

Effect of Temperature and Some Substrate on Teliospore Germination of Sugarcane Smut and Inoculation Methods on Infection by *Ustilago scitaminea*

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ABSTRACT

Temperature for teliospore germination of *Ustilago scitaminea* was ranging from 15 to 40 C and 15 to 35 C for mycelial growth, with 25 C for optimum germination and growth. Teliospores germinated within 3.50, 6.10, 4.00 and 4.50 hr after incubation in extracts of sugarcane bud, soil, sugarcane leaf and sugarcane root, respectively. Sugarcane bud showed highest percentage germination (20.16%) at 6 hr incubation. Teliospores germinated well in 1.5% dextrose and sucrose substrate and the fungus grew well on potato dextrose agar, V-8 juice agar, malt extract agar and glucose peptone agar at room temperature (30 ± 4 C) when inoculated with teliospores.

Six methods of artificial inoculation techniques were studied in both greenhouse and field conditions. The best method was injected weekly for 5 weeks with 2 ml teliospore or sporidial suspensions ($10^6 - 10^7$ spores/ml) into one week old shoot.

INTRODUCTION

The first report on smut disease of sugarcane caused by *Ustilago scitaminea* Syd. & P. Syd., characterized by a long whiplike sorus-bearing structure that developing from the apex of infected stalks was a serious disease in Thailand in 1963 – 1964 (Martin, 1964). The teliospores are formed only in the peripheral tissues of the whiplike structure while affected plants are stunted, produce slender and thin cane with widely spaced nodes (Chiengkul et al, 1980; Lee-Lovick, 1978; Martin, 1964). The Losses form the disease usually increase in stubble cane and it was up to 40 percent of the plants infected on our survey of the sugarcane disease in commercially cultivated areas last year. Several reports described the influence of temperature and humidity on teliospore germination (Appalanarasayya, 1964; Martin, 1964; Saxena and Khan, 1971) and the longevity of teliospores in small vial (Chona, 1956; Saxena and Khan, 1964). Teliospores germinated greater in the presence of 1.5 percent sucrose solution

(Lombal et. al, 1966; Saxena and Khan, 1971) particularly mannite and lactose but lesser in fructose (Lambal et al, 1966). The effect of host diffusates was also reported that the sugarcane bud extract increased teliospore germination (Saxena and Khan, 1971).

Teliospores of the causal agents are the principal inoculum that contributes to smut epidemics. The influence of temperature and some substrate on their germination and growth of the fungi that can limit the rate and extent of disease development.

The most suitable teliospore concentration was reported on the different methods of inoculation for disease development (Bock, 1964). The most effective method of inoculation with spore suspensions was the bud periphery pricked with needle before rubbing (Nasr, 1977), the cuttings pricked before soaking (Nasr, 1977; Towiriyawech, 1979) and young plants injected (Chiengkul et al, 1980). The report also described the method of buds injured before soaking gave more effective infection

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than young plants injected with teliospore suspension (Chiengkul et al, 1980).

The purposes of this research were to study : 1). The effects of temperature on germination and growth of the fungus teliospores 2). The influence of some substrate on their germination and 3). Inoculation method was also conducted for maximum disease development in the testing of sugarcane varieties for resistance to smut caused by *U. scitaminea* under environmental factors in both greenhouse and field conditions.

MATERIALS AND METHODS

Teliospores used for studies were collected from the middle of unopened smut whips grown in the commercial plantation and stored in small vials less than one month of age at room temperature ($30 \pm 4^\circ\text{C}$).

Plant material. One bud cutting piece of sugarcane variety Q 82 (about 1, 260) was used in this study for comparing inoculation techniques on infection by teliospores and sporidia of *Ustilago scitaminea*. The cutting pieces were treated in hot water at 52°C for 20 min to get disease free as described by Joshi (1954) before using. The 19 inch-diameter pots containing autoclaved soil (Bangkok clay series) were used for growing the plants under ordinary greenhouse conditions. The field trial was made in the experimental plots of department of plant pathology, Kasetsart University, Bangkok Campus, Bangkok 10900.

Effects of temperature on teliospore germination and fungus growth. Teliospore suspension was prepared on distilled water (0.1 g/10 ml) in 9.0 cm diameter petri dish. Each of 4 plates were incubated at eight different temperatures (10, 15, 20, 25, 30, 35, 40 and 45°C). Plates were kept in sealed plastic bags to obtain the moisture. To determine the effect of temperature on teliospore germination made by mounting them on slide with one

drop of medicine dropper after 12 and 24 hr-incubation.

