PHYSIOLOGICAL SPECIALIZATION

OF

UROCYSTIS AGROPYRI (PREUSUS) SCHROT.=U. TRITICI KOERN., AND TILLETIA FOETIDA (WALLR.) LIRO.

BY

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INTRODUCTION

Wheat is considered to be the most important winter crop in A.R.E. It occupies annually about 1.25 million feddans which yield approximately 2 million tons of grains. The balance amount is therefore imported from the principal wheat-producing countries (*ca*. 2.5 million tons annually). This amount is subject to progressive annual rise to meet the rapid increase in population, (Anonymous, 1976).

Wheat is liable to be attacked with many diseases in Egypt, i.e., rusts, smuts, powdery mildew, and other diseases of minor importance, which affect the yield of the susceptible varieties.

Bunt and flag smut, caused by *Tillertia foetida* (Wallr.) Liro, and *Urocystis agropyri* (Preusus) Schrot., respectively were reported in Egypt, El-Helaly (1948), Jones and Seif El-Nasr (1940). Both species are belonging to the family Tilletiaceae of the Ustilaginales. Bunt was found throughout Egypt on the durum varieties, i.e., Dakar and Baladi, whereas, flag smut was introduced to Egypt with the massive importation of the Australian wheats during the First Great War.

Most of the vulgar varieties were found to be susceptible to the flag smut, whereas the durum varieties were immune (Jones & Seif El-Nasr, 1940). On the other hand, recently these diseases are less distributed because of the distribution of the resistant varieties.

The discovery of the physiological specialization phenomenon in the fungi is considered one of the most important developments in the field of plant pathology, since it is closely correlated with the development of the new varieties. The roots of the problem of race and specialization in the smut fungi, are sharply restricted to the dynamic nature of the causal organisms.

The main object of this investigation is to identify the pathogenic races of Tilletia foetida(Wallr.) and Urocystis agropyri (Preusus) Schrot., on the basis of genetic and cultural purity, since the identification according to the spore collection is doubtfull from the scientific point of view (Fischer & Holton 1957). Moreover, the haplo plase nature of growth on different artificial media was studied to reveal the types of compatibility in the two tested species.

The present study focussed spot light on the foregoing aspects, since it has not been studied before in Egypt.

REVIEW OF LITERATURE

Physiological Specialization

Definition and terminology :

According to Fischer & Holton (1957), the term "Physiological specialization" refers to the occurrence of entities within morphologic characters. In the fungi, such entities have been designated variously as physiologic strains, physiologic forms; biologic forms, biologic species, biotypes, varieties, and races. These terms were used for a long time more or less synonymously until "physiologic race" was adopted officially, and became generally accepted as standard.

Christensen & Rodenhiser (1940) pointed out that the term in the smuts was wider in its application than in the rust fungi, since it included in the former not only the pathogenically different dikariophytes but also culturally different haplonts.

Objection may be referring smut cultures (sporidial or mycelial colonies), and the diploid spore population to the common category of the physiologic race. In most cases such cultures represented the haplo phase, which retained culturally distinct characteristics indefinitely, barring mutations.

When cultures were combined in sexually compatible combinations, they produced the diplo phase (teliospores), usually on the host. Thus if cultures may properly be designed as physiologic races, the spores, are then, hybrids between races, and as such; are potentially variable in a wide range of characteristics Holton (1931, 1942).

Theoritically, therefore, in any given spore collection of smut, each new generation of spores, might be different from the proceeding generation in one or more respects. Consequently, it may be said that no teliospore population can be genotipically the same in all characteristics for more than one generation. Therefore, the validity of designating teliospore collection as physiologic race may be in doubt, (Holton, 1930; Churchward, 1938; Christensen & Rodenhiser 1940).

Later, Holton et al., (1968) reported that pathogenic specialization is the reflection of the interaction of virulence genes in the parasite, and resistance genes in the host.

I- *Tilletia foetida* (Wallr.) Liro.

1- Pathogenic specialization :

The first systematic race classification in the bunt species, developed by Rodenhiser & Stakman(1927), who showed the existance of five forms (three of *Tilletia levis* and two of *T.tritici*) using collections from Minnesota and several foreign countries. These forms were identified by the reaction of spring wheats, Kota, Marquis, and Einkorn to seven spore collections, some of which were of European origin. Reed (1928), using similar methods proved that collections of bunt spores from various states in (U.S.A), some European countries, and Egypt, were composed of various physiologic forms of *Tilletia tritici*, he also demonstrated that there was vast difference in the reaction of varieties of wheat to bunt collected from different localities, but he did not attempt to determine number represented by his collections.

Gaines (1928) reported three strains of *Tilletia tritici* and two of *T.levis*, and showed that varieties which were resistant to bunt in a geographical area are not to be necessarily resistant in another. *Tilletia* tritici from Germany was found to have different capabilities from that, which was common in Eastern Washington. The American wheats were susceptible to the German forms, while the German wheats were succumbed more readily to the American forms of T.tritici. But in 1930 (Heald & Gaines) found that the number of T.levis strains had been increased to four, making a total of seven strains, Also Reichert (1930) obtained six different strains of T.tritici in Palestine.

Rodenhiser & Holton (1942) demonstrated that different environmental conditions may affect the response of some spring wheats to certain races of Tilletia levis and T.tritici, they suggested that such effect varied according to the species and /or race of pathogen and also to the host. Also they concluded that the most probable explanation, of the differential response of varieties to individual races of the smut fungi,under different environmental conditions was the expression of genetic factors of protoplasmic resistance in the host was modified in the different varieties by environment.

Holton (1930, 1931) stressed the existance of numerous physiologic strains, and their appearance of their introduction from other locality in the explanation for epidemics of bunt in varieties formerly though to be resistant, as for example, *Tilletia caries* comprised new forms attacked epidemically the durum wheats in Minnesota. Evidence was also presented that Vernal emmer and Marquis were more heavily bunted in 1930 than in 1929, it was concluded from results that new and more virulent forms attacked the two varieties in 1930 . Bonne (1931) found that *Tilletia tritici* from different varieties of the same origin showed equally as great differences in capacity to infect various varieties as did collections from different and widely separated localities.

Rodenhiser (1931) pointed out that *Tilletia leavis* and *T.tritici*, affected the length of colums of wheat plants and caused also different degrees of stunting according to the physiologic form of the species of bunt infected the plant. Also differences in the host reaction i.e., the general shape of the infected heads shape of the bunted balls, and consistency of the chlamydospore mass were observed, but were not constant for a single species. On the other hand Aamodt (1931) mentioned that there were several physiologic forms of both Tilletia tritici and T.leavis, and added that the dangers arising from importation and growing of spring varieties such as Kota, Ceres, and Progress. Even though they were resistant to stem rust, the bunt reaction of these varieties had not been determined prior to their introduction. He pointed out that the new varieties of durum wheats had greatly aggravated the bunt problem because they were considered to be the medium through which the pathogen had become more thoroughly and widely distributed.

Smith (1932) found that the different reactions exhibited by Hope variety in fall and spring plantings seemed to be mainly due to the respective temperatures subsequent to the emergence of seedling from the soil. He suggested that the resistance of Hope at higher temperatures was dependent on unfavorable nutritional conditions or an organization of the protoplasm that this temperature retarded, or the growth of the fungus.

Flor (1933) established a standard classification system for races of Tilletia caries and T.foetida in the United States initiated in the Pacific North west. Thirteen races (seven of T.tritici and six of T.levis) were identified with other previously known races on on seven winter wheat varieties, including Turkey , Ridit, Oro, White Odessa, Hybrid 128, Hohenheimer , and Albit.

Rodenhiser & Holton (1937) in an attempt to standardize race classification in relation to breeding new varieties, added Hussar, Martin, and spring wheat varieties Ulka, Marquis, Canus, and Mindum (durum), to the previous varieties.

Holton (1942) reported that pathogenicity of Tilletia tritici and T.levis was genetically controlled and apparentely inherited on a multiple factor basis. Factors of pathogenicity and spore morphology were inherited independently. The selective influence of the host variety was important in the establishment of new pathogenic types resulting from hybridization.

Kendrick & Holton (1958) recorded new physiologic races of Tilletia caries in the Pacific North west on ten differential varieties. Cherewick (1958) suggested that a smut collection may consist of a pure strain, but more frequently of mixed or heterozygous strains. Repeated passage of a variable culture through selected hosts, occasionally yielded a stable strain which may be called a race. The majority of variable cultures continued variable through the generations of selections. Results of selfing some of the variable smut cultures indicated a possibility of obtaining races stable for pathogenicity on the differential hosts.

Kendrick (1961) reclassified 28 races of <u>T.caries</u> and <u>T.foetida</u> into 17 pathogenic types, on the basis of the differential reactions of 7 wheat varieties. These 17 pathogenic types were further classified into 6 groups on the basis of their pathogenicity aginst 1 of 6 generally recognized resistant types. This system aimed to facilitate testing for varietal resistance to the bunt fungi. Later on, (1964) he also studied solopathogenicity in <u>Tilletia caries</u> on 17 monosporidial lines, that initially failed to behave as normal unisexual lines. One of the neuter lines proved to be solopathogenic by producing bunt when used singly to inoculate "Red Bobs" wheat.

Hoffmann, <u>et al</u>. (1967) revised the classification of some pathogenic races of <u>T.controversa</u>, and concluded that race D-9 was pathogenic to all the pathogenic factors commonly used in breeding for resistance to bunt, thus it was the most broadly race of many species of *Tilletia* occurring on wheat.

Metzger & Kendrick (1967) demonstrated that it is necessary for race identification to add varieties with new sources of resistance to the set of the differentials.

Hoffmann & Kendrick (1968) identified a new race of *T.caries* belonging to the Omar group of races as classified by Kendrick (1961). It attacked varieties possessing combined Martin, Turkey, and Hussar resistance, and in addition exhibited some virulence against the Ridit resistance.

2- Compatibility relationships :

Sexuality in the smut fungi had begun with the first controversy on the subject between DeBary and Brefeld nearly a century ago (Fischer & Holton 1957). They reported that when a number of monosporidial cultures from the same of from different spores of a species were mated together in all possible combinations, and the results of these matings indicated the existance of only two different sex groups, then the species concerned exhibited simple "bipolar" sexuality. If, however these matings disclosed the existance of three or more types, then the species exhibited complex "multipolar" sexuality.

They reviewed that Kniep (1949) was the first, to demonstrate heterothalism in the smut fungi, he pointed out that sexuality in *Ustilago violaceae* was controlled by simple bipolar system.

On the other hand, Bauch (1923) and Kammerling (1929) proved the existance of multiple sexuality in *Ustilago longissima*. Likewise Eddins (1929) reported that sexuality in *Ustilago maydis* (*U.zeae*) was controlled by multiple system. Compatible combinations of lines in this case were detected by using five monosporidial cultures as inoculum in all possible combinations of two each, with the results expressed in sporulation on the host, resolving the five cultures into four sex groups.

Concerning *Tilletia* spp., Flor (1932) reported that *Tilletia caries* (DC.) Tul., and *T.foetida* (Wallr.) Liro had a complex relationships in their sexuality, whereas Hanna & Pop (1934), Holton (1951, 1953),and

Holton & Kendrick (1957), reported the existance of simple sexuality in the two species of the common bunt of wheat.

Holton (1951) pointed out that fusion between primary sporidia as an index for compatibility had definite limitations, since a primary sporidium can not be paired but once, and thus tests with that particular meiotic products can not be repeated. Therefore, in addition to mating primary sporidia, haploid secondary sporidia from monosporidial cultures were also paired, and the results there interpreted as an index of compatibility. He later (1953) found that primary sporidia from three collections of Tilletia controversa fused with secondary sporidia of both mating types of T.caries. Compatibility was determined on the basis of percentage of fused pairs.

