in the cluster became fragmented, forming teliosporogenic hyphae and, later, teliospores. Dimensions of the teliospore sori averaged 1×5 to 6 mm, but the longest one measured 18 mm in length.

"Kah-peh-sung" (local name), or "water shoot" (given name in this article), is a stem gall of *Zizania latifolia* Turcz. that is used as a summer and autumn vegetable in Taiwan, East China, and many Southeast Asian countries. The gall is formed by a hypertrophy of the first three to four nodes immediately beneath the apical growing point, averaging 3 to 4 cm in width and 20 cm in length. The water shoot was first reported from Hanoi by Hennings (2) in 1895. In 1916 Sawada (6) briefly described from Taiwan the symptoms of the diseased plants and morphology of the fungus. The galls they described are mostly harvested galls. Su (10) studied the physiology and cytology of the fungus in 1961. Leu et al (5) recently developed a method for preservation of the water shoot.

Ustilago esculenta Henn. induces the gall and is distributed systemically in *Z. latifolia*. Cultivars of the vegetable are green, red, and white according to the color of the outer skin of the gall. A basal part of the stalk of the plant is used for replanting. The stools are dug in November and dried in the field for 2-4 wk after galls are harvested. Around 20 December, the dried heads of the stools, each containing two to three stalks, are planted in a 90-cm grid pattern. The field is flooded up to late October or early November of the next year. The leaves are nearly erect in mature stalk, which contains six to eight nodes and measures 2 m from the basal node to the apical leaf tip. Each stool comprises 20 to 30 stalks. The gall includes mostly three and sometimes four nodes below the apical meristem. The green cultivar forms galls from April to November in the Puli area; for red and

white cultivars, *Z. latifolia* is planted later and the galls are harvested in October. Water shoot is harvested manually at 2-day intervals. In the late season plants and galls are small due to shorter growth period. The gall is retained after removing the leaf sheath and usually is cooked by boiling or frying within 1-3 days after harvest. Sori of *U. esculenta* develop within the gall if it remains in the field for a long time. Under such conditions, the gall decomposes gradually. Palatability of the gall decreases as the sori increase in number.

This article reports observations on the formation of galls in the field and histological studies to clarify the distribution of the fungal hyphae in the host plant and the development of teliospore sori in the galls. Artificial inoculation with the fungus also was conducted.

MATERIALS AND METHODS

Observations of gall formation induced by *U. esculenta* in *Z. latifolia* were made at 1- to 2-wk intervals in the fields with the green cultivar infected with the mycelia-teliospore (M-T) strain except as otherwise mentioned. The study was made with infected material from the townships of Puli and Wufeng in central Taiwan.

For histological studies, tissues from various parts of the host plant, including galls of different stages of development, were collected from the fields. They were fixed Formalin/acetic acid/alcohol, serially dehydrated with alcohol, infiltrated with xylene, and embedded in paraffin. The tissues were sectioned at $10 \mu\text{m}$ with a rotary microtome, stained with safranin, counterstained with fast green, and then mounted in Canada balsam (3). Freehand sections also were examined.

Tissue isolation, except for the gall, was conducted by sterilizing pieces of tissues 2-3 mm thick with 0.1% HgCl_2

