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OVERWINTERING OF *PUCCINIA HORDEI* IN THE NETHERLANDS

BY J.E. PARLEVLIET AND A. VAN OMMEREN

Institute of Plant Breeding, Agricultural University, Wageningen, The Netherlands

It is generally assumed that leaf rust, *Puccinia hordei*, overwinters in Western Europe as a sporulating or as a dormant uredo-mycelium in winter barley (Gassner and Pieschel, 1934; D'Oliveira, 1939; Simkin and Wheeler, 1974a).

To obtain more insight into the overwintering, two experiments were carried out, while the occurrence of leaf rust on barley volunteer plants was recorded at intervals.

MATERIALS AND METHODS

Recording. In four successive years (1971 - 1975) barley volunteer plants were evaluated for the amount of leaf rust at many sites in the period October to March in the eastern part of the Netherlands.

Experiment 1. Eight winter barley cultivars were sown on two sites at Wageningen on 23 September 1972. The seed rate was 120 kg per ha. The soil at the two sites consisted of heavy clay and sand respectively. The randomized block design had four replicates at the clay site and two at the sandy one. Each plot consisted of eight rows, 4.5 m long and 0.25 m apart. Surrounding each block were four rows of spring barley sown in August and inoculated with leaf rust in September to simulate infection coming from barley volunteer plants. The level of leaf rust was never high on this August-sown barley (< 1%), which was killed in the second half of December by nearly two weeks of freezing weather.

The numbers of uredosori or clusters of uredosori were counted on six sampling dates on six plants per plot. Many uredosori were apparently individual lesions. Sometimes, however, several to many uredosori were closely clustered together, suggesting an origin from a single lesion either as secondary sori or due to germination of spores around the primary sorus in a drop of stagnating water. Such clusters were scored as single lesions.

At the first two sampling dates plants were taken from the sites, planted in small pots and transferred to a greenhouse to measure the number of non-sporulating infections in the field, it being assumed that these infections would form uredosori at the higher temperatures in the greenhouse.

Experiment 2. Ten winter barley cultivars were sown in plack plastic pots, 12 cm square and 12 cm deep, on 2 October 1974 and kept outside. On 2 December the leaves present were marked and the plants inoculated with leaf rust spores. The proportion of marked leaves still alive and the number of lesions were recorded on three dates.

RESULTS AND DISCUSSION

Leaf rust was abundantly present on barley volunteer plants in all four years on many sites recorded. Plants without leaf rust were in fact hard to find. These rusted volunteer plants remained present until the first serious freezing spell and sometimes even after that. In two out of the four years leaf rust was found on barley volunteers in late winter and early spring. These were very mild winters.

The volunteer plants form an abundant source of inoculum over an extended period to infect the winter barley. In mild winters the volunteer plants themselves may serve as a limited source of overwintered inoculum.

The infection of the winter barley from the imitation volunteers succeeded very well, the level being kept moderate by restricting the inoculum source on the pseudo-volunteers through the use of a partially resistant cultivar. Table 1 gives the data averaged over all cultivars.

Table 1. Number of sporulating lesions per plant in winter barley at two sites on six sampling dates.

Site	Sampling date					
	7/14 Dec.*	31 Jan.	19 March	24 Apr.	9 May	28 May
Clay	3.8	1.7	1.0	13.8	32.4	37.5
Sand	6.7	1.3	0.2	3.9	8.3	12.6

* The clay site was sampled on 7 December, the sandy site a week later.

At both sites the infection level drops through the winter to reach a minimum in early spring. The rate of decrease was much higher on the sandy site than on the clay site. The cultivars seem to differ in the rate of decline through the winter and also in the time and rate of recovery of the rust population in the spring (Table 2).

Table 2. Number of sporulating lesions per plant on 8 winter barley cultivars at six sampling dates. Clay site.

Cultivar	Sampling date					
	7 Dec.	31 Jan.	19 March	24 Apr.	9 May	28 May
Herfordia	2.8	0.8	0.2	2.0	1.5	3.9
Hauters	2.8	1.5	0.6	0.3	1.6	4.0
Pella	5.0	1.1	0.3	2.1	3.2	4.0
Hillman	4.5	2.3	0.9	1.8	3.6	3.6
Tschermacks-2 row	1.9	1.7	1.0	1.0	8	12
Maris Otter	3.5	0.4	0.3	0.5	10	40
Hudson	2.9	1.9	1.9	35	41	43
Dominator	6.8	3.7	2.8	70	190	190

The differences in decline through the winter are small compared with the differences in recovery. For example, the leaf rust on Hudson and Dominator started to recover in late March or early April, the increase being quite rapid. On Maris Otter the increase was rapid too, but the start was about a month later. The recovery was slow and relatively early on Herfordia and Pella, on Hauters it was slow and late.

The differences in decline through the winter caused by the cultivars do not seem to be related to the differences in recovery, suggesting a different cause. The inverse of the recovery rate in the spring most likely represents for a large part the slow rusting (partial) resistance of the cultivar, which can differ greatly (Parlevliet and van Ommeren, 1975). The decline was thought to be caused by the fairly high rate at which winter barley replaces its older, infected leaves by new ones. Leaf rust can reproduce at low temperatures but does so very slowly. Simkin and Wheeler (1974b) showed, that at 5 °C sixty days were needed for reproduction. In this period winter barley may replace most of its leaves as was shown in experiment 2. Table 3 shows that, 70 days after inoculation, only 4% of the leaves present at inoculation were still alive.

Table 3. Proportion of leaves, present at inoculation on 2 December, still alive and number of sporulating lesions on three sampling dates.

	Sampling date		
	2 Jan.	10 Feb.	25 March
% leaves still alive	76	4	0
Lesions per plant	> 300	10.8*	1.2

* on newly formed, unmarked leaves

The number of lesions on the newly formed leaves was far lower than on the inoculated leaves as measured on 2 January. On 25 March the infection level had dropped further, some of the infections appearing on leaves not yet present on 10 February, indicating at least two cycles of reproduction after inoculation.

Since in early spring practically all leaves present in the late autumn have been replaced by new ones, dormant uredo-mycelium does not seem to play a role in overwintering. Dormant mycelia may, however, occur as our data suggest. The proportion of non-sporulating infections, measured at the first two sampling data in experiment 1, varied little if at all with cultivar and site, but strongly with sampling date. At the first sampling date non-sporulating infections formed about 20% of all infections, at 31 January this was much higher, 70%, suggesting a considerable proportion of almost dormant mycelia.

In normal and mild winters the winter barley can grow, replacing its leaves slowly. This necessitates the pathogen reproducing through uredospores in order to overwinter. Only in severe winters with continuous snow cover may a more static situation exist where almost dormant mycelia may help to bridge the winter. Under our normal winter conditions it is the interaction between the rate of leaf replacement by the barley and the rate of reproduction by the pathogen, which may largely determine the size of the rust population in early spring. Factors

enhancing the rate of leaf replacement therefore may have a profound effect on overwintering of the pathogen. The differences between the two sites indeed suggest this. The number of leaves per plant on the sandy site was always less than on the clay site, the difference becoming larger during the winter.

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POTENTIALS FOR STABLE RESISTANCE TO *PUCCINIA POLYSORA* IN LOCAL (NIGERIAN) AND EXOTIC MAIZE VARIETIES

BY J. M. FAJEMISIN

Maize Pathologist, Federal Department of Agricultural Research, Ibadan, Nigeria

Rust caused by *Puccinia polysora* Underw. is one of the most important endemic diseases of maize (*Zea mays* L.) in West Africa. The historic epidemic of this disease in the early fifties dealt a great blow to farmers. Total crop failure was experienced. Yield loss as high as 44% is still common (Craig, 1966). The said epidemic gave birth to the West African Maize Rust Research Unit at Moor Plantation, Ibadan, Nigeria. Furthermore, it served as the stimulus for vigorous maize research efforts in the West African zone.

