DETECTION AND CONTROL OF *Tilletia* spp. ON WHEAT FROM LEBANON AND SYRIA

by

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BUNT OF WHEAT

Abu-Shakra

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ABSTRACT

In the summer of 1957, a study was made of *Tilletia* spp. on 20 wheat samples obtained from villagers in the Bekaa Plain and flour mills, located in Beirut, which import wheat from Syria for milling purposes. Seed treatment tests, spore load and morphological identification of the spores of *Tilletia* were done.

In 1958, five lots of wheat were used, namely Florence Aurore, Hourani, Mishrikani and Senator Capelli, which are commonly grown in Lebanon and Syria, plus an imported Cyprus variety B x IPI all of which were free from bunt and hence were artificially infested with spores of *Tilletia* for seed treatment and yield tests. In addition a set of 50 varieties local and newly introduced to the area were used as a study of smut resistance to species of *Tilletia*.

Among the 20 lots of wheat that were used in the seed treatment tests, the percentage of smutted heads in the 10 samples of Part "A" ranged from 11.7 to 70.0 and 4.6 to 49.4 percent in the remaining 10 samples of Part "B". The fungicides Panogen 15, Setrete, Mema 4, Anticarie and Phygon at the two rates of application gave good control of the smut. Granosan M in Part "A" allowed 4.4 percent smut when applied at the rate of

50 mg and only 0.5 at the rate of 75 mg per 100 g of seed.

Samples with heavy and light spore load were respectively high and low in the percentage of smutted heads.

The results of the seed treatment tests of the year 1958-59 indicated a low percentage of smutted heads in the non-treated, and practically nill in the treated. Such results were due to variation in resistance in the varieties used plus the inadequate rainfall in December after the infested seeds were planted.

No reduction in the germinability of the seed resulted from any of the chemicals used, except with the variety Mishrikani when treated with Phygon at the rate of 400 mg per 100 g of seed.

Estimates of losses from bunt based on the percentages of smutted heads was at least 4 to 16 percent among the varieties Hourani, Mishrikani, Senator Capelli and B x IPI and practically none in the variety Florence Aurore.

Among the 50 varieties used in the resistance test the percentage of smutted heads ranged from 0.0 to 42.3 which suggests the possibility of selecting smut resistant types adapted to the Middle East.

A mixture of _Tilletia foetida_ and _T,_ _Caries_ was cound in the 20 Lebanese and Syrian wheat samples that were used in the tests of 1957-58.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

In the fall of 1956, when the writer was beginning his senior year as a student in the School of Agriculture, American University of Beirut, one of the requirements was that a particular problem be selected for study on which a report was to be made at the close of the academic year. An application was made to the Professor of Seed Technology for aid in selecting a problem. It was learned that in the summer of 1956 the Seed Technology Section had obtained a number of samples of cereal grains from villagers near the University Farm in the Bekaa Plain. Among them were several lots of wheat to be used in teaching seed technology and also for an examination as to the presence of seed-borne organisms. Three of the samples were infested with stinking smut which was of interest because the Lebanese Ministry of Agriculture regularly imports seed wheat for distribution to farmers and treats it before any lots are released for planting. In addition to the lots supplied by villagers a collection of wheat samples was obtained from a flour mill which regularly imports wheat from Syria. These were examined and found to be heavily infested with spores and smut balls of stinking smut.

Two sets of experiments were undertaken (1) a study of Syrian samples for the problem required by the curriculum and (2) a study of Lebanese and Syrian samples to be used as a part of a research problem by Professor R. H. Porter in charge of the Seed Section. A report of the latter study is given in Citation No. (42) in the Bibliography and referred to frequently in this thesis. The first study was made with 10 Syrian samples and a report was prepared and approved. Reference to this report is made in the section on "Effect of Stinking Smut on Yield of Wheat".

The results of these 2 initial studies indicated that stinking smut is a serious problem in Syria and reports from Iran and Iraq gave evidence of a similar situation in those countries. In addition the discovery of stinking smut in farmer's samples in Lebanon was of sufficient interest to warrant a study of stinking smut by the School of Agriculture using such materials as were available. Consequently, the writer chose this problem for a thesis as part of the requirements for a Master's Degree.

Beginning in the summer of 1957 a second series of experiments was undertaken with wheat samples infested with stinking smut using different dosages of fungicides. In 1958 the experiments were continued but expanded to include (1) a study of the effect of stinking smut on yield of wheat and (2) the reaction of 50 different varieties or selections to the smut pathogen. In addition a microscopic study of the species of Tilletia found on wheat in Lebanon and Syria was included.

REVIEW OF THE LITERATURE

Bunt or stinking smut of wheat is one of the first plant diseases that was observed by ancient people. Woolman and Humphrey (60) state that "without question it was known to Theophrastus, as well as to Virgil, Pliny and other ancient Romans". It was probably the first smut disease to attract attention. In 1733 Tull (22) described smuttiness as a condition in which the wheat grains are full of a black stinking powder. Tillet (56) in 1755 differentiated between stinking smut (la carie) and loose smut (le charbon). He also proved that the disease was the result of contaminated seed with the black dust as the true agent but he did not mention the origin and nature of this dust. Tessier (22) in 1783 considered this black dust a degeneration of the grain, a concept which was accepted for a long time probably due to the bad odor that comes from the diseased grains. It was not until 1807 that Prevost (14) discovered that a fungus was the primary cause of stinking smut and in 1858 Kuhn (9,22) demonstrated the penetration of the fungous mycelia into wheat seedlings. Since then bunt has received more attention by investigators and especially so following Brefeld's detailed experiments on spore germination (22). In the numerous contributions about the disease that appeared later on, the main emphasis was on means of control. Before the nature of the causal agent was discovered salt brine, lime, wood ashes and other materials were tried as a means of control (56). In 1866 Khun (22) discovered the toxicity of copper sulfate on spores of bunt. Jensen (22) in 1888 recommended the use of hot water, and a few years later formaldehyde and copper sulfate were introduced to control bunt. Even though these latter fungicides were effective in the control of the disease they caused much seed injury. In 1917 Darnell-Smith (38) introduced copper carbonate which caused less seed-grain injury. Numerous organic-mercury dusts later became available as seed disinfectants. Ethyl mercury chloride (Ceresan) was the first adopted mercury seed disinfectant followed by volatile mercuric compounds such as ethyl mercury phosphate (New Improved Ceresan). The volatile compounds proved more effective in killing smut spores than non-volatile compounds. Recently liquid mercury compounds have been developed such as Panogen and Setrete.

Bunt of wheat is prevalent in almost every area where wheat is grown. It is however, less common in warm areas than in the cold humid ones - for example in southern and eastern parts of India and in the south part of the United States. Epiphytotics occurred in Nebraska and Kansas in years when the temperature was low during the seeding time (22). Rodenhiser and Holton (49) found Tilletia foetida to be more prevalent in the northern humid areas of the United States than T. caries, while the latter is more common in dry areas and namely in wheat areas west to the Rocky mountains.

Recently in 1956 Fischer and Duran (15) reported a new record of the incidence of the dwarf bunt from all over the world. According to the report Tilletia caries was discovered around the middle of the last century in Europe, 1890 in the United States (New York State), and in 1908 in West Persia.

Bunt of wheat causes serious losses in wheat growing areas all over the world. Losses are mainly due to (1) reduction in yield (2) lowering of the grain quality (3) fire hazards and (4) extra costs resulting from seed treatment, the production of bunt-resistant varieties, washing the grain before milling and adoption of certain cultural practices as preventive measures (9,14,22).

Smutted heads with all grains completely destroyed by the fungus cause a loss roughly estimated to be equal to the percentage of smutted heads in the crop (31). According to Heald and Woolman 1915 (23) stinking smut of wheat was the most destructive disease in the wheat areas of the Pacific north-west of the United States. They estimated the sum total of losses ~~in~~ many millions of dollars. Tisdale et al (57) estimated losses from bunt in the United States for the period 1917 to 1924 by an average of 14,000,000 bushels per year. Gaines' (16) estimate of losses from bunt in 1926 alone is $40,000,000. In 1918 Ehrenberg (11) reported a severe epiphytotic of bunt in Germany. In the fields of Denmark, Gram and Rostrup (18) in 1922 reported up to 78.6 percent bunt.

Winter wheat varieties suffer most from bunt. With the use of seed treatment, losses from bunt in 1950 in the Pacific North-west were estimated to have been reduced to 10 million dollars (31). As reported by Hanna and Popp (20) in 1929 losses from the reduction in grain quality only, in western Canada, was estimated at $400,000.

Price and McCormick (44) reported losses from fire explosions in southern Washington in 1916 at $100,000, including machinery and grain. Cardiff et al (7) concluded that such fire explosions are the result of static electricity which ignites the accumulating smut dust in the combining machines. Smut spores may contain up to 4 percent oil and about the same percentage of water. Such a composition of the spore is the important factor in the inflamability of the smut dust.

Bunt infection results in a number of morphological and physiological modifications of the wheat plant. The most evident symptoms are shown in the heading stage. Just before the emergence of the heads from the boot, a microscopic study of the florets has shown a deviation of the infected florets from the normal ones. Barrus (3) noticed that the bunt infected florets have larger ovaries and longer pistils than the healthy ones. The ovaries of the smutted florets were green instead of white under normal conditions. The diseased florets have the stamens reduced in length and width with pale yellow anthers in contrast to the healthy green ones.