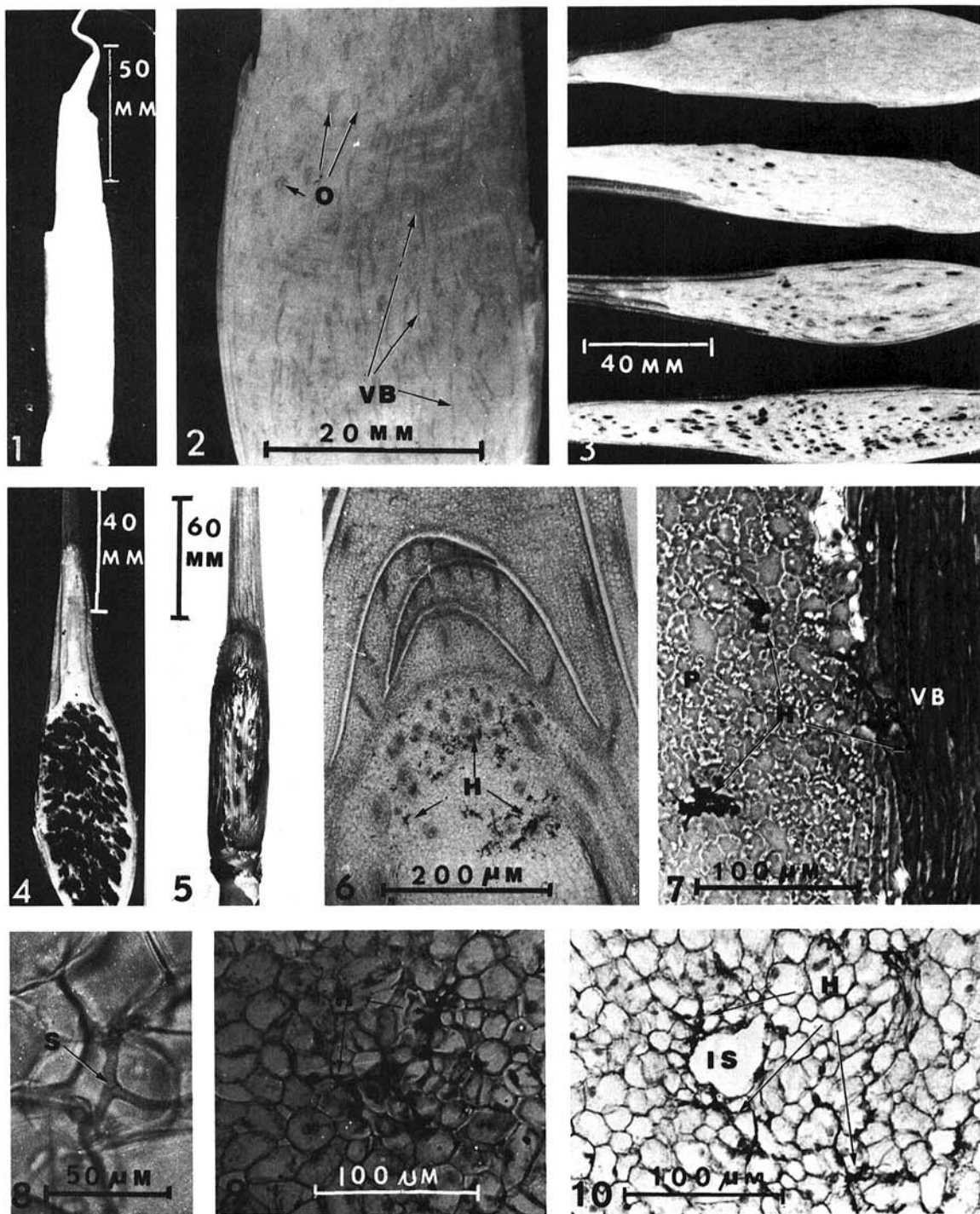


Fig. 1-10. Stages of formation of galls on *Zizania latifolia* infected with *Ustilago esculenta*. 1) Gall induced by *U. esculenta* on *Z. latifolia* with an S-shaped growing point. 2) Section of gall in edible stage showing oval structures (O) filled with hyphal strands or teliosporogenic hyphae of *U. esculenta*. Vascular bundles (VB). 3) Progressive stages (top to bottom) in development of sori in gall incited by mycelia-teliospore (M-T) strain of *U. esculenta*. 4) Gall incited by T strain of *U. esculenta* showing stem tissues full of sori. 5) Outer streaks, composed of sori in gall incited by T strain of *U. esculenta*, evident from outside. 6) Hyphae (H) of *U. esculenta* are abundant beneath apical meristem. 7) H of *U. esculenta* invade parenchyma tissue (P) and VB. 8) Branched hyphae of *U. esculenta* in parenchyma cells. Branching occurs near septum (S). 9) H of *U. esculenta* digesting cell walls of gall. 10) Void in intercellular space (IS) after cells of gall were digested by H of *U. esculenta*.