This paper reports the reaction of local, exotic, and improved local varieties to infection by *P. polysora* together with some data relevant to breeding such as maturity type and grain characteristics.

MATERIALS AND METHODS

In March 1974, 137 maize cultivars were planted each into a single non-replicated 20-plant row. The rows were spaced 0.9 m apart and the plants 0.3 m within the row. The maize entries comprised:-

A. Sixty-two open-pollinated varieties collected from across the maize growing areas of Nigeria ('unimproved locals').

B. Twenty-one maize synthetics or composites developed at various Agricultural Research Institutions in Nigeria ('improved locals').

C. Fifty-four exotic cultivars including a batch of 49 sent by CIMMYT in 1972 for the International Maize Adaptation Nursery (IMAN) Trial and which consisted of varieties from Asia, the Carribean, West Africa, and South Africa ('exotic varieties').

Four weeks after planting, and again 10 days later, each plant was artifically inoculated by dropping a 5 ml urediospore suspension of *P. polysora* into the leaf whorl. The percentage of leaf area covered by pustules on each cultivar was recorded 2 weeks after mid-silk. Data were also taken on the colour and endosperm characteristics of the grains of each cultivar and the number of days taken by each to reach 50% anthesis.

RESULTS

Secondary infection occurred within three weeks of the date of the first inoculation.

For easy categorization of the maize varieties for disease reaction, five classes were established (see Table 1).

Eighty-nine (65%) of the cultivars tested showed either a moderately susceptible or a more susceptible disease reaction (Table 1). Most of the entries were moderately susceptible.

Table 1. Frequency distribution (percent) of maize varieties for reaction to rust (*Puccinia polysora* Underw.)

Maize groups	Rust scale *				
	1	2	3	4	5
A. Overall	7	28	46	18	1
B. Origin					
Unimproved locals	3	13	58	24	2
Improved locals	29	9	43	19	0
Exotic varieties	2	53	35	8	2
C. Maturity **					
Early	0	6	47	35	12
Medium	7	29	36	28	0
Late	8	32	47	13	0
D. Grain colour					
White	3	30	46	21	0
Yellow	5	42	38	10	5
Mixed	19	9	53	19	0
E. Endosperm type					
Floury	0	17	66	17	0
Flint	0	47	35	15	3
Floury-flint	17	13	49	21	0

* Where 1 = resistant = 0-10% total leaf area infected
2 = moderately resistant = 11-30% total leaf area infected
3 = moderately susceptible = 31-50% total leaf area infected
4 = susceptible = 51-75% total leaf area infected
5 = highly susceptible = 76-100% total leaf area infected

** Early = takes less than 50 days from planting to anthesis
Medium = 50-59 days from planting to anthesis
Late = 60 days and above from planting to anthesis

The distribution pattern of the maize cultivars on the rust reaction scale was different for each of the three varietal groupings. Eighty-four, 70 and 45 percent of the unimproved locals, improved locals and exotic varieties, respectively, exhibited a moderate or more susceptible reaction. Thus, the distribution was skewed more to the resistant class in the introduced varieties than in either the improved or unimproved locals.

Six of the nine maize varieties in the resistant class exhibited hypersensitive resistance. The other three, comprising two unimproved locals and one exotic cultivar expressed quantitative resistance, that is, low pustule density.

The cultivars varied in grain characteristics and maturity rating. Sixty-seven were white, 38 yellow, and 32 had mixed (white and yellow) seed. Eighteen percent of the maize had grains with a floury texture while 44% were flinty and the remaining 38% floury-flint. Most of the maize types were of medium maturity (50-59 days from planting to anthesis); 12% were early (less than 50 days to anthesis) and the other 16% were late-maturing (60 or more days to anthesis).

DISCUSSION

There was a wide variability in the reaction of the various maize types to *P. polysora* from highly susceptible to resistant. Although the bulk of the varieties were susceptible, those in the moderately resistant class can successfully form the source materials for incorporating stable horizontal resistance into susceptible high yielding varieties.

The greater resistance of the exotic varieties than the improved locals indicatesthat these latter varieties could be improved further. The distribution of these improved varieties is skewed more to the resistant class than the unimproved locals because the former contain six varieties fortified with a hypersensitive resistance gene. Thus, in regards to rust reaction the six varieties should be regarded as one because of the common gene; the protection given by this qualitative resistance cannot be relied upon because of its vulnerability to new races of *P. polysora*.

Early maturing cultivars were more susceptible than either medium or late maturing varieties. Therefore, a larger maize population would need to be screened in order to obtain rust resistant early-maturing cultivars than to obtain rust resistant medium or late maturing cultivars.

There was a higher frequency of maize cultivars moderately resistant to rust among the flinty than among the floury grain type. There were also more moderately resistant maize cultivars with a yellow grain colour than those with a white grain colour.

A successful breeding programme must not be structured to produce only resistant and high yielding varieties. The finished product must be acceptable to farmers who must grow maize that will satisfy the consumers. In south western Nigeria, where most of the maize is grown and consumed, grain characteristics are important (Ebong & Raghunathan, 1970). Maize with white floury grain is preferred. Unfortunately in regards to rust reaction, the odds appear to be against all the features that are favoured by the consumers viz: white *vs* yellow, floury *vs* flint and early *vs* late. But even though the frequency of favourable characters appearsto be low, the potentials for improvement do exist.

Maize types with such desirable characters as high yield, disease resistance and specific maturity range and grain type can be identified through an elaborate screening exercise embracing cultivars from many parts of the maize growing world. In order to produce a maize variety fulfilling such specific criteria, selection for the source materials should not be too strict. For each character, cultivars in the 'moderate' (middle) class of the disease reaction scale might be selected instead of strict adherence to the desired end of the scale. This would help to widen the genetic base of the would-be variety and therefore guarantee a genetic reserve or insurance for future needs. Since each of most of the characteristics desirable to the consumers in the released crop variety is controlled by many genes, a recurrent selection technique is suggested for accumulating or pyramiding the favourable genes.

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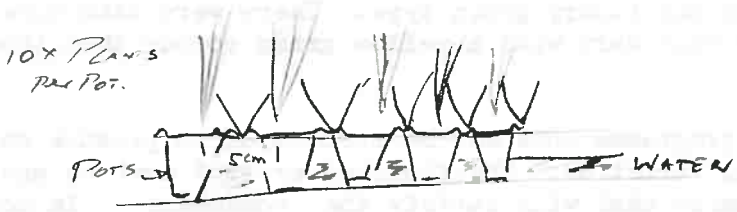

TOWARDS MASS PRODUCTION OF UREDOSPORES OF BROWN RUST ON WHEAT (*PUCCINIA RECONDITA* F. SP. *TRITICI*)

BY A.F. VAN DER WAL AND J.C. ZADOKS

Laboratory of Phytopathology, Agricultural University, Wageningen, the Netherlands

SUMMARY

A technique for the mass production of uredospores of *Puccinia recondita* f. sp. *tritici* on wheat seedlings in a controlled environment is described. The course of spore production during the first fortnight of sporulation is presented. Some effects of light intensity and cultivar on spore yield are discussed. The cost of production of one gram of uredospores is estimated at Dfl. 100 (US $ 35).