With respect to the pollen grains of the bunt-infected flowers, Barrus stated that such grains contain little or no protoplasm.

After emergence from the boot, infected heads are deep bluish green in contrast to yellowish green in the non-infected heads (3,31). Potter and Coons reported that spikes infected with Tilletia caries look enlarged and crowded in contrast to the healthy ones, while heads infected with Tilletia foetida look slender and rather open. As to the shape of the sori (smut balls), Potter and Coons stated that sori of Tilletia caries are rounded while those of T. foetida are elongated. Other investigators (1,12,25) reported that the shape of the sori is influenced by the wheat variety as well as by the physilogic races of the species of Tilletia.

Barrus (3) 1916, Kajanus (33) 1923 and Milan (40) 1933 reported that there are more smut balls in infected spikelets than there are seeds in non-infected spikelets. This symptom was also noticed by Fischer and Holton (14) in 1957 on wheat, in the Pacific Northwest, infected with dward bunt Tilletia controversa.

A wheat plant may be completely or partially infected as are individual heads and seeds. In the dough stage the smutted grains are filled with a black soft pastry mass. As the green heads mature, this mass changes into a brown to black oily powder after it has occupied the whole seed inside the pericarp. Smutted fields can be detected from the stinking odor of the trimethylamine which the spores contain. During threshing some of the "smut balls" break spreading the spores to the grains which are then dark in color. Combining the wheat heavily infected with bunt produces "smut showers" that contain clouds of smut spores. On windy days such "smut showers" may spread to fields that are miles away (9,14,22).

Potter and Coons (43) in 1918 reported that Tilletia Foetida and T. caries induce dwarfing in the plant but the latter species does this to the greater extent. Following that report the bunt caused by Tilletia foetida was called common or high bunt and that caused by T. caries dwarf or low bunt. Further studies by Rodenhiser (48) 1931, Bressman (5) 1932 and Aamodt et al (1) 1936 did not confirm clear differences between the two species of Tilletia. In 1953 Holton and Tapke (31) said that dwarfing depends on host variety, the fungus itself and the growing conditions. Not long ago Tilletia controversa was discovered in the United States. This discovery has thrown some light on the dwarfing exhibited by the host. Fischer and Holton (14) summarize this principle in that Tilletia foetida causes little, T. caries moderate and T. controversa maximum stunting.

Bunt caused by Tilletia caries has been observed to produce excessive tillering in wheat plants. In general the degree of shooting is influenced by the growing conditions, host and race of the species of Tilletia (29).

Sampson and Davies (51) in 1927 and Hely et al (24) in 1938 reported that bunt-infected wheat plants suffer a reduction in root development.

In 1934 Angell (2) found that seeds infested with Tilletia spp. produced twisted and curled seedlings in certain wheat varieties.

Fischer and Holton (14) 1957 reported the appearance of mottling and chlorotic striping on wheat leaves infected with Tilletia controversa.

According to Zade (61) latent or invisible infection is observed in resistant varieties. Even though there is no sporulation in the heads, this kind of obscure infection results in loss of vigor and yield.

In 1917 Lang (35) noticed that seed of varieties resistant to stripe rust, when infested with spores of Tilletia sp. produced susceptible plants. Lang's observation has been repeatedly confirmed by other investigators (10,55,59) who worked on this problem. Angell (2) in 1934 and Hanson (21) in 1946 reported that bunt infected seedlings were more susceptible to seedling blight and root rot caused by species of Helminthosporium and Fusarium than were those not infected. On the other hand, Sempio (53) in 1938 recorded the observation of an increase in resistance to powdery mildew (Erysiphe graminis) of wheat plants infected with Tilletia caries.

According to the results obtained by Holton and Heald (27) in 1936 winter injury is increased with Tilletia caries and T. foetida. Schlehuber (52), using freezing rooms, recorded that winter hardiness decreased in plants infected with species of Tilletia.

Stinking smut of wheat is caused by three related species, namely (1) Tilletia foetida (Wallr) Liro (T. levis), (2) T. caries (D.C.) Tul. (T. tritici) and (3) T. controversa Kuhn (9,14). The way this fungus infects the wheat plant was first demonstrated by Kuhn (34) in 1874. He found that the infection hyphae gain entrance thru the coleoptile of the young seedling. According to the numerous contributions by plant pathologists and other investigators in the field of plant science the life cycle of the organism may be summarized as follows: Viable spores in the proximity of the seed in the soil, whether carried on the seed or found in the soil, will germinate under favorable conditions to produce a promycelium with 4-12 H-shaped sporidia at the free end. These sporidia will produce infection threads that penetrate the susceptible seedling to grow systemically in the plant with little or no external symptoms until the time of heading to maturity. The organism attacks the ovaries changing them into smut balls each full of millions of spores. During threshing a large percentage of these smut balls break open, part of the spores spread on the kernels and a large portion is blown by the wind to infest lands in the neighbourhood.

Common bunt caused by Tilletia foetida and T. caries is dependent to a large extent on seed-borne inoculum. Exceptions to this condition are in regions of the Pacific

Northwest where the soil gets a tremendous amount of "smut showers" during threshing operations (14). According to Holton et al (30) 1949, soil infestation with smut spores of *Tilletia controversa* is almost the main source of infection to wheat seedlings.

Species of *Tilletia* were found to attack grasses other than wheat. In 1923 Gaines and Stevenson (17) reported *Tilletia tritici* on rye (*Secale cereale*). With reference to the kinds of wheat that are susceptible to *Tilletia* spp. Heald (22), in the Manual of Plant Diseases listed the following: *Triticum vulgare*, *T. compactum*, *T. turgidum*, *T. durum*, *T. polonicum*, *T. monococcum*, *T. spelta* and *T. dicoccum*. In addition to wheat as a host Butler and Jones (6) 1949 reported *Agropyron* and *Lolium* spp. as susceptible. In 1953 Fischer (13) listed a separate host range for each species of *Tilletia*. The different species that he used are *Tilletia foetida*, *T. caries* and *T. brevifaciens* later designated as *T. controversa* (Fischer and Holton 1957). Fischer's lists of susceptible hosts to *Tilletia* spp. are conveniently tabulated as follows:

Susceptible host	*T. foetida*	*T. caries*	*T. brevifaciens*
Agropyron cristatum		+	
A. intermedium			+
A. subsecundum			+
A. trachycaulum		+	
Arrhenatherum elatius			+

Susceptible host	*T. foetida*	*T. caries*	*T. brevifaciens*
Bromus rigidus		+	
B. tectorum		+	
Festuca rubra		+	
Secale cereale	+	+	+
Triticum aestivum	+	+	+
T. dicoccum	+	+	
T. durum	+	+	
T. spelta		+	

Meiners and Hardison (39) in 1957 reported a new record of susceptibility for *Tilletia controversa* in the Pacific Northwest on the following grasses: *Agropyron amurense*, *A. dasystachyum*, *A. repens*, *A. riparium*, *Elymus glaucus*, *E. triticoides*, *Festuca idahoensis* and *Koeleria cristata*.

MATERIALS, METHODS AND PROCEDURES

The wheat samples used in the studies herein described were obtained from two sources, namely (1) Lebanese villagers who live near to or a considerable distance from the Experimental Farm of the American University of Beirut located in the Bekaa Plain, and (2) flour mills, located in Beirut, which import different lots of wheat each year from Syria for milling into flour.

Each sample of wheat was examined in the laboratory to determine if "smut balls" or spores of species of *Tilletia* were present. Insofar as possible the spore load was determined for each lot by the method which will be described later. Following the initial examination each sample was subdivided into several different subsamples for treatment with different seed fungicides at different rates of application, based in part on the spore load. Samples that were bunt-free were infested with spores of *Tilletia* and then treated with different fungicides. In the 1958 experiments germination tests were made in the laboratory and about one month after the field plantings were completed, counts for germination were obtained.

Each year field plantings were made in November according to different designs using 3 or 5 replications for each treatment depending on the type of experiment. In June of each year the heads in each row were examined for smut infection and the number of infected and non-infected heads was recorded.

The trade name and the active ingredients of each compound used in the experiments herein described together with the name of the manufacturers are listed as follows:

Anticarie - 40% HCB (hexachlorobenzene) Agrochem Div. H.P. Rossiger and Co. Inc., 55 Vandam Street, New York 13, N.Y.

Granosan M - 7.7% Ethyl mercury P-Toluene sulfonanilide. E.I. Du Pont De Memours and Co., (Inc.) Grasselli chemicals Dept. Wilmington. Delaware.

Mema 4 - 4% mercury as methoxy ethyl mercury acetate. Plant Protection Ltd. Res. Sta. Fernhurst near Haslemere Surrey. England.

Panogen 15 - 2.2% methyl mercury dicyan diamide. AB Casco, Stockholm 11, Sweden, and Panogen Inc., Res. Dept., Woodstock, III.

