THE BEHAVIOR OF SOIL-BORNE SPORES OF THE COVERED SMUTS OF BARLEY AND WHEAT, *Ustilago hordei* (Pers.) Lagerh, and *Tilletia foetida* (Wall.) Liro

by

Sardar Mohammed Moghal

A Thesis Submitted to the Faculty
of Agricultural Sciences in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE IN AGRICULTURE

Major: Plant Pathology - Entomology

Minor: Agronomy

Approved:

In Charge of Major Work

Chairman, Graduate Committee

American University of Beirut
1964

Covered smuts of Cereals

Moghal

ACKNOWLEDGEMENTS

The author wishes to express his deep appreciation to Dr. Heinrich C. Weltzien for his invaluable suggestions, constructive criticism, and efforts in correction of this manuscript. The facilities and encouragement given by him throughout the course of this investigation are also gratefully acknowledged.

My thanks are due to Mr. Hikmat Nasr for his help in the field work.

Mr. Pierre Gamous needs special thanks for typing this manuscript.

Sardar Mohammed Moghal

ABSTRACT

The behavior of soil-borne spores of covered smuts of barley and wheat was studied under the laboratory and field conditions with respect to germination, longevity, inhibitory and stimulatory effects and infection. Experiments on spore germination indicated that Ustilago hordei spores could germinate on media with and without nutrients in a temperature of 5-30°C. They failed to germinate in moist soil at 25°C. Chlamydospores of Tilletia foetida germinated freely in moist soil at 15-20°C.

When U. hordei spores were reisolated from the inoculated field soil, they were found to retain their viability under natural field conditions for about oneyear. They lost their viability gradually with the age in spite of great variations in temperature and rainfall. It seemed that temperature mostly controlled their viability in the soil. Under constant conditions of temperature (25°C) and soil moistures (25, 50 and 75% field capacity), spores lost their viability more rapidly at higher than at low soil moisture. From these results, an active inhibition of U. hordei germination was assumed. Chlamydospores of T. foetida remained viable in the soil under dry conditions only, but after rains, they germinated and no spores could be reisolated at accumulated rainfall of 209.9 mm.

The inhibitory effect of soils on germination of U. hordei was proved by agar disk technique. Four different soils completely inhibited spore germination on water agar and Potato-dextrose agar disks, when placed on these soils 24 hours before inoculation. The inhibitory activity of soil was partly made ineffective by autoclaving the soil. Germination

of U. hordei was increased significantly when some stimulating materials as plant tissues or chemicals were placed on disks after inoculation. The well known stimulatory effect of soil on germination of T. foetida was also demonstrated by agar disk technique. This technique was found most suitable for observing and calculating the germination in percentage.

Soil-borne infection in covered smuts of barley and wheat was found possible, under the Beqa'a conditions. It was very low in covered smut of barley but rather high in covered smut of wheat. Since soil-borne infection can occur in the Beqa'a, seeds of barley and wheat must be treated before planting and seed treating chemical should be based on organo mercurials plus HCB preparations which are known to be effective against seed-borne or soil-borne infection in covered smuts.

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INTRODUCTION

Smuts have been associated closely with the historical development of Plant Pathology and are among the earliest reported plant diseases causing considerable losses in economic crops. Of all the cereal smuts, covered smut of barley caused by Ustilago hordei (Pers.) Lagerh, and covered smut of wheat (bunt) caused by Tilletia foetida (Wallr.) Liro, are widely distributed throughout the world, and in particular its arid and semi-arid regions. Many reports from different parts of the world indicate that losses due to covered smuts exceed the losses from loose smuts of barley and wheat. Both these diseases have been reported as important in Lebanon by Abu Shakra (1) and Weltzien (63).

The seed-borne spores of the covered smuts can easily be killed by fungicidal seed treatment, but infections after seed treatment have been frequently reported. Thus the possibilities for soil-borne infection of Tilletia species were studied in U.S.A. (40), Canada (28), and Germany (64). Extensive monoculturing of wheat and use of combine threshers are now thought to provide increased opportunities of soil-borne infection with wheat bunt (40). The effectiveness of soil contamination depends, however, on the longevity and viability of spores in the infested soil under the prevailing climatic conditions.

The infection of barley by covered smut from soil-borne spores, has received very little attention so far. Some workers (11, 33) believe or at least feel that soil-borne infection in covered smut of barley is possible in drier areas. However, these possibilities have not been worked out, and the disease remains economically important in many countries.

We may therefore assume that Lebanon provides good conditions for soil-borne infection of these diseases. Therefore, the behavior of soil-borne spores of the covered smuts of barley and wheat was studied with the following objectives:

1. To determine the longevity and pathogenicity of smut spores in soil under natural field conditions.
2. To investigate the influence of soil on spore germination with respect to inhibitory or stimulatory action.
3. To study the possibilities of soil-borne infection under the local conditions.
4. To suggest control measures against these diseases.

REVIEW OF LITERATURE

I. Spore Germination

Germination of the smut fungi has been considered as the key to their investigation and a basic and essential process for infection under the influence of various factors (21).

1. Ustilago hordei

Enomoto (16) and Lobik and Dahlstrem (42) reported that freshly harvested spores germinated less rapidly than those kept at room temperature for several days. However, no dormancy period was reported by Hassenbrank et al. (33).

In general, the spores of Ustilago species may be germinated on solid or liquid, nutrient or non-nutrient media. The best production of sporidia is obtained on nutrient media as Potato-dextrose agar or Malt agar (21). According to Schaffnit (56) sucrose, glucose and maltose are the best carbon sources. On biomalt agar, he obtained chlamydospores in three weeks.

Lobik and Dahlstrem (42) found 5 - 32°C as the temperature range for germination, and the optimum from 15-25°C. However, Huttig (36) noted for U. hordei, 30% germination at -1°C, 99% at 10°C, 50% at 30°C and no germination at 35°C. Rump (54) reported that in a dry atmosphere at 60°C, over 50% spores were killed in 15 minutes and all but 2% in 5 hours. After one hour at 75°C, only 5% of the spores germinated and only isolated individuals could withstand a temperature of 100°C. In a saturated atmosphere, the death rate was accelerated. In water at 52°C, the spores were destroyed in 10 minutes. After 48 hours exposure to a temperature of -17°C

in ice, 49.4% of the spores were still viable. The minimum, optimum and maximum temperatures for germination were reported by Rump as 5–6°C, 20°C and 34–35°C respectively.

Clayton (10) showed for the first time that the chlamydospores of U. hordei, when kept on glass or paraffin, were capable of germination at relative humidities of 95–100%, but not below 93%.

Remarks on germination of U. hordei in soil have been reported by two workers only. Mundkur (47) mentioned that the spores could germinate readily in water or damp soil. Rump (54) reported that germination of U. hordei was most profuse in a soil with a water content of 20%.

2. Tilletia foetida

Grasso (26) and Hulea (35) carried out germination tests for chlamydospores of T. foetida in agarized water medium at 15°C or in soil after 3–4 days. Kienholz and Heald (39) suggested plain agar or soil extract agar as the most suitable solid media. Benlloch (3) obtained the best results on germination by placing the spores on filter paper over a solution of calcium nitrate. A new nutrient medium was described by Keil (38) based on combination of sterilized agar, oats extract, charcoal and potassium hydroxide. Lobik and Dahlstrem (42) got the spores germinated on disks of white blotters kept on the surface of moist soil. Niemann (48) concluded that horse dung decoction agar was a suitable medium for germination of Tilletia species.

Obviously, many attempts were made to design simple or special media for the germination of Tilletia species, but still a

good medium is lacking where a constant and high germination can readily be obtained for calculating the germination in percent.

Several workers (35, 43, 61, 65) reported that spores of T. foetida germinated in the soil, and factors as soil moisture and soil temperature have been considered as important for germination. Gassner and Rabien (61) introduced soil plate from unsterilized soil as standard method for germination. Gimesi and Frenyo (25) found that soil has a stimulating effect on germination of T. caries and T. foetida. Lungren and Durrell (43) reported that bunt spores germinated best at a soil moisture content of 15-20% and a temperature of 55°F. 20-30% soil humidity and an optimum temperature of 15-18°C or 40-60°F are considered good for germination of bunt spores (8, 40, 41). According to Lobik and Dahlstrem (42) and Lungren and Durrell (43), germination was depressed by soil humidities above 20-30% and higher temperature. At 40-50% soil humidity with 25°C as soil temperature, only a few spores germinated.

According to Holton (34) chlamydospores of T. foetida germinated readily at 18°C. He also reported that different races required different temperatures and different germination times. Niemann (49) observed that spores of T. foetida germinated at both 3 and 15°C, while Hahne (29) got the minimum, optimum and maximum temperature for germination of Tilletia species as 4°, 18-25°C and 36°C respectively.

3. Inhibitory and stimulating action of soil on spore germination

Studies on the aspect of soil fungistasis on different organisms are not very old. Dobbs and Bywater (12) described that spores, capable of germinating in distilled water, failed to germinate in soil solution and he attributed this effect to the presence of a diffusible inhibitor in the surface soils. This fungistatic factor has been studied by Jackson (37) and Weltzien (62) in different soils and with different test organisms. Weltzien (62) reviewed 79 fungi including Ustilago zea (Beckem.) Ung and U. avenae Pers. which are inhibited by soil fungistasis. It was reported by him that this inhibition could be broken by some stimulating material as swelling seeds, tissues of leaves, leaf-stems, roots and straw and it could be totally or partly made ineffective by heating the soil or by addition of nutrients including some carbohydrates and amino acids. Dobbs and Bywater (12) also reported that 0.1% glucose solution when added to the soil resulted in disappearance of fungistasis effect.

Soil has also a stimulating effect on the germination of some fungi, especially on Tilletia species. Gimesi and Frenyo (25) investigated for the first time that soil has a stimulating effect on germination of T. caries and T. levis. Stimulation was attributed to the absorption of trimethylamine contained in the spores, by soil. Hanna and Vickery (31) and Ettel and Halbsguth (15) confirmed these findings and studied the trimethylamine effect in detail.

II. Longevity and Viability of Smut Spores

1. Ustilago hordei

Fischer (20) found U. hordei spores from a herbarium viable after 23 years. Pal (50) reported that U. hordei spores remained viable under the laboratory conditions at Delhi and Pusa for four years without any loss in germinability, and Rump (45) found them viable after 5 years in Germany.

Gussow and Conners (28) reported from Canada that certain smuts, as covered smut of barley, were long lived. The spores might retain life for 12 years and under dry conditions, they were exceedingly resistant to frost. Viability was not even affected in the bodies of animals fed with smutted food, and the spores could be disseminated over considerable area with manure. However, longevity of soil-borne chlamydospores of U. hordei under natural field conditions has not been reported.

2. Tilletia foetida

Fischer (20) found viable chlamydospores of Tilletia species in 25 years old herbarium specimens. Under dry field conditions, the fungus remained viable for 7-8 years but under favorable conditions the spores germinated readily as reported by Guest (27) from Iraq. While studying the physiology of bunt fungus, Woolman and Humphrey (65) reported that the spores remained viable under laboratory conditions for 12 years, but they lost their viability after one or two months in the soils. Unbroken bunt balls retained their viability throughout the winter, and in spite of alternate freezing and thawing, infection occured even after two years under dry soil conditions. It was determined by Weltzien

(61) that spores of T. tritici and bunt balls disintegrated rapidly in damp soil and they lost their viability within one and two months respectively. When Hanna and Popp (32) artificially infested small plots with bunted wheat heads, bunt balls and sifted spores of T. tritici, the relative amount of bunted ears in the new crop was 45, 29, and 11 respectively. They concluded that some of the spores in all plots survived the winter and spores in the bunted heads or balls retained their viability for longer than those left over-wintering in direct contact with the soil.

Gussow and Conners (28) reported that wheat bunt spores could germinate in the bodies of animals fed with infected ears and were destroyed. Vilkaitis (60) could not kill bunt spores completely by dry heat up to 90°C for one hour. Freezing for 10 hours at -27°C had no adverse effect on their viability. Berend (4, 5) discovered a pronounced stimulation of the germination of old spore samples of T. foetida by X-rays, ultraviolet and radio-active rays, ultrasonic vibrations or deep-freeze treatments.

III. Soil-borne Infection

1. Covered Smut of barley

Almost all the studies made in the covered smut of barley are based on seed-borne infection (21, 52, 58). It appears that soil-borne infection in covered smut of barley has not received its due attention. Gussow and Conners (28) suggested that possible source of infection, other than seed-borne, especially in covered smuts of cereal, may be soil-borne spores which presented difficulty in control. Dickson (11) suspected that in some drier areas, the

infection might occur from the spores in the surface soil, and also Hassebrank et al. (33) believed that soil-borne infection in covered smut of barley might be possible in dry areas. Only Bleck (6) mixed the chlamydospores of U. hordei in the soil in eight small plots, but obtained 5% infection in one plot only.