INTRODUCTION

Large quantities of inoculum are needed for some newer types of experiments. Among those are: (1) experiments designed to analyse "components of resistance" (Zadoks, 1972a) and to detect partial resistance and tolerance; (2) experiments on crop losses, in which the relation between yield and an amount of disease (severity) is studied (James, 1974); and (3) ecophysiological experiments on crop losses, in which not only the amount of disease but also the amount of spores deposited on the leaves has to be known (Van der Wal & Cowan, 1976).

Commercial breeders asked for a technique of direct mass inoculation of selection material in the field with known amounts of spores, comparable to that used for *Fusarium* spp. and *Septoria nodorum*, where a spore suspension is sprayed over the plants. The results of such inoculation techniques can be evaluated relatively quickly, i.e. about one to three latent periods after inoculation. This rapid evaluation of an inoculation result is in striking contrast to the delayed evaluation of early inoculations with small amounts of material as used in the "rust nursery" and the "microfield" techniques (Zadoks, 1972b).

MATERIALS AND METHODS

Seedlings of wheat (*Triticum aestivum* L.) were sown in "multipots" (plastic sheets of 30 x 51 cm each with 51 pots of 5 cm diameter) ten seeds per pot. The multipots were placed on benches measuring 4.0 x 0.5 m. The result was about 3,000 plants per square metre, that were arranged in drills at 5 cm intervals. The "rust factory" consisted of a 30 m^3 walk-in growth cabinet with two benches (one above the other) on each side of a corridor. Light was provided by either fluorescent lamps (Philips TLMF 40 W/33 RS) or high pressure mercury lamps (Philips HPLR 400 W). When the seeds had been sown and covered by about

0.5 cm of soil, Cycocel was sprayed over the seeded pots (3.8 ml Cycocel in 300 ml of water per "multipot") in order to obtain short, springy seedlings. During the first week after sowing the "multipots" were placed under TLMF lamps, at about 10 Klux at soil level, at a light period of 16 hours at 20°C alternating with a dark period of 8 hours at 15°C, and 80% RH. When the second leaf became visible, the "multipots" with plants were transferred to an incubator (Van der Wal & Cowan, 1974). Prior to inoculation the plants were wetted by a mist of water. Uredospores of leaf rust, about 8 mg spores per "multipot" with 500 seedlings, were mixed with an equal volume of *Lycopodium* powder in order to increase the regularity of the spore deposit, and dusted over the seedlings. The spore deposit was about 4 spores per mm^2 leaf area. Inoculated plants were incubated during about 14 hours in the incubator, in the dark, in a water saturated atmosphere at 18°C. The germination percentage of the spores, determined at the end of this incubation period, was always over 85. After incubation, the plants were returned to the growth cabinet. Watering was controlled so that the pots always stood in a water layer, of up to 2 cm depth.

During the latent period, V-shaped smooth cardboard strips were placed between the rows of seedlings. The lower side of the strips was covered with laminar plastic, to prevent the absorption of water from the wet soil by the cardboard. Three days after finding the first open pustules, the tips of the leaves were gently touched so that the ripe spores fell on the strips. The spores were then harvested by means of a cyclone collector (Cherry & Peet, 1966). Harvested spores were weighed and transferred into glass ampoules for cryogenic storage (Loegering *et al.*, 1966). An ampoule of 1 ml usually contained not more than 100 mg of spores. This procedure was repeated at two to three days' intervals, three times a week, over a period of 10 months.

RESULTS

Spore production. The course of the spore production is given in Fig. 1. The curve peaks on day 3 after the beginning of sporulation. This indicates that the spore production rate was maximal during the first three to four days. After 12 days of sporulation the spore production rate decreased below 25% of the initial value, and harvesting was discontinued. By then, the plants showed large chlorotic areas and were often wilted.

Effect of light and cultivar on spore production. When the plants were placed under TL lamps at 10 Klux towards the end of a latent period of one week, the spore production on cv. Orca followed the lower curve of Fig. 2. On cv. Felix the production of spores by the same isolate was higher (Fig. 2, middle curve). Seedlings of cv. Felix, placed under HPLR-lamps during the sporulation period at a light intensity of about 20 Klux produced about 60 per cent more spores than those under TL-lamps at 10 Klux. The spore production rates decreased faster, and the wilting of the infected plants started earlier under HPLR-lamps than under TL. These results suggest that spore production rates are related to light intensity during the sporulation period, a high light intensity leading to a high spore production rate, probably indirectly via the rate of photosynthesis.

The heat load imposed upon the rusted plants by the 400 W HPLR-lamps must have accelerated transpiration. The greater transpiration of rusted plants as compared with healty plants under the same circumstances (Van der Wal *et al.*, 1975) combined with this extra heat load probably brought the water potential in the leaf down to the wilting point after about 10 days of sporulation. The plants wilt, and the spore production ceases.

A spore deposit of about 4 spores per mm^2 leaf area appeared to be optimal for the race-cultivar combinations used. This deposit almost equals one germinating spore per 10 stomata at the upper leaf side. Halving or doubling the spore deposit led to lower spore yields. NB

Some production data. One seedling produces up to 1 mg. but on the average 0.7 mg of spores during a sporulation period of 12 days. The inoculation density was 17 µg (= approx. 4,000 spores) per seedling; the multiplication factor was 700/17 = approx. 40. The production was organized in batches of 6,000 seedlings each, inoculated with 100 mg of spores, and yielding well over 4 g of spores on the average. Four batches were handled simultaneously with intervals of one week between successive batches. During a period of continuous production the average yield of uredospores per day was approx. 6,000 x 0.7/14 = 4,200/14 = 300 mg per day. The total spore production during the three winter seasons 1972/1975 was 120 g. DJS what is our capa

Costs of mass uredospore production. The costs of the growth cabinets at the Laboratory of Phytopathology were calculated to be about Dfl. 2.-- per day per m^3 (including depreciation, inflation correction, maintenance, energy consumption, but excluding overhead costs and taxes). The fluctuation in temperature of the inblown air was ± 0.2 °C, notwithstanding the considerable heat load due to 15 HPLR 400 W-lamps. The fluctuations in the relative humidity were up to ± 5 per cent. A growth chamber of this type is rather too sophisticated for the purpose of uredospore production. It seems unlikely that wider tolerances of temperature and humidity control considerably reduce spore production. Total costs per gram rust produced appeared to be about Dfl. 100,--, or US $ 35.--. Of the costs of uredospore production 75 per cent were attributed to growth chamber facilities and 25 per cent to labour.

Larger technical tolerances, combined with a more refined technique of harvesting the spores, might cut down the production costs in controlled environment to 50 or even 25 per cent of the present estimates.

DISCUSSION

The pattern of spore production shown in Fig. 1 is similar to that obtained by Mehta & Zadoks (1970). Results of tentative experiments using mature plants indicate a less prominent peak in the spore production curves that occurs later than that using seedlings. The production of spores in a controlled environment is safe. It does not depend on weather. Races can be kept separate at least when they are grown one after the other with intermittent cleaning of the growth chamber. The quality of the product as expressed in purity and in germination percentage is high and constant. Because of the reproducibility of the uredospore production by a given race-cultivar combination, labour and growth chamber availability can be planned in accordance with the amount of inoculum needed, and with other departmental activities. It must be admitted the isolation and other aspects (e.g. negative pressure arrangements) of the growth cabinet used were so poor, that the environment of the growth cabinet was heavily infested by brown rust.

The method of mass production of uredospores in a controlled environment during a limited period of the year is profitable only in conjunction with good storage facilities for the spores produced. At the Laboratory of Phytopathology, storage takes place in sealed vacuum-tight ampoules under liquid nitrogen (Loegering *et al.*, 1966). The nitrogen stored inoculum, when treated adequately, is of high quality, but occasional failure must be admitted.