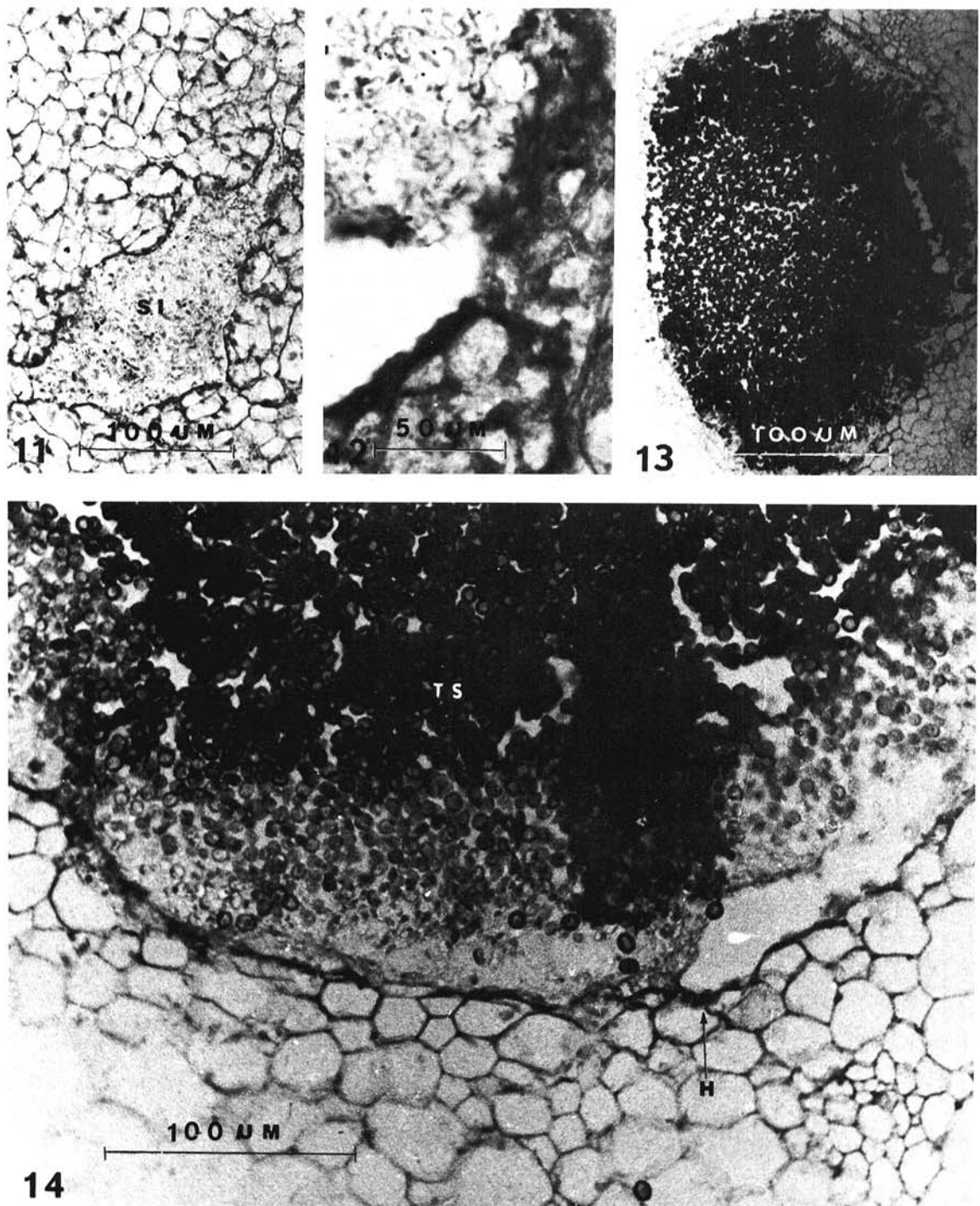


Fig. 11-14. Photomicrographs of galls on *Zizania latifolia* infected with *Ustilago esculenta*. 11) Longitudinal section of gall in *Z. latifolia* showing hyphal mass of *U. esculenta* filling fusoid sorus initial (SI). 12) Teliosporogenic hyphae of *U. esculenta* in SI, showing early developmental stage of teliospores in gall of *Z. latifolia*. 13) Cross section of single matured sorus of *U. esculenta* in gall of *Z. latifolia*. 14) Teliospore sorus (TS) showing pattern of centrifugal development and maturation of teliospores. Hyphae (H) of *U. esculenta* are still observable around sorus.

for 30 sec and washing them with sterile distilled water three times before transferring them to potato-dextrose agar (PDA) slants. For isolation from the gall, pieces of inner tissues were transferred directly to PDA slants after surface sterilization with 70% alcohol. The slants were incubated at 20 C.

For inoculation, the colonies obtained from tissues on PDA were used. Young buds of one to two eye cuttings obtained from the flowering plants were wounded and then smeared with thick "sporidial" suspension prepared from the colonies. After inoculation the cuttings were planted in pots and observed for gall formation.

RESULTS

Gall formation.—Galls usually form at 15–20 cm above the ground, but occasionally appear as high as 1 m or as low as 10 cm above the ground. The gall is compact, spindle-shaped, wider near the base, and tapers toward the apical end. The end containing the growing point appears S shaped (Fig. 1). Total length ranges from 4.5 to 32 cm, but is typically about 20 cm long. The widest diameter measures 2–5 cm (3.5 cm on the average). The gall commonly weighs 30 g, with exceptionally large ones weighing up to 100 g. The buds on all three or four nodes of the gall usually disappear except for some degenerated ones.

The actual time needed for the tissues to enlarge to an edible form of gall is usually not more than 15 days. One strain of the fungus starts to form sori during the initial stages of gall formation. It is designated as the teliospore