The centers of 9-cm diameter petri dishes containing 20 ml of PDA (200 g potato, 10 g dextrose and 18 g of agar per litre of distilled water) were inoculated with the hyphal tip from 7-to 10-day-old cultures of *Ustilago scitaminea* On PDA for growth studies. Each of 5 plates were incubated at 10, 15, 20, 25, 30, 35, 40 and 45°C . Radial growth was measured after 20 day.

Effects of some nutrients on teliospore germination and growth. The weight of 0.1 g teliospores were seeded in petri dish containing 20 ml of nine different media and water agar as control. They were PDA, V-8 juice agar (200 ml V-8 juice, 2 g CaCO_3 and 18 g agar per litre of distilled water), soil extract agar (3 g malt extract, 2 g yeast extract, 0.5 g KH_2PO_4 , 0.5 g MgSO_3 and 18 g agar per litre of distilled water), Czapek's solution agar (3 g NaNO_3 , 1 g K_2HPO_4 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g KCl, 0.01 g $\text{FeSO}_4 \cdot \text{H}_2\text{O}$, 30 g Sucrose and 18 g agar per litre of distilled water), corn meal agar (50 g corn meal and 18 g agar per litre of distilled water), glucose peptone agar (10 g glucose, 2 g peptone, 0.5 g KH_2PO_4 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 18 g agar per litre of distilled water), yeast extract peptone agar (10 g yeast extract, 10 g peptone, 0.5 g KH_2PO_4 and 18 g agar per litre of distilled water) and yeast extract peptone agar added with trace elements (trace of KI, MgSO_4 , FeSO_4 and MnCl_2). Each of 4 plates were prepawed and kept in room temperature ($30 \pm 4^\circ\text{C}$). The growth density of mycelia produced was determined after 3 and 5 days.

Effect of some natural substrate on teliospore germination. Fresh sugarcane buds, leaves and roots were blended with distilled water (10 g/ 100 ml) for 30 sec at low speed in a Waring Blender. The extract was squeezed from the mixture through cheesecloth

and filter paper No. 2 for 2-fold. Soil from the field of sugarcane plantation (Bangkok clay type) was used for extract similarly (2.5 g/100 ml). All extracts were freshly prepared before use.

To determine percentage of germination 0.1 g teliospores were placed on 10 ml extracted in 9.0-cm diameter petri dish and incubated at room temperature ($30 \pm 4^\circ\text{C}$). The germination was determined on slide as described above and recorded after 6, 12, 24 and 48 hr-incubation. Incubation period of teliospore germination was also observed after incubation.

Effect of various concentrations of some substrate on teliospore germination. Solutions of carbon sources (dextrose, fructose, lactose, sucrose and mannitol) and amino acids (glycine, glutamic acid and phenylalanine) were used for study the stimulating on germination of teliospores at four various percentage of concentrations (0.5, 1.0, 1.5 and 2.0). Each of 4 plates were seeded by teliospores on the solution in 9.0-cm diameter petri dish (0.1 g/10 ml). They were incubated at room temperature ($30 \pm 4^\circ\text{C}$) for observation after 6 and 12 hr. The method of observation and in incubation study were made as mentioned above.

Six inoculation techniques on infection. The trial was conducted in both greenhouse and field conditions with randomized block design in three replications. Two sets of 450 pots were used for inoculation techniques by teliospores and sporidial inocula (300 soil pots for planting inoculated pieces and 150 healthy plant pots with one week old) under greenhouse studies.

Teliospore and sporidial inocula were used in the concentration of 10 g in 1 litre of distilled water about 10^6 – 10^7 spores per ml as used in Bock's experiment (1964) for all methods in this study. Teliospores were collected from the method as mentioned before, but spordia were produced in teliospores

seeded on PDA after 20 days of incubation at room temperature ($30 \pm 4^\circ\text{C}$).

Greenhouse. Each set of seventy pots were placed in each replication. Each treatment of 10 pots was inoculated by different methods used as follows:

1. Cutting soaked in spore suspension for 24-hr before planting.

2. Cutting injured with needle prickle at bud periphery and soaked in spore suspension as mentioned above before planting.

3. Hypodermic injection with 2 ml spore suspension at growing point regions weekly for 5 weeks.

4. Rolled top leaf tube dropped with 1 litre spore suspension weekly for 5 weeks.

5. Buds rubbed with spore suspension (5 g/10 ml) before planting.

6. Soil mixed with fungus spores (10 g/pot soil).

Field condition. The methods of inoculation tested were made as the procedures under greenhouse studies. Plot size was $15.5\text{m} \times 3.0\text{m}$ and a spacing of 0.5 m interval of 5 rows was maintained. Thirty budded cutting pieces were planted in each row of 15.5 m. Five plants between treatments were used as control.