Silbernagel (1964) reported that some of the lines of Tilletia controversa fused with both plus and minus lines of T.caries.

Hoffmann & Kendrick (1969) proved that matings between primary sporidia and between secondary sporidia from monosporidial lines showed that sexual compatibility in T.controversa was controlled by multple alleles at one locus. Five alleles at the locus controlling fusion were detected in 13 collections of T.controversa from the Pacific Northwest.

3- Nutritional requirements :

Fischer & Holton (1957) indicated that the real pioneer in the study of the development of the smut fungi on artificial media was Brefeld,1938. He used simple sterilized dung decoction and found that the addition of sugars improved the medium to favour the multiplication of sporidia.

Sortoris (1924) reported that the mycelium of Tilletia caries and T.foetida developed best on a heavy oatmeal agar, which would certainly have a high starch content. Kienholz & Heald (1930) showed that sucrose at a concentration of 4 per cent was the best source of carbohydrates. In addition to sucrose Halbsguth (1949) reported that glucose and levulose were satisfactory as sources of carbohydrates. Zscheile (1951) attributed the stability of the previous sugars for the growth of the smuts to that the medium was more stable when autoclaved in the presence of amino acids and other constituents beside the formentioned sugars. The nitrogenous requirement of the smut fungi has been also the subject of some investigations. Lange de la Camp (1939) obtained very poor results with T.caries using various inorganic sources of nitrogen, whereas, allantoin and asparagine, were the best sources of nitrogen for the growth of T.caries, followed by nucleic acids and pepton. Similar results were reached by Halbsguth (1949), who reported that allantoin, alanine, and asparagine were considered as superior sources of nitrogen for the growth of T.caries.

On the other hand Zscheile (1951), obtained the best results using L.asparagine at 0.05 M concentration. He also found that the optimum ion concentration of the necessary elements were : phosphate = 0.0024 M, calcium = 30 p.p.m., manganese = 0.16 p.p.m., zinc= 1.2 p.p.m., iodine = 1.27 p.p.m.

Concerning vitamine requirements, Defago (1939) clarified that T.caries was auxoheterotrophic, as aneurin was found to be essential to its development. Such result was confirmed by Zscheile (1951) who revealed that thiamine produced a pronounced stimulation of the growth in cultures of T.caries, and that pyrimidine and

and thiazol together were just as effective as thiamine.

II- Urocystis agropyri (Preusus) Schrot.

1- Pathogenic specialization :

Verwoerd (1929) was pioneer in studying the pathogenic specialization in *Urocystis agropyri*. He compared the pathogenicity of the American and South African collections on several varieties of wheat. The latter collections were more virulent than the former ones.

The first positive indication of the presence of pathogenic races in *U.agropyri* was given by Yu *et al.* (1936), who indicated the existance of five pathogenic types of this pathogenic fungus in China.

Yu *et al.* (1945), described additional seven races from China, based on differential reactions of five wheat varieties, four of the common wheat, *Triticum vulgare*, and one a poulard wheat, *Triticum turgidum*. Six of these varieties as well as the five previously described by Yu *et al.* (1936), were pathogenic to the common wheat tester varieties, while race 12 was distinguished by its unique pathogenicity to the poulard wheat. All the common testers were resistant to race 12, and the poulard wheat was resistant to races 1 to 11, inclusive.

Holton & Johnson (1943) differentiated the causal agent of the flag smut in Washington from that prevalent in the Midwest (Illinois, Kansas, and Misouri)by differential reactions to certain wheat varietie. The Washington collection, was designated as race 2, had a wider pathogenic range than the Midwest collection designated as race 1.

Hafiz (1951) determined four race groups of flag smut on the basis of reactions of 11 collections on seven tester varieties in Pakistan, Cyprus, and China. He identified five physiologic races, U.S.A. races 1, 2, Chinese races 2, 3, and a new race from Pakistan.

Samra (1952) in Egypt, identified five physiologic races by using seven differential varieties to compare them with those in U.S.A., and China. He described their distribution in the Egyptian provences. On the other hand Abdel-Hak & Ghobrial (1966) identified ten physiologic races, five of them were similar to those previously identified by Samra (1952), the other five were new ones.

Johnson (1959) established an international system of designating flag smut races, on the basis of the results obtained from tests conducted over five years period. He differentiated 21 collections from different countries including the United States, China, Australia, Chile, Japan, India, and South Africa. Thirty three tester varieties of wheat were secured from these locations. He compared the identified races with those previously identified as U.S. races.

Fischer & Holton (1943) showed that two species of agropyron (A.canenum F 138 and A.spicatum W 739), were susceptible to race 1 and resistant to race 2, and therefore were potentially suitable as differential hosts for flag smut races.

Purdy (1965) reviewed all the aspects connected with the flag smut of wheat in addition to the physiologic specialization.

2- Germination :

High percentage of germination of smut spores, was obtained by floating the spores on the surface of water, and even those that sank germinated well (Wolff 1873). On the other hand Mc Alpine (1910) failed to germinate fresh smut spores from an infected plants, but after being held in contact with soil for seven days and then transferred to water, for further 30 days, the spores germinated well. Verwoerd (1929) reported that a period of 42 to 82 days or longer is needed for their maturation after spores were collected.

Noble (1923) showed that the environment greatly influenced the after ripening period required for germination. He collected flag smut sori, which had turned to the leadengray color and dried them at room temperature over concentrated sulfuric acid for 48 hr, then were removed from the leaf tissue, and floated for three days on distilled water to which young fresh tissues were added. In 18 hours 60-70 per cent of germination had occurred. In addition to plant products Noble (1924) reported the stimulatory effects of several volatile oils and chemicals on germination of flag smut spores. Benzaldehyde and butyric acid at concentrations 1.5 and 2 p.p.m., stimulated the germination considerably. Presoaking in distilled water 3 to 10 days at 20°C, made the spores more responsive to the various investigated stimuli. He also postulated that the action of stimulation was excerted on the spore contents, bringing about their physical conditions, that observed in the spores of other fungous species, with gel-like contents in the dormant stage that at germination changed to hydrosol as a result of imbibition of the water. He reported also that optimal germination occurred between pH 5.1 and 5.7, and at 18 to 20°C. Similar results were obtained by Sttar & Hafiz (1952) in Pakistan.

Verwoerd (1929) observed that the addition of some plant tissues to the water medium increased the spore germination. Expressed sap from six-day-old seedlings, grown at 20C at a concentration of 1 part per 10.000 stimulated the germination of the presoaked spores. Higher concentrations of sap reduced germination and caused deformation of the promycelia.

Sattar & Hafiz (1952) obtained good results on the flag smut spores germination when they used a solution of expressed freshly germinated wheat seedlings diluted to 1 to 200 in water. The same spores failed to germinate by Noble's (1923) method, suggesting that the Australian and Pakistan collections represented distinct physiologic strains, at least with regard to their requirement for spore germination.

3- Axinical growth of Urocystis agropyri on articicial media :

Although germination of flag smut can be stimulated by various treatments and under various conditions, a pure culture of this fungus has been reported once by Wu (1949).

Verwoerd (1929) failed to culture U.agropyri and concluded that this species can not adopt saprophytic habits of growth as do some of other fungi.

Wu (1949) successfully obtained cultures of U.agropyri, from infected plant material. He cut 0.5 cm. sections of unbroken fresh smutted host material and sterilized them in either 20 per cent solution of bleaching powder or in mercuric chloride (1:1000) for 10 minutes followed by rinsing in distilled water. The surface-sterilized tissue sections were then placed on nutrient media in test tubes. Cultures were obtained from collections from three provinces of China, and after 60 days the colonies reached a diameter of 15.6 to 26.5 mm., reflecting a relatively slow rate of growth. Although Wu (1949) did not describ the growth in details, and did not investigate the infectivity of the obtained cultures, it seemed evident that. *U.agropyri* resembled in some respects other smut fungi The color of the colonies ranged from white to dark olive, and they reflected a tough mycelial growth, that produced scant sporidia.

MATERIALS AND METHODS

Sources of the studied fungi :

Samples of wheat local varieties showing bunted heads, were collected from the different governorates of Egypt. On the other hand, the flag smut samples were obtained from the stock collections at the cereal Disease Section, at Giza. Such samples were collected from the different governorates in the period from 1962 to 1972.

I- Identification of the causal agent of wheat bunt :

Identification of the spores collected from bunted wheat heads, was accomplished by microscopical examination, and dimensions of teliospores were recorded. The keys given by Fischer & Holton (1957) and Duran(1973) were followed, type of germination was also observed.

Isolation of the bunt sporidial cultures :

The process of culturing bunt of wheat was early found to comprise naturally two main phases; germination and growth.

Germination :

The end of unbroken smut ball was cut off and about one fourth to one half of the contents was immersed in 10 ml., of one per cent copper sulfate solution from 12 to 72 hours to eliminate surface bacterial contaminants. They were then rinsed in distilled sterile water. The double plate method developed by Bodine (1931) was adopted. A loop of the washed spore suspension was placed on the upper layer of water agar, while the bottom medium was Potato 4 per cent sucrose agar. The inoculated plates were incubated at 5-10 C for 4-10 days.

Growth :

The small visible white "colonies"formed on the surface of the bottom medium (PSA), were carefully picked out with a sterile needle and transferred to another more suitable medium in flasks to retain sufficient moisture and to lessen the possibility of contamination. The isolates were concentrated only in five monosporidial cultures, were used for further studies. The cultures were maintained in the flasks described above as a source of stock culture. Subculturing was carried out every two months. The following items were investigated :

1- Cultural studies.

2- Compatibility studies.

3- Pathogenicity studies.

1- Cultural Tests

The object of these experiments is to study the behaviour of the haplophase of Tilletia foetida, in addition to the nature of growth on different media including, i.e., diameter of the colony, color, topography, margin, and consistency following the method suggested by Christensen & Stakman (1926), and Rodenhiser(1928), to describe the growth of the smut fungi on artificial media. Different synthetic, semi synthetic, and natural media were tested. Some of which were tested for experimentation, but the critical studies were performed only the common media; potato 2 per cent glucose agar, Potato 4 per cent sucrose agar, and the specific media: Sartoris, Ranker, and Huskins.

For studying the growth, 20 ml. of the medium in quistion were poured in petri dishes. An agar disc,5 mm.

in diameter, cut from a 20 days old culture served for inoculation. The cultures were incubated at 20-24C. Data were recorded 48 hours after inoculation. Each experiment was replicated three for each isolate and medium tested.

2- Compatibility Tests

The aim of these experiments is to know the type of systems, that control sex in this species (simple bipolar, or complex multiple allelic system).

These studies were conducted on the basis of two methods :

a) Compatibility in vitro, b) Compatibility in vivo.

a) Compatibility in vitro :

Since the micromanipulator is not available, this part has been performed according to the macroscopic test (= Bauch test), with some modifications (Fischer & Holton, 1957). Water agar was poured as a thin film in 5 cm. petri plates, every two isolates with all the possible combinations, were mixed thoroughly by means of sterile platin looped needle, in the presence of few drops of distilled sterile water. The plates were incubated at room temperature 20-24 C, for three days. The plates were examined microscopically every six hours throughout this period. The presence of clamp connections was the criterion of compatibility between the mating isolates.

b) Compatibility in vivo :

These experiments were directed to test the infectivity of the paired cultures with all possible combinations.