(T) strain, to distinguish it from the common M-T strain described above. Sorus formation by the M-T strain occurs only in the later stages of gall formation (Fig. 3). The gall and sori induced by the T strain are shorter and wider than those induced by the M-T strain (Fig. 4). The gall of the T strain is not harvested, and streaking is clearly noticeable on the skin (Fig. 5). Streaking is not observed with the M-T strain. Because of this, plants infected with the T strain are not used as planting material.

When the gall attains its full size for marketing, the inner tissues appear white and are rich in hyphae. During this stage, some oval and threadlike patches, both light yellowish, can be seen easily against the background of white tissues. The former consists of a mass of hyphae of the fungus, and the latter, the vascular bundles of the host plant (Fig. 2). Later, the hyphae in the oval patches produce a spindle-shaped sorus of teliospores. Sori are scattered throughout the gall tissues except within the 10-mm length immediately below the apical growing point. Presence of the hyphae, however, were observed up to 50–80 μ m beneath the apical growing point.

Histology of gall.—In infected *Z. latifolia*, hyphae of *U. esculenta* were distributed profusely in young stem tissues, particularly near the apical meristem (Fig. 6), but less in matured stem tissues. Hyphal growth seemed to keep pace with the growth of the apical meristem tissues. Thus, the hyphae can be distributed systemically in a diseased plant and can persist in the plants after replanting without need for further infection. No hyphae were observed in leaves except occasionally in the basal part connected with stem tissues near the meristematic region. Root tissues also were free of hyphae.

The hyphae averaged 2.5 μ m in diameter. They grew intracellularly and intercellularly. They were found mostly in the parenchyma, but invasion of vascular bundles in young tissues was quite common (Fig. 7). The hyphae were septate, and branches formed near the septa (Fig. 8).