RESULTS

Effects of temperature on teliospore germination and fungus growth. Teliospores of *U. scitaminea* germinated over a wide range (15–40 °C) of temperature with the optimum at 25 °C (between 20 and 30 °C) as shown in Table 1. The highest temperature (40 °C) tested was less favorable for teliospore germination than was the lowest temperature (15 °C). Incubation of teliospores at 15 °C delayed germination in comparison with 20–35 °C.

Optimum radial growth (5.0 cm after 20 day) was occurred on PDA with the optimum temperature of 25 °C. After incubation for 20

days, there were not much differences between 25 and 30 C.

Effects of some nutrients on teliospore germination and growth. Teliospores germi-

nated and grew well on PDA, V-8 juice agar, malt extract agar and glucose peptone agar at room temperature (30 ± 4 C) after 3 and 5 day incubation (Table 2).

Table 1 Effects of temperature on teliospore germination of *Ustilago scitaminea* in distilled water and on fungus growth on potato dextrose agar

Temperature (C)	Germination (%) after plating spore ^{a/}		Radial growth on PDA ^{b/} after 20 days (cm in diameter)
	12 hr	24 hr	
10	0	0	0
15	11.2	15.4	2.1
20	31.6	35.1	3.2
25	<u>33.4</u>	<u>39.5</u>	<u>5.0</u>
30	32.8	35.8	4.5
35	25.4	27.4	4.0
40	8.6	10.5	0
45	0	0	0

^{a/}Each value represents mean of 4 replications and average from 400 spores

^{b/}Average of 5 replications

Table 2 Effects of some nutrients on teliospore germination and growth of *Ustilago scitaminea* at room temperature (30 ± 4 C) after 3 and 5 days

Nutrient	Mycelial growth after incubation ^{a/}	
	3 days	5 days
Potato dextrose agar	+++	++++
V-8 Juice agar	++	++++
Soil extract agar	0	++
Malt extract agar	++	++++
Czapek's solution agar	+	+++
Corn meal agar	0	++
Glucose peptone agar	++	+++
Yeast extract peptone agar	+	+
Yeast extract peptone and trace elements agar	+	+
Water agar	0	+

^{a/}Degrees of growth density: 0 = No growth; + Very slightly; ++ = Slightly; +++ = Moderately; ++++ = Abundantly

Effect of some natural substrate on teliospore germination. The highest germination of teliospores on substrate tested was the sugarcane buds extract.

The greater germination of teliospore in the solution of sugarcane buds extraction was found by increasing the time incubation

(Table 3). The shortest incubation period of germination was also observed after 3.50 hr incubation. There were not much difference in germination by using the extracts of soil from sugarcane plantation, sugarcane leaves and sugarcane roots when compared with distilled water as substrate.

Table 3 Effect of some natural substrate on teliospore germination of *Ustilago scitaminea* at room temperature (30 ± 4 C)

Substrate (extracted)	Incubation period (hr)	Germination (%) after incubation ^{a/}			
		6	12	24	48
Sugarcane buds	3.50	20.16	39.38	46.06	49.19
Soil from sugarcane plantation	6.10	0.80	13.60	27.00	29.64
Sugarcane leaves	4.00	1.13	13.63	28.56	31.94
Sugarcane roots	4.50	0.99	1.72	23.06	33.69
Distilled water	6.20	3.13	16.19	21.69	32.69

^{a/} Each value represents mean of 4 replications and average from 400 spores.

Effect of various concentrations of some substrate on teliospore germination. The teliospores germinated very well on carbon source solutions and less for their germination on nitrogen source solutions (Table 4). The highest percentage of germination was sucrose substrate, but none in phenylalanine. There are not much differences on germination of teliospores that was influenced by the concentration of substrate. The shortest incubation period on the germination was found on dextrose substrate. There are also no evidence of any different concentrations of substrate concerned.

Germination of teliospores in the presence of carbon source solution was higher than in the nitrogen source solution. No effect of various concentrations of substrate were used on germination of teliospores. In this study, sucrose was found as very important substrate. The percent of germination in the presence of sucrose was highest but none in phenylalanine

substrate. The shortest incubation period on the germination was found in the presence of dextrose substrate.