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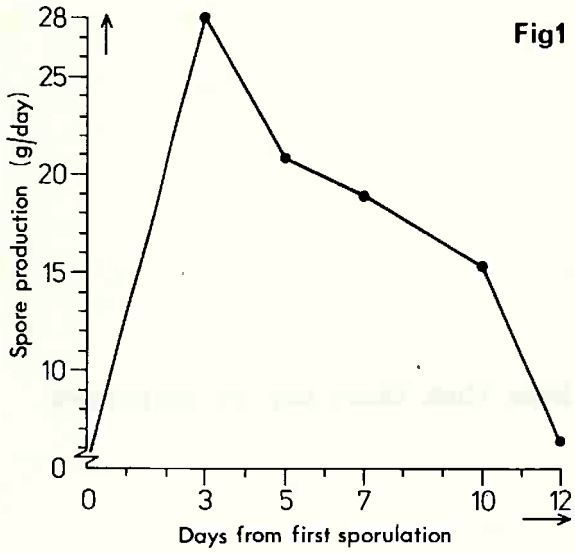
TEXT - FIGURES

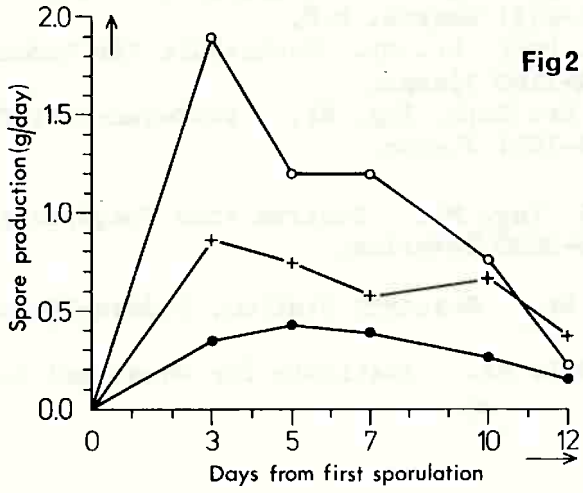
Fig. 1. The course of spore production (grams) on seedlings during 12 days following the observation of the first open pustules. Data refer to spores harvested at the indicated times, averaged over cultivars, light treatments, and spore deposits. The yield is expressed in grams harvested on day indicated: total yield from 127,000 seedlings.

Fig. 2. The effects of cultivar and light on spore production. In all experiments, the same isolate (from cv. Flamingo) was used.

-	=	cv. Orca ;	TL-lamps (10 Klux)
+ -	=	cv. Felix;	TL-lamps (10 Klux)
o -	=	cv. Felix;	HPLR-lamps (20 Klux)

The yield is expressed as grams per batch harvested on day indicated. Figures are averages of 8 batches.





LIST OF CEREAL RUST WORKERS IN THE EUROPEAN AND MEDITERRANEAN AREA

An attempt has been made to compile a list of rust workers in the European and Mediterranean area. Information has been gathered from National Correspondents and from lists of those who have attended Rust Conferences. I do not know whether the list is complete or whether addresses are accurate but hope that, despite any shortcomings, the list will prove useful. I have tried to indicate the main areas of interest of those listed according to the following key:

1. Epidemiology
2. Disease assessment - variety testing
3. Estimation of losses due to disease
4. Genetics of host resistance
5. Physiologic specialisation
6. Genetics of pathogen virulence
7. Host-pathogen physiology - Mechanisms of resistance
8. Resistance breeding
9. Disease control especially by fungicides
10. Taxonomy and Evolution of rusts

Rust species

a. *Puccinia graminis* on any host
b. *P. recondita*
c. *P. striiformis* on any host
d. *P. hordei*
e. *P. dispersa*
f. *P. coronata*

I apologise for any misclassifications and hope that these may be corrected in future lists.

R. Johnson

AUSTRIA

ADAM, J. Dipl. Ing. Mr. Pflanzenzucht Neuhof-Rohrau, Schloss Rohrau,
" A-2471 Rohrau, N.Ö. (8,a,b)

HANSEL, H. Prof. Dr. Mr. Hochschule für Bodenkultur, Gregor Mendelstrasse 33,
A-1180 Vienna. (8,a,b)

ZWATZ, B. Dr. Dipl. Ing. Mr. Bundesanstalt für Pflanzenschutz, Trunnerstrasse 5,
A-1021 Vienna. (2,9,a,b)

BELGIUM

NICLAES, J. Ing. Mr. Centrum voor Toegepaste Biologie, de Croylaan 6,
B-3030 Heverlee.

BULGARIA

DIMOV, A. Mr. Research Station, Sadovo-Plovdiv,
(2,5,b)

DONCHEV, N.I. Mr. Institute for Wheat and Sunflower, Near Tolbuhin.
(2,5,8,a,b)

BULGARIA (cont.)

GOSPODINOVA, E. Mrs. Institute for Plant Protection, Kostinbrod-Sofia. (1,d)

KHINKOVA, C. Mrs. Botanical Institute of the Bulgarian Academy of Sciences, Sofia 13. (10,a,b,c,d,e)

KARDJIN, H. Mr. Institute for Plant Protection, Kostinbrod-Sofia. (1,5,a)

KRISTEV, K. Prof. Mr. Agricultural College "V. Kolarov", Plovdiv. (7,a,b,c)

KONOVSKI, J.S. Mr. Institute of Genetics and Plant Breeding, Sofia 13. (4,5,8,a)

MALINSKI, K.G. Mr. Institute for Wheat and Sunflower, Near Tolbuhin. (8,a,b)

CYPRUS

DELLA, A. Mrs. Agricultural Research Institute, Nicosia. (8,a,b,c,d)

HADJICHRISTODOULOU, A. Dr. Mr. Agricultural Research Institute, Nicosia. (8,a,b,c,d)

CZECHOSLOVAKIA

BARES, I. Ing. Mr. Research Institute of Crop Production, 16106 Praha-Ruzyňe 507. (2,4,8)

BARTOŠ, P. Ing. Mr. Research Institute of Crop Production, 16106 Praha-Ruzyňe 507. (2,4,5,6,8,a,b)

BENADA, J. Dr. Ing. Mr. Research Institute of Cereal Crops, 76741 Kroměříž, " Havlíčkova 2787. (7,9)

BRUCKNER, F. Ing. Mr. Research Institute of Cereal Crops, 76741 Kroměříž, Havlíčkova 2787. (2,4,5,8,d)

CAGAŠ, B. Ing. Mr. Research Station for Grasses, 75661 Roznov pod Radhoštém. (g)

HANIŠ, M. Ing. Mr. Plant Breeding Station, 25084 Stupice p. Sibřina. (2,8,a,b,c)

KNYTL, V. Ing. Mr. Plant Breeding Station, 25084 Stupice p. Sibřina. (2,8,a,b,c)

KOŠNER, J. Ing. Mr. Research Institute of Crop Production, 16106 Praha-Ruzyňe 507. (4,a,b,c)

ONDRÁČKOVÁ, J. Dr. Miss. Charles University, Faculty of Science, 12801 Praha 2, Benátská 2. (10)

PAULECH, C. Prof. Dr. Mr. Institute of Experimental Biology and Ecology SAS, Dubravska 26, Bratislava 80900. (1,2,3,4,5,8,9)

RUSNAK, J.M. Mr. Slovosivo, Zahradnicka 21, Bratislava 800 00. (8)

ŠEBESTA, J. Ing. Mr. Research Institute of Crop Production, 16106 Praha-Ruzyňe 507. (1,3,4,8,9,a,f)