The experiments were repeated twice at two successive season (1973/74 and 1974/75) with two replications for each treatment.

Germinating seeds of the variety Baladi 116 were inoculated with two paired cultures, then sown at the depth of 1-1.5 cm., in plastic containers (10 cm. in diameter), containing sterilized soil, then irrigated with sterile water. Every container was cultivated with 20 germinating seeds, contaminated thoroughly with a single paired culture : 1 x 2, 1 x 3... etc. In the same time there five containers were cultivated with germinating seeds, contaminated with single cultures (1, 2, ... 5).

The leaves, crowns, and washed roots of the emerged seedlings, 5 to 10 days old were examined microscopically. The examination was carried out by macerating the parts under investigation, with fine glass needles on glass slides, and then stained with Hohenhein's dye to show the dikaryotic mycelium.

3- Pathogenicity Tests

The object of these studies is to give every pathogenic pure strain or race, with its components (compatible monosporidial cultures), the oppurtunity to express its self individually from the pathogenic point of view, since the identification of races originated from the collection of teliospore is doubtfull.

Thereby, the cultural races were grown on sterilized wet barley seed, for 20 days at 20-24 C. Thereafter, the following trials were conducted in the field, greenhouse, and laboratory.

a) Greenhouse experiments :

Seeds of the variety (Baladi 116) were disinfested, washed with distilled water, dried in air, and sown in sterilized soil potted in plastic pots (15 cm. in diameter), and irrigated with distilled water. The experiment consisted of two treatments 15 pots each. Ten pots were inoculated with the combinant cultures, and five with the single cultures.

Inoculation was carried out, in both treatments,by injecting the seedling with a suspension of two mixed cultures, each grown separately. The injection was performed at two different growth stages, (20 and 40 days old seedlings). Then inocula were prepared by two means. In the first one water was used as a suspensive agent, while Zscheile's liquid medium (MT_2) replaced water in the second one.

b) Field experiments :

The individual cultures grown on barley medium were mixed together in all posible combinations in sterilized soil. The field was devided into plots 2x2.5m., each contained 14 rows, sown with the seeds of the differential varieties contaminated with infested soil. The wet method (Heraty) was followed. The rows were covered and pressed with 3 cm. layer of soil. Irrigation was conducted after 15 days from sowing.

c) Laboratory experiments :

The five cultural isolates of *Tilletia foetida* were mated in the possible combinations on Potato 2 per cent glucose agar in test tubes by inoculating every tube with two different isolates and incubated at 15-20 C. for 15 days. The contents of every tube were emptied into milk bottles containing barley medium by the help of a macro needle, and distilled sterile water. The inoculum was mixed carefully with the grain medium and incubated at room temperature (for about 2-3 weeks) till the fungal growth covered the grains.

Autoclaved soil was distributed in plastic containers (10 cm. diameter). Surface disinfested seeds of the susceptible variety (Baladi 116) were permitted to germinate in distilled sterile water. The soil's surface of each plastic pot was covered with a layer of 1-1.5 of the inoculum. Germinated seeds were embeded in the inocula layer, and covered with a thin layer of sterile soil. The same procedure was performed on the individual cultures to test the solopathogenicity of the tested species. After nine days the emerged seedlings were transplanted into the field with some of the infested soil adhering to the roots. Afterward irrigation and fertilization were carried out in the proper times and rates.

Control treatment was a plot containing the differential varieties without inoculation.

The following varieties were used as differentials:

Variety	C.I.
1- Ridit	6703
2- Oro	8220
3- Hohenhimer	11458
4- Hussar	4843
5- Albit	8275
6- Martin	4463
7- White Odessa	4655
8- Ulka	11478
9- Marquis	3614
10- Canus	11637
11- Mendum	5296
12- Dakar 52	Locally variety
13- Baladi 116	" "
14- Bauhi	" "

Seeds of the varieties (1-11) were supplied by J.C. Craddock-Blag. 046 United States Department of Agriculture (A.R.S.) Beltsville, Maryland, 20705, while the varieties 12, 13, 14 were obtained from the Cereal Disease Research Section, Giza-Cairo.

II- Identification of the causal agent of flag smut :

Identification of the spore balls collected from wheat plants diseased with flag smut, was accomplished by microscopical examination, and dimensions of spore balls were recorded. The keys given by Fischer and Hoton (1957) and Duran (1973) were followed :

Isolation of the flag sporidial cultures :

All the steps previously followed in part I, were conducted typically herein, except for the germination methods and subsequently the isolation methods, since this organism requires certain treatments for stimulation of the dormant teliospores to germinate.

Germination tests :

Teliospores were obtained from the infected parts by teasing. Surface sterilization was carried out using a solution of mercuric chloride (0.1%) for ten minutes followed by rinsing in distilled water for a period 3-5 days (Noble 1924). Young fresh leaf tissues of wheat (Mokhtar variety) were added to the water medium as stimulator. In another treatment, a thin film of benzaldehyde was added to the water medium to act as stimulator.

Four days after germination and before the formation of sporidia, a loopfull of the spore suspension was transferred to petri plates for isolation of sporidia.

Isolation was performed by the double plate method (Bodine 1931). A petri dish containing germinating teliospores placed on water agar layered on the cover of the plate. The plate cover thereafter was inverted over the bottom containing a sterile nutrient agar. The developed secondary sporidia from the germinating teliospores were settled down on the surface of nutrient agar. Plates were incubated at 24 C for 3 to 8 days then examined microscopically for the presence of secondary sporidia which marked for later transfer by macro needle, when the colony became visible to the naked eye. This method originally developed by Bodine (1931) was conducted for the isolation of Tilletia levis. The modifications in this work were that soil extract agar and Potato 2 per cent dextrose agar, or Potato 4 per cent sucrose agar were substituted by water agar and nutrient agar, respectively, and the teliospores were transferred to the plates after germination.

The cultures were tentatively identified by J.A. Hoffman, Utah State University U.M.C., as cultures having the appearance of the smut fungi. Detailed identification was carried out in the Plant Disease Research Laboratory, at Sakha, on the basis of the formation of the characteristic teliospores by the consequent methods in vivo and in vitro.

Compatibility tests :

a) In vitro :

Microscopical determination of sex compatibility or sporidial fusion must be performed, by the aid of micromanipulator. If it is not desired, the macroscopic test

(= Bauch test) will be the reliable test, but it is not positive for all the species, (Fischer & Holton 1957). Therefore, the following modified procedure was conducted.

Four monosporidial cultures obtained from teliospores of <u>Urocystis agropyri</u> were mixed in pairs each in 5 cm. diameter petri plate, containing a thin layer of water agar. Distilled sterile water was added in few drops to promote the mating between the uniting pairs. Plates were incubated for 5 days at 24 C. Microscopic examination started after three days from inoculation and continued for further ten days. The same combinations were conducted identically using slide culture technique (the coating medium was water agar).

b) <u>In vivo</u> :

Compatibility studies were carried out in vivo using the local susceptible variety, Mokhtar". The pathogenicity tests were performed in the laboratory and in the field using the following differential varieties :

Variety	C.I.
1- Oro-Federation	11914
2- Baart	12386

3- Federation	4734
4- Ngochen	149805
5- Tsing haue	149807
6- Giza 139	Local variety
7- Mabrook	" "
8- Mokhtar	" "
9- Giza 148	" "
10- Giza 155	" "

The varieties (1-5) were supplied by J.C.Craddock Bldg. 046 United States Deparrment of Agriculture(A.R.S.) Beltsville, Mayland 20705, while the varieties (6-10) were obtained from the Cereak Diseases Research Section Giza-Cairo.

The following media used during this study :

(1) Ranker's Medium :

K_2SO_4	0.3 g
NH_4NO_4	0.1 g
$CaCl_2$	0.1 g
$Mg_3(PO_4)_2.4\ H_2O$	0.1 g
Dextrose	10.0 g
Distilled water tomake	100.0 ml

(2) Sartoris "Best" Medium :

Calcium nitrate	0.4 g
Potassium nitrate	0.2 g
Potassium nitrate dihydrogen phosphate	0.2 g
Magnesium sulfate	0.01g
Peptone	0.1 g
Dextrose	2.0 g
Malt extract	3.0 g
Distilled water to make	100.0 ml

(3) Haskin's MB-50 :

Monobasic potassium phosphate	0.1%
Magnesium sulphate crystals	0.04%
Ferrows sulphate crystals	0.003%
Yeast extract	0.06 %
Urea	0.06 %
Commercial sucrose	5.0 %

(4) Zscheile's "MT_2" Medium :

$MgSO_4$(0.2 M sol.)	2.5 ml.
KH_2PO_4(0.2M sol.)	4.5 ml
K_2HPO_4(0.2M sol.)	7.5 ml.

$CaCl_2$ (0.5 M sol.)	0.5 ml.
Ferric tartarate (0.5 per cent sol.)	0.5 ml.
$MnSO_4$ (0.03 M sol.)	0.1 ml.
$ZnSO_4$ (0.009M sol.)	2.0 ml.
KI (0.005 M sol.)	2.0 ml.
L^-(-) asparagine (0.1 M sol.)	500.0 ml.
Thiamine chloride (I g/1.0.01 M HCl)	1.0 ml.
Sucrose	16.0 g.
Dis. Water to make	1000.0 ml.

Agar was added at 2% to the above media to solidify them.

RESULTS

Identification of the causal agent of wheat bunt :

Teliospores were ranged from 20-50 um in diameter exospores were smooth, spore germination followed the Tilletiaceous type, and the color ranged from light to dark brown.

According to the keys suggested by Fischer & Holton (1957) and Duran (1973), it could be concluded that the examined spores are : *Tilletia foetida* (Wallr.)Liro.

1- Cultural tests on *Tilletia foetida* (Wallr.) Liro.

The monosporidial isolation revealed the existence of five characteristic isolates. Preliminary results showed that the best growth of the studied isolates and their combinations was achieved on barley medium, therefore it was applied, throughout the course of this studies especially for the pathogenicity tests.

Table (1) and Figure (1) indicate the colony diameter of the five isolates of *T.foetida*, maintained on different tested media. The following could be concluded from both table (1) and figure (1) :

1) Isolate 1, showed the highest rate of growth, on the tested media except on Huskin's medium, as compared with the other isolates. All the tested media except Huskins medium resulted in moderate growth in the case of isolate 3. However, the other isolates were favoured by one or two media in this respect, i.e., Sartoris medium supported the growth of isolate -2, Potato -4 per cent sucrose agar proved to be superior for the growth of isolate 4, and Potato 4- per cent sucrose agar and sartoris media favoured isolate 5.

The characteristics of the tested isolates of Tilletia foetida (Wallr.) Liro., on five different media are listed in Tables 2 (A-E), ffom which the following could be deduced :

1. Isolate-1, has the same color on the five tested media, it ranged from light brown on Ranker's and Potato 2-per cent dextrose agar, to intermediate brown on the other three media. Isolate 2 showed pure white color on Ranke's and Potato dextrose agar media, and very light pinkish on Potato sucrose agar and Huskin's media. However, the color was vinaceous with light Pinkish margin on Sartoris medium. Isolate

3 showed very light brown color on all the media, except for Huskin's medium, since it has the avellanous color. Isolate -4 showed dull white color with brown knobs on all the media except for, sartoris medium, on which it was pure white. Isolate 5 has the same pure white color on Ranker's,Potato dextrose agar, and Potato sucrose agare, and difered on Huskins and Sartoris medium.