2. Covered smut of wheat

a. Failure in seed treatment

Brygalova (9), Fajersson (17) and Savulescu et al. (55) reported that seed grain disinfection was not always effective in protecting the wheat crop from bunt. Dry climatic conditions, cold springs and inefficiency of some fungicide preparations were mostly held responsible for infections which were as high as 80-90% in exceptional cases. Muller (45), Muller and Schuhmann (46), and Weltzien (61) correlated failure of seed disinfection in Germany to the coincidence of several adverse environmental factors including soil moisture at high levels, soil fertility, type of soil, specific varietal reaction, highly pathogenic bunt samples and the possible development of mercury-resistant biotypes of pathogens. Weltzien (61) reported a significant increase of bunt infection from soil-borne spores after seed treatment with mercury compounds.

b. Soil-infestation with bunt spores and soil-borne infection of wheat bunt

Several workers (21, 23, 24, 28, 30, 66) proved that soil-borne spores of wheat bunt were the cause of failure in

seed treatment and subsequent infection. Winkelmann (64) reported that extended cultivation of wheat and combine threshing provided increased opportunities of soil infestation, and these were responsible for increasing the prevalence of wheat bunt in Germany and elsewhere. Young (66) also observed a range of 0.2-36.7% infection due to soil infestation by smut spores.

It is now well known that due to combine harvesting of wheat, the chances of soil contamination are great. Leukel et al. (40) have reported that during harvesting and threshing operations of wheat by mechanical means, smutty heads are broken, spores are blown away with the wind and settle on the ground. Contamination mainly occurs where summer fallowing is a common practice. The problem was more serious in mountainous and isolated valleys and it was suggested to get a better knowledge of local seasonal influences upon infection. Similar statements were made by Zobrist and Thiolliere (67). Fischer and Holton (21) have published pictures showing clouds of smut dust pouring from the combine thresher and resulting in soil contamination. According to Gussow and Conners (28) Heald and George found in the State of Washington, that a spore trap, located a mile and a half away from the nearest threshing machine, collected over 1000 spores per square inch during one week.

The evidence of soil-borne infection in wheat bunt

was made by several workers. Eastham (13, 14) reported that winter wheat became infected by soil-borne spores resulting in heavy losses. Galloway (22) obtained the evidence of soil-borne infection of wheat bunt, T. caries, T. foetens and T. indica at Kernal in India. At Utah when healthy soil was heavily infected with T. foetens and wheat was planted at 10-day intervals, infection could occur throughout the period of planting but incidence of bunt declined soon after spore dissemination (2). These findings were confirmed by Weltzien (61) who demonstrated that spores did not last longer than four weeks in moist soil in Germany.

IV. Factors Affecting the Development of Infection in Covered Smuts

1. Covered smut of barley

According to Leukel and Tapke (41) and Tapke (58) when infected barley seed is planted, smut spores and seed germinate at the same time and germ tubes invade the young seedlings before their emergence from the soil. Infection was favored by certain environmental factors. Slightly acidic soil, medium moisture content of soil and soil temperature between 60-70°F, were considered more conducive factors for infection. Popp (52) suggested that the incubation substrate influenced the degree of infection. Under constant conditions of temperature and moisture, he obtained the highest infection in perlite or vermiculite, moderate in greenhouse silica sand and green house potting soil, and light or absent in heavy clay loams.

Faris (18, 19) conducted a series of experiments with different temperatures, soil pH and soil moisture, and found the lowest infection (14.8%) at 5°C, pH 5 and 40% water holding capacity of soil. The highest infection (83.3%) developed at 20°C, pH 5 and 50% water holding capacity of soil. At 25°C, there was a distinct drop in infection and changing the temperature between 10 and 25°C gave higher infection than at constant temperature tests. Tapke (57) confirmed these results and concluded that pH effect was however less marked at 10 and 15°C than 20 and 25°C.

Taylor and Zehner (59) found that 115 times more smut appeared in barley sown at a depth of 3" than when sown at a depth of only $\frac{1}{2}$" due to the longer susceptibility stage of seedlings.

2. Covered smut of wheat

It is known that the degree of infection of wheat with T. foetida is dependent upon a number of environmental factors. Soil type, soil temperature and soil moisture are all involved and any one of these factors may limit infection (53). Several workers (2, 24, 40, 53) showed that the wheat bunt fungus can infect the young seedlings more readily when soil temperature is 40-60°F, and soil moisture 20-30%. Exceedingly high amount of moisture in soil inhibited infection. Gibs (24) conducted a series of experiments with different temperatures and soil moisture levels and found that bunt was the lowest in the highest temperature, and infection was more at low moisture content. Gibs concluded that disease occurence was greater between the lowest and medium water levels than it was

between medium and highest water levels. At medium temperature, spores germinated quickly and wheat seeds slowly resulting in severe infection and deficiency of soil moisture favored spore germination and prolonged the susceptible stage of grain. Rodenhiser and Taylor (53) studied the effect of environmental factors, soil type and reaction and soil sterilization on bunt infection at different incubation temperatures by using L-2 race of T. levis. Soil and soil temperature during incubation period were closely related in the determination of degree of infection contracted by a given variety . They could not draw any general conclusion from the effect of steam sterilization of soil on bunt infection.

Melchers (44) surveyed the relation of environmental factors to plant diseases and cited the example of influence of soil and meteorological conditions on the development and distribution of T. caries and T. foetida. The occurence of disease in one field and its absence in others, assuming that the same seed was used, was undoubtedly attributed to variation in soil temperature resulting from sowing on different dates. They suggested that such a knowledge has an important bearing on the program of breeding bunt resistant varieties having planting period as an essential feature, otherwise bunt resistant varieties may become susceptible. Bohus and Podhradszky (7) reported that there was a definite connection between the distribution of fungi and quantity of rainfall in Hungary. The incidence of T. foetida was over 95% in regions with rainfall below 600 mm.

V. Control Measure Against Covered Smuts

Seed treatment has been the main practice for combating the smut diseases, whether seed-borne or soil-borne, (21). Zobrist and Thiolliere (67), Pichler (51) and Holton (34) suggested to use hexachlorobenzene (HCB) preparations for seed treatment where soil-borne infection could occur. Fischer and Holton (21) reported that HCB and its preparations are highly effective against infection of wheat bunt from soil-borne inoculum. In the Pacific Northwest, clean seed, treated with HCB and planted in contaminated soil, showed only 2% infection as compared to 55% in untreated plots. When inoculated seed was treated and planted in contaminated soil, HCB preparations reduced the infection from 80% in untreated plots to 3% in plots grown from treated seeds.

Weltzien (61) demonstrated the superiority of Pentachloronitrobenzene (PCNB) over mercury preparations under laboratory and field conditions as seed disinfectant against T. tritici. The average incidence of infection dropped from 66% (Mercury treatment) to 0.75% (PCNB treatment).

MATERIALS AND METHODS

Field experiments and laboratory investigations were undertaken from summer 1963 to summer 1964. The field experiments were conducted at the A.U.B. Agricultural Research and Education Center, located in the Beqa'a about 80 kilometers east of Beirut. All laboratory experiments were conducted in the A.U.B. Plant Pathology laboratory.

I. Collection and Preparation of Inoculum

During spring 1963, covered smut of wheat was multiplied in the field by planting 4000 bunt contaminated seeds of "Mishrakani" wheat. This yielded about 300 bunted heads. In june 1963, about 600 ears of "Baladi" barley, infected with covered smut, were collected from one barley field adjacent to the A.R.E.C. The infected ears of wheat and barley were air-dried. Some of the infected material was crushed and passed through a 0.023" mesh. Sieved spores of Ustilago hordei and Tilletia foetida were kept in glass bottles. The remaining infected material was kept as such in paper bags.

II. Standardization of Techniques

Some of the techniques that were to be used frequently were standardized throughout the course of study.

1. Preparation of disks of media

Whenever the germination of U. hordei or T. foetida was to be examined on agar disks, 10 ml of melted medium was poured into clean, selected, sterilized, flat bottomed 90 mm petridishes, kept on a levelled surface and given a layer of medium about 1.5 mm in

thickness. The disks were cut with a flamed cork-borer, 9 or 12 mm. in diameter. Disks were then removed by a flame-sterilized needle, and kept on glass slides or on moist soil, depending on the nature of the experiment.

2. Preparation of soil samples

For the field inoculation experiments, sand passed through a 0.023" sieve was mixed with spores. Volume-weight of sand, and weight of spores were recorded. In the laboratory experiments, different types of soils were used. Samples were collected, thoroughly mixed, air-dried and passed through a 0.023" mesh. A known amount of soil was mixed with spores and distilled water was added to bring it to the desired fraction of field capacity. Mostly field soil, brought from the A.R.E.C. was used in the experimental work. This is characterized by pH of 7.6, gravel clay and having 30% moisture at field capacity.

3. Counting of spore germination

All countings were made under a compound microscope with a magnification of 160 x. At least 100 spores were counted under a random microscopic field on each disk, and the results of four disks were averaged.

III. General Experiments on Germination of U. hordei and T. foetida

1. Germination of U. hordei on different media

Three different media, Water agar (WA), Potato-dextrose agar (PDA) and Malt agar; all from dehydrated "Bacto" agar, were

tried. The surfaces of 9 mm disks of each medium, kept on glass slides, were inoculated with a drop of spore suspension, dried for 5 minutes and placed in moist chambers. These were kept at room temperature (26-27°C) for 24 hours and counting of spore germination was made.

2. Temperature studies of U. hordei and T. foetida

Temperature levels from 0-35°C with an interval of 5°C were studied. The incubators were adjusted for each temperature 24 hours before the beginning of experiment. For U. hordei, three sets of moist chambers were prepared and in each set, 4 PDA disks were inoculated. These were placed at the desired temperature and after a period, when maximum germination was assured, countings were made. T. foetida spores were dusted on moist soil in three petridishes, which were placed in incubators and were frequently checked. Germination was graded by the intensity of mycelial growth and not by spore countings.

3. Germination of T. foetida and U. hordei on soil

Spores of T. foetida and U. hordei were dusted on the surface of moist soil, prepared from 100 gms farm soil from the A.R.E.C. and 12-15 ml distilled water, in each of two petridishes. These were incubated at 20 and 25°C respectively and frequently observed for germination.

IV. Studies on Longevity and Viability of Smut Spores in Soil

1. Inoculation of soil

To observe how long the chlamydospores of U. hordei and T. foetida remain viable in soil under natural field conditions, two patches as shown in figure 1, were made in a 2-years fallow land on 2.9.63. Each patch was 50 cm x 50 cm and was 2 cm deep, giving a capacity of 5000 cc. 5000 cc sand weighing 7.5 kgs, and 8 gms spores of U. hordei (approximately 15×10^8 spores) or T. foetida (approximately 64×10^6) respectively, were thoroughly mixed for 5 minutes in separate flasks. Each patch was filled with the sand-spore mixture so prepared. The inoculated patches were levelled and protected from external disturbances as far as possible. Throughout the experiment, these were kept clean and free from weeds.

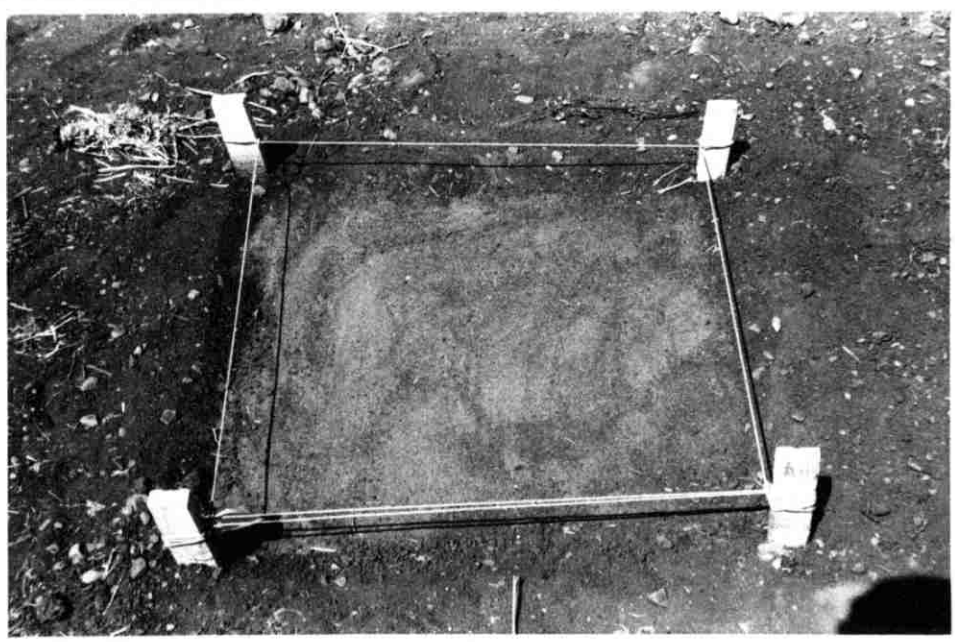

Photo by S.M. Moghal

Figure 1: Inoculated patch in field soil, filled with sand spore mixture.

2. Sampling and reisolation of spores

Random samples were collected from these patches fortnightly or monthly. Spores were reisolated from the soil by preparing a soil suspension in water. At first, the spores floating at the water surface, were collected with a pipette. Later, the spores were separated from the soil by centrifuging at 3000 rpm. for 5 minutes followed by three washings with sterile water.