After the plants were 4–5 mo old or older, the fusoid gall, containing mostly three, sometimes four nodes, was formed beneath the apical meristem. The hyphae first digested the parenchymatous cell wall (Fig. 9). The digestion proceeded more or less synchronously in nearby tissues, which caused an increase in the intercellular space (Fig. 10). When cell masses were digested, the hyphae grew quickly into the increased space and formed a hyphal cluster, the initial stage of the sorus. The hyphae grew outward by digesting more cells and fusing with other nearby hyphal clusters. Sometimes disintegrated cells could be seen mixed with the hyphal clusters in the initial developmental stage of the sorus. Occasionally, the hyphae did not fill all of the increased space. This space sometimes was left empty even after the sorus formed. A fusoid sorus was formed by the more rapid growth of hyphae in the longitudinal than in the transverse dimension (Fig. 11). Usually sori are black when mature, but brown sori also were observed in the southern part of Taiwan.

Hyphae in the wefts within the increased space fragmented to 10–20 μ m in length to form teliosporogenic hyphae, then teliospore fundamentals (Fig. 12). Sori and teliospores developed and matured

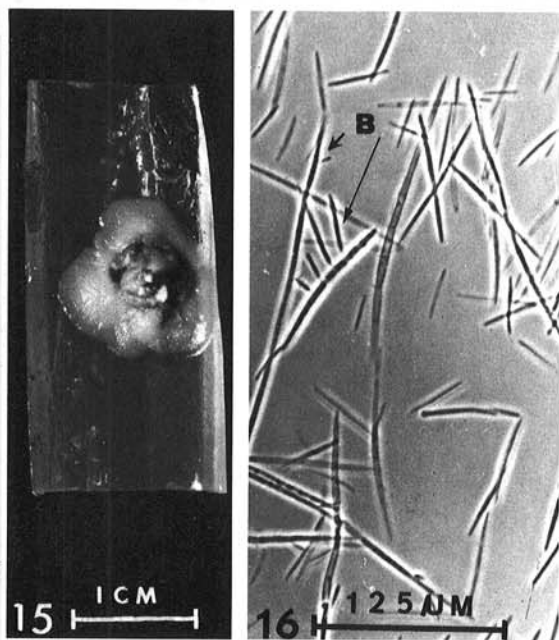


Fig. 15–16. *Ustilago esculenta* in pure culture. 15) Slimy colony developed 20 days after isolation from apical meristem tissues incubated at 20 C. 16) Sporidia obtained from tissue isolation. Budding (B) from septa of sporidia was observed.

centrifugally (Fig. 14). The formation and maturation of sori in the gall were gradual. If the gall was not harvested in the proper time, sori were numerous but scattered within the prevalent white tissues (Fig. 3). All tissues in a gall formed by the T strain were full of sori as soon as the gall was initiated (Fig. 4). Most sori measured 1×5 to 6 mm, but some attained a length of 18 mm (Fig. 13). Teliospores were brownish black (rarely brown), spherical, thick walled, and spiny, and measured 5–6 μ m in diameter.

Tissue isolation.—Isolation from tissues confirmed the distribution of the fungus observed in the histological studies. Sections of tissues taken from near the apical meristem, gall, and stem gave rise to the slimy colonies (masses of sporidia) of the pathogen 10–20 days after isolation. The slimy colony was at first whitish-yellow, later turning brown (Fig. 15). Occasionally hyphae spread out from the tissue sections and eventually covered the slimy colonies. The sporidia are filiform, mostly two to three septa. Some sporidia had no septum; the longer sporidia had up to five septa. They are measured 15–220 μ m long, with 92 μ m average, and 2.5 μ m for the widest part. Budding from septa usually was observed (Fig. 16). The frequency of isolation was 100% from tissue near the apical meristem and gall tissues, and 30–40% from stem tissues. No colonies of the fungus developed from leaf and root tissues.

The fungus was not isolated from flowering plants without galls. A teliospore suspension obtained from galls and a sporidial suspension obtained from the isolated colony were smeared on one or two eye cuttings obtained from flowering plant specimens. The inoculated cuttings were planted in a pot. Galls formed in May (4 mo after the inoculation) in all six and 20 cuttings, respectively, for two separate tests. Tissue sections except leaf and root tissues taken from plants with galls yielded slimy colonies. None of the 20 cuttings not inoculated formed galls. Instead, they bloomed again. The results of all attempts to isolate the fungus from plants not inoculated were negative.