2. As for the consistency isolate -1 was velvety on Sartoris, Huskin's, and Potato sucrose agar, and differed on Potato dextrose agar and Ranker's media. Isolate 2 was velvety on Sartoris, Ranker's, and Potato dextros agar media, and waxy on the other media. Isolate 3 showed similar consistency on Huskins and Sartoris media, and different on the other media, while isolate 5 was cottony on Potato sucrose agar and Potato glucose agar, velvety on Ranker's, Huskin's media and differed on Sartorid medium.

3. Colony characters includes the presence of exudates, sporidial discharge and furrowing. The general shape of the margin, and the hight of the colony was.

also observed, the following could be concluded : All the isolates did not form exudates except isolates 4, 5 on Rankers, Potato dextrose agar, isolate 2 on sartoris, and 5 on potato sucrose agar. Isolate 1 has sporidial discharge on all the tested media, while isolates 4, 5 has no sporidial discharge on all the tested media. Isolate 3 has sporidial discharge on Huskins medium. However, isolate 2 has no sporidial discharge only on sartoris.

All the isolates were not furrowed on Ranker's and Potato dextrose agar media, furrowing was clear on Sartoris medium for all the tested isolates, on Huskin's for isolates -2,5 and on Potato sucrose agar for isolate 2 only.

The hight of the tested cultures ranged from 2 to 4 mm., on all the tested media except for potato dextrose agar and Rankers media.

All the tested isolates were ranged from circular to nearly circular on the different media, however, the tested media did not changed in color by the different isolates except isolate 5 that was surrounded by brown hallow. Sartoris medium did not changed with all the isolates.

The margin in isolate 1 was entire on the different media. Isolate 2 was entire on Ranker's, Huskins and Potato dextrose agar, lobate on sartorid, and Potato sucrose agar. Isolate 3 was undulate on all the media except Sartoris medium. Isolate 4 was entire on Sartoris, Huskins, and Potato sucrose agar and was undulate on Ranker's and Potato dextrose agar. Isolate 5 was entire on all media except Huskins medium.

The differences in colony characters are shown in Figure 2.

2- Compatibility tests :

Compatibility of five monosporidial cultures derived from different promycelia, from different teliospores, and from different bunted heads, were tested alone, and in pairs in all the possible combinations in vivo and in vitro. As for the individual combinations the results of inoculation by single monosporidial culture revealed no infection or formation of dikaryotic mycelium, in vivo and in vitro, respectively.

Secondary sporidial matings between every two pairs of monosporidial cultures in all possible combinations, revealed that isolate 2 has the largest combining ability, however isolate 3 has the least one in this respect (Figure 3). The formation of clamp connections in vitro (slide culture) is clarified (Figure VII-10).

3- Pathogenicty tests :

Generally it was observed that there were no visible symptoms either on the seedling or on the adult stage. Throughout the course of this investigation the unique procedure to prove the existence of the parasite was the detection of the dikaryotic mycelium in the infected tissues.

A) Greenhouse experiments :

The microscopic examinations showed that the injection with water suspension of the combinations of cultures gave negative results, whereas the spore suspension in "MT_2" revealed the formation of clamp connections in some cases.

B) Field experiments :

There was no difference between the infected plants and the checks from the symptomatological and anatomical points view, since in both cases, clamp connections could not be detected.

C) Laboratory experiments :

Although no observed symptoms were assessed, the microscopic examinations has elucidated the formation of the clamp connections into the infected plants by the in vitro compatible combinant isolates. On the other hand, Incompatible paired cultures in vitro has given negative reaction in vivo (Figure VII-a).

D) Tests of the differential varieties :

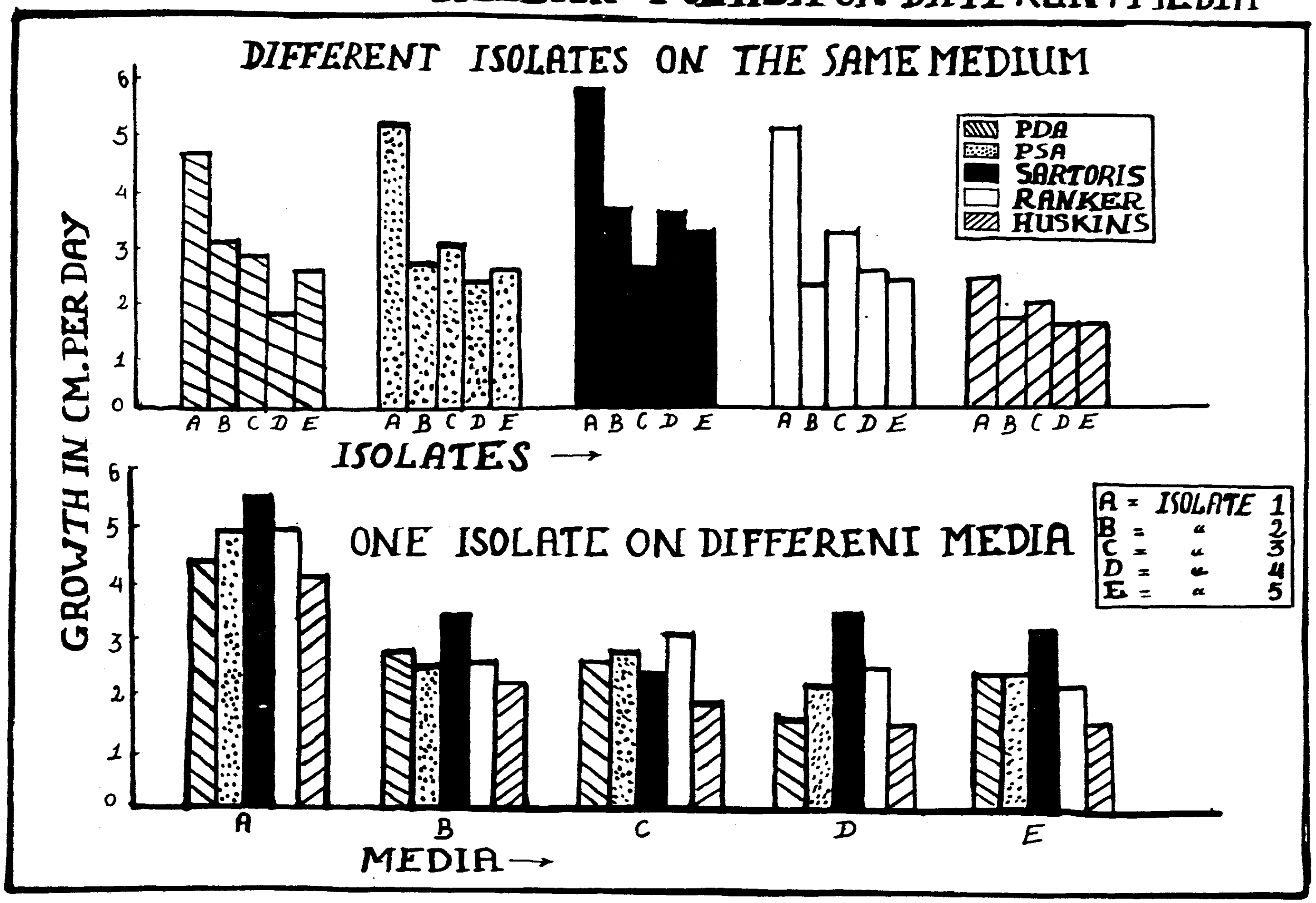
Every two compatible paired cultures gave different reactions on the tested fourteen differential varieties, on the basis of the formation of clamp connections in their tissues. It was observed that the pathogenic race 6 (2x4), formed clamp connections in twelve varieties out of the fourteen, whereas pathogenic race 10 (4x5) produced the clamps only in six varieties out of the fourteen. On the other hand the incompatible combinations : 2 (1x3),8(3x4) and 9(3x5) did not form any clamp connections in any of the tested varieties, (Table 3).

Table 1- The growth, in cm of five different isolates of <u>Tilletia foetida</u> on different media, incubated at 20 C for 6 days.

…o …ates …ys	T_1					T_2					T_3					T_4					T_5				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
1	2.1	2.0	2.1	2.5	1.2	1.5	1.1	1.7	1.0	1.2	1.7	1.2	1.4	1.6	0.6	0.6	1.1	1.7	1.2	0.7	1.6	1.2	1.7	1.2	1.2
2	2.3	2.5	3.1	2.8	1.4	1.7	1.4	2.1	1.2	1.2	1.9	1.4	2.5	1.8	0.2	0.8	1.3	2.2	1.4	0.7	1.8	1.6	2.1	1.3	1.1
3	2.5	3.5	3.9	3.5	1.6	1.9	1.7	2.3	1.4	1.3	2.1	1.9	3.0	2.0	1.0	1.3	1.4	2.4	1.6	0.8	2.0	1.9	2.4	1.6	1.2
4	3.0	4.0	4.6	3.9	1.8	2.1	2.0	2.4	1.6	1.4	2.3	2.1	2.2	2.5	1.2	1.4	1.8	3.0	2.0	1.0	2.2	2.1	2.6	1.7	1.3
5	3.5	4.5	5.1	4.3	2.0	2.8	2.3	2.9	1.9	1.5	2.5	2.6	2.3	2.2	1.4	1.5	2.0	3.2	2.3	1.3	2.3	2.2	2.8	2.0	1.4
6	4.5	5.0	5.7	5.0	2.3	2.9	2.6	3.5	2.2	1.6	2.7	2.9	2.5	3.2	1.9	1.7	2.2	3.5	2.5	1.5	2.4	2.4	3.2	2.3	1.5

T (1-5)= different isolates obtained from monosporidial cultures
A = Potato dextrose agar.
B = Potato sucrose agar.
C = Sartoris's medium.
D = Ranker's medium.
E = Huskins medium.

FIG. I GROWTH OF *TILLETIA FOETIDA* ON DIFFERENT MEDIA



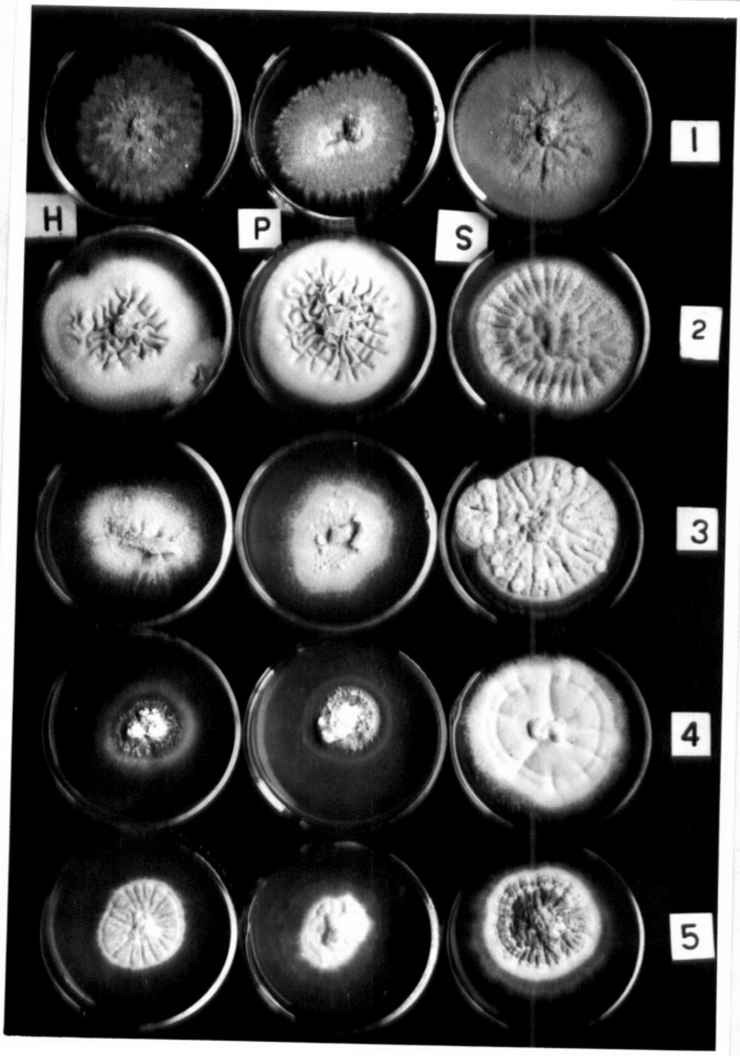


Fig. (2) The growth of five isolates of <u>Tilletia foetida</u> (1, 2, 3, 4 and 5).