SLOVENČÍKOVÁ, V. Ing. Mrs. Research Institute of Crop Production, 16106 Praha-Ruzyňe 507. (2,4,5,8,9,c)

STUCHLIKOVA, E. Ing. Miss. Research Institute of Crop Production, 16106 Praha-Ruzyňe 507. (2,4,8,a,b)

URBAN, Z. Dr. Mr. Charles University, Faculty of Science, 12801 Praha 2, Benátská 2. (10)

VALKOUN, J. Dr. Mr. Institute of Genetics and Plant Breeding, 16106 Praha-Ruzyne 507. (4,5,6,a,b)

ZADRAŽIL, K. Ing. Mr. Plant Breeding Station, 53832 Uhřetice. (2,4,8,a,b,c)

DENMARK

ANDERSEN, A.H. Mr. Landbrugets Kornforaedling, Sejet, 8700 Horsens. (2,8,a,b,c,d)

BAGGER, O. Mr. State Plant Pathology Institute, Lottenborgvej 2, Lyngby D-2800. (1,8,c)

HERMANSEN, J.E. Dr. Mr. Royal Veterinary and Agricultural University, Department of Plant Pathology, 1871 Copenhagen V. (1,2,4,c)

JENSEN, J. Dr. Mr. Danish A.E.C. Research Establishment, Risø, 4000 Roskilde. (4,c,d)

JØRGENSEN, J.H. Dr. Mr. Danish A.E.C. Research Establishment, Risø, 4000 Roskilde. (4,c,d)

LEITZKE, J. Mr. Pajbjergfonden, Overbygaard, 7080 Børkop. (2,8,a,b,c,d)

MUNK, A. Mr. Landbrugets Kornforaedling, Sejet, 8700 Horsens. (2,4,a,b,c,d)

RASMUSSEN, J. Dr. Miss. Government Experimental Station, Tystofte, 4230 Skaelskør. (2,4,a,b,c,d)

VIVE, K. Mr. Abed Planteavlsstation, 4920 Søllested. (2,4,a,b,c,d)

EGYPT

ABDEL-HAK, T. Dr. Director General, Department of Plant Pathology, Giza, Orman. (1,2,5,9,a,b,c)

CHAUDHRY, A.H.M. Dr. Mr. F.A.O. Regional Office for the Near East, P.O. Box 2223, Cairo. (2,8,a,b,c)

KAMEL, A.H. Dr. Cereal Disease Section, Giza, Orman. (2,8,9,a,b,c)

SAARI, E.E. Dr. Mr. The Ford Foundation, P.O. Box 2344, Cairo. (1,2,8,a,b,c)

SRIVASTAVA, J.P. Dr. Mr. The Ford Foundation, P.O. Box 2344, Cairo. (2,4,8,a,b,c)

STEWART, D.M. Dr. Mr. Liaison Officer and Acting Project Manager, c/o Resident Representative of U.N.D.P., P.O. Box 982, Cairo. (2,5,a,b)

FRANCE

AURIAU, P. Mr. I.N.R.A., Station d'Amélioration des Plantes, Route de Saint-Cyr, 78000 Versailles. (8,c)

BERBIGIER, A. Mr. I.N.R.A., Station d'Amélioration des Plantes, Domaine de Mon Désir, 63000 - Clermont Ferrand. (8,d,e)

CAUDERON, Y. Mrs. I.N.R.A., Station d'Amélioration des Plantes, Route de Saint-Cyr, 78000 - Versailles. (4,b,c)

CHERY, J. Mr. E.N.S.A.M., Laboratoire d'Amélioration des Plantes, 9 Place Viala, 34000 - Montpellier. (8,d)

DOUSSINAULT, G. Mr. I.N.R.A., Station d'Amélioration des Plantes, E.N.S.A.R., 65 Route de Saint-Brieux, 35000 - Rennes. (8,c,f)

GRIGNAC, P. Mr. E.N.S.A.M., Laboratoire d'Amélioration des Plantes, 9 Place Viala, 34000 - Montpellier. (8,b)

de LARAMBERGUE, R. Mr. I.N.R.A., Station d'Amélioration des Plantes, 21000 - Dijon Epoisses. (8,d)

MASSENOT, M. Mr. I.N.A. Laboratoire de Pathologie Végétale, 78850 - Thiverval-Grignon. (5,a)

PAUVERT, P. Mr. I.N.R.A., Station d'Amélioration des Plantes, Route de Saint-Cyr, 78000 - Versailles. (1)

RAPILLY, F. Mr. I.N.R.A., Station Centrale de Pathologie Végétale, Route de Saint-Cyr, 78000 - Versailles. (1,9,a,c)

TROTTET, M. Mr. I.N.R.A., Station d'Amélioration des Plantes, B.P.29 35650 le Rheu. (8)

GERMANY - EAST

HERDAM, L. Dr. Mrs. Institut für Getreideforschung, Bernberg - Hadmersleben, DDR 3234 Hadmersleben. (5,8,c)

MULLER, H.J. Dr. Mr. Institut für Phytopathologie, Theodor-Roemer-Weg 1-4 DDR 431 Aschersleben. (7,c)

NOVER, I. Dr. Mrs. Institut für Phytopathologie, Martin-Luther-Universität, Ludwig-Wucherer Str. 2, DDR 401 Halle (Saale). (4,5,b,c,d)

SCHMIEDEKNECHT, M. Dr. Mr. Institut für Phytopathologie, Theodor-Roemer-Weg 1-4 DDR 431 Aschersleben. (7,c)

UNGER,O. Mr.VEG - Saatzucht, Saatzuchtstation, DDR 3721 Bohnshausen, üb Blakenburg (Harz). (5,8,b,c)

GERMANY - WEST

AHLBORN, D. Mr. BASF AG, Hemshof Center 1413, Ludwigshafen 67. (9,a,b,c,d)

BUCHENAUER, H. Dr. Mr. Institut für Phytopathologie und Pflanzenschutz, Universität Hohenheim, Otto-Sander-Strasse 5, 7 Stuttgart 70. (9,a,b,c,d)

EFFLAND, H. Dr. Mr. Institut BASF, Holtsten Strasse 88, Kiel 23. (9,a,b,c,d)

FISCHBECK, G. Prof. Mr. Institut für Pflanzenbau und Pflanzenzüchtung, 8050 Freising - Weihenstephan. (4,5,a,b,c,d)

FROHBERGER, P. Dr. Mr. Pflanzenschutz AT, Biologische Forschung, Bayer AG, Bayerwerk, 509 Leverkusen. (9,a,b,c,d)

FUCHS, E. Dr. Mrs. Biologische Bundesanstalt für Land- und Forstwirtschaft, Messeweg 11/12, Braunschweig 33. (5,c)

FUCHS, W.H. Prof. Mr. Nicklausberger Weg 144, 34 Göttingen.