DISCUSSION

Many *Ustilago* spp. are meristematic invaders. The hyphae usually concentrate in the apical dome, but keep a small distance from the growing point. After a period of interaction, sorus formation takes place. Forms of sori differ according to the host-parasite combination. In loose smut of wheat (1), barley (9), and millet (11), most organs of the spikelet are replaced by teliospores. In culmicolous smut of sugarcane, the apical dome is transformed into a whiplike appendage (4). In *Z. latifolia*, the gall is formed only from the first three to four nodes beneath the apical meristem, and the growing point remains normal. Growth of the plant ceases for a while

after the gall formation is initiated. Limited leaf expansion, however, takes place from the apical meristem of galls that either remained in the field or were harvested and stored for 2 wk (5). No other kind of host-smut combination seems to behave as *Z. latifolia* in regard to the type of gall.

Gall development and formation of teliospores of *U. esculenta* have much in common with the behavior of *U. crameri* in millet (11) and *U. nuda* in barley (8). The formation and maturation of teliospore in water shoot and in the barley inflorescence is centrifugal (7), but it is centripetal in sugarcane smut (4).

The physiology of water shoot formation is under study. Rarely is a diseased plant of special value to farmers of many places. This may well be an added impetus for more attention to be given water shoot for further study.

LITERATURE CITED

- BATTS, C. C. V., and A. JAEATER. 1958. The development of loose smut (*Ustilago tritici*) in susceptible varieties of wheat, and some observations on field infection. *Trans. Br. Mycol. Soc.* 41:115-125.
- HENNINGS, P. 1895. Neue und interessante Pilze aus dem Koniglich Botanische Museum in Berlin III. *Hedwigia* 34: 10.
- JOHANSEN, D. A. 1940. *Plant microtechnique*. McGraw-Hill, New York. 523 p.
- LEU, L. S. 1971. Culmicolous smut of sugar cane in Taiwan. II. Pathological histology of diseased cane. *Plant Prot. Bull. (Taiwan)* 13:6-9.
- LEU, L. S., H. C. YANG, and S. K. SUN. 1977. Preservation of wateroot, a crown gall of *Zizania latifolia* induced by *Ustilago esculenta*. *Plant Prot. Bull. (Taiwan)* 19:238-244.
- SAWADA, K. 1916. Descriptive catalogue of the Formosan fungi. [Japanese] *Spec. Bull. No. 19. Gov. Agric. Exp. Stn., Formosa* 1:319-326.
- SHINOHARA, M. 1973. Anatomical studies on barley loose smut (*U. nuda* (Jens.) Rostrup). III. Developmental observations of the fungus in the tissue of the seedling plants growing from the infected embryos. [Japanese] *Bull. Coll. Agric. Vet. Med., Nihon Univ.* 30:46-60.
- SHINOHARA, M. 1973. Anatomical studies on barley loose smut (*U. nuda* (Jens.) Rostrup). IV. Cytological observations of the smut spore formation processes in the young spikelet. [Japanese] *Bull. Coll. Agric. Vet. Med., Nihon Univ.* 30:61-75.
- SHINOHARA, M. 1973. Anatomical studies on barley loose smut (*U. nuda* (Jens.) Rostrup). V. Characteristic symptoms and anatomical observations of the adventitious young ear, the ratoon, from the smutted adult plant. [Japanese] *Bull. Coll. Agric. Vet. Med., Nihon Univ.* 30:76-84.
- SU, H. J. 1961. Some cultural studies on *Ustilago esculenta*. *Coll. Agric. Nat. Taiwan Univ. Spec. Publ.* 10:139-160.
- WANG, C. S. 1943. Studies on cytology of *Ustilago crameri*. *Phytopathology* 33:1122-1133.