H = Huskins.

P = Potato sucrose 4% agar.

S = Sartoris.

Table 2(A-E) The characteristics of the tested isolates of *Tilletia foetida* maintained on five different media after two weeks at 20°C.

(A) Potato 4% sucrose agar

Isolate	Colony diameter	Consistency	Color	Colony characters	Color of medium	Margin
1	9.0	Velvety	Intermediate to light brown	No exudates, sporidial discharge, no furrowing, flat nearly circular	Unchanged	Entire
2	5.1	Waxy	Very light pinkish	No exudates, sporidial discharge, radial redges slight circular. Furrows disappearing near the margin circular; raised 2-3 mm.	Unchanged	Undulating
3	5.0	Cottony to leathery.	Very light brown	No exudates, no sporidial discharge, flat no furrows, scattered small cottony knobs nearly circular.	Unchanged	Undulating
4	7.1	Velvety to ivory	Dull white to brown	No exudates, no sporidial discharge, no furrow, surrounding with light hallow raised 1-2 mm.	Slightly brown around the colony	Entire
5	6.5	Pure white	Cottony	Small buff exudates, no sporidial discharge, raised 4 mm., circular.	Unchanged	Entire

(B) Huskins medium

Isolate	Colony diameter	Color	Consistency	Colony characters	Color of medium	Margin
1	4.7	Interme-mediate brown.	Velvety	No exudates, sporidial dis-charge, no furrowing flat, nearly circular.	Unchanged	Entire
2	3.0	Very light pinkish	Waxy	No exudates, sporidial dis-charge, radial ridges, slight circular furrows disappearing near the margin, nearly circu-lar, raised 2-3 mm.	Unchanged	Entire
3	3.9	Very light brown on avellanous	Chalky	No exudates, sporidial dis-charge, flat, no furrowd, scattered small cottony knobs, nearly circular	Unchanged	Undula-ting
4	2.9	Dull white with brown knobs	Velvety to ivory	No exudates, no sporidial discharge, no furrows, sur-rounding with light brown zone.	Slightly brown around the colony	Entire
5	3.0	Very light vinaceous	Velvety	No exudates, no sporidial discharge, radial furrows, raised, 3 mm. circular.	Unchanged	Lobate

(C) Sartoris medium

Isolate	Colony diameter	Color	Consistency	Colony characters	Color of medium	Margin
1	9.0	Intermediate brown	Velvety to cottony	No exudates;sporidial discharge,slightly radial furrows. Flate margin, nearly circular	Unchanged	Entire
2	6.9	Light vinaceous with light pinkish margin	Velvety	Exudates very light brown droplets, no sporidial discharge; surface radialy furrowed; curcular,raised 3 mm.	Unchanged	Lobate
3	5.1	Avellaneous to very light brown	Chalky	No exudates, no sporidial discharge,scattered cottony knobs,raised 4 mm; surface radialy furrowed and circular.	Unchanged	Lobate
4	7.0	Pure white	Cottony	No exudates, no sporidial discharge,slightly radial furrowing; nearly circular raised 2 mm.	Unchanged	Entire
5	6.5	Light brown center and white margin	Waxy center & cottony margin	No exudates,no sporidial discharge,raised 2-3 mm; high radial ridges, nearly circular.	Unchanged	Entire

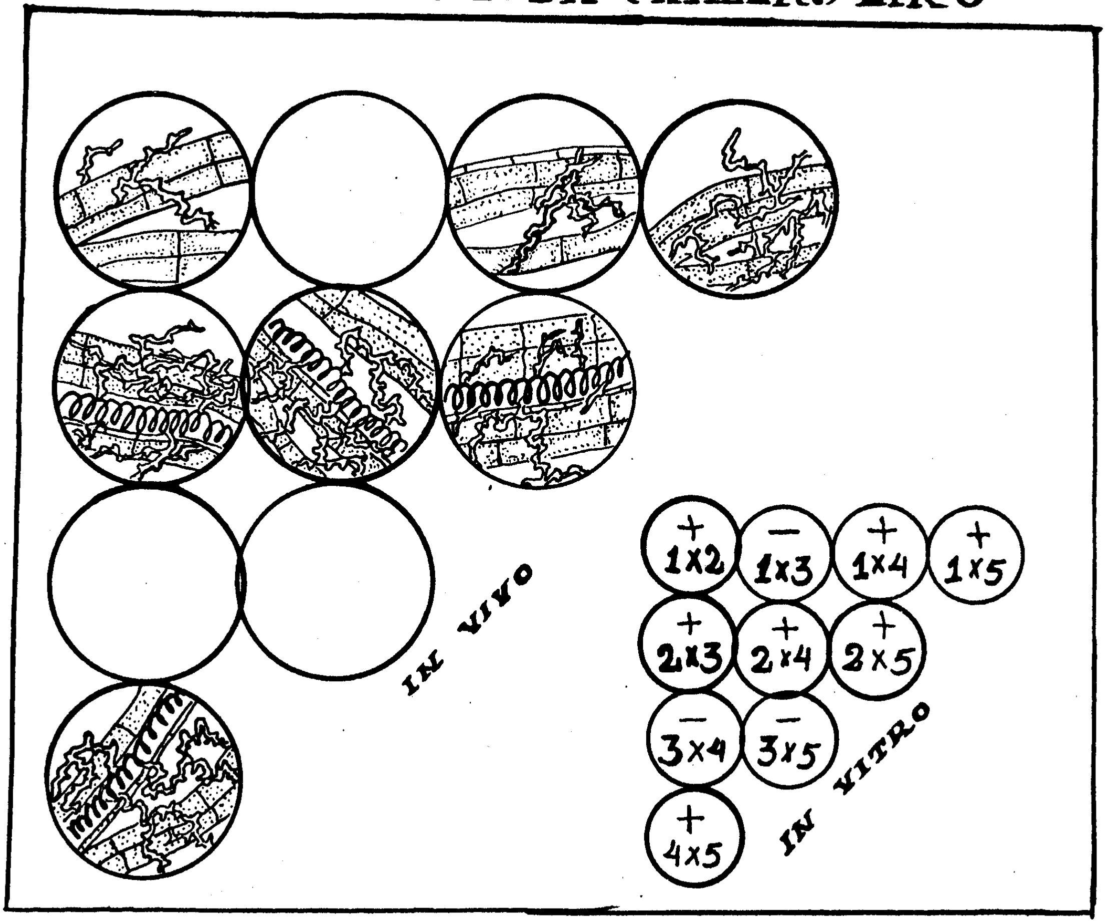
(D) Potato 2% - Dextrose agar

Isolate	Colony diameter	Color	Consistancy	Colony character	Color of medium	Margin
1	9.0	Light brown	ccottony	No exudates, sporidial discharge, no furrowing, flat nearly circular.	Unchanged	Entire
2	3.8	Pure white	velvety	No exudates, sporidial discharge,no furrowing, flat nearly circular.	Unchanged	Entire
3	5.4	Very light brown	Leathery	No exudates no sporidial discharge,slight furrowing flat, circular.	Unchanged	Undulate
4	3.4	Dull white with brown knobs	Velvety to ivory	Small buff exudates, no sporidial discharge, no furroing	Brown hallow	Undulate
5	4.8	Pure white	Cottony	Small buff exudltes, no sporidial discharge raised 3-4 mm, nearly circular.	Unchanged	Entire

(E) Ranker's medium

Isolate	Colony diameter	Color	Consis-tancy	Colony characters	Color of medium.	Margin
1	9.0	Light brown	Powdery	No exudates, sporidial discharge,no furrowing, flat, nearly circular.	Unchanged	Entire
2	3.8	Pure white	Velvety	No exudates, sporidial discharge,no furrowing flat, nearly circular.	Unchanged	Entire
3	5.4	Very light	Velvety	No exudates, no sporidial discharge,slight furrowing flat, circular.	Unchanged	Undula-ting
4	3.4	Dull white with knobs	Velvety to ivory	Small buff exudates, no sporidial discharge, no furrowing,flat,circular.	Brown hallow around colony	Undula-ting
5	4.8	Pure white	Velvety	Small buff exudates, no sporidial discharge, raised 3-4 mm., nearly circular	Unchanged	Entire

FIG. III. COMPATIBILITY BETWEEN FIVE ISOLATES OF *TILLETIA FOETIDA* (WALLR.) LIRO



⊕ = COMPATIBLE ISOLATES, ACCORDING TO THE FORMATION OF CLAMP CONNECTIONS.

⊖ = INCOMPATIBLE ISOLATES.

Table 3- Reaction of pathogenic races of <u>Tilletia foetida</u> according to the formation of clamp connections.

Races varieties	R_1^{++} 1x2$^+$	R_2 1x3	R_3 1x4	R_4 1x5	R_5 2x5	R_6 2x3	R_7 2x5	R_8 3x4	R_9 3x5	R_{10} 4x5
Ridit	M		M	nM	M	M	nM			nM
Oro	M		M	nM	M	M	M			nM
Hohen	nM	Incompatible	M	M	M	nM	M	Incompatible	Incompatible	M
Hussar	M		nM	M	M	M	nM			M
Albit	nM		nM	M	M	M	M			M
Martin	M		nM	nM	M	nM	M			nM
W.Oddessa	nM		M	M	nM	M	nM			nM
Ulka	nM		M	nM	M	M	M			nM
Marquis	M		M	M	M	M	M			M
Canus	M		M	M	M	M	nM			nM
Mendum	M		nM	M	M	M	nM			nM
Dakar 52	nM		nM	M	nM	M	M			M
Baladi 116	M		M	M	M	M	M			nM
Bauhi	nM		nM	M	M	nM	M			nM

M = Compatible strains formed clamp connection in the differential varieties.

nM = Compatible strains did not form clamp connections in the differential varieties.

+ = Cultural isolates.

++ = Pathogenic races.

Identification of the causal agent of flag smut of wheat :

Teliospores (spore balls), were ranged from 40-55 u in diameter, sterile cells of the outer cortex were smaller than the spores, the spore ball consisted of (1-4) spores in the tested collections. The germination followed the Tillatiaceous type, and the color of the spore balls ranged from dark brown to dark olivaceous whereas the surrounded sterile cells were lighter in color.

According to the Keys suggested by Fischer and Holton (1957) and Duran (1973), it could be concluded that the examined spores are :

Urocystis agropyri (Preusus) Schrot.= U tritici Koern.,

Germination tests :

The application of the methods discribed by Noble (1924), gave positive results by using benzaldehyde and some plant tissues, i.e., barley, wheat, and bean as stimulators. About 40 and 25 to 30 per cent of germination were obtained by the formentioned methods respectively.