HAMPEL, M. Dr. Mr. Landwirtschaftliche Versuchsstation, BASF AG, Postfach 220 Limburgerhof 6703. (9,a,b,c,d)

HASSEBRAUK, K. Prof. Mr. Waterloostrasse 21, Braunschweig 33. (4,5,a,b,c)

HEITEFUSS, R. Prof. Mr. Institut für Pflanzenpathologie und Pflanzenschutz, Grisebachstrasse 6, Göttingen 3400. (7)

HOESER, K. Mr. Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Vöttinger 38, Freising 8050. (8,a,b,c)

LEIN, A. Dr. Mr. F. von Lochow-Petkus GMBH, Zuchtstation Wetze 3411 Stöckheim. (8,a,b,c)

OPPITZ, K. Dr. Mr. Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Vöttinger 38, Freising 8050. (8)

POMMER, E.H. Dr. Mr. Landwirtschaftliche Versuchsstation, BASF AG, Postfach 220, Limburgerhof 6703. (9,a,b,c,d)

REHSE, E. Dr. F. Von Lochow-Petkus GMBH, Zuchtstation Wetze, 3411 Stöckheim. (8,c,d)

RINTELEN, J. Dr. Mr. Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, " Menzingerstrasse 54, 8000 München 19. (5)

ROBBELEN, G. Prof. Dr. Mr. Institute of Plant Breeding, von Siebold 9, Göttingen 34. (4,8)

TAN, B.H. Dr. Mr. Max Planck Institut für Züchtungsforschung, 5 Köln-Vogelsang. (4,5,8,d)

ZELLER, F.J. Dr. Mr. Institut für Pflanzenbau und Pflanzenzüchtung, 8050 Freising-Weihenstephan. (4,a,b,c)

GREECE

MICHAILIDIS, V. Mr. Agricultural Research Station Ptolemais, Martion 25 84, Ptolemais.

SKORDA, E.A. Dr. Miss Plant Breeding Institute, Thessaloniki. (5,8,9,a)

HUNGARY

BALAZS, E. Dr. Mr. Research Institute for Plant Protection, 1525 Budapest, P.O.B. 102. (7,a,b)

BARABÁS, Z. Dr. Mr. Cereal Research Institute, 6701 Szeged, P.O.B. 391. (2,8,a,b)

BARNA, B. Dr. Mr. Research Institute for Plant Protection, 1525 Budapest, P.O.B. 102. (7,a,b)

BEKE, F. Dr. Mr. Plant Breeding Station, Tàplànszentkereszt. (2,8,a,b)

BOCSA, E. Mrs. Research Institute for Plant Protection, 1525 Budapest, P.O.B. 102. (5,a,b)

KIRÁLY, Z. Dr. Mr. Research Institute for Plant Protection, 1525 Budapest, P.O.B. 102. (7,a,b)

MANNINGER, M. Mrs. Institute of Agriculture of the Hungarian Academy of Science, Martonvásár. (2,8,a,b)

MESTERHÁZY, A. Dr. Mr. Cereal Research Institute, 6701 Szeged, P.O.B. 391. (2,8,a,b)

SZIRÁKI, I. Mr. Research Institute for Plant Protection, 1525 Budapest, P.O.B. 102. (7,a)

IRAN

BAMDADIAN, A. Mr. Plant Pests and Disease Research Institute, P.O. Box 3178, Evin, Tehran. (5,a,b,c)

DADAIN, M. Mr. Seed Improvement Institute, Ahwaz. (2,8,a,b,c)

DANESHPAGHOOH, P. Mr. Plant Pest and Disease Research Institute, P.O. Box 3178, Evin, Tehran. (5,a,b,c)

KHAZRA, H. Mr. Seed Improvement Institute, Eslahe Nebatat, Karaj. (2,8,a,b,c)

SADRI, B. Mr. Seed Improvement Institute, Eslahe Nebatat, Karaj. (2,8,a,b,c)

YAZDI-SAMADI, B. Mr. Agriculture Faculty, Karaj. (2,4,a,b,c,d)

ISRAEL

DINOOR, A. Prof. Mr. Faculty of Agriculture, P.O. Box 12, Rehovot. (4,5,6,9,f)

GERECHTER-AMITAI, Z.K. Prof. Mr. Agricultural Research Organization, The Volcani Center, P.O.B.6 Bet Dagan. (4,5,6,a,f)

WAHL, I. Prof. Mr. Department of Botany, University of Tel Aviv, Ramat-Aviv. (4,5,8,a,b,c,d,f)

ITALY

BASILE, R. Dr. Miss Istituto Sperimentale di Patologia Vegetale, Via Casal de' Pazzi 250, 00156 Roma. (5,a,b)

CARRIELLO, G. Dr. Mr. Istituto di Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari. (2,5,f)

PARADIES, M. Dr. Mrs. Istituto di Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari.

PIGLIONICA, V. Dr. Mr. Istituto di Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari. (2,5,c)

SISTO, Dr. D. Mr. Istituto di Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari. (2,5,a)

TARANTINI, P. Dr. Mr. Istituto di Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari. (2,5,b)

VALLEGA, J. Dr. Mr. Istituto di Patologia Vegetale, Università degli Studi, Via Amendola 165/A, 70126 Bari. (2,4,5,8,a,b,c,d)

ZITELLI, G. Dr. Miss Istituto Sperimentale per la Cerealicoltura, Via Cassia 176, 00100 Roma. (4,8,a,b)

NETHERLANDS

BOOMSTRA, A. Dr. Mrs. 'De Haaff' Foundation for Agricultural Plant Breeding, P.O. Box 177, Wageningen. (8)

DIJKHUIS, J.P. Mr. Breeding Station 'Luidenburg', Baron van Asbeckweg 2, Warfhuizen. (8,c)

FEEKES, W. Dr. Ir. Mr. Breeding Station 'G. Geertsma', Kleiweg 9, Emmeloord. (8,c)

GELING, K.B. Ir. Mr. Breeding Station 'Van de Have', Fredericapolder, Rilland-Bath. (8,c)

GROENEWEGEN, L.J.M. Ir. Mr. Breeding Station 'Zelder', Ottersum (L.) (4,8,c)

MASTENBROEK, C. Dr. Ir. Breeding Station 'Cebeco', Lisdoddeweg 34, Lelystad. (8,b,c)

MESDAG, J. Dr. Mr. Foundation of Agricultural Plant Breeding, Droevendaalsesteeg, Wageningen. (8,b,c)

PARLEVLIET, J.E. Dr. Ir. Mr. Agricultural University, Department of Plant Breeding, Lawickse Allee 166, Wageningen. (2,4,5,8,d)

PRUMMEL, W. Ir. Mr. Breeding Station 'Verenigde Kweekbedrijven', Burchtweg 17, Emmeloord. (8,c)

RIJSDIJK, F.H. Mr. Agricultural University, Laboratory of Phytopathology, Binnenhaven 9, Wageningen. (1,2,b,c)

SCHELLING, P. Ir. Mr. Breeding Station 'Cebeco', Lisdoddeweg 34, Lelystad. (8,c)

SLOOTMAKER, L.A.J. Ir. Mr. Foundation of Agricultural Plant Breeding, Droevendaalsesteeg, Wageningen. (2,8,b,c,d)

STUBBS, R.W. Ir. Mr. Institute for Phytopathological Research, Binnenhaven 12, Wageningen. (2,5,c)

TEN KATE, R.M. Ir. Mr. Breeding Station 'Wiersum', Lunaweg 4, Dronten. (8,c)

VAN DER WAL, A.F. Ir. Mr. Agricultural University, Laboratory of Phytopathology, Binnenhaven 9, Wageningen. (1,2,b)

VAN SILFHOUT, C.H. Ir. Mr. Institute for Phytopathological Research, Binnenhaven 12, Wageningen.

WOUDA, K.P. Mr. Breeding Station 'Mansholt', Westpolder 8, Ulrum (gr.) (8,c)

ZADOKS, J.C. Dr. Mr. Agricultural University, Laboratory of Phytopathology, Binnenhaven 9, Wageningen. (1,2,5,b,c)

NORWAY

HANSEN, L.R. Dr. Mr. Norwegian Plant Protection Institute, Division of Plant Pathology, 1432 Ås-NLH. (2)

RØED, H. Dr. Mr. Norwegian Plant Protection Institute, Division of Plant Pathology, 1432 Ås-NLH.