Phygon - 50% 2,3 - Dichloro - 1,4 - Naphthoquinone 3% Dichloro-diphenyl-trichloroethane. United States Rubber Co. Rockefeller Center, New York 20, N.Y.

Setrete - 7% phenyl mercuric ammonium acetate (mercuric equivalent 4.0%). W.A. Cleary Corp. (Canada) Ltd. Belleville, Ont. Canada.

Inasmuch as the materials, methods and procedures were not the same each year, a brief discussion is given for each of two years as follows:

1. <u>Experiments in 1957-58</u>

In the summer of 1957, 5 samples of wheat were collected from different farms in the Bekaa and 15 additional samples taken from Syrian wheat lots obtained from flour mills in Beirut.

a) <u>Seed treatments</u>

On the basis of the number of smut balls present in each sample the seed treatment experiment was divided into 2 parts A and B as shown in Table 1.

Part "A" (heavy infection) included 10 samples each containing any number of "smut balls" above 50 per 100 g of seed.

Part "B" (light infection) included the remaining 10 samples each having less than 50 "smut balls" per 100 g of seed.

Only pure seed, as described in the International Rules for Seed Testing, was used in the experiments. Four fungicides were used for the samples in Part "A", namely Panogen 15, Anticarie, Granosan M, and Phygon. Two rates of application were used for each treatment as shown in Table 2. In Part "B" a check and 6 fungicides were used, namely Panogen 15, Setrete, Mema 4, Anticarie, Granosan M, and Phygon. As indicated in Table 3 only one rate of application was used for each fungicide.

Table 1. Origin, smut ball load and classification of samples used in the seed treatment test 1957-58

Sample number	Origin	Number of smut balls per 100 g of seed	Part
1	Syria	93	A
2	"	95	"
3	"	115	"
4	"	132	"
5	"	152	"
6	"	169	"
7	"	176	"
8	"	239	"
9	"	93	"
10	"	400	"
11	Lebanon	12	B
12	"	14	"
13	Syria	15	"
14	Lebanon	17	"
15	"	18	"
16	Syria	27	"
17	Lebanon	30	"
18	Syria	27	"
19	"	44	"
20	"	46	"

Table 2. Fungicidal dosage used in the treatment test. Part "A" - 1957

Fungicide	Form	Rate of application per 100 g of seed	
		First	Second
Panogen 15	Liquid	0.10 ml in 8 ml H_2O	0.14 ml in 8 ml H_2O
Anticarie	Dust	90 mg	200 mg
Granosan M	Dust	50 mg	75 mg
Phygon	Dust	300 mg	400 mg

Table 3. Fungicidal dosage used in the treatment test Part "B" - 1957

Fungicide	Form	Rate of application per 100 g of seed
Panogen 15	Liquid	0.10 ml in 8 ml H_2O
Setrete	Liquid	0.06 2/3 ml in 8 ml H_2O
Mema 4	Liquid	0.10 ml in 0.6 ml H_2O
Anticarie	Dust	100 mg.
Granosane M	Dust	50 mg
Phygon	Dust	200 mg

In Part "A" 9 x 50 g subsamples were prepared from each lot of wheat by means of a mechanical divider, whereas 7 such subsamples were prepared in Part "B" each to be treated by a different fungicide. With respect to the liquid treatments, namely Panogen 15, Setrete and Mema 4, the seeds were retained in a corked Erlenmeyer flask for 48 hours at room temperature then taken out and dried. In the case of dust treatments the amount of fungicide required per sub-sample was weighed on an electrical balance and the dust was spread over the seed in a glass jar which had its walls previously coated with a thin layer of the dust before any seed was put in. The jar was covered and the mixture shaken by hand until the dust adhered as uniformly as possible over the entire surface of the grains. In preparing the seed for planting 5 x 200 seeds were used from each treatment. The samples were planted at the American University Farm, in the Bekaa Plain in a field in which the soil was plowed and smoothed then furrows were opened by means of markers pulled by a tractor. The furrows were 5 meters in length at intervals of 50 cm. The seeds were planted on Nov. 22 in the open furrows directly from the small envelopes without touching the treated seed by hand, after which each row was separately covered by means of a rake.

About the middle of June each head in each row was examined for the presence of bunt. The number of non-infected and infected heads was recorded in each row in which the partially smutted were counted with those completely smutted. Smutted heads were distinguished from the healthy ones by means of one or more of the following features: (1) pale green colored spikes, (2) feathery-shaped spikes and (3) exposed "smut balls" where the lemma and palea of a floret had separated at the apex. In addition to these features and especially in heads that were difficult to classify from outside appearance the nature of one or more kernels per head was checked separately.

b) Spore load

Shortly after planting, the spore load test was made on each of the 20 wheat samples that were used. The microscopic method of counting washed spores from the surface of the seed was used on a 5 gram representative sample of each lot. Each sample was washed in a glass test tube with 20 ml of distilled water. The number of spores in a volume of 1/4000 ml of the washings was counted under the microscope, using a blood counting chamber, from which the total number of spores on one gram of seed was calculated.

c) Morphological study of *Tilletia* spp. under the microscope.

Soon after the smut count of the seed treatment test was made in June 1958, 5 smutted heads were collected from each lot or kind of wheat for their spores to be studied and identified. In the laboratory only one "smut ball" was removed from each head making 5 "smut balls" for each kind of wheat. With the help of a tweezer and a probe one slide of spores from each "smut ball" was prepared and examined under the microscope; this microscopic study was based simply on morphology of Tilletia spores taking into consideration Fischer's species concept on smut classification (13).

2. Experiments in 1958-59

Seed treatment tests in 1958 were similar to those of the previous year, except that the 5 lots of wheat used, namely Florence Aurore, Hourani, Mishrikani and Senator Capelli which are commonly grown in Lebanon and Syria and a Cyprus variety B x IPI, all obtained from the American University Farm in the Bekaa were free from bunt and hence were artificially infested with Tilletia spores. In addition, a study of the effect of bunt on yield was made on the 5 varieties that are mentioned above. A third study of smut resistance as a means of control was initiated this year on a set of 50 samples of wheat both local and newly introduced to the Bekaa Plain.

a) Seed treatments

The spores of Tilletia that were used to infest the 5 lots of wheat selected for the seed treatment test were collected from the smutted heads in the spring of 1958. The smut balls of the infected heads were removed, crushed and sieved to provide a black powder of spores which were applied at the rate of 5 mg per 1 g of seed. The spores were weighed on the electrical balance, spread on the seed in a glass jar and then shaken by hand in all directions until the spores adhered completely to the surface of the kernels. Afterwards, the infested samples were prepared for planting in the same manner as described for the 1957 test. The different fungicides and the rates at which applied are shown in Table 4.

Table 4. Fungicidal dosage used in the seed treatment test of 1958

Fungicide	Form	Rate of application per 100 g of seed
Panogen 15	Liquid	0.14 ml in 8 ml H_2O
Setrete	Liquid	0.066 ml in 8 ml H_2O
Anticarie	Dust	200 mg
Phygon	Dust	400 mg

On Nov. 25 the seeds were planted as described for 1957 at the American University Farm in the Bekaa using a Latin square design.

Soon after the seeds had germinated and before the seedlings had started to tiller a field germination count was made for each treatment and in each row.

A laboratory germination test was also made to provide data on the effect of the different fungicides on the germinability of the seed. Using special blotters, as artificial media for germination, 2 x 100 seeds of each treatment including the check were counted by means of a vacuum counter. The blotters were kept moist with no excess of water in a germinator equipped with automatic control of humidity and a temperature of 18-20°C. The test period ranged from 6 to 8 days during which time preliminary counts were made whereby molded seeds were separated to prevent any contamination of the fungus-free seed.

About the first of June the smutted heads in the field were counted and the number of infected and non-infected was recorded as was done in the previous year.

b) Effect of bunt on yield

Each sample of the 5 varieties that were used in this test, namely Hourani, Florence Aurore, Mishrikani, Senator Capelli and B x IPI, was divided into two main subsamples one of which was infested with spores of *Tilletia* as described before, at the rate of 5 mg per 1 g of seed; the other subsample was used as a check. In the preparation of the seeds for planting the infested as well as the non-infested samples were replicated 5 times using 4 rows per replicate or block and 15 g of seed for each row. On Nov. 25 the seeds were planted along with the seed treatment test as described for 1957 on the American University Farm in the Bekaa using the randomized block design. About the first of June the wheat plants were counted for the smutted heads, as described for spring 1957, in which the number of the infected and non-infected heads was recorded and the percentage of smut was calculated to be considered as an index of reduction in yield, which could not be obtained until threshing time in July.

c) Study of smut resistance

Along with the seed treatment and yield tests a study of smut resistance was made on 50 lots of local and newly introduced wheat. The list of these varieties with the origin and weight of each sample used in this test are shown in Table 5. Each sample was infested with smut spores, from smutted heads of the seed treatment test of 1957, at the rate of 6.7 mg per 1 g of seed. The procedure used in infesting seeds with *Tilletia* spores and preparing the samples for planting was similar to that previously described. The seeds were planted on Nov. 25 at the American University Farm in the Bekaa in a completely randomized block after replicating each sample 3 times. On the first of June the smutted heads were counted and the number of infected and non-infected heads was recorded.