Isolation of the flag smut sporidial cultures :

The double-plate method, for sporidial isolation developed for *Tilletia levis* by Bodine (1931), was successfully used in this investigation. Four monosporidial isolates were obtained.

1- Cultural tests on *Urocystis agropyri* (Preusus)Schrot:

Preliminary results showed that the best growth of the tested isolates, and their combinations was achieved on barley medium therefore, it was applied, throughout the course of this studies especially for the pathogenicity tests.

Table 2 and Figure (II) indicate the colony diameter of the four isolates of *U.agropyri*, maintained on different tested media. The following could be concluded from Table (2) and Figure (II).

Isolate-2, showed the highest rate of growth on the tested media as compared with the other isolates. All the tested media except Potato dextrose agar resulted in moderate growth in the case of isolate-1. Isolates 3, 4 resulted in related growth except for Ranker's medium which supported the growth of isolate-4. On the other hand isolates 3,4 did not develop on Potato glucose agar. Sartoris and Ranker media proved to be superior for the growth of isolate -1, and Potato sucrose agar and Ranker media favoured isolate-4,. Generally all the isolates developed best on Sartoris medium.

The characteristics of the tested isolates of *Urocystis agropyri*, on five different media are listed in Table 5 (A-E) from which the following could be concluded :

The color of isolate-1, did not change on the different media. Isolate -2 ranged from light brown to avellaneous color on the tested media. Isolate 3 ranged from dull white on Ranker, Huskins and Potato sucrose agar, to pale olive on sartoris medium, however isolate-4 has a pure white color on Huskins and Potato sucrose agar, and differed on the other media.

As for consistency, isolate-1 was powdery on the five tested media, however isolate-2 was mycelioid on Sartoris, Huskins and Potato sucrose agar, and velvety on the two others.

On the other hand, isolates -3,4 has yeast like and bacterioid consistency on the tested media, respectively.

Concerning the topography,,it was observed that all the tested isolates developed flat on Potato dextrose agar and Ranker's media, however it was ranged from raised, verrucose to rugose on the other media, except isolate-2 on Sartoris which has a pulvinate centre and was zonate. Also isolates 3,4 has developed warty on Potato sucrose agar.

The margin of isolates 1, 2 was entire on Ranker and Potato dextrose agar media, and ranged from,lobate, undulate, to erose. On the other hand, isolates 3,4 has erose margin on Rankers, Sartoris, and Huskins and undulate margin on potato sucrose agar.

It must be remembered that there were no exudates observed on the cultures and the color of media did not change throughout the course of this investigations.

Differences in colony characters are shown in Fig. (V).

2- Compatibility tests :

Compatibility of four monosporidial cultures derived from different promycelia, different spore balls, and different locations were tested alone and in all possible combinations in vivo and in vitro.

Inoculation by single isolates, failed to incite infection and to form dikaryotic mycelia or teliospores in vivo and in vitro, respectively.

As for the paired combinations, it was observed in vivo and in vitro tests that isolate-3 was more compatible than the others, whereas isolate-4 was the least one (Figure V).

The microscopical examination revealed the development of the teliospores in vivo after inoculation with paired compatible cultures, and in vitro in petri-plates.

On the other hand, the study showed that, in the same pathogenic race (two compatible cultures) the formed spore balls varied in the number of cells in every ball (Fig. VII 1-4). This study has also proved that *Urocystis agropyri* can complete its development in vitro (on cultural media) Figure VII (5-8). Spore ball differed even on the level of the race Figure VII (1,2,3 and 4). The shape, color, constituents, and dimensions of the different spore balls originated from the different combinations, were similar in vivo and in vitro Figure VII (1-8). Also, spore characters agreed with the keys suggested by Fischer & Holton (1957) and Duran (1973).

The microscopical examination also clarified the formation of the dikaryotic mycelium on water agar in slide culture. This mycelia were formed only from two paired compatible monosporidial cultures, and not from single or incompatible cultures, Figure VII (9-11).

3- Pathogenecity tests :

Irrespective of the disppearance of any visible symptoms either in seedling or in the adult stage, the microscopical examination revealed the formation of teliospores in the seedling tissues of the infected plants.

A) Greenhouse experiments :

The microspical examination showed that injection with spore suspension and "MT_2" spore suspension has led to the formation of teliospores after 10 days from inoculation in the variety Mokhtar injected at the 20 and 40 days old, respectively.

B) Field experiments :

The mixed isolates separately grown, failed to form teliospores either in the seedling or in the adult stage. Microscopic examination revealed also the absence of the dikaryotic mycelium.

C) Laboratory experiments :

Microspical examination showed the formation of teliospores in the inoculated differential varieties in the seedling stage, but the spores has disappeared after transplantation in the adult plants.

D) Differential varieties tests :

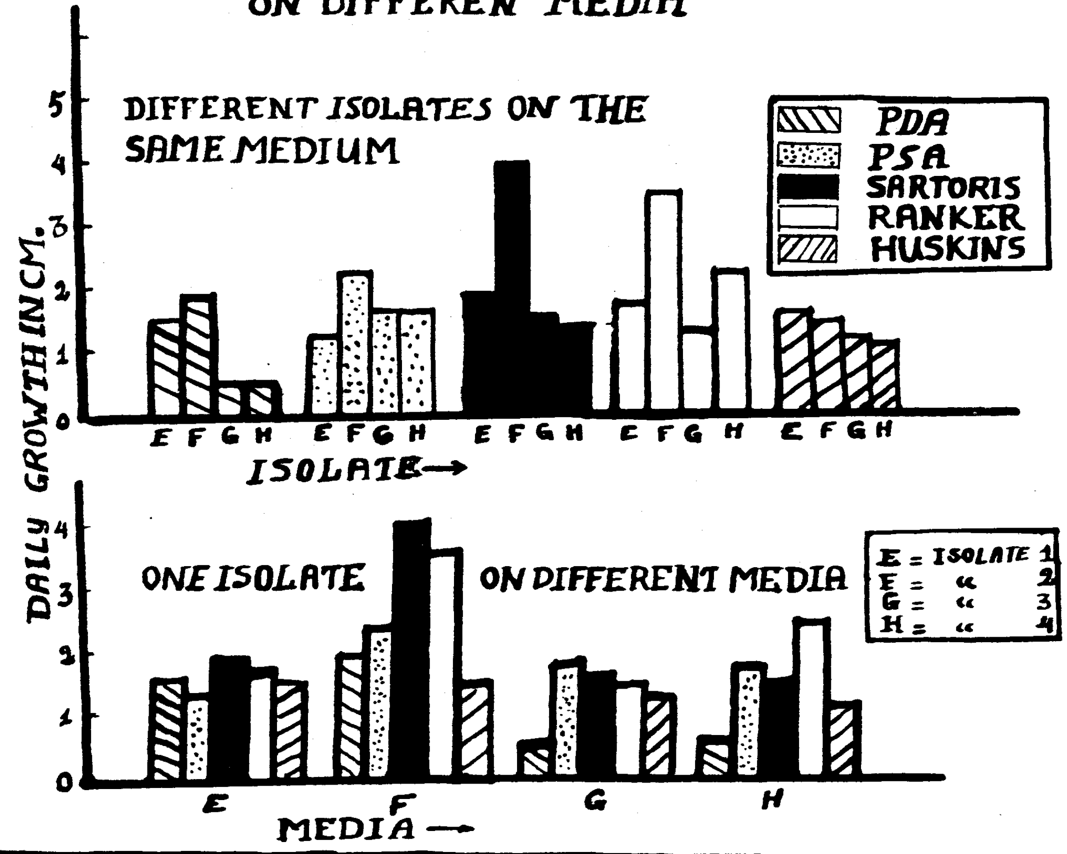
Every two compatible paired cultures, differed in the formation of teliospores in the infected tissues of the differential varieties. Race 2 (1x3) formed spores in nine varieties out of ten, whereas in race 5 (2x4), the spores were formed in three varieties only. The other compatible races ranged between these limits. On the other hand R_3 and R_6 failed to be compatible in any of the differential varieties. Table (6).

Table 4- The growth, in cm. of four isolates of <u>Urocystis agropyri</u> on five different media maintained at 20 C,after 6 days.

Isolate	U_1					U_2					U_3					U_4				
days	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
I	0.9	0.8	1.2	1.2	1.1	0.8	1.0	2.2	1.6	1.1	-	1.1	1.1	1.1	0.8	-	1.2	1.1	1.1	0.7
2	1.1	0.9	1.4	1.3	1.3	0.9	1.4	2.6	2.2	1.1	-	1.2	1.3	1.2	0.9	-	1.3	1.2	1.2	0.7
3	1.2	1.0	1.6	1.4	1.3	1.3	1.5	2.9	2.4	1.2	-	1.4	1.4	1.2	1.1	-	1.4	1.2	1.4	0.8
4	1.4	1.1	1.7	1.5	1.4	1.6	2.1	3.4	2.9	1.3	-	1.5	1.5	1.3	1.1	-	1.5	1.3	1.8	0.9
5	1.5	1.2	1.8	1.7	1.5	1.7	2.2	3.9	3.2	1.4	-	1.6	1.6	1.3	1.2	-	1.6	1.3	2.2	1.0
6	1.6	1.3	1.9	1.8	1.6	2.0	2.9	4.1	3.6	1.5	-	1.7	1.6	1.4	1.2	-	1.7	1.4	2.4	1.1

A = Potato 2 per cent glucose agar.
B = Potato 4 per cent sucrose agar.
C = Sartoris's medium.
D = Ranker's medium.
E = Huskin's medium.

FIG. IV GROWTH OF UROCYSISTIS TRITICI ON DIFFEREN MEDIA



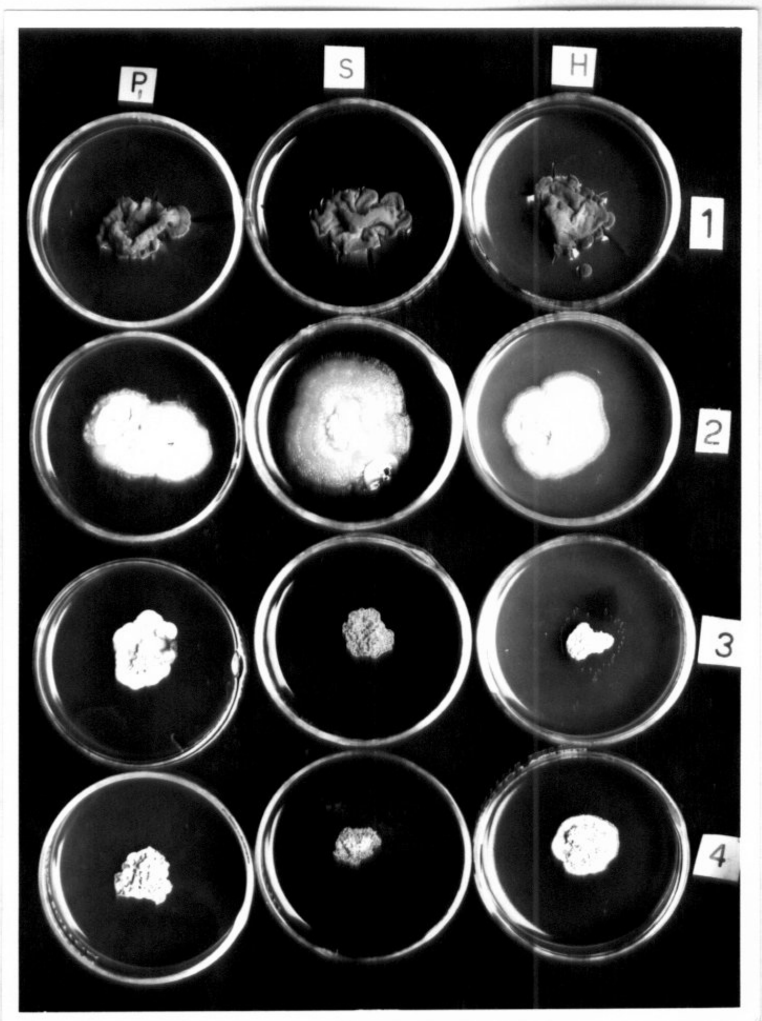


Fig. (V) The growth of four isolates <u>Urocystis agropyri</u> Koern, on three different media.