The germinability of reisolated spores of U. hordei was tested on PDA disks at 25°C. Air-dry spores were included as check. For spores of T. foetida, no germination tests were carried, only the availability of spores was recorded. Meteorological data were also recorded.

3. Longevity studies on U. hordei under constant conditions of temperature and soil moisture

600 gms. of air-dried farm soil and 2 gms. spores of U. hordei (approximately 36 x 10^7 spores) were thoroughly mixed and taken in clean beakers. The soil in beakers was brought to 25, 50 and 75% moisture of field capacity with the addition of distilled water. Beakers were covered by cheese cloth to check evaporation. Their weights were taken daily or on alternate days and water was added to each beaker to maintain the moisture level. Three replicates at each moisture level were incubated at 25°C throughout the experimental period. Samples were taken weekly, fortnightly or monthly and spores of U. hordei were reisolated as

described above and their germinability was tested on PDA disks at 25°C.

V. Studies on the Inhibitory and Stimulatory Actions of Soil on Spore Germination

1. General technique

To detect the inhibitory or stimulating effect of soil on germination of U. hordei and T. foetida, the agar disk technique (figure 2) as described by Jackson (37) and Weltzien (62), was employed. It should be emphasized here that this technique is depedent upon diffusion of soil activity into agar disks when placed on moist soil.

For each test, soil paste was prepared from 100 gms of

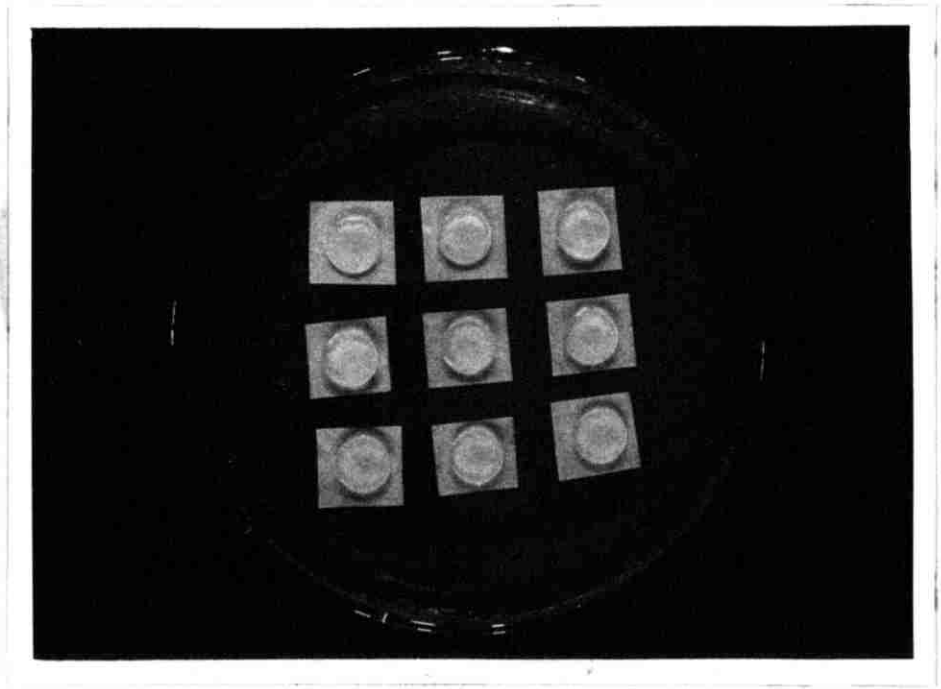

Photo by H. Chohmelian
A.U.B. Photography Dept.

Figure 2. Agar disk technique for soil-inhibition and stimulation studies.

soil and 25-30 ml of distilled water. This was transferred to dishes and the surface of soil paste was smoothened and levelled. 1 cm squares of filter paper, Whatman No. 1, were placed on the moist soil in dishes and agar disks were kept on them to make a good contact between filter paper squares and disks. Either immediately or after a period of incubation at 25°C to allow diffusion of soil activity into the disks, their surfaces were inoculated with a drop of spore suspension or the spores were directly dusted on the surfaces. The dishes were kept at the desirable temperature and removed after a period for examination.

2. Inhibitory effect of different soils on germination of U. hordei

Four different soils, fresh farm soil, two months air-dried farm soil, green house soil and sandy clay soil were tested for assessing the fungistatic activity on U. hordei by the method as described above. Disks of two media, WA and PDA were inoculated after allowing four diffusion times, 0, 6, 12, and 24 hours. Germination was counted after 48 hours. In controls, the disks were kept on moist filter paper squares on glass.

3. Germination of U. hordei on agar disks over autoclaved soils

Farm soil and green house soil were brought to about 50% of water holding capacity and autoclaved without pressure for 45 minutes on 3 successive days. Filter paper squares were oven sterilized at 160°C forl hour. After the sterility test on PDA at 27-28°C, soil paste was prepared as usual under sterile

conditions. Agar disk method was used, and 0 and 24 hours diffusion times were allowed to disks on the soil before these were inoculated. Control tests in the presence of unsterilized soil or without soil were also included.

4. The effect of different stimulating materials on germination of U. hordei

To study the stimulatory effect of different plant tissues or chemicals on germination of U. hordei, 12 mm WA disks were kept on moist soil for 24 hours to allow soil activity to diffuse into the disks. After their inoculation with spores, stimulating materials were placed in the center of the disks. The following materials were tested: barley seeds broken and unbroken, hulled seeds, seed hulls, tissues of barley stems and leaves, dextrose, sucrose and starch. Two controls were prepared, one with the same materials placed on WA disks on moist filter paper on glass slides. The second had no stimulating materials on the disks over the soil.

5. Studies on the stimulatory effect of soils on germination of T. foetida

To notice the stimulatory effect of soils on germination of T. foetida, farm and green house soils were used. Soil pastes were prepared in the usual way, and the WA disk method was employed. The surfaces of disks were dusted with chlamydospores of T. foetida, and the petridishes were incubated in light at 18-20°C for 5 days. Control tests were run in the absence of soils.

VI. Soil-borne Infection in Covered Smuts of Barley and Wheat

In a two-years fallow land, a plot measuring 21 x 6 meters was prepared. A central alley 2 meters wide was allowed, and on each side of it, 42 rows, each 2 meters long, 5 cms wide and 2 cms deep , giving a capacity of about 2000 cc were made. One side was alloted to barley and the other to wheat.

For each row, 2000 cc of sand weighing 3 kg,and 1.5 grams of spores of U. hordei (approximately 27 x 10^7 spores) and T. foetida (approximately 12 x 10^6 spores) were mixed thoroughly for 5 minutes in separate flasks. Starting from 26.7.63 and up to 19.10.63, 5 random rows at a time were inoculated with the sand spore mixture at 15 day intervals. Control rows were filled with pure sand only. The rows were levelled by hand and protected as far as possible.

Planting of barley and wheat was done at one time on 19.10.63 after the first rains. 100 seeds of each, barley or wheat,using the susceptible varieties "Baladi" and "Mishrakani" respectively, were planted in the inoculated or uninoculated rows. Weeding was done throughout the experiment.

At maturity, plants of barley and wheat were checked for infection. Healthy and infected heads were counted. All the heads were then cut by sickles and a second counting was done in the laboratory. Morphological characters as plant height, length of heads, number of tillers and internodes and thickness of stem of 20 healthy or infected plants were recorded.

VII. Methods of Statistical Analysis

The results were mostly expressed in percentages. The data were statistically analysed using the "t-test" and Analysis of Variance Method, according to Snedecor from the book "Statistical Methods".

EXPERIMENTAL RESULTS AND DISCUSSION

I. General Experiments on Spore Germination

1. Germination of Ustilago hordei on different media

The results of germination of U. hordei on disks of three media, Water agar, Potato-dextrose agar and Malt agar are presented in table 1.

Table 1: Germination of U. hordei on disks of three different media at 26–27°C.

(Average of 400 spores)

Medium	Nature of medium	pH	Mean germination %
Water agar	Non-nutrient	6.5	97.3
Potato-dextrose agar	Nutrient	6.3	98.5
Malt agar	Nutrient	5.7	95.0

Differences between media not significant at P = .05

The spores germinated freely on these media with and without nutrients. As shown in figure 3, spores started germinating after six hours. This indicates that the collected spores had high viability and they did not have any dormancy period. Two media, WA and PDA were selected for further studies.

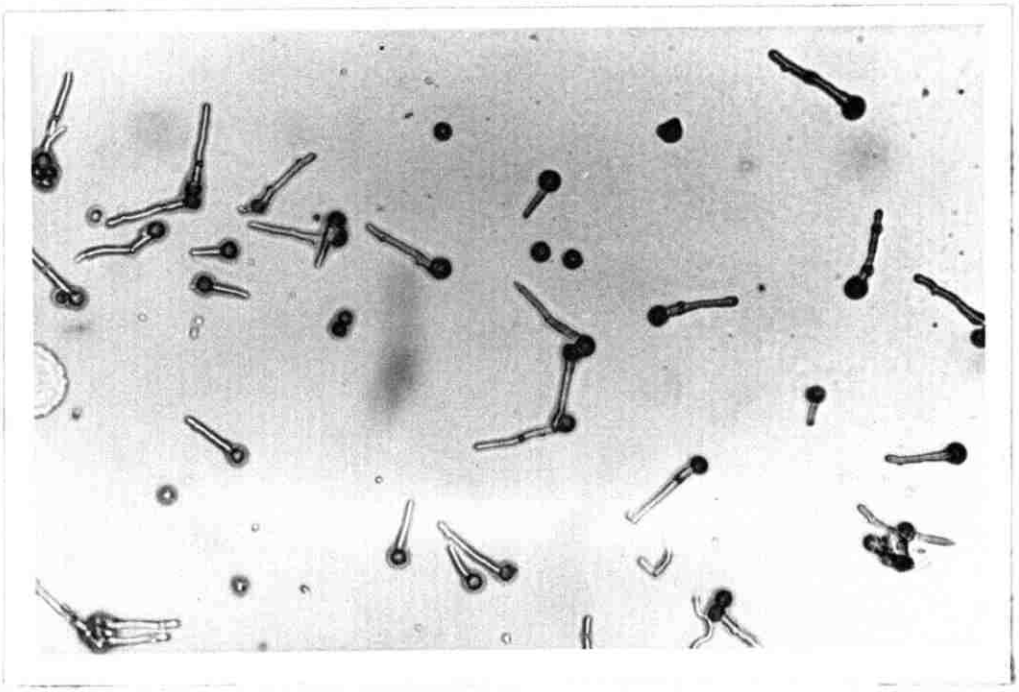

Photo by K. Jabbur

Figure 3. The germination of U. hordei spores on water agar disks after 8 hours at 26°C. x 425 and fluorescent filter.

2. Temperature studies with U. hordei and T. foetida

The effect of different temperatures on germination of U. hordei and T. foetida was quite prominent as shown in table 2. Germination of U. hordei spores was counted on PDA disks, but for T. foetida it was observed by the intensity of mycelial growth on moist soil.

Spores of U. hordei failed to germinate at 0 and 35°C. At 5°C, little germination was obtained even after 9 days. Best germination was observed between 15-25°C. At lower temperatures, spores took longer time for germination, and in many cases germ tubes and sporidia were not well developed. Spores produced long slender germ tubes at 30°C. The germination process was normal

Table 2. The influence of different temperatures on germination of U. hordei and T. foetida.

Temperature °C		0	5	10	15	20	25	30	35
U. hordei[+]	Mean germination %[*]	0	29	65	87	89.5	91.5	53	0
	Germination time days	-	9	4	2	1	1	1	-
T. foetida	Germination	No germination	Little	Adequate	High	High	Very little	No germination	-
	Germination time days	-	14	9	6	8	6	-	-

[+] In germination of U. hordei, each figure represents an average of 400 spores.

[*] Significant difference between means for P = .05 is 7.43.

between 10-25°C and production of sporidia was abundant. 25°C was selected for futher studies as the highest germination was obtained at this temperature.

These results are in agreement with those of Lobik and Dahlstrem (42) and Rump (54). Results disagree from those of Huttig (36) who got 30% germination at -1°C. However, in this experiment spores failed to germinate even at 0°C.

Spores of T. foetida failed to germinate on soil at 0 and 30°C. Some spores germinated at 5°C after 14 days. 10°C gave good germination, but a clear maximum germination was obtained between 15-20°C. At 25°C, only few spores germinated, but some colonies of actinomycetes developed. The morphology of germinating spores was not affected by lower or higher temperatures. Germ tubes, sporidia and fused sporidia were always observed. Temperatures between 15-20°C were selected for futher investigations on T. foetida.

Grasso (26), Lungren and Durrell (43), Holton (34) and Niemann (49) reported the similar results.

3. Germination of U. hordei and T. foetida on soil in petridishes

After the spores of U. hordei were dusted on the surface of moist soil in petridishes, they remained unchanged and no germ tubes were observed for the first two days, although spores on disks of any medium in the absence of soils started germinating within six hours. When some dusted material was examined under the microscope, an insignificant number of spores were observed to produce germ tubes. A majority of the spores were dormant, and

no indication of shrinkage or disintegration of spores could be seen. After 3 days many whitish colonies of actinomycetes, as shown in figure 4, developed and covered the spores on surface. The mycelium of actinomycetes was clearly observed.