POLAND

RALSKI, Prof. Mr. Agricultural Academy Cracow, al. Michiewicza 24. (1,a,b,c)

RYSZ, M. Miss Institute of Plant Breeding and Acclimatization, Cracow, ul. Zawita 4. (2,b)

DWURAZNA, M. Mrs. Immunology Laboratory, Institute of Plant Breeding and Acclimatization, Cracow, ul. Zawita 4. (7,a,b)

GAJDA, Z. Mrs. Institute of Plant Breeding and Acclimatization, Cracow, ul. Zawita 4. (2,5,a)

MUSZYNSKA, K. Miss Institute of Plant Breeding and Acclimatization, Cracow, ul. Zawita 4. (2,4,8,b)

POKACKA, Z. Mrs. Phytopathology Laboratory, Institute of Plant Protection, Poznań, ul. Grunwaldzka 189. (3)

PORTUGAL

ANTUNES, M. M.A. Eng^{a} Agr. Miss Lab. de Fitopatologia, Estação de Melhoramento de Plantas, Elvas. (5,a,f)

FREITAS, A. P.C. Eng^{o} Agr. Mr. Dept. de Fitopatologia, Estação Agronómica National, Oeiras. (1,4,5,8,b)

FREITAS, L. C.L.C.R.A. Dr. Mrs. Dept. de Fitopatologia Estação Agronómica National, Oeiras. (4,5,b)

ROMANIA

BONTEA, V. Dr. Mrs. Research Institute for Plant Protection, Miciurin 48, Bucharest 7000. (1)

DUMITRAS, L. Dr. Mrs. Research Institute for Plant Protection, Bd. I. Ionescu de la Brad 8, Bucharest.

IONESCU-COJOCARU, Ing. Mr. Research Institute for Cereal and Industrial Plants, Fundulea, Jud. Ilfov. (2,4,5,8,a,b)

ITTU, M. Mrs. Research Institute for Cereal and Industrial Plants, Fundulea, Jud. Ilfov. (2,8,a)

MUNTEANU, I. Dr. Mr. Experimental Station Turda, Jud. Cluj. (1,a,b,c)

NEGULESCU, F. Dr. Mrs. Research Institute for Cereal and Industrial Plants, Fundulea, Jud. Ilfov. (4,5,8,c)

SPAIN

BRAÑAS, M. Mr. Instituto nacional de Investigaciones agronomicas, Avenida Puerta de Hierro, Madrid-3. (5,7,a,b)

MARTINEZ, M. Dr. Mrs. Instituto nacional de Investigaciones agronomicas, Avenida Puerta de Hierro, Madrid-3. (5,8,a,b)

SALAZAR, J. Dr. Mr. Instituto nacional de Investigaciones agronomicas, Avenida Puerta de Hierro, Madrid-3. (5,8,a,b)

SWEDEN

LEIJERSTAM, B. Dr. National Institute for Plant Protection, Fack, S-171 07 Solna. (1-9)

LUNDIN, P. Mr. Weibullsholm Plant Breeding Institute, Fack, S-261 20 Landskrona. (2,4,5,6)

MAC KEY, J. Prof. Mr. Agricultural College of Sweden, Department of Genetics and Plant Breeding, S-750 07, Uppsala. (2,4,6,7,8)

MATTSSON, B. Mr. Swedish Seed Association, S-268 00 Svalöv. (2,4,6,8)

MEYER, J. Mr. National Institute for Plant Protection, Laboratory for Plant Resistance, S-268 00, Svalöv. (1-9)

SWITZERLAND

BRONNIMANN, A. Dr. Mr. Swiss Federal Research Station for Agronomy, Zurich-Reckenholz, 8046 Zurich. (8,a,c)

KERN, H. Prof. Dr. Mr. Swiss Federal Institute of Technology, Institute of Special Botany, Universitätstrasse 2, 8006 Zurich.

SCHMIDT, D. Dr. Miss Station Fédérale de Recherches Agronomiques de Changins, Bourgogne 11, Nyon 1260. (7,a)

ZOGG, H. Prof. Mr. Swiss Federal Research Station for Agronomy, Zurich-Reckenholz, 8046 Zurich.

TUNISIA

DJERBI, M. Dr. Mr. Institute Nationale Agronomique de Tunisie, Avenue Charles Nicolle 43, Tunis.

TURKEY

AKTAS, H. Mr. Bölge Zirai Mücadele ve Arastirma Enstitüsü, Diyarbarkir.

AKTUNA, I. Dr. Bölge Zirai Mücadele ve Arastirma Enstitüsü, Diyarbakir.

ALTAY, F. Mr. Zirai Arastrim Istasyonu, Eskisehir.(2,5,b,f)

ATAY, T. Dr. Ege Bölge Zirai Arastirma Enstitüsü, Menemen, Ismir. (2,5,b,f)

TURKEY (contd.)

BABAOĞLU, B. Mr. Bölge Zirai Mücadele ve Arastirma Enstitüsü, Ankara.

BAYSAL, O. Dr. Mr. Bölge Zirai Arastirma Enstitüsü, Yesilköy, Istanbul. (2)

BICICI, M. Mr. Bölge Zirai Arastirma Enstitüsü, Adana. (2,9)

BILGIN, O. Mr. Bölge Zirai Arastirma Enstitüsü, Samsun. (5)

ÇÖPCÜ, M. Mr. Bölge Zirai Mücadele ve Arastirma Enstitüsü, Bornova, Izmir. (a,b,f)

DAMGACI, E. Mr. Bölge Zirai Mücadele ve Arastirma Enstitüsü, Ankara. (2)

DUTLU, C. Mr. Wheat Research and Training Center, P.K. 226, Ankara. (2,5,c)

FINCI, S. Mrs. Bölge Zirai Mücadele ve Arastirma Enstitüsü, Ankara. (2,5)

INCE, S. Dr. Ege Bölge Zirai Arastirma Enstitüsü, Menemen, Izmir. (2,5,c)

IREN, S. Prof. Dr. Mr. Ankara Üniversitesi, Ziraat, Fakültesi, Ankara. (a,f)

OZKAN, M. Dr. Mr. Bölge Zirai Mücadele ve Arastirma Enstitüsü, Ankara. (5,c)

PARLAK, V. Dr. Mr. Bölge Zirai Mücadele ve Arastirma Enstitüsü, Diyarbakir. (5,c)

PRESCOTT, J.M. Dr. Mr. CIMMYT, Rockefeller Foundation, P.K. 226, Ankara. (2,5,9)

SARGIN, E.S. Mr. Bölge Zirai Arastirma Enstitüsü, Antalya. (2)

SAYDAM, C. Mr. Bölge Zirai Mücadele ve Arastirma Enstitüsü, Bornova, Izmir. (5)

TEKINEL, N. Mr. Bölge Zirai Arastirma Enstitüsü, Adana. (5,b,c,f)

USSR

AZBUKINA, Z.M. Dr. Mrs. Biology and Soil Biology Institute, Prospect of the Century 159g, Vladivostok 22. (5,10,a)

BABJANC, L.T. Dr. Mr. All-Union Institute of Genetics and Plant Breeding, Odessa. (5,b)

FEDORENKO, V.S. Dr. Mr. Ukranian Institute of Plant Protection, Vasilkovskaja 33, Kiev 252022. (7,b)

KHOKHRAJAKOV, M.K. Dr. Mr. All-Union Plant Protection Institute (VIZR), Gercen Str. 42, 190000 Leningrad.