Table 5. Wheat samples for study of resistance to stinking smut with the origin and amount of seed used per sample

Sample number	Variety	Origin	Grams used per sample
1	Gazor-Sang	Iran	30
2	10815 (Taban)	"	27
3	1185 (DMID)	"	24
4	1-27-5906	"	21
5	1-30-13331	"	10.5
6	1-31-14391	"	30
7	1-29-11666	"	30
8	1-27-5480	"	24
9	1-27-5844	"	30
10	1-29-11695	"	30
11	4779	---	30
12	7201	---	30
13	C.I. 8629	---	30
14	5089	---	30
15	7092	---	21
16	Qued Quenati	---	30
17	1-33-223	Iran	30
18	Italy	"	30
19	1-29-12035	"	30
20	1-28-9289	"	30
21	Tabasi No. 7	Iran	30
22	1-33-36	"	30
23	Shahpasand	"	24
24	1-30-13274	"	30
25	4820	"	30
26	Blé node lignée 3355	"	30
27	Blé node lignée 4915	Iran	30
28	1-31-14623	"	30

Table 5. Cont'd

Sample number	Variety	Origin	Grams used per sample
29	093,44	---	30
30	P26 x Florence	---	30
31	5249	---	30
32	7064	---	30
33	Florence x Pusa 9095	---	29
34	Mentana	---	30
35	# 13	---	30
36	Briaji	Local	30
37	Zaraa	"	21
38	Biathi	"	30
39	Hamari	"	30
40	Biadi	"	30
41	Hourani	"	30
42	Meshrakani Abiad	"	30
43	Senator capelli	"	30
44	Florence aurore	"	30
45	Bekai	"	30
46	Psathas Cyprus	Cyprus	30
47	Kyperounda Cyprus	"	30
48	S_2	"	30
49	1-27-5489	Iran	30
50	B x IPI	Cyprus	30

RESULTS AND DISCUSSION

The Lebanese and Syrian samples that were obtained during the summer of 1957 to be used in this study of stinking smut of wheat were initially examined in the laboratory to determine the presence of "smut balls" and spores of species of Tilletia. Subsequently, each sample was subdivided into different subsamples each of which was treated with a different fungicide at different rates of application as described in the section dealing with Materials, Methods and Procedure. The spore load of each sample was further determined and the spores of Tilletia were identified by microscopic examination.

During the summer of 1958, 5 lots of wheat that are commonly grown in Lebanon and Syria were selected for seed treatment tests similar to those of the previous year together with a study of the effect of the disease on yield. A laboratory seed germination test was made and field counts were also obtained after the plantings were completed and the seeds had germinated. In addition, a set of 50 samples of wheat both local and newly introduced to the Bekaa Plain were selected for a study of resistance to the bunt pathogen.

Each year field plantings were made in November according to different designs and in June smut counts were obtained as described in the previous section on Materials and Methods. The results of these several studies are discussed separately for each year.

1. Seed treatment tests of 1957-58

The samples used in this test were divided into 2 parts "A" or heavily infested and "B" or lightly infested on the basis of the number of "smut balls" present in each sample. This method however, does not give a correct estimation of the extent of infestation because the number of "smut balls" present in each infested sample depends to a large extent on the methods of threshing and handling which each had received before it was brought to the laboratory. A superior method of estimation is by an actual count of the spores, under the microscope, per unit volume of spore suspension but this was postponed until after planting because of the shortage of time. Samples that contained more than 50 "smut balls" per 100 g of seed were considered as heavily infested and those below 50 as lightly infested. Two rates of application of seed disinfectants were used on the former and only one on the latter group of samples. The heads in each row were studied in June for smut infection and the number of infected and non-infected heads was recorded.

The percentage of smutted heads in part"A" was calculated and the results are shown in Table 6. Two comparisons can be made (1) between the check and each treatment and (2) among the different fungicides at the 2 rates of application. Smut infection of the 10 samples of wheat which are of Syrian origin ranged from 11.7 percent

Table 6. Percentage of smutted heads in the treatment test, Part "A" - Spring 1958

Sample No.	Check	Anticarie 1st rate	Anticarie 2nd rate	Phygon 1st rate	Phygon 2nd rate	Granosan M 1st rate	Granosan M 2nd rate	Panogen 15 1st rate	Panogen 15 2nd rate
1	11.7	0.0	0.0	0.0	0.2	1.5	0.0	0.4	0.2
2	39.6	0.0	0.1	0.6	0.2	5.3	0.0	0.0	0.2
3	70.0	0.1	0.0	1.0	0.2	12.0	2.3	1.8	0.4
4	43.5	0.0	0.0	0.2	0.1	7.7	1.0	0.1	0.0
5	45.8	0.0	0.0	0.8	0.7	6.2	0.5	0.0	0.0
6	42.1	0.0	0.0	0.0	0.1	3.8	0.0	0.2	0.0
7	40.4	0.0	0.0	0.0	0.0	1.4	0.7	0.2	0.4
8	37.3	0.0	0.0	0.3	0.2	0.6	0.3	0.3	0.3
9	13.0	0.0	0.0	0.1	0.0	0.3	0.0	0.0	0.0
10	55.1	0.0	0.0	0.6	0.5	4.7	1.0	0.4	0.6
Mean	40.8	0.01	0.01	0.4	0.2	4.4	0.5	0.3	0.2

in sample number 1 to 70.0percent in sample 3. The mean percentage of smut infection in the 10 samples is 40.8 which is relatively high to be expected from seed-borne spores. In general the 4 seed treatments, namely Anticarie, Phygon, Granosan M and Panogen 15 that were used in this part of the seed treatment test resulted in an effective control of the disease. The mean percentage of infection with the treatment of Anticarie used at the rate of 90 and 200 mg per 100 g of seed was reduced from 40.8 in the check to practically 0.0 at either rate of application. Similar results were also obtained by Holton (26), Holton and Purdy (28) and Siang and Holton (54). For Phygon, Granosan M and Panogen 15 in the first and second rates the percentages were reduced to 0.4 and 0.2, 4.4 and 0.5, and 0.3 and 0.2 respectively. With respect to Phygon only sample number 3, of the 10 samples used, showed 1.0 percent smut infection in contrast to the check that had 70.0 percent smut; the remaining 9 samples that were treated at the rates of 300 and 400 mg per 100 g of seed ranged from 0.8 to zero percent smut.

On the average the first rate of application allowed twice as many smutted heads as the second rate. Granosan M used at the rate of 50 mg was the least effective among the 4 fungicides yet on the average it reduced infection considerably compared to the check. It is also shown, that there is much fluctuation in the percentage of smutted heads at both rates which means that the amount of dust used was not enough to cover the entire surface of the seed as was shown by Purdy (46) on seeds that were treated with HCB. According to Purdy and Holton (47) Granosan M (also called Ceresan M) does not show a vapor action on Tilletia caries to compensate for the uneven coverage when used at low rates. However by increasing the rate of application from 50 to 75 mg per 100 g of seed, infection was reduced from 4.4 percent in the first rate to 0.5 percent in the second. The sample that has the highest percentage of bunt in the check has also the highest percentage at the first and second rates of application as shown with respect to sample number 3 which indicates that even at the second rate Granosan M was not very effective. Panogen 15 was very effective in controlling bunt a result also shown in other experiments by Machacek (37) Leukel (36) and Holton and John (32). It gave similar results to Phygon in reducing smut to 0.3, when used at the rate of 0.1 ml in 8 ml of water for every 100 g of seed. This result may be compared to 0.4 percent for Phygon at the rate of 300 mg of dust and to the percentage of 0.2 for each one at the rate of 0.14 ml of Panogen 15 and 400 mg of Phygon per 100 g of seed. Here again at the light rate number 3 showed the highest percentage of smut (1.8) compared to the check that had 70.0 percent.

The data in Table 7 show the percentages of smutted heads in the seed treatment test for Part "B". The 5 sample numbers 11,12,14,15 and 17 are of Lebanese origin, the remaining samples are Syrian. The lowest percentage of smut from non-treated seed is 4.6 and the highest is 49.4 with a mean for the 10 samples of 21.9 percent. Five fungicides, namely Panogen 15, Setrete, Mema 4, Anticarie and Phygon used at the rates of 0.10, 0.066, 0.10 ml, 100 and 200 mg per 100 g of seed respectively gave complete control of bunt. Granosan M used at the rate of 50 mg per 100 g of seed resulted in good control of bunt but results were variable as was shown in the data for Part "A".