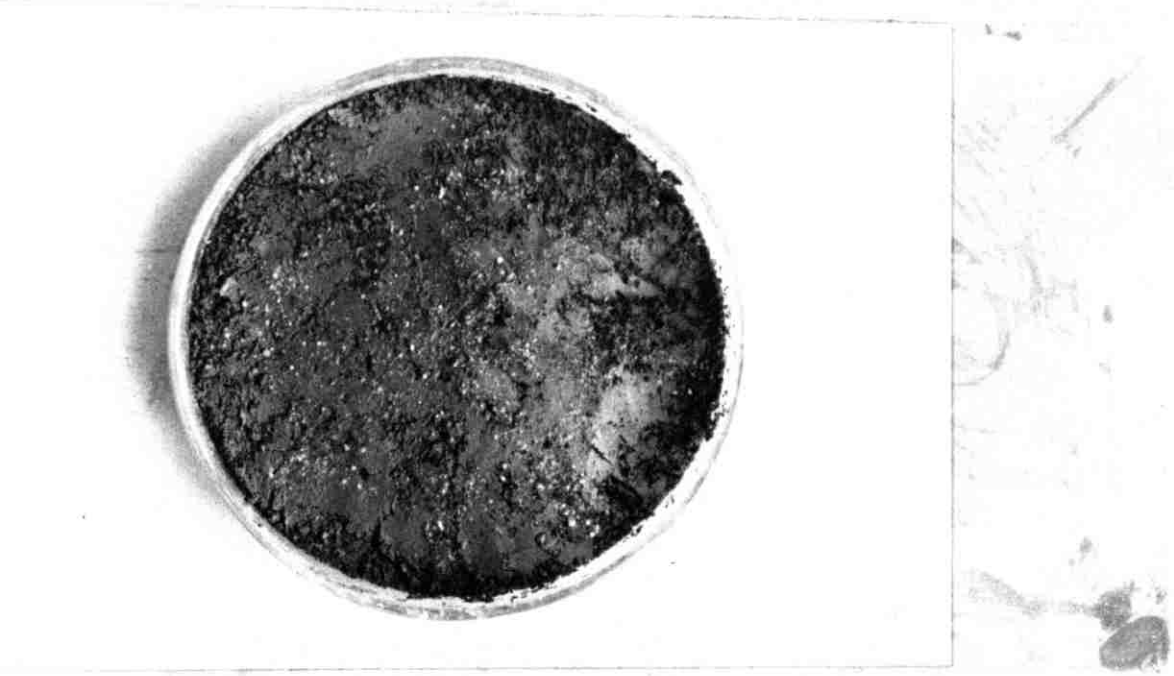

Photo by Dr. H.C. Weltzien

Figure 4. Soil plate dusted with U. hordei spores. The whitish colonies are those of actinomycetes that appeared 3 days after incubation at 25°C.

The results of this experiment disagree from those obtained by Mundkur (47) or by Rump (54) who reported that U. hordei spores could germinate in water or damp soil. Rump reported that germination was profuse in a soil with 20% moisture content. All further attempts, made to germinate U. hordei in farm soil or green house soil with low or high moisture contents, failed to confirm the findings of Mundkur or Rump.

When T. foetida spores were dusted on the surface of moist soil, germ tubes were observed after four days of incubation at 18-20°C. Germination was very clear and profuse as it covered the whole surface of soil in about 8 days. On microscopic examination, germinating spores with germ tubes and sporidia were readily observed.

These results are in agreement with all earlier reports made by Lungren and Durrell (43), Holton (34), Weltzien (61) and Woolman and Humphrey (65).

II. Field and Laboratory Studies on the Longevity and Viability of U. hordei

1. Under natural field conditions

The data on the viability of U. hordei under natural field conditions in the Beqa'a are presented in table 3. Included is the age of spores in the soil at the time of sampling, mean minimum and maximum temperatures of air 165 cm above ground, mean soil temperatures at a depth of 5 cm (temperatures are calculated for the period between two reisolations), accumulated rainfall and germination of reisolated spores in contrast to that of air-dried spores.

The results reveal that the chlamydospores of U. hordei remained viable in the inoculated field soil at the A.R.E.C. for a considerable period. Immediate reisolation of U. hordei spores gave 91% germination as compared to 92.7% in the air dry spores. After four weeks, germination of reisolated spores was significantly reduced from that of the first reisolations as well as from

Table 3. The longevity and viability of U. hordei spores reisolated from infected field soil at the A.R.E.C. during 1963-64.

(Spore germination on PDA in %, average of 400 spores)

Dates of Reisolations	Age of spores in soil weeks	Mean air Temp °C		Mean soil Temp. °C	Accumulated Rainfall mm	Germination %	
		Max.	Min.			Reisolated	Air dry spores
2. 9.63	0	30.5	14.0	22.3	0.0	91.0	92.7
18. 9.63	2	30.3	12.7	23.8	0.0	88.7	92.0
5.10.63	4	28.1	11.4	21.9	0.0	86.0 *	92.0
19.10.63	6	27.4	10.7	18.9	7.1	84.2	91.2
2.11.63	8	16.7	6.8	16.4	63.7	83.2 **	90.0
16.11.63	10	19.2	4.9	15.1	63.7	80.0	90.5
4.12.63	13	15.4	3.2	13.3	94.8	86.2	89.0
19.12.63	15	11.2	-0.06	8.9	129.5	75.0 **	89.0
2. 1.64	17	10.7	-1.8	7.8	136.6	68.9	87.5
17. 1.64	19	7.4	-3.2	5.8	169.0	66.2	88.0
1. 2.64	21	5.1	-5.1	3.9	209.9	64.7	88.0
16.2. 64	23	5.1	-1.7	3.9	328.1	65.0	88.5
5. 3.64	26	11.5	1.1	9.1	329.6	62.5	87.5
4. 4.64	30	15.8	4.0	12.4	447.9	63.2 **	87.0
4. 5.64	35	15.5	2.9	15.3	471.4	54.5	88.0
5. 6.64	39	22.4	6.4	20.7	471.4	42.0	89.0
4. 7.64	44	29.4	12.8	22.8	471.4	31.7	88.0
6. 8.64	48	32.1	14.8	25.3	471.4	19.2	87.0

* Denotes significant differences at P = .05

** Denotes significant difference at P = .01

that of air dry spores. With the prolonged dormancy of spores in the soil, there was a gradual and significant decrease in germinability of the reisolated spores. On the onset of winter rains, this decrease in viability continued and was reduced to 63.2% in 30 weeks. However, between 17-30 weeks duration, there was some indication of stability in the viability of spores and no significant decrease was observed. This period coincides well with high amounts of rainfall and low temperatures. Figure 5 shows that the viability of the reisolated spores decreased sharply after 30 weeks. But even after 48 weeks, spores were reisolated and their viability was still 19.2%.

A decrease in the number of spores during the experimental period was also noticed, but high germination in initial stages and a rapid decrease in the latter half cannot be attributed to the fact that many more spores were available in the beginning than at the end, as the germination counts were always based on 400 spores. No observation was made if the reduction in spore number was due to germination or due to the death of ungerminated spores. However, data clearly indicate that the chlamydospores of U. hordei remained viable in the soil for about one year throughout the periods of drought, high rainfall, temperature as high as 35°C and as low as -11.0°C.

2. The Effect of Rains and Temperature on Viability of U. hordei

The relations between spore germination, accumulated rainfall and average soil temperature are illustrated in figure 6.

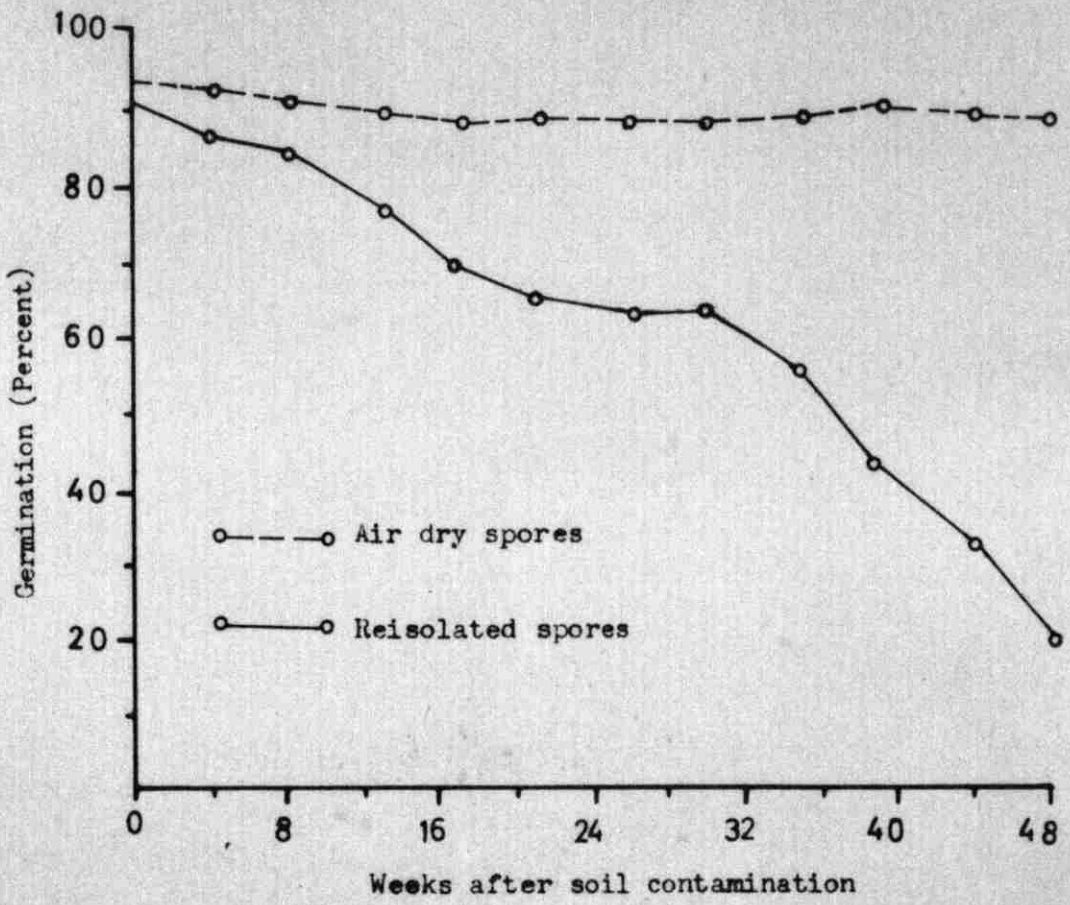

Figure 5. The germination of U. hordei spores reisolated from field soil in the Beqa'a and of dry stored spores.

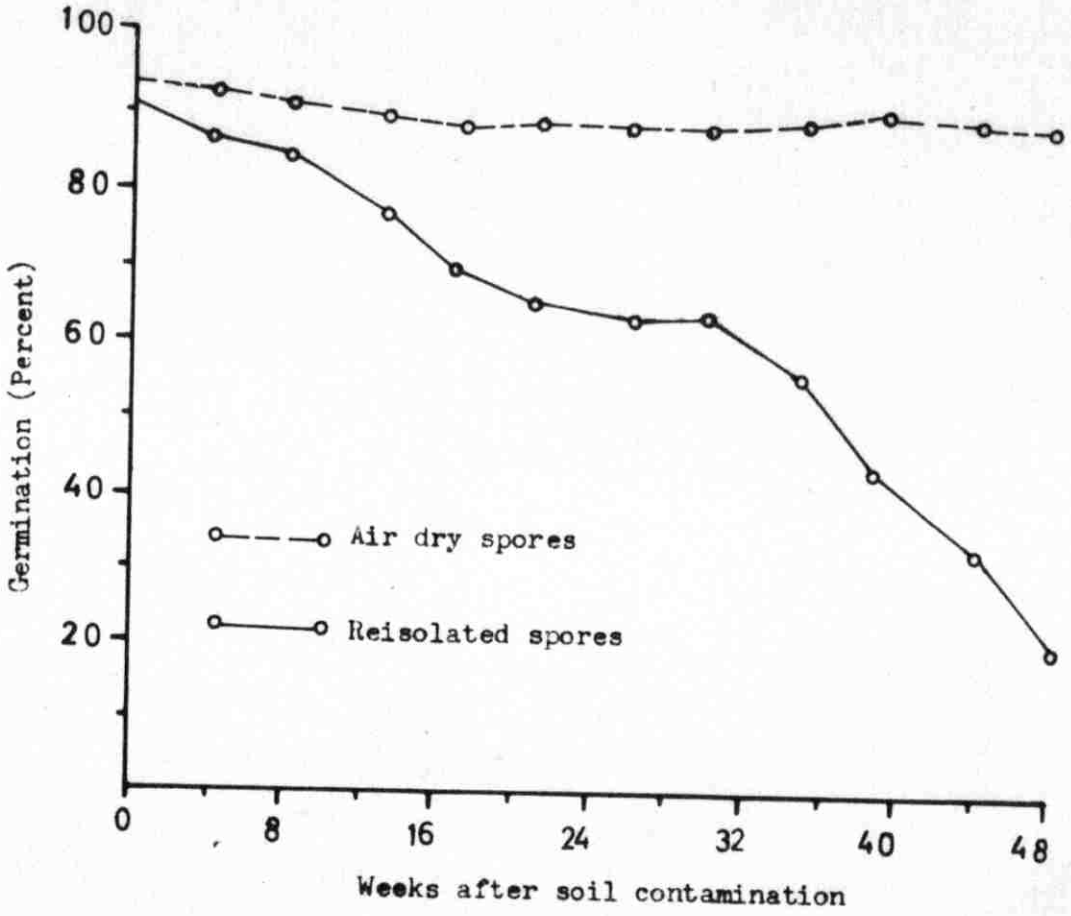

Figure 5. The germination of U. hordei spores reisolated from field soil in the Beqa'a and of dry stored spores.