Six inoculation techniques on infection. The result of this study was shown in Table 5. Teliospores of *Ustilago scitaminea* seem to be more effective than sporidia when used as inoculum for all inoculation studies. Hypodermic injection with teliospore or sporidial suspensions at the growing point region gave excellent infection (100%), 2 months after inoculation in both greenhouse and field conditions. The external leaf sheath and the growing point region of infected plants became brown on the 7th day of inoculation followed by crinkle and death of shoot. At this stage, one or two lateral buds may shoot out. By the end of disease development (2 months after inoculation), small and narrow leaves were formed with the swellings of young leaf sheath forming slightly raised galls. They were covered with a silvery-white

Table 4 Effect of various concentrations of some substrate on teliospore germination of *Ustilage scitaminea* at room temperature (30 $\pm$ 4 C)

Concentration (%)	Germination (%) in substrate solutions after inoculation (hr)																							
	Dextrose			Fructose			Lactose			Sucrose			Mannitol			Glycine			Glutamic acid			Phenylalanine		
	IP ^a /			IP			IP			IP			IP			IP			IP			IP		
	6	12		6	12		6	12		6	12		6	12		6	12		6	12		6	12	
Distilled water	5.6	1.6	30																					
0.5	2.3	47	49	3	43	43	4	20	22	3	54	55	6	1	4	6	1	1	6	1	3	0	0	0
1.0	2.0	64	68	3	45	45	3	30	34	3	67	77	6	1	8	6	1	2	6	1	5	0	0	0
1.5	2.3	73	72	2	57	57	3	26	25	2	63	70	6	1	5	6	1	1	6	.3	8	0	0	0
2.0	2.0	55	60	2	66	66	4	21	28	2	35	48	6	1	3	6	1	1	6	.3	4	0	0	0
Average	2.2	60	62	3	53	53	4	24	27	3	55	63	6	1	5	6	1	1	6	1	5	0	0	0

^a/Incubation period of teliospore germination (hr)

Table 5 Comparison of 6 inoculation techniques on smut infection by spore suspensions, *Ustilago scitaminea* on sugarcane variety Q 82 under greenhouse and field conditions

Inoculation method	Source of inoculum			
	Teliospore		Sporidia	
	Infected plant (%)		Infected plant (%)	
	Greenhouse	Field	Greenhouse	Field
Cuttings soaked	36.67(11) ^a	25.00(15)	20.00(6)	5.00(3)
Cuttings injured and soaked	40.00(12)	35.00(21)	16.67(5)	10.00(6)
Hypodermic injection at growing point region	100.00(30)	100.00(60)	100.00(30)	90.00(54)
Rolled top leaf tube dropped	10.00(3)	15.00(9)	0	0
Buds rubbed	3.30(1)	10.00(6)	0	10.00(6)
Soil mixed	0	5.00(3)	0	.0
Control	0	0	0	0

^a/Figures in parentheses represent numbers of plants infected

membrane which ultimately flaked off exposing masses of dark powdery teliospores.

From the sixed methods of inoculation studied, the hypodermic injection method was considered to give the best result followed by the cutting injured before soaking, the cutting soaked, the rolled top leaf tube dropped, the buds rubbed and soil mixed, respectively.

DISCUSSION

In the experiments presented above, the optimum temperature for germination of teliospores was 25 C as reported by Lambal et al. (1966), Appalanarasayya (1964) and Towiriyawech (1979). The most favorable temperature range for germination was 20 – 30 C that was different from the report of Saxena and Khan (1963) with 25 – 30 C. The result was different on wider range of temperature depending on the collection, locality, longevity and variety of sugarcane as reported by Singh, K. et al. (1966) on the variation in the longevity of teliospores. The relative humidity might get involved on germination because the teliospores could

not germinated if the relative humidity lower than 100 percent (Saxena and Khan, 1964).

Studies on the teliospore germination in different substrate and different concentrations, the best substrate was sucrose (63% germination) when compared with distilled water (30% germination). The teliospore germinated in dextrose solution as well as fructose that was agreed with Lambal et al. (1966) and Towiriyawech (1979). The carbon compounds gave more effective germination than distilled water and the nitrogen compounds tested. This was assumed that the carbon compounds were not necessary needed for the germination of teliospores as exogenous sources except for increasing germination.

The highest germination of teliospores on natural substrate was sugarcane buds extract to be similar to Saxena and Khan (1971) that sugarcane bud extract increased teliospore germination. The result showed much differences among substrate in their germination. It might be the stimulation of some growth substances presented in the bud tissue.

From our data indicated that the most suitable method for infection of sugarcane by *U. scitaminea* was hypodermic injection at growing point region in contrast to the reports of Nasr (1977), Towiriyawech (1979) and Chiengkul (1980). This might be differences in the collection and longevity of teliospores, sugarcane variety and environmental conditions.

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