Table 7. Percentage of smutted heads in the treatment test, Part "B" - Spring 1958

Sample No.	Check	Panogen 15	Set-rete	Mema 4	Anti-carie	Granosan M	Phygon
11	6.6	0.0	0.0	0.0	0.0	0.1	0.0
12	12.0	0.0	0.0	0.0	0.0	0.0	0.0
13	4.6	0.0	0.0	0.0	0.0	0.0	0.0
14	18.7	0.0	0.0	0.0	0.0	0.0	0.0
15	18.9	0.0	0.0	0.0	0.0	0.0	0.0
16	22.4	0.0	0.0	0.0	0.0	0.8	0.0
17	49.4	0.0	0.0	0.0	0.0	0.1	0.0
18	26.1	0.0	0.0	0.0	0.0	0.2	0.0
19	28.2	0.0	0.0	0.0	0.0	0.0	0.0
20	31.7	0.0	0.0	0.0	0.0	0.0	0.0
Mean	21.9	0.0	0.0	0.0	0.0	0.1	0.0

The percentages of smutted heads from the non-treated seed of samples 17,18,19 and 20 were not dependable to a great extent because the stand of the 4 samples in each row was very poor with weak plants due to poor soil conditions of the plot in which they were planted. This may apply also to the rows from treated seeds. Granosan M used at the rate of 50 mg per 100 g of seed allowed only 0.1 percent smut in sample 17 which had 49.4 percent in the check, a result contradictory to that obtained in Prt "A". The value 0.1 is not too dependable because it was calculated from only 3 replications since 2 were destroyed in the irrigation operation that was done in spring. Inadequate rainfall in the late spring of 1957-58 made it necessary to supply water by irrigation, a practice not normally used in dry land wheat culture.

The liquid fungicides Mema 4, Panogen 15, and Setrete were effective and uniform in controlling bunt in the 20 samples when used at very low rates compared to the dust fungicides Granosan M and Phygon that were used at higher rates as shown in Tables 2 and 3. Such good control and uniformity of the liquid fungicides are due to the contact action of their vapor which spreads over the seed into cracks and crevices destroying any spores wherever they may be hidden. Fungicides that were applied in the form of a dust were very effective when used in amounts large enough to cover the entire surface of the treated seed as in Anticarie and Phygon, however when the amount of dust was small as in Granosan M the control of the Pathogen was incomplete and variable due to incomplete coverage of the seed by the dust. This advantage of the liquid over the dust fungicides becomes very prominent under commercial seed treatment conditions when large quantities of seed are used. Purdy (46) reported that seed, commercially treated with HCB, had about 26 percent of their surface uncovered which is responsible for the incomplete death of the spores of Tilletia. However, with respect to liquid fungicides when used at low rates the vapor that is produced is able to kill the spores on the seed surface if kept in a closed atmosphere for a recommended period of time.

In the seed treatment experiments that were described soil infestation was lacking and spores of Tilletia adhering to the surface of the seed were the only source of infection. Therefore, the smut control which resulted from the treatments described in this thesis was due to fungicidal action on spores present on the surface of the seed. Consequently their effective control of bunt at different rates of application is applicable only when seed-borne inoculum is the source of infection. It is highly probable that the long, dry summer climate of Lebanon and Syria has a detrimental effect on the viability of soil-borne spores which should minimize the possibility of soil-borne inoculum. This phase however should be investigated under controlled conditions.

2. The relationship of spore load to smut infection.

Each of the 20 samples that were used in the seed treatment test was examined for spore load by counting the number of spores under the microscope as described in the section on Materials and Methods. The data in Table 8 show the calculated spore load in number of spores per one gram of seed and the percentage of smut infection in the non-treated of each sample.

The calculated number of spores on one g of seed is not an exact value of the spore load of each sample because the method used in this study as well as the remaining 2 methods (50), namely the centrifugal method and the binocular method of counting the spores on individual grains, give only approximate results. Besides, spore load against infection is not a direct straight line relationship but it is more likely a curve that reaches a constant at high spore load after which any increase in number of spores will not increase the percentage of smut infection proportionally. Therefore, keeping also in mind the large number of spores that is necessary to result in a considerable percentage of smut infection, we should not expect an increase in percentage of smut resulting from an increase of a few thousand spores. Accordingly and for the simplicity of the discussion the spore load values in Table 8 can be divided into 3 groups, (1) heavy spore load which includes those that are above 3×10^5 spores per g of seed, (2) low spore load that includes any value below 2×10^5 and (3) medium spore load for those values between 2×10^5 and 3×10^5 spores per g of seed.

In general, samples with heavy spore load are high in percentage of smutted heads namely samples 2,3,4,5,6, 7,8 and 10 whereas samples with light spore load have a low percentage of smutted heads as shown in samples 11, 12,13,14,15 and 16. Sample number 3 with the heaviest

Table 8. Spore load and the percentage of smut infection in the seed treatment test.

Sample No.	Estimated number of spores on one gram of seed	Percentage of smutted heads
1	256800	11.7
2	376800	39.6
3	503200	70.0
4	420800	43.5
5	385600	45.8
6	320000	52.1
7	360000	40.4
8	326400	37.3
9	210400	13.0
10	446400	55.1
11	10400	6.6
12	17600	12.0
13	10400	4.6
14	18400	18.7
15	22400	18.9
16	68800	22.4
17	201600	49.4
18	140800	26.1
19	163200	28.2
20	218400	31.7

spore load of 503,200 spores per g of seed has also the highest infection with 70.0 percent smutted heads. On the contrary, samples 11 and 13 have the lowest spore load of 10,400 which corresponds to the lowest percentage of smutted heads, namely 6.6 and 4.6 respectively. Samples 18, 19 and 20 are medium in spore load and percentages of smutted heads. Exceptions to these 3 groups are samples 1,9 and 17, the spore loads of which do not correspond to their respective percentages of smut infection. Samples 1 and 9 have low percentages of smut infection whereas sample 17 has a high percentage of smutted heads. Such indirect relationships plus the occurrence of fluctuations within groups 1 and 2 probably result from one of the following causes:

1. The use of mixed kinds and varieties of wheat which vary in susceptibility to species of Tilletia.
2. The nature of the organism, from the standpoint of species and races, was undoubtedly different from sample to sample as well as within the same sample.
3. Probable differences in spore germinability of each species and race in the field due to different germination requirements.
4. Differences in spore viability for each sample at the time of planting.

3. Seed treatment tests of 1958-59

The seed treatment tests of 1958 were similar to those of the previous year using the fungicides Panogen 15, Setrete, Anticarie and Phygon at the rates of 0.14 and 0.066 ml in 8 ml of water for the liquid and 200 and 400 mg for the dust fungicides per 100 g of seed respectively. The wheat samples for this year were purer as to variety than those of last year and all were free from spores of Tilletia which made artificial infestation in the laboratory necessary before planting in the field.

After the seeds had germinated and seedlings had emerged sufficiently, a field germination count was made for each treatment and in each row. A laboratory germination test was also made to provide data on the effect of the different fungicides on the germinability of the seed. The results of both tests for each variety of wheat are shown in Table 9. The data indicate no beneficial effect from chemical treatments on the seeds that were planted in the fall which confirms the results that were obtained by Porter et. al. (42) in their study on Chemical Treatments of Crop Seeds in Lebanon. This condition is probably due to the absence of seed-borne pathogens like species of Helminthosporium and Fusarium and of soil-borne organisms in soils that are exposed each year to a long, dry and hot summer period. There was no harmful effect from the fungicides that were used for the control of spores of Tilletia on the surface of the seed except with Mishrikani which was planted in the field and treated with Phygon at the high rate of 400 mg per 100 g of

Table 9. Percentage of the laboratory and field germination of the 5 lots of wheat that were treated with different fungicides in the seed treatment test of 1958-59.

Treatment	Florence Aurore		Hourani		Mishrikani		Senator Capelli		B x IPI	
	Lab.	Field	Lab.	Field	Lab.	Field	Lab.	Field	Lab.	Field
Anticarie	89.5	85.0	85.0	78.2	90.0	84.9	88.5	86.8	87.5	80.8
Phygon	90.5	80.7	89.5	76.3	85.0	70.0	88.0	83.9	90.0	73.8
Setrete	94.0	78.5	86.5	76.0	91.5	76.7	90.5	84.7	91.5	76.0
Panogen 15	89.5	79.9	88.5	76.8	83.5	78.3	89.0	87.0	89.5	78.6
Check	87.5	85.2	81.5	79.3	87.5	81.5	86.5	87.9	86.5	76.6
Mean	90.2	81.8	86.2	77.3	87.5	78.2	88.5	86.0	89.0	77.1

seed. This treatment decreased germination to 70 percent as compared with 81.5 percent for the check.

Early in June the heads in each row were studied for smut infection and the number of infected and non-infected heads was recorded. The results in percentage of smutted heads for each wheat lot are shown in Table 10 in which no smut infection was observed in any of the rows planted with the 4 treatments, namely, Panogen 15, Setrete, Anticarie and Phygon. The mean percentage of smutted heads from non-treated seed is 7.9 compared to percentages in the 1957-58 tests of 40.8 and 21.9 in Parts A and B respectively. This condition of low infection could not be due to low viability in the spores of Tilletia because they were collected in the spring of 1958, stored in the laboratory for 3 months and then applied on the seeds at a high rate of 5 mg per 1 g of seed which is enough to cause high infection on susceptible plants under favorable conditions for spore germination. Furthermore, considerable infection occurred in 2 varieties and in a subsequent section it may be noted that infection as high as 42 percent occurred. The factor of resistance in the relatively pure varieties that were used for this year as shown in the different percentages of smutted heads in each sample, could be partly responsible for the low infection - for example Florence Aurore has 1.4 percent while Mishrikani has 16.8 with all factors and environmental conditions being the same. However none of the varieties included have been reported as resistant and all are grown in Lebanon where stinking smut occurs unless the seed is treated. It is believed that variation in resistance is not the only factor responsible for the generally low infection in the 5 varieties because environmental conditions were the same, namely rainfall, temperature and soil. Possibly interaction of the 3 environmental factors was of some importance in its influence on the results.