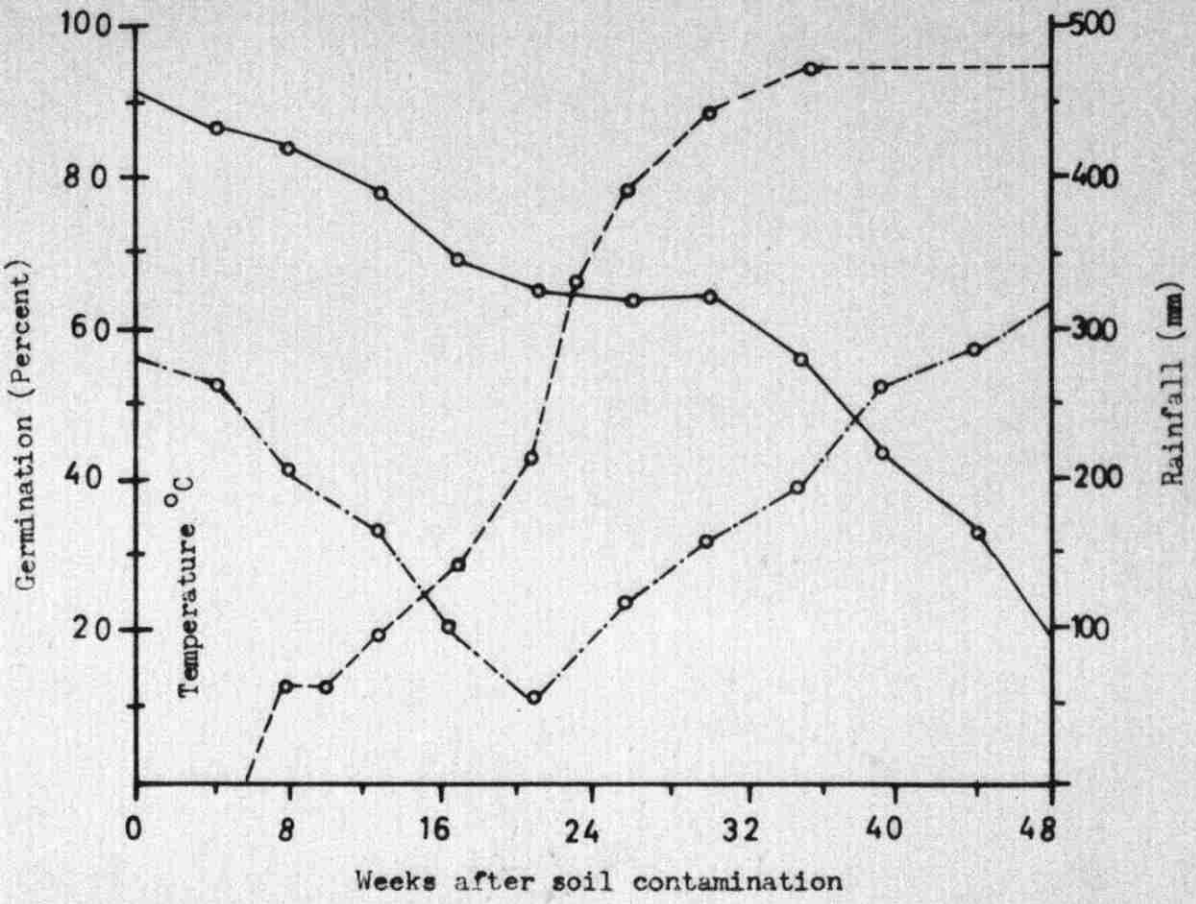

Figure 6. The longevity of U. hordei in soil, accumulated rainfall and average soil temperature in the Beqa'a during 1963-64.

o---o Accumulated rainfall

o-.-o Average soil temperature

o——o Reisolated spores

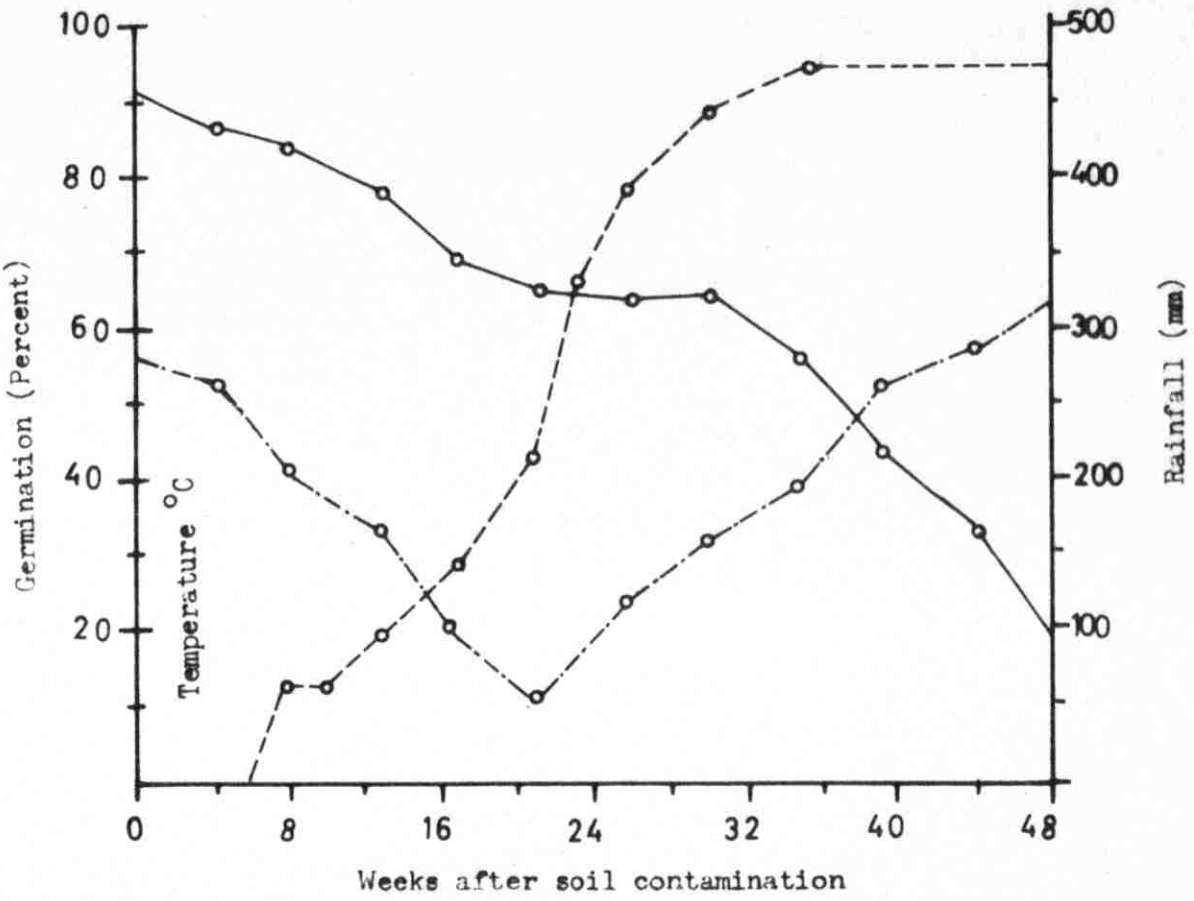

Figure 6. The longevity of U. hordei in soil, accumulated rainfall and average soil temperature in the Beqa'a during 1963-64.

o- - - -o Accumulated rainfall

o—.—o Average soil temperature

o——o Reisolated spores

Unexpectedly, chlamydospores of U. hordei in the soil do not seem to have been much affected by high amount of rainfall. After inoculation, conditions remained dry for 6 weeks and germination of reisolated spores was significantly reduced by about 8%. Along with the rains, gradual decrease in germinability of reisolated spores continued until the accumulated rainfall was 129.5 mm in 15 weeks. There was no significant decrease in germination of reisolated spores between 17-30 weeks and it should be observed that maximum rainfall of 241.9 mm occured in this period. At the end of winter rains, germination of reisolated spores was sharply decreased. Throughout the period, the trend of germination was not much changed but there was a marked decrease in the number of spores available in reisolations which may be due to leaching. The loss of spores continued throughout the rainy period.

During the experimental period there were great temperature variations. For the first 17 weeks, mean soil temperatures remained above 7°C, a temperature known to be favorable for germination of U. hordei, but within this period, viability gradually decreased from 91% to 69%. For the following 13 weeks there was a continuous low mean soil temperature and germination did not drop significantly. After 30 weeks, the mean soil temperature rose again above 10°C, and the germinability of reisolated spores was significantly decreased.

In general,it is evident that the sharp decrease of germination of reisolated spores after 30 weeks coincides well with the end of winter rains and a rise of temperature above 10°C. It seems therefore, these climatic factors greatly influence the viability of

U. hordei spores in the soil, but more experimental data are needed to confirm these findings.

3. Under constant conditions of temperature and soil moisture

The results of germination of reisolated spores of U. hordei, held in laboratory at 25°C in soil with constant moisture contents of 25, 50 and 75% of field capacity are given in table 4.

Throughout the experimental period, there was again a decrease in the germinability of reisolated spores with the increase of age. After one week, germination of reisolated spores from all the samples decreased significantly from that of air dry spores. Throughout the experiment, the decrease in viability was considerably faster in those samples with higher moisture contents. As shown in figure 7, at 75% of F.C. all spores were dead after 30 weeks, while at 50 and 25% of F.C., 29.5 and 40.2% of the spores were still viable respectively after 35 weeks. The decrease of the availability of spores was negligible for 25% of F.C. medium for 50% of F.C. and very pronounced for 75% of F.C.

The data indicate that ungerminated spores of U. hordei can survive in moist soil for a longer time at 25°C, a temperature known to be the most favorable for spore germination. They lose their viability faster in soil of higher moisture contents.

Field and laboratory investigations on the longevity and viability of U. hordei spores reveal such similarities as gradual decrease in germinability of the reisolated spores, and reduction in spore numbers. It may be concluded that the chlamydospores of

Table 4. The longevity of U. hordei under constant conditions of temperature (25°C) and soil moistures (25, 50 and 75% F.C.).
(Spore germination on PDA in %, average of 400 spores.)

Dates of Reisolation	Age of spores in soil - weeks	Germination at soil moistures of 25%	50%	75%	Air dry spores
5.12.63	0	84.6	83.3	84.0	89
12.12.63	1	81.0	80.0	80.0 **	88.5
				*	
19.12.63	2	80.0 *	76.3	74.6	89.0
		*			
5. 1.64	4	76.3	72.0	72.0	88.0
				**	
12. 2.64	9	74.3	68.6 *	63.0	87.7
2. 3.64	12	71.3	69.0	61.6	87.0
			*		
2. 4.64	16	70.6	63.0	57.6	87.0
		*	**		
9. 5.64	21	64.5	55.0	42.0	88.0
4. 6.64	25	57.2	43.0	31.0	88.5
5. 7.64	30	48.5	36.7	0.0 (Few spores)	89.0
5. 8.64	35	40.2	29.5	0.0 (Few spores)	87.0