H = Huskins.
P = PSA
S = Sartoris.

Table 5(A-E) The characteristic behaviour of the tested isolates of _Urocystis agropyri_ on five different media after two weeks, at 20 C.

(A) Potato- 4% sucrose agar

Isolate	Colony diameter	Consistency	Coloration	Topography	Margin
1	2.7	Powdery	Dark olivacious	Raised, verrucose to rugose.	Erose, lobate
2	5.0	Mycelioid	Avellaneous, to very light brown	Raised furrowing verrucose to rugose.	Erose, lobate
3	3.3	Yeast-like	Dull white to very light vinaceous	Warty centre, falt margin.	Undulate
4	3.3	Bacterioid	Pure white to light avellaneous	Raised, warty to rugose	Undulate

(B) Huskins medium

Isolate	Colony diameter	Consistency	Coloration	Topography	Margin
1	2.9	Powdery	Dark olive	Raised, verruscose to rugose	Undulate
2	3.1	Mycelioid	Light brown	Pulvinate, slight rugose	Lobate erose.
3	2.3	Yeast like	Dull white	Slight rugose	Erose
4	2.1	Bacterioid	Pure white	Worty to rugose	Erose

(C) Sartoiris medium

Isolate	Colony diameter	Consistency	Coloration	Topography	Margin
1	3.5	Powdery	Dark olive	Raised, verucose to rugose	Lobate
2	5.2	Mycelioid	Light brown	Pulvinate centre, zonate.	Lobate
3	3.0	Yeast	White to pale olive	Verrucose to rugose	Erose
4	2.8	Bacterioid	Dull white	Verrucose to rugose	Erose

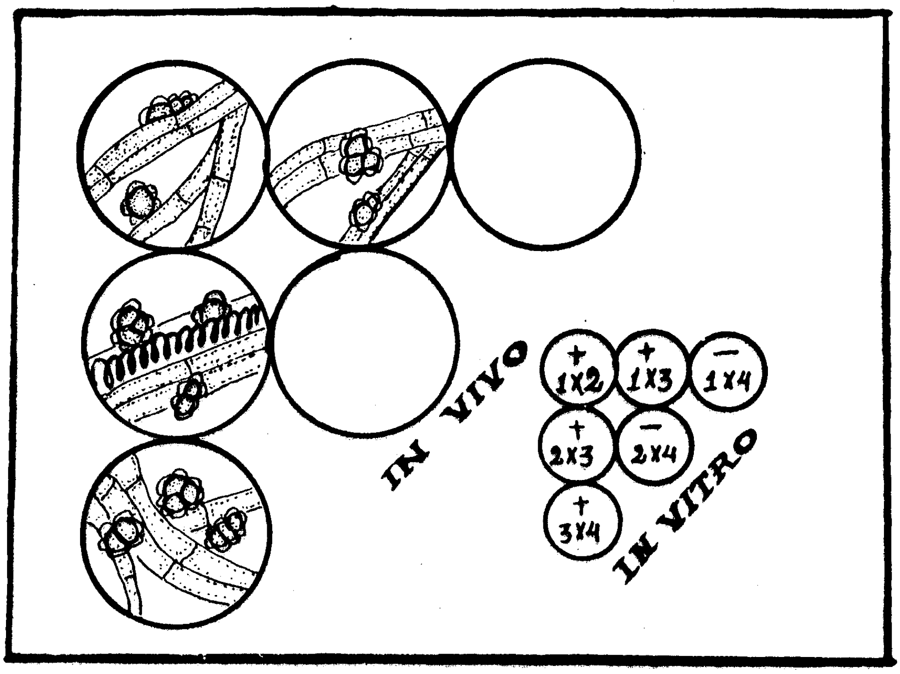
(D) Potato 2% glucose agar

Isolate	Colony diameter	Consistency	Coloration	Topography	Margin
1	2.2	Powdery	Dark olive	Flat	Entire
2	4.1	Velvety	Light brown	Flat	Entire
3		Could	not develop		
4		Could	not develop		

(E) Ranker's medium

Isolate	Colony diameter	Consistency	Coloration	Topography	Margin
1	3.4	Powdery	Dark olive	Flat	Entire
2	6.5	Velvety	Avellaneous	Flat	Entire
3	2.5	Yeast like	Dull white	Flat	Erose
4	4.1	Baterioid	Light vinaceous	Flat	Erose

FIG.VI COMPATIBILITY BETWEEN FOUR ISOLATES OF UROCYSTIS TRITICI KOERN



⊕ = COMPATIBLE ISOLATES ACCORDING TO THE FORMATION OF THE SPORE BALLS

⊖ = INCOMPATIBLE ISOLATES

Table 6- Reaction of pathogenic races of <u>Urocystis agropyri</u> on the differential varieties according to microscopic examination.

Races varieties	R_1 [+]	R_2	R_3	R_4	R_5	R_6
	1x2 [++]	1x3	1x4	2x3	2x4	3x4
Oro-Federatio	S	S	Incompatible	S	nS	Incompatible
Baart	nS	S		S	S	
Federation	S	S		nS	S	
Ngochen	nS	S		S	nS	
Tsing haue	nS	nS		S	nS	
Giza 139	S	S		nS	nS	
Mabrouk	nS	S		nS	nS	
Mokhtar	S	S		S	S	
Giza 148	S	S		S	nS	
Giza 155	S	S		S	nS	

S = Compatible isolated formed spore balls in the differential varieties.

nS= Compatible isolates did not form spore balls in the differential varieties.

+= Pathogenic races.

++= Cultural isolate.

Fig. (VII) showing different shaps of spore balls of *Urocystis tritici* obtained from the different combinations between the compatible isolates (1 x 3) (X 1125).

1,2- Three celled spore ball (X 1125).
3- Two celled spore ball (X 1125).
4- One celled spore balled (X 1125).

Different shapes of balls of *Urocystis tritici* obtained from different combinations in vitro.

5- Combination 1 x 2 (X 800).

6- Combination 1 x 3 (X 800).

7- Combination 2 x 4 (X 800).

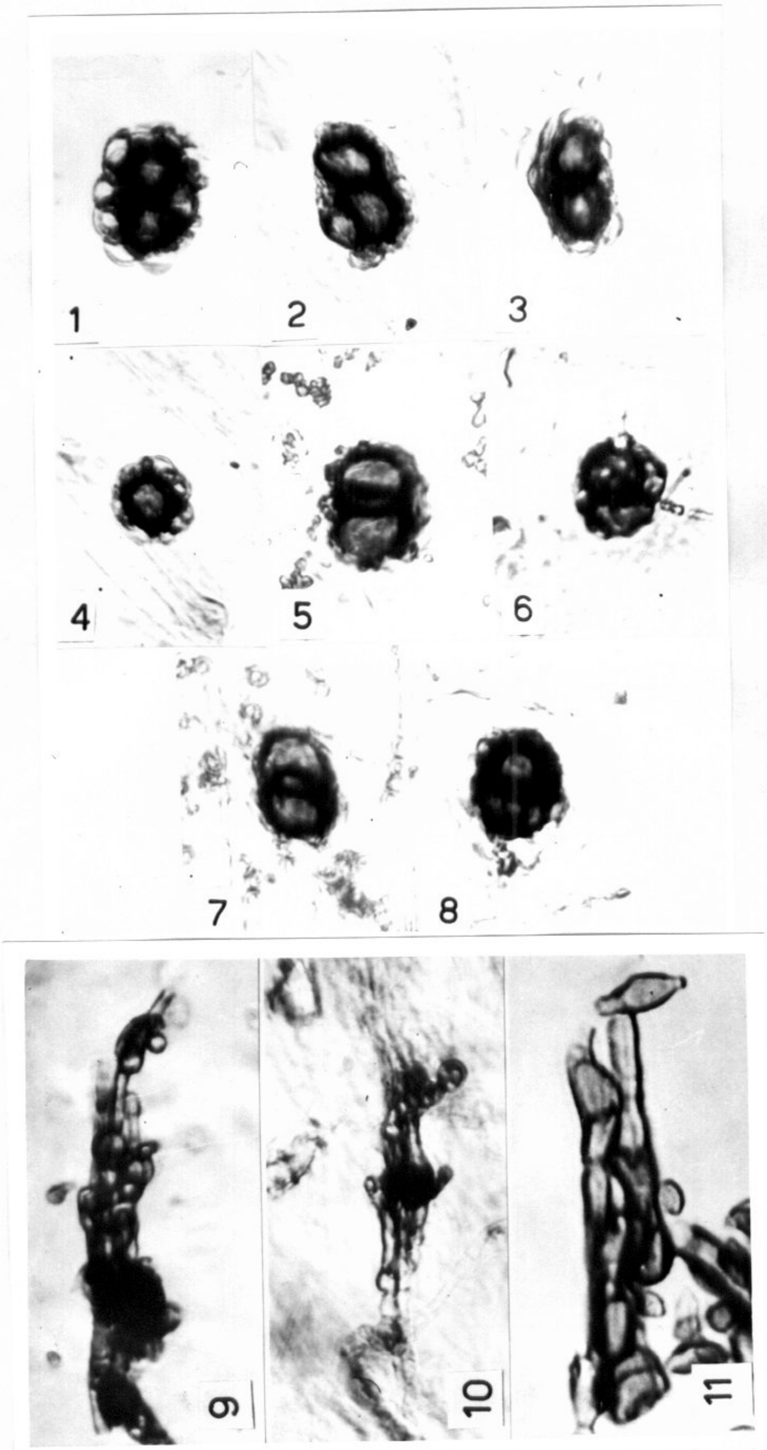
8- Combination 3 x 4 (X 800).

Dikariotic mycelia developed on slide culture.

9- Urocystis tritici combination 1 x 3 (X 800).

10- Combination of *Tilletia foetida* 2 x 5 (X 800).

11- The same combination 2 x 3 (X 800) revealed the uniting haplonts in *Urocystis tritici*.


1
2
3
4
5
6
7
8
9
10
11

DISCUSSION

The smuts contain fewer obligate parasites than the rusts, for, many have now been grown on artificial, particularly in the haploid phase, and much is known of the behaviour and genetics of these fungi (Halisky 1965). In the smut disease, the principal host-parasite interactions known to be subject to genetic interpretation are symptoms expression, and host variety reaction to physiologic races of the various smut species (Holton, 1959).

The goal of this work is to consider some of the genetic aspects in *Tilletia foetida* and *Urocystis agropyri* regardless their relative economic significance.

The results obtained showed that, the five monosporidial cultures of *Tilletia foetida* (Wallr.) Liro., and the four monosporidial cultures of *Urocystis agropyri* (Preusus) Schrot., were more stable on the tested media. Every individual race has a characteristic growth habits on each of the tested media, regardless of the differences between the individual isolates on the different media, the growth was more rapid than that reported elsewhere, for culturing of the diploid phase. The same findings has been reported by Rodenhiser (1928), Kienholz & Heald (1930), Melchers (1934), and Wu (1949).