Table 10. Percentage of smutted heads in the treatment test - spring 1959

Wheat lot	Check	Panogen 15	Setrete	Anticarie	Phygon
Florence Aurore	1.4	0.0	0.0	0.0	0.0
Hourani	13.6	0.0	0.0	0.0	0.0
Mishrikani	16.8	0.0	0.0	0.0	0.0
Senator Capelli	3.0	0.0	0.0	0.0	0.0
B x IPI	4.7	0.0	0.0	0.0	0.0
Mean	7.9	0.0	0.0	0.0	0.0

Following the tabulation of the data from the field plantings made in November 1958 a comparison was made with results obtained in 1956-57 and 1957-58. In plantings made in 1956 by Porter <u>et</u>. <u>al</u>. (42) the incidence of infected heads in rows planted with the non-treated seed ranged from 15.6 to 50.3 percent among 8 lots of wheat naturally infested. The writer also planted 10 samples in 1956 and obtained infection ranging from 34 to 69 percent in rows planted with non-treated seed.

The strikingly low percentages of smutted heads in the plantings made in November 1958 suggested that a climatic factor might have operated. Accordingly an examination of the rainfall pattern which occurred in the 3 years under consideration was made and the data are summarized in Table 11. The rainfall in 1956-57 was recorded at Ryak which is about 12 km from the University Farm whereas the data for the 2 subsequent years were recorded at the weather Station on the University Farm.

A careful study of the precipitation data for the 3 years as recorded reveals a striking similarity for the years 1956-57 and 1957-58 whereas the total for December 1958 is 1/3 that of 1957 and 2/5 that of 1956. Plantings were made at about the same time in November each year and seed germination of wheat occurs even at 0°C, a condition which is abnormal for December. The frequent heavier rains during December in 1956 and 1957 as compared with what occurred in 1958 seem to be correlated with the higher incidence of smut in the first 2 years under discussion.

It must be kept in mind that rainfall in the Bekaa Plain is practically nil from May to November with the result that non-irrigated land is exceptionally dry when wheat is planted and a heavy rain is required to moisten the soil sufficiently for seed germination to occur. In December 1956 the total rainfall between December 4 and 20 was 98.9 mm, in 1957 between December 1 and December 20 the total was 130.9 mm whereas in 1958 the total for December was only 52.7 mm and no rain fell during December after the 12th of the month.

Table 11. Summary of rainfall for December and January from 1956 to 1958 in the Bekaa Plain near to or at the University Farm.

1956-57		1957-58		1958-59	
Date	Rainfall (mm)	Date	Rainfall (mm)	Date	Rainfall (mm)
Dec. 4	6.5	Dec. 1	0.02	Dec. 3	8.4
Dec. 6	39.9	Dec. 2	6.7	Dec. 4	3.0
Dec. 7	17.4	Dec. 3	5.8	Dec. 8	9.8
Dec. 8	16.0	Dec. 4	10.5	Dec. 9	10.03
Dec. 9	16.0	Dec. 5	15.7	Dec. 12	21.5
Dec. 10	0.6	Dec. 6	31.0		
Dec. 19	1.4	Dec. 7	26.5		
Dec. 20	1.1	Dec. 8	21.0		
Dec. 21	10.0	Dec. 9	9.7		
Dec. 26	0.9	Dec. 20	4.5		
Dec. 27	8.0	Dec. 24	22.0		
Dec. 28	2.0	Dec. 25	0.7		
Dec. 30	4.0				
Dec. 31	2.4				
Totals	126.0		154.0		52.7

Table 11 (Cont'd)

1956-57		1957-58		1958-59	
Date	Rainfall (mm)	Date	Rainfall (mm)	Date	Rainfall (mm)
Jan. 4	1.0	Jan. 2	6.0	Jan. 5	13.0
Jan. 5	13.0	Jan. 3	25.5	Jan. 7	0.7
Jan. 12	5.4	Jan. 4	18.3	Jan. 8	12.8
Jan. 13	1.0	Jan. 5	5.6	Jan. 9	1.7
Jan. 18	29.5	Jan. 6	15.1	Jan. 18	41.9
Jan. 19	1.0	Jan. 7	0.4	Jan. 24	2.6
Jan. 29	38.0	Jan. 10	25.5	Jan. 31	27.3
Jan. 30	51.5	Jan. 11	0.5		
Jan. 31	19.3	Jan. 16	0.6		
		Jan. 17	2.0		
		Jan. 24	1.3		
		Jan. 26	3.8		
		Jan. 27	19.0		
		Jan. 28	5.9		
		Jan. 29	1.2		
		Jan. 30	17.0		
		Jan. 31	8.5		
Totals	159.7		154.0		116.8

The relative effect of small amounts of moisture on seed germination and bunt spore germination is not known, nor is the interaction of the temperature-moisture effect known. It is conceivable that the light rains in 1958 initiated spore germination more rapidly than seed germination and since infection apparently takes place through the seedling coleoptile the spores may have suffered drying after the initial germination sooner than the wheat grains. The wheat kernels are larger in comparison with the smut spores and presumably would not lose moisture so rapidly as the spores. Whatever the actual phenomenon that occurred it seems evident that the rainfall pattern in 1958 was unfavorable for smut infection of wheat seedlings.

4. Effect of Stinking Smut on Yield of Wheat.

In the Review of the Literature as presented in this thesis reference was made to the heavy losses caused by smut in the United States and Canada. In general, investigators have concluded that the percentage loss in any one field can be measured by the percentage of infected heads. No careful estimates of losses from stinking smut in the Middle East have been made but on the basis of data obtained by Porter et al (42) and by the writer, in his senior year working on a special problem, the loss from stinking smut could easily be 50 percent in some samples. For example, on 8 samples of wheat infested with smut and planted in 1956 (42) the percentage of smutted heads ranged from

15.6 to 53.5 with an average of 41.4. Five of the 8 samples were from Syria and the average percentage of smutted heads was 45. The percentages of smutted heads obtained by the writer with 10 Syrian samples ranged from 34 to 69 with an average of 48.7. If we assume a loss equal to the percentage of smutted heads it is evident that losses in Syria are extremely high and the same probably occurs in Iraq and Iran. The average percentage of smutted heads for 3 farmers' samples in Lebanon (42) was 21.6.

In one test in Lebanon (42) planted in December 1957 the total number of smutted heads was 99 in 4 rows grown from non-treated seed and the total yield was 467 grams. Four rows from seed treated with Granosan M contained 17 heads of smut but yielded 657 grams, representing a yield increase of 40.7 percent. No counts were made for total heads but the percentage of smutted heads could not have exceeded 25, yet the loss was much greater.

The high incidence of smut in 1957 and again in 1958 suggested that a further study of losses from this disease should be made. Unfortunately the political situation in Lebanon in the summer of 1958 were such that no wheat was imported by millers from Syria and no samples of naturally infested seed could be obtained. It became necessary, therefore, to artificially infest seed for this study.

Samples of the 5 varieties Florence Aurore, Senator Capelli, Mishrikani, Hourani and B x IPI were collected and infested with spores of Tilletia as described in the section on Materials and Methods. The rate of application was 5 mg of spores per gram of seed. Plantings of infested and non-infested seed were made in Blocks in November, 1958 using 4 rows per block with 3 replications.

On June 5, 1959 infected and non-infected heads of the 2 middle rows of each replicate were counted and the percentages of smutted heads calculated and recorded as follows:

Florence Aurore	1.0	percent	smut
Senator Capelli	4.9	"	"
Mishrikani	16.2	"	"
Hourani	10.5	"	"
B x IPI	4.3	"	"

The incidence of smut was similar to that reported in the seed treatment test and was much less than expected. The cause for the low infection has already been discussed. It may be concluded that yield reduction will be at least 4 to 16 percent among 4 of the varieties. The harvest could not be made before late June and yield data will not be available before July hence actual loss in yield from smut will be available later in the summer for inclusion in a paper to be published, based on the total study covered by this thesis.

5. Reaction of wheat selections in Lebanon to Tilletia spp.

Smut control by selection and breeding of wheat has been investigated for many years in countries where agricultural research has been well organized and much progress has been made in the study of different kinds and varieties of wheat as reported by Conners (8) and Bressman (4). In addition the nature of resistance to species and races of Tilletia has been investigated by Griffith et al (19) and many others. Geneticists and plant breeders naturally look upon smut control by resistance as the most permanent and acceptable method. Resistance exists in species and varieties of Triticum and as combinations of these are made new varieties and types are produced. Unfortunately this approach to the problem of control is not so simple as it would appear to a casual observer. Hybridication among species of Tilletia occurs, new physiologic races are constantly being produced either by hybridization or mutation and such races do not necessarily possess the same virulence as either of the parents. It is a common experience among pathologists to find new races of parasitic fungi to which established varieties and species of crop plants react differently than to previously recognized races. For this reason plant pathologists generally do not completely abandon chemical or other control methods in favor of resistance as the sole method.