* Denotes significant differences at P = .05

** Denotes significant differences at P = .01

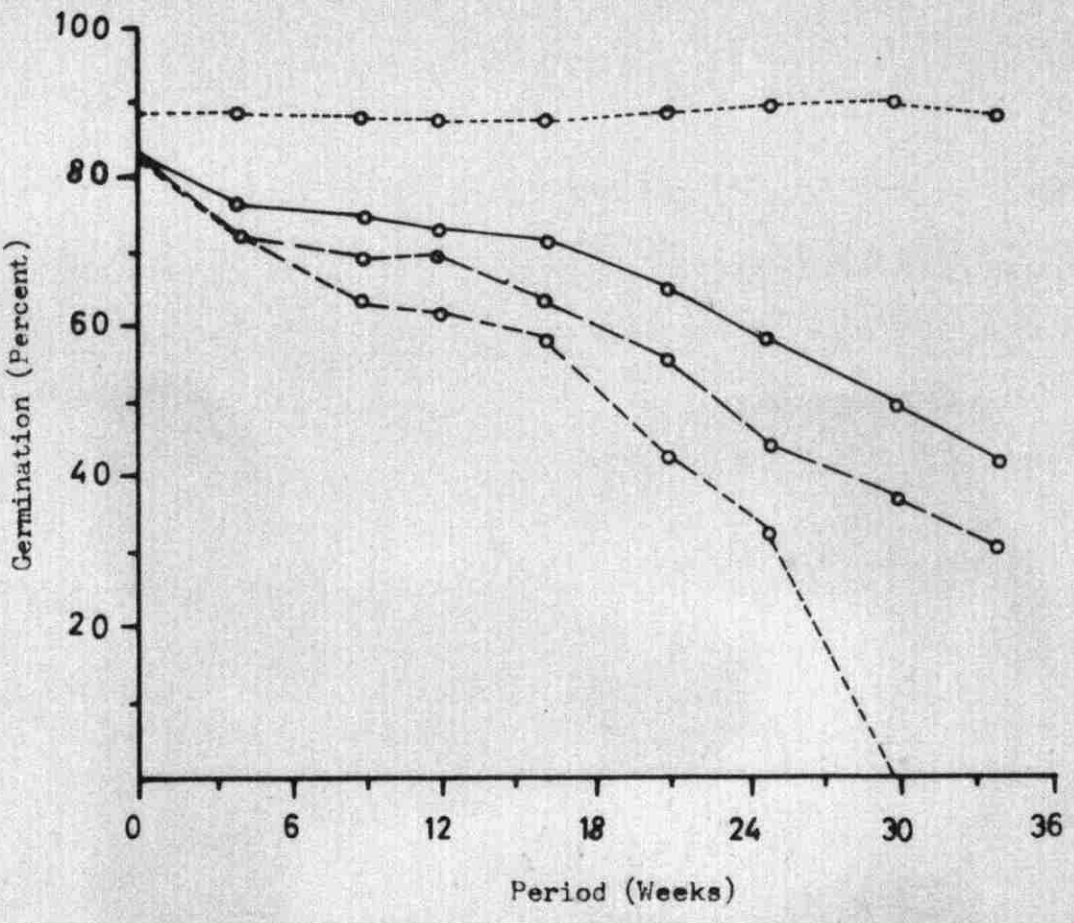

Figure 7. The longevity of U. hordei in soil under constant temperature (25 °C) and soil moistures (25, 50 and 75% Field Capacity) conditions.

o------o Air-dry spores o——o Spores at 25% FC

o— — —o Spores at 50% FC o— — —o Spores at 75% FC

U. hordei remain viable in the soil under varying climatic conditions for at least one year, and their viability is reduced by high soil moisture together with temperature above 10°C. Air dry spores do not lose their viability below 87% within the same period.

It is suggested that these experiments should be continued for a longer time. A quantitative study on the number of reisolated spores should be made in order to get a true picture of the various factors involved. Detailed temperature and moisture relationships should be studied for the further analysis of their effects.

Rump (54) reported that 48 hours exposure of U. hordei spores to -17°C in ice did not much affect their viability, and the spores were resistant to drought as mentioned by Gussow and Conners (28). Some workers (20, 50, 54) reported the longevity of U. hordei spores from 5 to 23 years without any appreciable loss in germination if stored dry. We may therefore expect their longevity in dry soil for some time at least. However as viable spores could be reisolated from the moist soil at favorable temperatures, we may assume an active inhibition of spore germination by soil.

III. Longevity and Viability of Tilletia foetida Under Natural Field Conditions

As no germination tests for the reisolated spores of T. foetida were carried out, only their availability at each reisolation was determined as indicated in table 5.

Table 5. The longevity of T. foetida spores under natural field conditions at the A.R.E.C. during 1963-64.

Dates of Reisolations	Age of spores in soil weeks	Mean air temp. °C	Mean soil temp. °C	Accumulated rainfall mm	Availability of spores
2. 9.63	0	22.3	22.3	0.0	++++
18. 9.63	2	21.6	23.8	0.0	++++
5.10.63	4	19.8	21.9	0.0	++++
19.10.63	6	19.1	18.9	7.1	++++
2.11.63	8	11.7	16.4	63.7	+++
16.11.63	10	12.0	15.1	63.7	+++
4.12.63	13	9.3	13.3	94.8	++
19.12.63	15	5.5	8.9	129.5	++
2. 1.64	17	4.4	7.8	136.6	+
17. 1.64	19	2.1	5.8	169.0	+
1. 2.64	21	0.0	3.9	209.9	-
16.2.64	23	1.7	3.9	328.1	-

++++ Many spores of T. foetida reisolated

+++ Less number of spores

++ Few spores

+ Very few spores

- No spores available in reisolations

For six weeks after inoculation of soil with chlamydospores of T. foetida, conditions remained dry, and many spores could be reisolated. As soon as the rains started, the number of reisolated spores decreased. They completely disappeared within 11 to 13 weeks after the first rainfall. Figure 8 shows the ungerminated reisolated spores of T. foetida from the inoculated soil.

Since no spore counts were made in any reisolation availability of spores is based only on theoretical and comparative numbers of spores

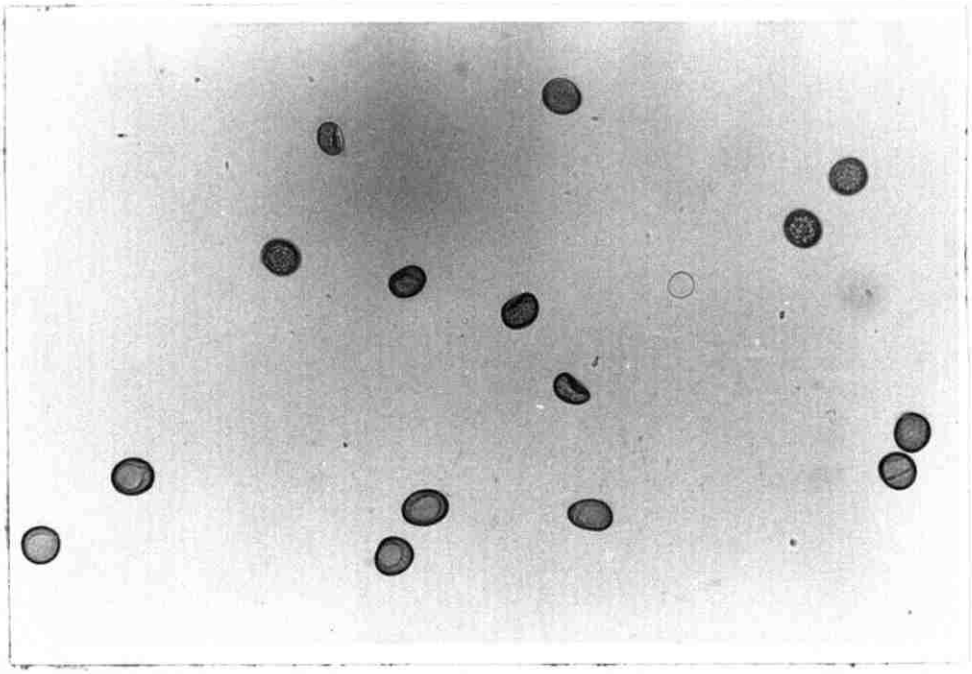

Photo by S.M. Moghal

Figure 8. Reisolated spores of T. foetida from inoculated soil, at 10 weeks age and after 63.7 mm rainfall. Note 5 spores at bottom of picture which show signs of beginning germination. x 265 and fluorescent filter.

in subsequent reisolations. However, complete disappearance of spores at 209.9 mm rainfall clearly points out that these spores germinated in the moist soil. The soil temperature

during the first 19 weeks of the experiment varied between 22 and 6°C, and was thus favorable for germination.

These results, that the spores remained viable in soil under dry conditions, but germinated readily when moisture became available, agree at large with those of Guest (27), Hanna and Popp (30) and Weltzien (61).

IV. Studies on Inhibitory and Stimulatory Actions of Soil on Germination of U. hordei and T. foetida

1. Inhibitory activity of four different soils on U. hordei

The results of this experiment are presented in table 6. The data show that germination of U. hordei was reduced on disks of two media when kept on moist soils. The differences between soils and media were not significant. With the increase of diffusion time, germination on all four soils was significantly decreased below the germination % of the control disks. It was reduced to 54.7%after 0 hours, 12.1% after 6 hours, and 3.1% and 1.1% after 12 and 24 hours, irrespective of any soil or medium. Figure 9 shows clearly that soil activity diffuses into the disks mainly in the first 6 hours. Then diffusion slows down, probably due to saturation of the disks.

Morphology of the germinating spores, particularly after 12 and 24 hours diffusion activity of soil, was very much affected. Germ tubes were not well developed, septation was not clear and sporidia were not produced. Figure 10 shows non germinated U. hordei spores inhibited by farm soil after 24 hours diffusion

Table 6. The inhibitory effect of four different soils on germination of Ustilago hordei on WA and PDA disks on soil with different diffusion times.

(Spore germination in % - average of 400 spores)

Soils	Green House Soil			Fresh Farm Soil			2 months Air-dried farm soil			Sandy Clay			=	Disks on Glass	
pH	7.5			7.6			7.6			7.1			X **		
Diffusion time	WA	PDA	X	WA	PDA	X	WA	PDA	X	WA	PDA	X		WA	PDA
0 - Hour	45.2	54.2	49.6	49.7	66.2	57.9	48.5	60.2	54.3	47.5	66.7	57.1	54.7	88.0	89.0
6 - Hours	9.0	13.0	11.0	10.2	13.7	11.9	11.2	13.7	12.4	11.2	15.2	13.2	12.1	87.5	88.0
12 - Hours	3.0	5.2	4.1	2.7	4.0	3.3	2.0	4.0	3.0	2.2	2.0	2.1	3.1	88.0	89.0
24 - Hours	1.2	1.5	1.3	1.0	1.5	1.2	1.2	1.5	1.3	1.0	0.7	0.8	1.1	87.0	87.0
$\bar{X}$	14.6	18.4	16.5	15.9	21.3	18.6	15.7	19.8	17.7	15.4	21.1	18.3	17.7	87.6	88.2

X Denotes mean germination percent on different soils.

$\bar{X}$ Denotes mean germination percent on different media.

$\bar{\bar{X}}$ Denotes mean germination in different dissufion times.

** Denotes significant differences in diffusion times at P = .01

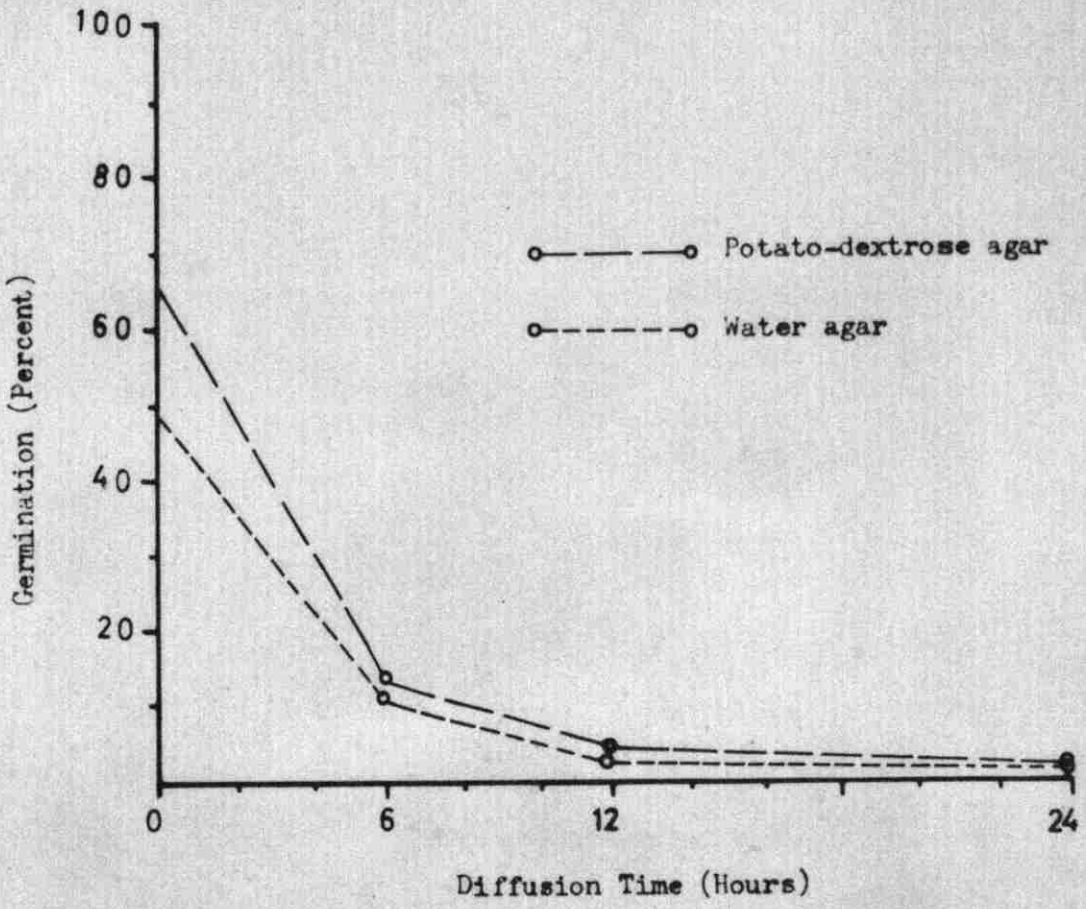

Figure 9. The inhibitory effect of farm soil on the germination of U. hordei on water agar and PDA disks after different diffusion times.