According to the stability of the haplophase of T.foetida and U.agropyri (at least in the tested cultures) on the tested artificial media, it is suggested that the term (physiologic race) must describ the haplophase regardless of its pathogenic behaviour. This view poin was determined by Holton (1930). On the other hand, all the differences in cultural characteristics could be considered as variability, since any culture retained its own characteristic behaviour when recultered on any of the tested media.

The methods applied herein on the germination of spore balls of U.agropyri, agreed with Noble's methods (1924).

The isolation of U.agropyri was performed from spore material, and not from infected plaht tissues as reported by Wu (1949). The double-plate method has proved its efficiency as a reliable procedure when micromanipulator is inavailable.

The foregoing study proved that *U.agropyri* could form teliospores (spore balls) in vitro, originating only from paired compatible monosporidial cultures, and not from single cultures. This reflected the relation between sexual compatibility and parasitism. On the other hand, this point has been exploited in the compatibility indication tests throughout the course of this investigation.

This investigation gave evidence that, the number of cells in spore ball was characteristic for the species, but not for the pathogenic race, since many spore balls differed in their number of cells,although they were obtained from the same race. The obtained results also indicated that the two tested species of *T.foetida* and *U.agropyri* are considered heterothallic, since the monosporidial cultures of each, failed either, to cause infection or to form clamp connections, on the other hand, the tested cultures did not exhibit the so-called solopathogenicity. Similar results were reached by Flor (1932) in case of *Tilletia tritici* and *T.levis*.

The presence of a relation between sex compatibility and parasitism is doubtedless. The failure in the appearance of the symptoms of the concerned diseases could be attributed to any technical or environmental factors especially for Tilletia foetida.

The problem in Urocystis agropyri may deal with the quantity of the inoculum, since the inoculum potential by this method has not been determined. Hoffmann (Personal communications) attributed the disappearance of the symptoms to the procedure applied, since the inoculation by paired cultural lines, leads to the production of collections which lack aggressiveness in subsequent generations.

The results also give evidence, that pathogenic race tends to become characteristic in its reaction on the differential varieties. In spite of the absence of symptoms, the dikaryotic mycelium, behaved as if it was a sporic material. Such behaviour forces the author to hyposize or assume that the pathogenic race as a term must be used to describe the dikaryotic mycelium derived from two distinct (culturally), compatible (sexually) cultures, or two physiologic races.

The identification of pathogenic races according to the populations of teliospores was a subject of conflections, Holton (1930, 1931) Aamodt (1931), Fischer &

Holton (1957) and Chcrewick (1958). On the other hand Hoffman (Personal communications) supported this assumption, he showed that multiple infection with subsequent exchange of nuclei between dikaryons and other parasexual phenomena may add to the variability of pathogenicity from teliospore inoculations.

SUMMARY

In the course of this study, the physiological specialization of *Tilletia foetida* (Wallr.) Liro and *Urocystis agropyri* (Preusus) Schrot., was studied. Regardless the methods of isolation, the obtained sporidia were subjected to three kinds of studies i.e., cultural, compatibility, and pathogenicity studies.

Cultural studies were carried out using five different media for both isolates of the two species. Five isolates of *Tilletia foetida* and four isolates of *Urocystis agropyri* were investigated.

I- Cutlural studies revealed the following points :

1) All the isolates and their successive transfers were stable on the different tested artificial media.
2) On the same medium differences were observed in the growth characters for every isolate. These characters included colony diameter after two weeks from inoculation, color, consistency, topography, exudates, margin, and the color of medium.
3) With few exceptions, Sartoris and Potato 4 per cent sucrose agar were considered the best media for the isolates of both species.

4) All the differences in cultural characteristics were considered to be variability, since any culture has retained its own characteristics when recultured on any of the tested media.

5) Barley medium has proved its efficiency for growth of the individual and paired cultures of both *Tilletia foetida* and *Urocystis agropyri*.

6) The double plate method developed by Bodine (1931) for the sporidial isolation of *T.levis*, was modified to be favourable for the isolation of *U.agropyri*.

II- Compatibility studies revealed the following :

1) The two tested species are heterothallic. On the other hand, the tested isolates did not exhibit the so-called solo-pathogenicity.

2) The different isolates were different in the combining ability between themselves intraspecifically. Consequently the present study revealed that there were two systems or mating types controlling the sex in the tested species : simple bipolar and complex multiple allelic system.

3) Results in vivo were supported by the results in vitro.

4) Modifications in "Bauch" test, or the indication of compatibility test, led to the formation of teliospores axinically , or in other term, led to the completion of life cycle in vitro (in U.agropyri)

5) The indication of compatibility throughout this study was performed according to the formation of clamp connections in Telletia foetida, and the formation of teliospores in the U.agropyri.

6) On the other hand, the occurrance of teliospores as a result of infection by paired compatible cultures, is considered reflection to the relation between sexual compatibility and parasitism.

III- The pathogenicity studies revealed the following :

The inoculation by paired compatible cultures failed to produce visible symptoms. Therefore, the unique procedure for detecting the parasite, was carried out by macerating the infected tissues and examinating them microscopically.

On the light of the foregoing results the term pathogenic race must describe the dikaryotic mycelium originated from two distinct compatible monosporidial cultures. Also, the physiologic race must describe the haplophase which exhibits characteristic cultural behaviour.

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ARABIC SUMMARY

بسم الله الرحمن الرحيم

التخصص الفسيولوجي في فطرى
التفحم اللوائى والتفحم المغطى في القمح

ملخص البحث

يعتبر القمح أهم محصول شتوى فى جمهورية مصر العربية ، ويزرع منه سنويا ما يقرب من ١٫٢٥ مليون فدان تنتج حوالى ٢ مليون طن من الحبوب (وزاره الزراعة ١٩٧٦). ولـذا فيستورد النقص من الدول المنتجة للقمح (ما يقرب من ٢٫٥ مليون طن سنويا) وهذه الكمية عرضه للزياده بارتفاع عدد السكان.

ويتعرض القمح فى مصر للاصابه بالعديد من الامراض مثل الاصداء والتفحمات والبياض الدقيق ، وبعض الامراض الاخرى القليلة الاهمية التى تقلل من محصول الاصناف المنزرعة.

وقد سجل مرضى التفحم المغطى واللوائى على القمح فى مصر (جونس وسيف النصر ١٩٤٠) ، (الهلالى ١٩٤٨). ويصيب الاول أصناف durum مثل الدكر والبلدى ، بينما ورد الثانى الى مصر مع الاقماح الاسترالية خلال الحرب العالمية الأولى ، وقد لوحظ أنه يصيب أصناف القمح ال vulgare بينما كانت أصناف ال durum منيعة (جونس وسيف النصر ١٩٤٠) ويعتبر المرضان حاليا أقل أنتشارا من ذى قبل ، وذلك بفضل الاصناف المقاومة المنزرعة حديثا.

ويعتبر أكتشاف ظاهره التخصص الفسيولوجى بلاشك أحد التطورات الهامة فى علم أمراض النبات حيث أنها وثيقة الصلة باستخدام الاصناف الجديده.

وتكمن جذور مشكلة التخصص والسلالة فى فطريات التفحم فى الطبيعة المتغيره للكائنات المسببة للمرض.

وقد تم خلال هذا البحث دراسة للفطرين Tilletia foetida, Urocystis agropyri على ضوء ظاهره التخصص الفسيولوجي ، وبغض النظر عن التفصيلات طرق العزل للمسببات المرضية ، فان الاسبوريديات الناتجة قد تعرضت لثلاثة أنواع من الدراسة : دراسات مزرعية ودراسات توافقية ودراسات القدره المرضية فى الحقل والمعمل .

وقد تم تنفيذ الدراسات المزرعية على خمس بيئات صناعية لكل من عزلات النوعين المختبرين (خمس عزلات خاص بال Tilletia وأربع عزلات خاص بال (Urocystis) قد أظهرت الدراسات المزرعية النقاط التالية :

١ـ كل العزلات الاصلية والفرعية كانت ثابته فى صفاتها على البيئات المختلفة موضع الاختبار .

٢ـ لوحظ وجود اختلافات فى خصائص النمو لكل عزلة واحده على البيئات المختلفة ، وعلى مستوى البيئة بالنسبة للسلالات المختلفة وقد شملت الخصائص موضع الاختلاف : قطر النمو بعد أسبوعين من التلقيح ـ القوام ـ لون المزرعة ـ تضاريس السطح ـ الافرازات ـ شكل الحافة ـ التغير المحدث فى لون البيئة .

٣ـ تعتبر بيئتا Potato sucrose agar and sartoris أحسن البيئات المختبره لغالبية عزلات كلا النوعيين باستثناءات بسيطة .

٤ـ تعتبر كل الاختلافات فى الصفات المزرعية تنوعا لارجعيا . حيث أن أى مزرعة كانت تستعيد صفاتها المزرعية على بيئة معينة اذا ما نقلت اليها من بيئة أخرى .

٥ـ أثبتت بيئة الشعير الطبيعية كفاءتها كبيئة لاكثار السلالات الاحادية والمزدوجة لكلا النوعيين المختبرين .

٦ـ تم فى هذه الدراسة تحوير طريقة الطبق المزدوج التى اقترحها Bodine سنة ١٩٣١ لتعزل الفطر Tilletia levis بحيث تلائم عزل الفطر Urocystis agropyri

وقد أظهرت الدراسات التوافقيه ما يلى :

١ــ يعتبر النوعان المختبران متباينا الثالوس Heterothallic ولايملك أى من المزلات المختبره ما يسمى بالقدره على العدوى الفردية Solopathogenicity

٢ــ تختلف المزلات المختبره فى مقدرتها الاتحادية بالسلالات الاخرى ، وبناء على ذلك فقد أسفرت هموذه الدراسة عن وجود نوعين من النظم التى تحكم الجنس فى الفطرين موضع الدراسة الا وهما : النظام ثنائى القطبية البسيط ــ والنظام المعقد المتعدد الاليلات.

٣ــ أيدت النتائج لخطوط المائل النتائج داخل المائل.

٤ــ أدت التحويرات فى أختبار "Bauch" أو اختبار اثبات دليل التوافق بين السلالات أدت الى تكوين الجراثيم التيلتية أو بمعنى آخر استكمال دورة الحياه للفطر U. agropyri خارج المائل.

٥ــ تم اثبات وجود التوافق خلال هذا البحث طبقا لتكوين الميسليوم الثنائى فى حالة Tilletia foetida وتكوين الجراثيم التيليتية فى حالة الفطر U. agropyri

٦ــ ومن جهة أخرى فأن ظهور الجراثيم التيلتية كنتيجة للعدوى بعزلات مزدوجة متوافقة جنسيا ، يعتبر انعكاسا لمدى العلاقة بين التوافق الجنسى والتطفل.

وأوضحت الدراسات المرضية ما يلى :

١ــ فشلت العدوى بسلالات مزرعيين مزدوجة ومتوافقة فى أحداث أية أعراض مرضية.

٢ــ لذلك فقد كانت الوسيلة الوحيده للبحث عن الطفيل تتم عن طريق تمزيق الانسجة وفحصها ميكروسكوبيا .

وعلى ضوء نتائج الدراسات السابقة فان أصطلاح السلاله المرضية ينبغى أن يصف الميسليوم الثنائى المشتق من سلالتين محددتين مزرعيا ومتوافقتين جنسيا " وينبغى أن يصف اصطلاح " السلالة الفسيولوجية " الطور الاحادى الذى يمتلك سلوكا مزرعيا مميزا .