In spite of the inadequacy of disease resistance as a method of control it has its place because even partial resistance is of value, especially when the environmental factors for infection are considerably below the optimum and a highly resistant variety may serve an area for many years before a new virulent race of the fungus becomes established. Furthermore it is possible to produce synthetic varieties which combine resistance of many varieties to different races into one variety.

In the fall of 1957 a collection of wheat samples was received from Iran and the agronomy section had been testing a number of additional samples for adaptability to the Bekaa area. In the fall of 1958, the more promising members of these collections were selected and 50 of them were infested with spores of Tilletia, as described previously for other experiments, using 6.7 mg of spores per gram of seed. From each lot 3 subsamples were prepared and planted in a completely randomized block design with 3 replications of each lot. A key to the origin of the 50 varieties is given in Table 5. On June 5, 1959 the field smut count was made and the calculated percentages of smutted heads are shown in Table 12. The variation in resistance to stinking smut among the 50 varieties is well illustrated by the different percentages of smutted heads in each variety. Infection ranged from zero percent smut in sample number 4 to 42.3 in sample 35. Twenty two samples showed infection below 10 percent, 20 between 10 and 20 percent and 8 above 20 percent smutted heads. The results of this study are based only on one year with a rainfall pattern which probably influenced infection greatly as discussed in the previous test on seed treatment.

The relative percentages of infection that are shown in Table 12 are the result of interaction of the wheat plants of each sample with the different species and races of Tilletia under the environmental conditions of 1958-59. It is possible then, that sample 4 which has zero percent smut may be susceptible under differnet environmental conditions and to new hybrids that may develop among the mixed races of Tilletia.

Of major interest is the fact that out of 50 samples, 10 showed no more than 3 percent infection and 3 no more than one percent. On the other hand 8 varieties had more than 20 percent smutted heads of which 3 had more than 30 percent and one showed 42 percent smutted heads. These results, even though environmental conditions for smut infection were less favorable than in 1956 and 1957 as discussed in a previous section, are promising in that they indicate a great variation in susceptibility in a few selections.

6. Species of Tilletia in Lebanon and Syria

A morphological study of the spores of Tilletia present in the 20 Lebanese and Syrian samples was made under the microscope for the purpose of identification as to species. Five "smut balls" obtained in the spring of 1957 from different heads of each sample were examined and the spores were classified on the basis of Fischer's new concept (13) of smut species. According to him spores that were globose

Table 12. Percentage of smutted heads of 50 wheat samples selected for a study of resistance, 1959

Sample No.	Variety	Smutted heads	Sample No.	Variety	Smutted heads
1	Gazor-Sang	13.7	26	Ble node lignee 3355	2.1
2	10815 (Taban)	6.0	27	Ble node lignee 4915	15.2
3	11.85 (DMID)	4.9	28	1-31-14623	15.9
4	1-27-5906	0.0	29	093, 44	10.7
5	1-30-13331	16.4	30	P26 x Florence	11.6
6	1-31-14391	18.1	31	5249	15.0
7	1-29-11666	11.4	32	7064	27.5
8	1-27-5480	8.3	33	Florence x Pusa 9095	16.8
9	1-27-5844	1.0	34	Mentana	35.7
10	1-29-11695	14.9	33	# 13	42.3
11	4779	1.7	36	Briaji	12.7
12	7201	2.4	37	Zaraa	9.6
13	C.I. 8629	24.9	38	Biathi	4.8
14	5089	9.3	39	Hamari	9.4
15	7092	23.3	40	Biadi	15.6
16	Qued Quenati	16.8	41	Hourani	12.0
17	1-33-223	33.8	42	Mishrikani Abiad	24.4
18	Italia	1.0	43	Senator Capelli	5.0
19	1-29-12035	2.2	44	Florence Aurore	1.3
20	1-28-9289	4.0	45	Bekai	7.4
21	Tabasi No. 7	12.5	46	Psathas Cyprus	16.9
22	1-33-36	13.2	47	Kyperounda Cyprus	26.9
23	Shahpasand	9.5	48	S_2	5.2
24	1-30-13274	10.9	49	1-27-5489	17.9
25	4820	2.5	50	B x IPI	3.0

to subglobose and ranging from 14-23u in diameter with reticulations varying from 0.5-1.2u deep were classified as Tilletia caries; those which were globose to elongate ranging from 17-20 x 18-22u and with very shallow tubercles to completely smooth were classified as T. foetida.

The occurrence and abundance of spores of both species of Tilletia in each sample are shown in Table 13. A mixture of both species of Tilletia namely T. caries and T. foetida was found in each sample in which the spores of the former appeared to be somewhat more abundant than those of the latter. In many cases the same "smut ball" contained spores that apparently fit the description of each species as shown in Figure 1 in which many forms or possibly races appear to be present. The smooth spores of T. foetida which are shown in Figure 2 were found in small numbers compared to those of T. caries (Figure 3) which were more abundant. Up to the present time only one sample of spores that was identified as T. brevifaciens (T. controversa) was obtained by the laboratory from Turkey which is shown in Figure 4 for comparison with other spores found in this study. The spores of one "smut ball" of sample 15 that are shown in Figure 5 are equal in size and depth of reticulations to those of T. conroversa but the reticulations of the spores of the latter are more pointed and enclosed in deeper areolae than those of sample 15. These spores could be a race of either species, namely T. caries or T. controversa

Table 13. Relative prevalence of *Tilletia* spp. in Lebanese and Syrian wheat samples, 1958

Sample No.	Origin	Spores of *T. foetida*	Spores of *T. caries*
1	Syrian	Few	Abundant
2	"	"	"
3	"	"	"
4	"	Abundant	Few
5	"	"	"
6	"	Few	Abundant
7	"	Abundant	Few
8	"	Few	Abundant
9	"	"	"
10	"	"	"
11	Lebanon	Abundant	Few
12	"	"	"
13	Syria	Few	Abundant
14	Lebanon	Abundant	Few
15	"	Few	Abundant
16	Syria	Abundant	Few
17	Lebanon	"	"
18	Syria	Few	Abundant
19	Syria	"	"
20	Syria	"	"