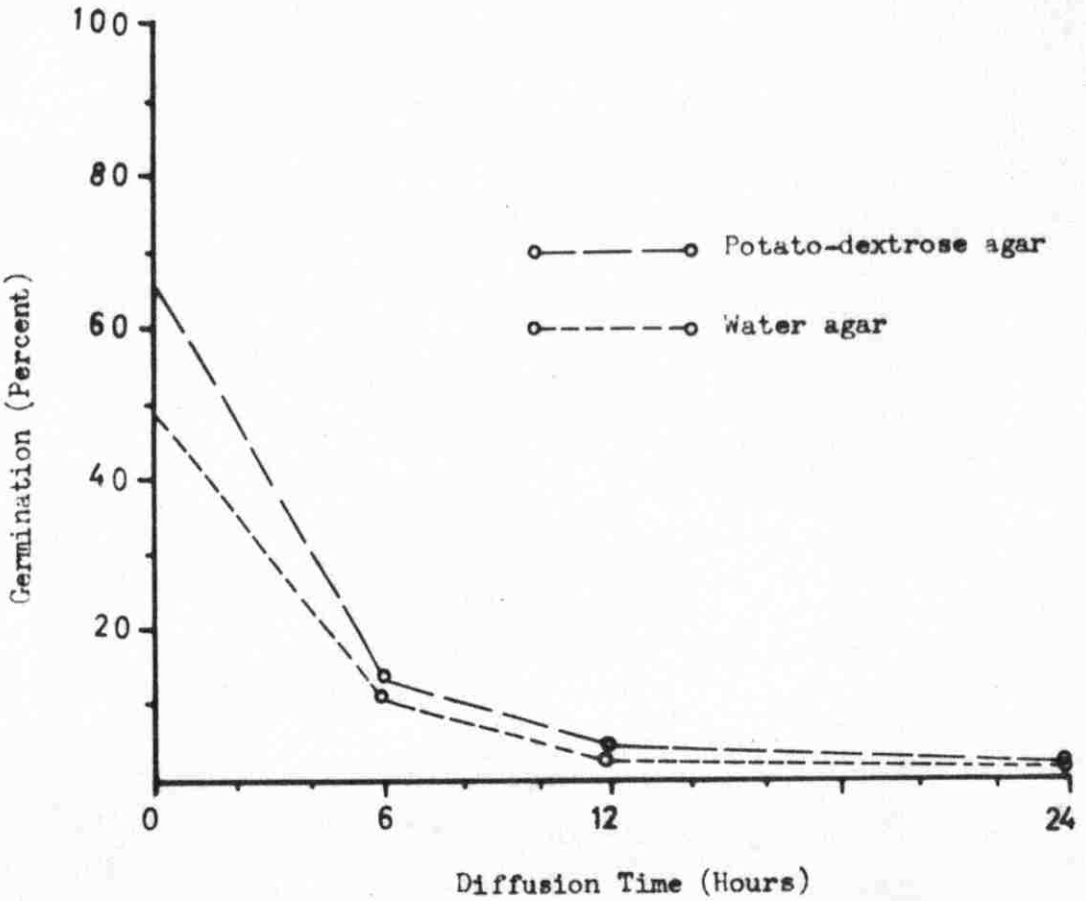

Figure 9. The inhibitory effect of farm soil on the germination of U. hordei on water agar and PDA disks after different diffusion times.

time. Strong inhibition was however found to be dependent on many factors, as the formation of soil paste with sufficient water saturation, a good contact of disks with the soil with no air bubble in between and a reasonable diffusion time.

The inhibitory action of soil is also very likely to account for the long viability of U. hordei spores, found in the earlier reported field and laboratory conditions.

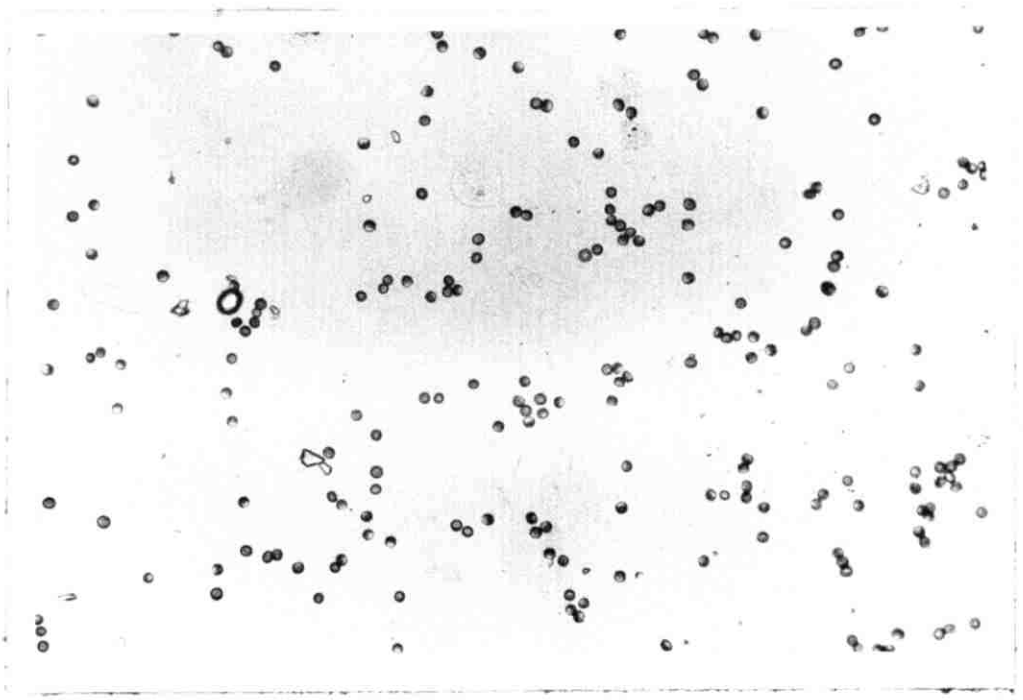

Photo by S.M. Moghal

Figure 10. Non germinated U. hordei spores on WA disks, and inhibited by farm soil after a diffusion time of 24 hours. x 285 and fluorescent filter.

While the inhibition of U. hordei has not been reported before, it is fully in line with the results of Weltzien (62)

who reported a strong inhibition of Ustilago avenae Pers., U. nuda Jens., U. perennans Rostr. and U. zeae (Beckm.) Ung. by natural soils. With the same technique, other organisms as Penicillium citrinum Thom. and Aspergillus fumigatus Fres. were also reported to have been strongly inhibited by natural soils (37, 62).

2. Effect of autoclaved soil on U. hordei

Some workers as Bywater (12), Jackson (37) and Weltzien (62), reported a loss of inhibitory activity by soil sterilization. Green house and farm soils were autoclaved and after a sterility test on PDA at 26-27°C, were tested for inhibitory activities. The results of this experiment are given in table 7.

Table 7. Germination of U. hordei on water agar disks on autoclaved and untreated soils.
(Spore germination in %, average of 800 spores).

Diffusion time	0 Hour		24 Hours		
Soils	Auto-claved	Un-treated	Auto-claved	Un-treated	Control on glass
Green house soil	61 **	48 *	15	0	88.0
Farm soil	60 *	53	13 **	1	88.0

* Denotes significant differences at P = .05

** Denotes significant differences at P = .01

Untreated soils in this experiment again inhibited spore germination after a diffusion time of 24 hours. On autoclaved soils, germination was significantly higher, but was still much less than in the controls.

As these results do not agree with earlier reports (12, 37, 62), a more detailed study of the phenomenon is suggested before some conclusions on the effect of soil sterilization on the inhibition of U. hordei spores can be drawn.

3. The effect of different stimulating materials on germination of U. hordei

Weltzien (62) has reported that inhibition of Aspergillus fumigatus Fres. could be broken by various plant tissues or chemicals. Thus stimulation studies were also undertaken on U. hordei with the same technique. Different tissues of barley and some carbohydrates were tested as they are most likely to come in contact with dormant and inhibited spores in the soil, and thus may stimulate germination and infection. Table 8 gives the results of this experiment.

Table 8. The effect of different stimulants on germination of U. hordei on agar disks over soil.
(Spore germination in %, average of 400 spores).

Material	Kind	Disks on soil	Disks on glass
Seeds	Dehulled barley seeds	54.0	87.7
	Hulled barley seeds	50.0	88.5
	Broken barley seeds	50.0	88.0
	Barley seed hulls	9.0	88.0
		**	
	Check	1.5	88.0
Plant Tissues	Barley leaf	25.0	85.0
		*	
	Barley stem	37.0	86.5
	Check	2.0	87.0
Carbohydrates	Starch	0.0	89.0
	Sucrose	61.5	88.0
		**	
	Dextrose	49.5	88.0
	Check	1.0	88.0

* Denotes significant differences at P = .05

** Denotes significant differences at P = .01

Barley seeds, dehulled, hulled and broken, strongly stimulated germination to 50-54%, and no significant differences

were found between them. Seed hulls however gave only 9% germination compared to 1.5% in checks on soil. Also, barley leaf and stem tissues stimulated germination, but stimulation from these plant tissues was less active. Stem tissue was significantly more active than leaf tissue.

Strong stimulation was observed in case of sucrose, which gave the highest germination of 61.5%. Dextrose showed comparatively less stimulatory effect, and differences in germination stimulated by sucrose and dextrose, were significant. Starch did not exhibit any stimulatory effect. However, none of the tissues or substances allowed a germination as high as 88% in the checks on glass.

These results demonstrate that the fungistatic effect of soil on germination of U. hordei can be overcome by the action of some stimulating substances available in barley seeds, barley leaf and stem tissues, and by some carbohydrates present during seed germination. No further study was undertaken, but it could be anticipated that the addition of manures, roots, stem and leaf tissues, nutrients or green manures into the field soils would also in some cases stimulate the germination of U. hordei. This aspect should be studied as any stimulating material may well either promote or counteract the infection of barley.

The results are in good agreement with those reported by Weltzien (62).

4. Stimulatory effect of soil on germination of Tilletia foetida

In contrast to U. hordei, T. foetida germination is stimulated by natural soils. The agar disk technique was found most favorable to exhibit this stimulatory effect of soils, and it was therefore employed with farm and green house soils. The results are given in table 9.

Table 9. Germination of T. foetida on disks, as stimulated by farm and green house soils.

(Spore germination %)

Replications	Disks on farm soil	Disks on green house soil	Disks on glass
1	62	55	8
2	53	70	10
3	50	68	12
4	51	72	11
5	53	61	15
6	60	70	8
7	42	64	16
8	48	60	9
$\bar{X}$	52.3 **	65.0 **	11.1

** Denotes significant differences at P = .01

Chlamydospores of T. foetida, dusted on the surface of water agar disks on glass gave 11.1% germination after 7 days. Germination was significantly increased when the inoculated disks were placed on moist farm and green house soils. The stimulatory effect of green house soil was significantly stronger than the one from farm soil. Thus soils with a higher biological activity seem to be more stimulatory than others. Germination was found rather uniform in all tests, and all the stages in the germination process were clearly visible up to the formation of dikaryotic conidia.

This experiment demonstrates that the known stimulatory effect of soil on germination of T. foetida, (Gimesi and Frenyo, 25 and Ettel and Halbsguth, 15) can well be studied with the agar disk technique, which allows to calculate the germination percent and thus represents a marked improvement above all previously used methods.

V. Infection of Covered Smuts of Barley and Wheat from Soil-Borne Spores

1. Covered smut of barley

The results of the 1963-64 infection experiment under field conditions in the Beqa'a are presented in table 10.

At planting time, spores were in the soil up to 12 weeks, and infection could occur from the soil-borne spores. The infection was always very low and never exceeded 0.77%. Differences between inoculating dates were insignificant.

These results again demonstrate the longevity, viability and pathogenicity of U. hordei spores in the soil, and in principal prove the possibility of soil-borne infection in covered smut of barley.

The low disease incidence may be attributed to the inhibitory action of soil on germination of U. hordei. Since soil-borne infection has been found possible, more detailed

Table 10. The incidence of covered smut of barley from soil-borne spores at the A.R.E.C.

(From 5 rows of 100 seeds each)

Date of inoculation of rows	Age of spores in soil at planting time weeks	Total heads counted	Infection		Mean infected heads %
			Infected plants	Infected heads	
26. 7.63	12 weeks	1386	2	9	0.65
9. 8.63	10 weeks	1500	0	0	0.0
23. 8.63	8 weeks	1750	1	4	0.24
7. 9.63	6 weeks	1375	2	9	0.66
21. 9.63	4 weeks	1490	1	5	0.34
4.10.63	2 weeks	1550	4	12	0.77
19.10.63	0 weeks	2200	0	0	0.0
Check	-	1852	0	0	0.0

Difference between means of infected heads not significant at P = .05

studies are needed to confirm these findings and to determine the relationships of various factors such as stimulating materials involved in the occurrence of the disease. This study confirms the results obtained by Bleck (6) who observed 5% infection in one of the eight inoculated plots.