KRIVCHENKO, V.I. Dr. Mr. Department of Disease Resistance, All-Union Institute of Plant Industry, Moskowskoe shosse 11, Leningrad-Pushkin 188620. (2,5,b)

LESOVOJ, M.P. Dr. Mr. Ukranian Institute of Plant Protection, Vasilkovskaja 33, Kiev 252022. (2,4,5,6,b)

MIKHAILOVA, L.A. Dr. Mrs. Department of Disease Resistance, All-Union Institute of Plant Industry, Moskowskoe shosse 11, Leningrad-Pushkin 188620. (5,b)

ODINTSOVA, I.G. Dr. Mrs. Department of Disease Resistance, All-Union Institute of Plant Industry, Moskowskoe shosse 11, Leningrad-Pushkin 188620. (5,b)

SHOPINA, V.V. Dr. Mrs. Department of Disease Resistance, All-Union Institute of Plant Industry, Moskowskoe shosse 11, Leningrad-Pushkin 188620. (5,8,b)

VORONKOVA, A.A. Dr. Mrs. Scientific and Research Institute of Krasnodar, Krasnodar. (2,5,8,a,b)

UNITED KINGDOM

BRADSHAW, J.E. Dr. Mr. Scottish Plant Breeding Station, Pentlandfield, Roslin, Midlothian, EH25 9RF (8,c,d)

CHAMBERLAIN, N.H. Dr. Mr. Rothwell Plant Breeders Ltd., Rothwell, Lincoln LN7 6BR (2,5,8,b,c)

CLIFFORD, B.C. Dr. Mr. Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed SY23 3DD (1,2,4,5,8,b,d)

COCK, L. Mr. ADAS, Block C, Government Buildings, Brooklands Avenue, Cambridge CB2 2DR (9,c,d)

DICKINSON, S. Dr. Mr. Department of Applied Biology, Pembroke Street, Cambridge CB3 3DX (7,f)

DOLING, D.A. Dr. Mr. The Lord Rank Research Centre, Lincoln Road, High Wycombe, Bucks, HP12 3QR (2,b,c,d)

DOODSON, J.K. Dr. Mr. National Institute of Agricultural Botany, Huntingdon Road, Cambridge CB3 OLE (2,b,c,d)

GAINES, R. Mr. Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ (4,c)

GILMOUR, J. Dr. Mr. Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG (2,9,b,c,d)

HARDING, S.C. Miss Dept. of Biology, Building 44, The University, Southampton SO9 5NH (7,c)

INGRAM, M. Miss Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG

JENKINS, J.E.E. Mr. ADAS Yorks & Lancs Region, Government Buildings, Lawnswood, Leeds LS16 5PY (2,9,b,c,d)

JOHNSON, R. Dr. Mr. Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ (2,4,5,8,b,c,d)

JUNG, K.U. Mr. BASF United Kingdom Ltd., Agricultural Division, Lady Lane, Hadleigh, Ipswich, Suffolk IP7 6BQ (9,b,c,d)

KAY, J.G. Dr. Mr. The Miln Marsters Group Ltd., Plant Breeding Station, Churton Road, Farndon, Chester CH3 6QP (8,b,c,d)

KING, J.E. Dr. Mr. Ministry of Agriculture Fisheries & Food, Plant Pathology Laboratory, Hatching Green, Harpenden, Herts. AL5 2BD (1,2,b,c,d)

LABRUM, K.E. Miss Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ (4,7,c)

MACER, R.C.F. Prof. Mr. Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG (1,4,8,9,b,c,d)

McGREGOR, A.J. Mr. Dept. of Biology, Building 44, The University, Southampton SO9 5NH (1,c)

McKEOGH, P.A. Miss Department of Botany & Microbiology, University College of London, Gower Street, London WC1E 6BT (7,c)

MANNERS, J.G. Dr. Mr. Dept. of Biology, Building 44, The University, Southampton SO9 5NH (1,2,5,6,7,c,d)

OSMAN-GHANI, N. Mrs. Department of Biology, Building 44, The University, Southampton SO9 5NH (1,c)

POTTER, J.E. Miss Department of Biology, Building 44, The University, Southampton SO9 5NH

PRIESTLEY, R.H. Dr. Mr. National Institute of Agricultural Botany, Huntingdon Road, Cambridge CB3 OLE (2,5,b,c,d)

RASTEGAR, M. Mrs. Department of Biology, Building 44, The University, Southampton SO9 5NH (1,d)

RUSSELL, G.E. Prof. Mr. Department of Agricultural Biology, The University, Newcastle upon Tyne NE1 7RU (7,c)

STRANGE, R.N. Dr. Mr. Department of Botany & Microbiology, University College of London, Gower Street, London WC1E 6BT (7,c)

UNITED KINGDOM (contd.)

TAYLOR, E.C. Miss Department of Biology, Building 44, The University, Southampton S09 5NH (6,c)

THOMAS, J. Miss Department of Biology, Building 44, The University, Southampton S09 5NH (7,c)

WALKER, A.G. Dr. Mr. ADAS South Western Region, Burghill Road, Westbury-on-Trym, Bristol BS10 6NJ (2,5,9,b,c,d)

WRIGHT, R.G. Mr. Edinburgh School of Agriculture, West Mains Road, Edinburgh EH9 3JG (2,5,c)

YUGOSLAVIA

BOROJEVIC, K. Prof. Dr. Mrs. Faculty of Agriculture, Department of Genetics, University of Novi Sad, Akademska 2, 21000 Novi Sad. (8,b)

BOŠKOVIĆ, M. Dr. Mr. Faculty of Agriculture, Institute for Plant Protection, 21000 Novi Sad, Akademska 2. (2,5,8,b)

ENGELMANN, M. Dr. Mrs. Institute for Plant Breeding and Agronomy, Maruličev Trg 5/1, Zagreb 41000.

KOSTIĆ, B. Dr. Mr. Institute for Agricultural Research, 21000 Novi Sad, Maksima Gorkog 30. (2,5,a)

MACEK, J. Prof. Dr. Mr. Biotechniska Fakulteta, Krekov Trg 1, Ljubljana 61001.

MOMCILOVIC, V. Mr. Institute for Agricultural Research, 21000 Novi Sad, Maksima Gorkog 30. (8,b)

POTAĆANAC, J. Dr. Mr. Institute for Plant Breeding and Agronomy, Maruličev Trg 5/1, Zagreb 41000. (8,a,b)

SPEHAR, V. Dr. Mrs. Institute for Plant Breeding and Agronomy, Maruličev Trg 5/1, Zagreb 41000.

VLAHOVIĆ, V. Mrs. Institute for Plant Breeding and Agronomy, Maruličev 5/1, Zagreb 41000.

EUROPEAN AND MEDITERRANEAN CEREAL RUSTS FOUNDATION

FINANCIAL REPORTS

Income and expenditure account, 1 January 1974 to 31 December 1974

RECEIPTS			PAYMENTS		
Subscriptions 1973	f.	516.10	Bank charges	f.	419.07
Subscriptions 1974	"	3817.-	Administration	"	875.55
Bank interest	"	61.38	Cereal Rusts Bull.	"	2220.38
Charter Members	"	352.74	Excess of income over expenditure	"	1546.95
Reprints	"	74.73			
Proceedings	"	240.-			
	f.	5061.95		f.	5061.95

Balance sheet as at 31 December 1974

CREDIT			DEBIT		
Funds at bank	f.	5119.22	Foundation costs	f.	171.-
			Capital 31.12.'73: f. 3401.27		
			Excess of income over expenditure 1546.95		
			Capital 31.12.'74	"	4948.22
	f.	5119.22		f.	5119.22

List of grants received (New Charter Members)

1974

I.P.O.	The Netherlands
Mrs. Dr. Eva Fuchs	Germany
Bayer	Germany
Lochow-Petkus	Germany
Dr. Karl Hoeser	Germany

1975

Dr. R. Johnson	United Kingdom

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