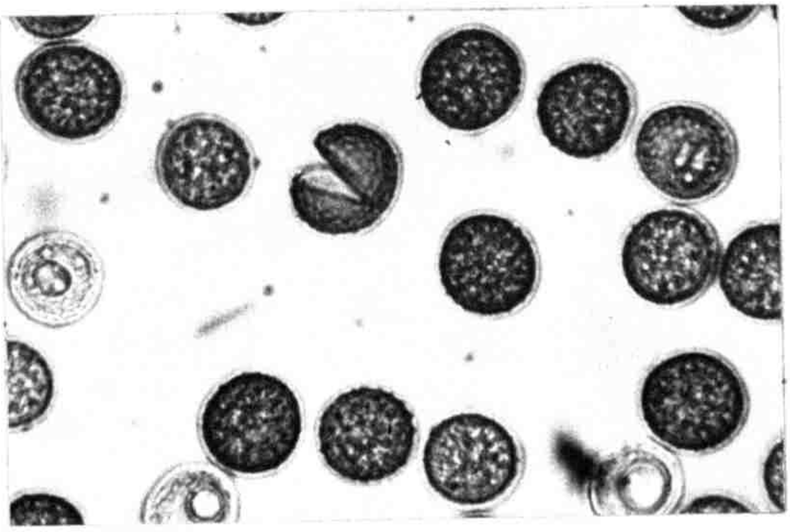

Fig. 1
Tilletia spp.
spores, x 1000

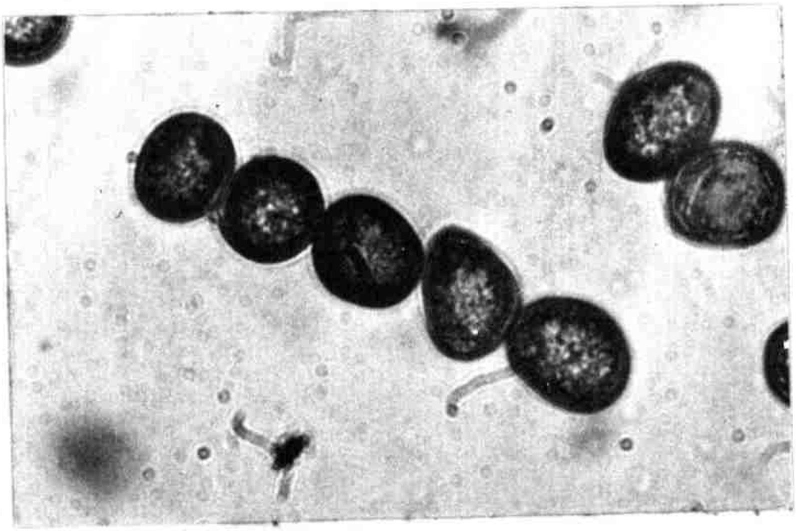

Fig. 2
Tilletia foetida
spores, x 1000

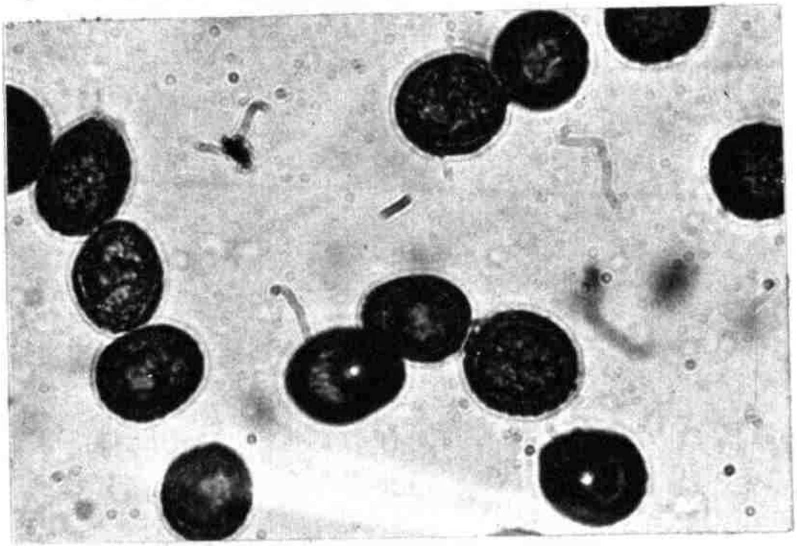

Fig. 3
Tilletia caries
spores, x 1000

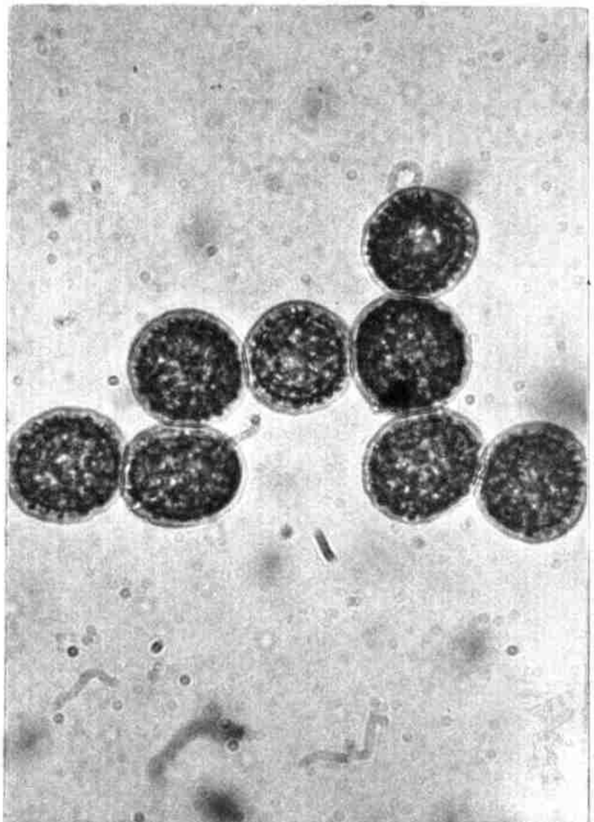

Fig. 4

Tilletia controversa

spores, x 1000

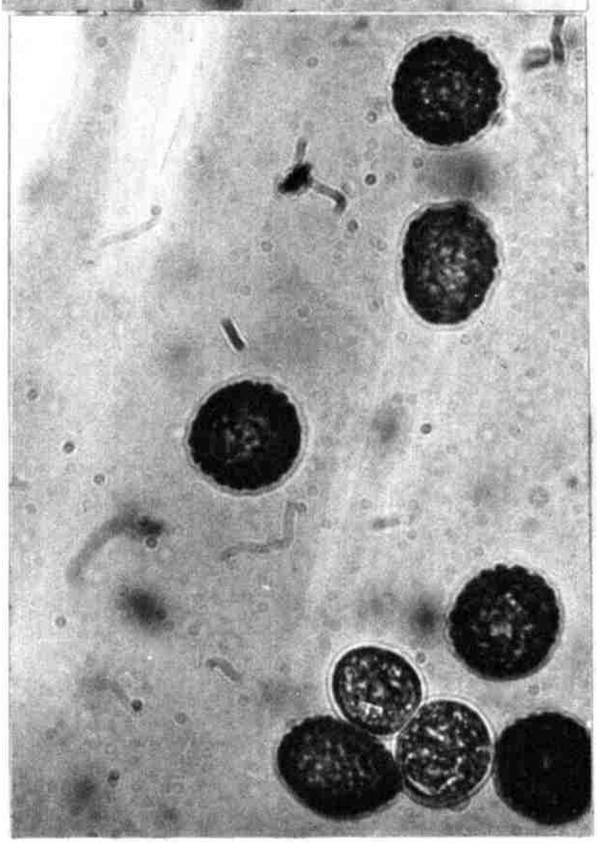

Fig. 5

Tilletia caries or

T. controversa

spores, x 1000

that are the causal agents of medium to severe dwarfing in smutted wheat plants respectively.

Field observations in 1958 and again in 1959 revealed the presence of many dwarfed plants, a condition which has been noted for many years in the north western region of the United States. Only in a few localities has the type of smut with severe dwarfing caused by T. controversa been found. It occurs in fields on the Western slope in Colorado and in Utah and Washington. As mentioned in a preceding paragraph it has been reported from the Anatolian Plateau in Turkey. Occurrence at high altitudes rather than lower elevations has also been noted. In addition the life cycle of T. controversa is considered different from that of the other 2 species that are common on wheat. Some investigators (41,45,58) have obtained evidence that infection occurs only after the seedlings emerge and probably not through the coleoptile as with other species. It is difficult to believe, therefore, that T. controversa occurs in Lebanon and Syria insofar as the examination of material thus far collected is concerned.

In 1957-58 extremely high percentages of infection occurred from naturally infested seed and spores from infected heads were employed to infest the seed used in the fall plantings of 1958. None of the spores microscopically examined were exactly like the spores of T. controversa obtained from Turkey. The low percentages of infection which occurred in the 1958-59 tests therefore, cannot be explained on the basis of species of *Tilletia*. Other factors must have operated as discussed in another part of this paper. Further investigation of this aspect of taxonomy and infection of the stinking smut fungi in the Middle East must be made.

SUMMARY

Twenty samples of mixed wheat naturally infested with spores of *Tilletia* and 5 local varieties which were artificially infested in the laboratory were obtained in 1957 and 1958 respectively. Seed treatment tests, spore load and identification of species of *Tilletia* were made on the samples of 1957 while a continuation of the seed treatment tests plus a study on the effect of the disease on yield were performed on the samples of the year 1958. An additional study on varietal resistance to species of *Tilletia*, in the fall of 1958, was initiated on 50 local and newly introduced varieties of wheat.

According to the work done by the writer and Porter *et al* (42) in 1956, stinking smut of wheat caused by species of *Tilletia*, was found to be present in Lebanon and to a larger extent in Syria. There are no published reports on this problem in either country, hence this study was undertaken with the idea of it being continued in the years that follow.

The results of this study are summarized as follows:

1. In 1957-58, the percentages of smutted heads of the non-treated, in general, were high ranging from 11.7 to 70.0 and 4.6 to 49.4 percent in Parts "A" and "B" respectively. All the fungicides at the 2 rates of application gave good

control of the smut except Granosan M (in Part "A") which allowed 4.4 percent smut when applied at the rate of 50 mg per 100 g of seed. Samples with heavy spore load were high in percentage of smutted heads, namely samples, 2,3,4,5,6,7,8, and 10 whereas samples with light spore load had a low percentage of smutted heads as shown in samples 11,12,13,14,15 and 16.

2. The results of the seed treatment test of the year 1958-59 indicated a low percentage of smutted heads from the non-treated seed with a mean of 7.9 compared to means of 21.6 and 40.8 percent infection the year before.
3. Variation in resistance in varieties Hourani, Florence Aurore, Mishrikani, Senator Capelli and B x IPI plus the inadequate rainfall in December after the infested seeds were planted were probably the main reasons that led to the low infection in 1958-59.
4. No reduction in the germinability of the seeds resulted from Panogen 15, Setrete, Anticarie, and Phygon when used at the rates of 0.14 and 0.066 ml in 8 ml of water, 200 and 400 mg per 100 g of seed respectively except for the variety Mishrikani treated with Phygon.
5. No actual yield data were available before July,

hence estimates on losses from the smut were based on the percentages of smutted heads in each variety. The incidence of smut was similar to that reported in the seed treatment test which was much less than expected possibly due to the varietal resistance and rainfall pattern which were discussed before. It may be concluded however, that yield reduction will be at least 4 to 16 percent among 4 varieties and little or none in the variety Florence Aurore.

6. Variation in resistance to stinking smut which ranged from 0.0 to 42.3 percent smutted heads among the 50 varieties indicates the possibility of selecting smut resistant types adapted to the Middle East. A test for only one year is by no means conclusive nor dependable but the different degrees of infection in the 1958-59 tests are striking.

7. A mixture of the spores of _Tilletia foetida_ and _T. caries_ was found in each of the 20 Lebanese and Syrian wheat samples that were studied in the samples used in 1957-58. The latter species appeared to be more abundant than the former. None of the spores that were microscopically examined were exactly like the spores of _T. controversa_. The dwarf bunt fungus, which was obtained from Turkey.

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