2. The Effect of disease on the morphology of plants

Some of the morphological characters, shown in figure 11, of U. hordei infected plants were studied by comparison of healthy ones. 20 healthy and 20 infected plants were selected at random for the study of morphological characters, except for the character 3 where only 5 healthy and 7 infected plants are taken. The results are given in table 11.

Table 11. The effect of U. hordei on infected plants of Baladi barley

No. in figure	Characters of plants	Healthy	Infected	't' value	P value
1.	Height of plant	70.4 cm	51.2 cm	11.17	.001**
2.	Length of heads	9.2 cm	2.7 cm	23.65	.001**
3.	Length of top internode	20.3 cm	9.4 cm	7.8	.001**
4.	Length of third internode	11.3 cm	11.3 cm	1.39	.1-.2
5.	No. of internodes	5	5	-	-
6.	No. of tillers/plant	6.35	6.89	1.27	.2-.3
7.	Diameter of third internode	0.4 cm	0.24 cm	2.12	.2-.05*
8.	Maximum length of leaf at third node of plant	15.8 cm	11.4 cm	4.75	.001**
9.	Maximum width of leaf at third node of plant	1.2 cm	0.8 cm	2.40	.2-.5*

* Denotes significant differences at P = .05

** Denotes significant differences at P = .01

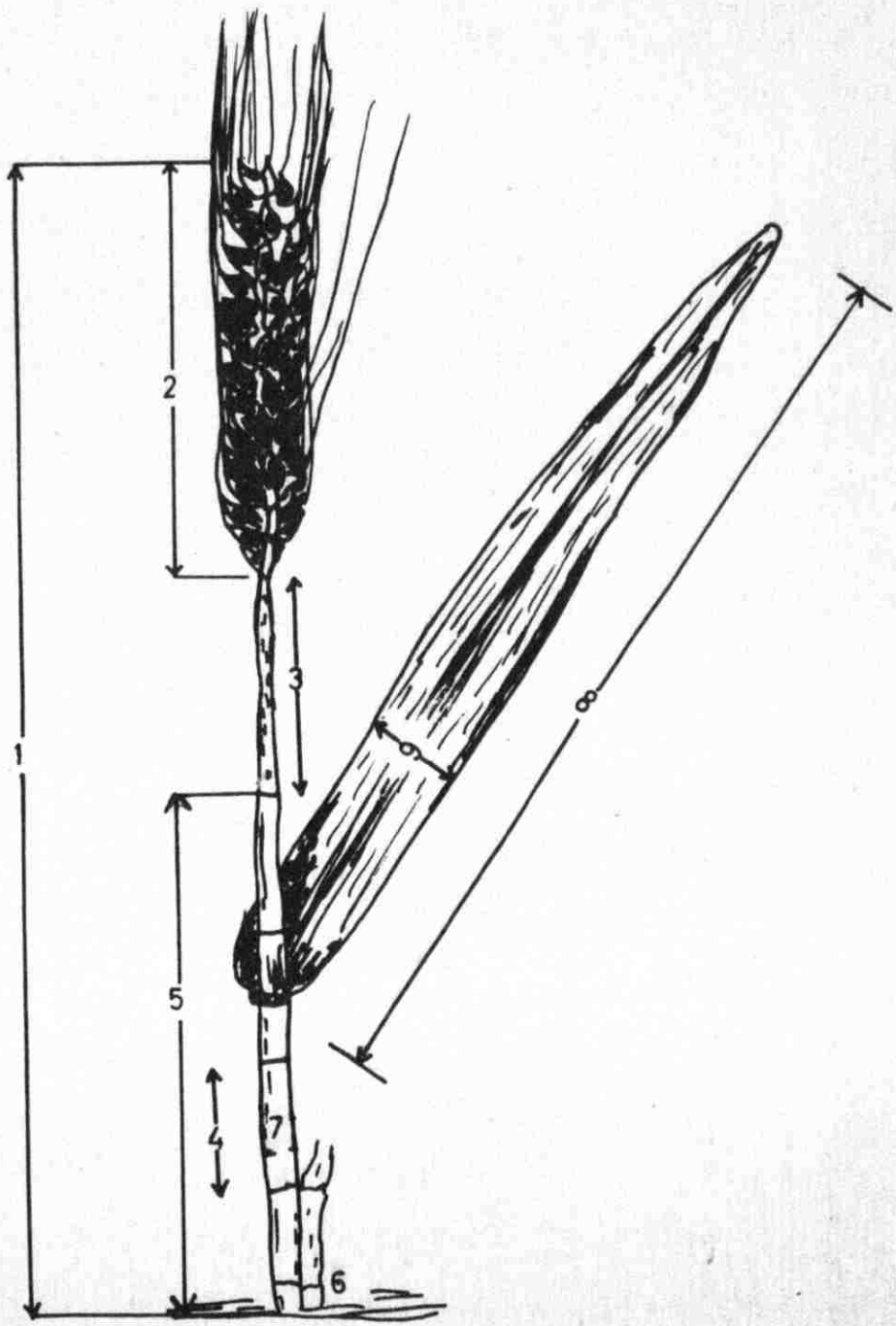

Figure II.The morphological characters studied in healthy and infected plants of barley.

The infected plants were very much stunted and their lengths were significantly reduced by 38%. Number of internodes or their lengths were not affected, hence only third internode was taken for length as representative. The reduction in the height of infected plants was due to the fact that the length of top internode, and length of heads were significantly reduced. There was a slight but insignificant increase in the number of tillers in infected plants. Culms of infected plants were thinner. Manifestations of the disease were very conspicuous on the leaves which were yellowish green. The leaves of the infected plants were much narrower than those of healthy plants. The measurements, recorded from the leaves at the third node of healthy and infected plants, indicated that both length and width of leaves on infected plants were significantly reduced and consequently leaf area was much decreased.

3. Covered smut of wheat

The results of the infection experiment with soil-borne spores of T. foetida are presented in table 12.

As high as 43% infection from the soil-borne spores was found under the Beqa'a conditions. The results clearly point out that longer the spores remained in the soil, less was the infection produced by them. 19% infection from the first inoculation made 12 weeks before planting, supports earlier investigations on the longevity and viability of T. foetida spores in the soil under dry conditions, and also shows their high pathogenicity.

It should be emphasized here that planting of wheat was done when the soil temperature was 19.5°C, rains had just started and

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It should be emphasized here that planting of wheat was done when the soil temperature was 19.5°C, rains had just started and

Table 12. The incidence of covered smut of wheat from soil-borne spores at the A.R.E.C.
(From 5 rows of 100 seeds each).

Date	Age of spores in soil at planting. weeks	Total heads counted	No. of infected heads	% infected heads *
26. 7.63	12	898	170	18.96
9. 8.63	10	984	225	22.84
23. 8.63	8	1023	459	25.52
7. 9.63	6	938	222	23.64
21. 9.63	4	968	323	33.38
4.10.63	2	854	295	34.60
19.10.63	0	991	427	43.04
19.10.63	Check	1036	0	0.0

* Significant difference between means of infected heads for P = .05 is 8.75.

moisture became available to seeds and spores. These conditions were found most suitable for germination under the laboratory conditions. Since no more plantings were made after the rains, it is suggested that further studies should be conducted to determine the longevity and pathogenicity of T. foetida spores also in moist soils. This might allow recommendations for good planting dates to check soil-borne infection in the Beqa'a.

The results of this study agree at large with those of Eastham (13, 14), Young (66) Winkelmann (64), and of many others

(28, 30, 40, 41, 46 and 61) in various parts of the world.

4. The effect of disease on the morphology of plants

Table 13 gives some of the morphological characters measured on 20 healthy and 20 diseased plants.

Table 13. The effect of T. foetida on infected plant of "Mishrakani" wheat.

Plant Character	Healthy	Infected	't' value	P value
Height of plant	76.2 cm	74.8 cm	0.87	0.4-0.5
No. of internodes	6	6	-	-
Length of heads	9.8 cm	9.3 cm	1.1	0.3-0.4
No. of tillers	6.90	6.4	1.21	0.2-0.3
Thickness of third internode	0.53 cm	0.47 cm	1.01	0.3-0.4

Plant height, size of heads and number of internodes in infected plants were not significantly affected. A slight decrease occured in number of tillers on the infected plants, but differences were not significant. Infected plants were looking thinner than the healthy ones, but again the differences were not significant.

SUMMARY AND CONCLUSIONS

Field experiments on the longevity and viability of U. hordei and T. foetida chlamydospores, and soil-borne infection in covered smuts of barley and wheat were conducted at the A.U.B. Agricultural Research and Education Center in the Beqa'a, Lebanon during 1963-64. Laboratory investigations on physiology of germination of these spores were made in the Plant Pathology Research Laboratory at Beirut.

The inocula of the two covered smuts were either multiplied in the field or collected from the fields adjacent to the A.R.E.C. Spore germination experiments indicated that U. hordei could germinate readily on three different media, Water agar, Potato-dextrose agar and Malt agar, in a temperature range of 5-30°C, and best at 25°C. However, they failed to germinate in moist soil at 25°C. Chlamydospores of T. foetida germinated in soil in petridishes in a temperature range of 5-25°C, with an optimum between 15-20°C.

From inoculated field soil, the spores of U. hordei were reisolated at 15-30 day intervals to observe their longevity and viability under natural field conditions. Chlamydospores of U. hordei remained viable in soil for about one year. During the experimental period, there were great variations in climatic conditions. After four dry weeks rains started. Thirty weeks after the start of rain, the accumulated precipitation reached 471.4 mm. The viability of spores was reduced by that time to 54.5%. Continuous low temperature for eight weeks did not adversely affect the spore viability, but at the end of winter rains and with an

increase of temperature above 10°C, viability of reisolated spores was sharply decreasing. It appears that these climatic factors control the viability of spores. When the spores of U. hordei were held under constant temperature conditions (25°C) and constant soil moistures (25, 50 and 75% field capacity) for 35 weeks, spores lost their viability more prapidly at higher than at lower soil moisture. In each case, spores lost their viability with the increase of age of spores in continuous moist soil. From these results it was assumed that soil has an inhibitory effect on germination of U. hordei spores.

On the other hand, T. foetida chlamydospores remained viable only under dry conditions. On the onset of winter rains, a significant decrease in the number of reisolated spores started and this decrease continued for 10 weeks. When accumulated precipitation reached 209.9 mm, no spores were available in reisolations. Their unavailability indicated that these spores had germinated in moist soil.

For the first time, inhibitory effect of soil on germination of U. hordei was observed by employing agar disk technique. This technique is dependent on the diffusion of soil activity into agar disk when placed on moist soil. Four different soils, farm fresh soil, two months laboratory stored farm soil, green house soil and sandy clay soil, were found to inhibit germination of U. hordei. Almost complete inhibition was obtained when Water agar or Potato-dextrose agar disks were placed on these moist soils 24 hours before inoculation with spore suspension. The morphology of germinating spores on disks over soil, was also affected as germ tubes were not well developed, septations were not clear

and there was no production of sporidia. A definite and complete inhibition of spore germination was found dependent on factors like sufficient soil moisture, good contact of disks with moist soil with no air bubbles beneath disks and a diffusion time before inoculation of disks. Germination of spores on disks of any media in the absence of soil was always above 80%.

The inhibitory effect of soils on germination of U. hordei spores was partly made ineffective by autoclaving the soil. Germination was increased by about 15%. However, inhibition could also be broken when some stimulating materials were placed on the disks after inoculation. Barley seeds gave active stimulation. The tissues of barley leaves and stems showed comparatively less stimulation. Sucrose crystals stimulated germination up to 61.5%. Starch had no such stimulatory effect. This demonstrates that the fungistatic effect of soil can be neutralized and spores may be allowed to germinate. This aspect needs detailed studies as the addition of such stimulating materials to soil may either promote or counteract the infection of barley.

The agar disk technique was found most suitable to study the well known stimulatory effect of soil on germination of T. foetida spores. When inoculated disks were placed on moist green house and farm soils, germination of T. foetida chlamydospores with germ tubes, fused sporidia and dikaryotic conidia was clearly observed after 5 days. The stimulatory effect of green house soil was significantly stronger than the one from farm soil. This method of spore germination represents a marked improvement above all the previously used methods as it allows to calculate the germination in percent.

Rows in the field at A.R.E.C. were inoculated with a sand-spore mixture at 15 day intervals between 26.7.63 and 19.10.63. Planting of barley and wheat was done at the latter date after the first rains had fallen. Soil-borne infection of coveredsmut of barley has seldom been reported before. However, in the present experiment 0.24-0.77% infection in the inoculated rows clearly demonstrates the possibility that infection of barley by covered smut can occur from the spores in the soil under the Beqa'a conditions. There was no correlation between the amount of infection and time of soil contamination. The infection of covered smut of wheat from soil-borne spores was as high as 43%. In the case of wheat covered smut infection decreased as the soil contamination time increased.

Since the covered smuts of barley and wheat can occur from soil-borne spores under the Beqa'a conditions, seeds of these crops should be treated with chemicals which allow control of soil-borne spores of the covered smuts. These chemicals should therefore be based on the combination of organo-mercurial and HCB preparations which have been shown effective against seed-borne or soil-borne spores of the covered smuts (34, 61).

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